CHARACTERIZATION AND PROCESS OPTIMIZATION STUDY ON PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES CHANGES IN OIL ISOLATES DRIED UNDER VARIOUS TEMPERATURE AND TIME

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DECLARATION

I hereby declare that this project report is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that it has not been previously and concurrently submitted for and other degree or award at UTAR or other institutions.

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APPROVAL FOR SUBMISSION

I certify that this project report entitled "**CHARACTERIZATION AND PROCESS OPTIMIZATION STUDY ON PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES CHANGES IN OIL ISOLATES DRIED UNDER VARIOUS TEMPERATURE AND TIME**" was prepared by **KANG YI JIN** has met the requirement standard for submission in partial fulfilment of the requirements for the award of Bachelor of Engineering (Hons.) Petrochemical Engineering at Universiti Tunku Abdul Rahman.

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Date : 29th April 2023

To my beloved parents, siblings, and supervisor.

I dedicate this thesis to you as a token of my profound gratitude for your unwavering support, encouragement, and guidance throughout my academic journey. Your constant love and belief in me have been my source of strength and inspiration, and I could not have reached this milestone without you.

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ABSTRACT

The increasing human population causing food supply to be insufficient. Due to high nutritional values of edible insects, it has been studied by researchers in the world to prevent the insufficient food supply. In this study, the objectives are to examine the effect of drying time and temperature of the oil properties, optimize the drying parameters by using Response Surface Methodology (RSM), and characterize the changes on the functional properties of oil. The targeted edible insect use in this study is Black Soldier Fly prepupae (BSFP) which were fed with fruit waste. Based on the optimization results, the moisture loss was 65.31 % at 100 ℃ and 34.70 hours, crude lipid yield was 49.79 % at 88.29 ℃ and 29.82 hours, browning index was measured to have a value of 294.70 at 100 ℃ and 48 hours, iodine value of 23.70 gI_2/ 100g at 90.89 ℃ and 34.82 hours. Meanwhile, the optimized peroxide value obtained was 8.20 mEq O_2/ kg at 100 ℃ and 48 hours, and DPPH Free Radical Scavenging Assay (antioxidant activity) of 76.14 % and 99.60 ℃ and 47.93 hours. In conclusion to this study, the oven dried prepupae undergo different drying parameters of 80 ℃ to 100 ℃ and 24 hours to 48 hours, produced a maximized crude lipid of 49.74 % at 88.29 ℃ and 29.82 hours.

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LIST OF SYMBOLS / ABBREVATIONS

°C	Degree Celsius
g	Gram
k	Numeric Factor
kg	Kilogram
M	Molarity
m	Metre
mEq	milliequivalent
mg	milligram
min	minutes
mL	Millilitre
mm	Millimetre
N	Normality
wt %	Weight Percent
α	Significance Threshold, Alpha Level
μm	Micrometre

3D	Three-dimensional
AFB1	Aflatoxin B1
ANOVA	Analysis of Variance
AOAC	Association of Official Agricultural Chemists
AP	Adequate Precision
BI	Browning Index
BSF	Black Soldier Fly
BSFC	Brake Specific Fuel Consumption

BSFL	Black Soldier Fly Larvae
BSFLP	Black Soldier Fly Protein
BTE	Brake Thermal Efficiency
C	Carbon
CO_2	Carbon Dioxide
CCD	Central Composite Design
CEW	Coconut Endosperm Waste
CN	Cetane Number
D	Drying Duration
DPB	Dried Prepupae Biomass
DPPH	2,2-diphenyl-1-picrylhydrazyl
DSC	Differential Scanning Calorimetry
FAO	Food and Agriculture Organization
H_2	Hydrogen
H_2SO_4	Sulphuric Acid
I_2	Iodine
IV	Iodine Value
K	Potassium
KOH	Potassium Hydroxide
LHG	Longhorn Grasshopper
MD	Microwave Dried
MUFA	Monounsaturated Fatty Acid
N	Number of Set
N_2	Nitrogen
OD	Oven Dried
O_2	Oxygen
PUFA	Polyunsaturated Fatty Acid
PV	Peroxide Value
R^2	Regression Coefficient
RSM	Response Surface Methodology
SFA	Saturated Fatty Acid
T	Drying Temperature
TAGs	Triacylglycerols

UFA	Unsaturated Fatty Acid
YMB	Yellow Mealworm Beetle
YML	Yellow Mealworm Larvae

CHAPTER 1

INTRODUCTION

1.1 Overview of Study

The nutritional requirements of animal, avian, and aquaculture feed vary based on the specific needs of the animal. As stated by Kiarie and Mills (2019), the nutritional content of the feed is vital in determining the growth, development, and overall health of the animals. The feed for animal typically consists of a combination of protein, energy, minerals, and vitamins. Protein is essential for muscle growth and repair, while energy is required for metabolic processes and body maintenance (Carbone and Pasiakos, 2019). Minerals and vitamins are also necessary for bone development, immune function, and hormone regulation (Tardy et al., 2020).

Avian feed, due to the unique digestive system of birds, contains a higher percentage of protein than animal feed (Mtei et al., 2019). Protein is critical for egg production, feather development, and overall growth and development of the bird. According to (Junqueira et al., 2006), the increase in dietary protein level in avian feed able to improve the egg production and feed efficiency in laying hens. Bartter et

al. (2018) emphasized that, avian feed includes minerals and vitamins, with calcium being particularly important for eggshell production. In aquaculture, feed plays a crucial role in the growth and development of fish and other aquatic organisms. Fish feed usually contains protein, fat, carbohydrates, as well as minerals and vitamins. The quality and quantity of protein in the feed are particularly significant, as it is essential for muscle growth and overall body composition. According to Ye et al. (2006), adequate dietary phosphorus supplementation positively influences bone development, overall fish health, and muscle composition. The nutritional value of the feed also affects the cost-effectiveness and sustainability of animal, avian, and aquaculture production. Poor nutritional value feed can result in poor animal performance, increased feed intake, and higher costs for the farmer. Conversely, high nutritional value feed can lead to better animal performance and lower feed costs (Weihe et al., 2018). In general, the nutritional value of animal, avian, and aquaculture feed is vital for the growth, development, and overall health of the animals. The quality and quantity of protein, energy, minerals, and vitamins in the feed are essential factors in ensuring optimal animal performance and sustainable production.

As a sustainable alternative to traditional animal feed ingredients, insects have gained momentum in animal, avian, and aquaculture production. Insect protein is highly nutritious and considered a cost-effective and environmentally friendly feed source (Vauterin et al., 2021). Insects such as black soldier fly, house fly, mealworm, and cricket are commonly used as feed due to their high crude protein content of up to 75% and presence of essential amino acids, minerals, and vitamins (Bessa et al., 2020). Cullere et al. (2018) also highlight the use of black soldier fly larvae as a dietary protein source and demonstrate its positive effects on growth performance, carcass traits, and meat quality in broiler quails which in line with the findings from vitamins (Bessa et al., 2020).

Studies show that using insects as feed can boost animal performance and reduce production costs (Sogari et al., 2023). For example, feeding broiler chickens with black soldier fly larvae resulted in better weight gain and feed conversion ratios than traditional feed (St-Hilaire et al., 2007). Rainbow trout fed with mealworms showed improved growth performance and higher protein efficiency ratios than those fed with fishmeal (Makkar et al., 2014). One of the primary reasons for the growing

interest in insect farming for feed production is its sustainability compared to traditional livestock farming. Insects can be raised on organic waste, emit fewer greenhouse gases, require less water and space than traditional farming, and have a smaller environmental footprint (van Huis and Oonincx, 2017). This makes insect farming a more eco-friendly and cost-effective alternative to traditional livestock farming. In addition, insects are widely available and easy to rear in small spaces, which makes them accessible and cost-effective (van Huis et al., 2013). They also provide a rich source of protein and essential amino acids, making them a valuable nutritional component in animal feed.

Moreover, animal feed commonly utilizes fish meal and insect meal as protein sources. Although fish meal has been traditionally used, insect meal has gained popularity as a sustainable alternative due to its high nutritional value and lower environmental impact (Vauterin et al., 2021). Fish meal production involves processing fish waste, which is then dried and ground into a fine powder (Fitri et al., 2022). The drying process comprises two stages: initial drying and final drying. The former involves sun-drying to remove surface moisture, while the latter employs specialized equipment such as rotary or flash dryers to decrease moisture content to less than 10% (Fitri et al., 2022). The dried fish meal is stored in airtight containers to prevent moisture intrusion and subsequent spoilage.

On the other hand, insect meal production involves the process of breeding, process of inactivation, and drying of insects. The drying stage is crucial as it reduces moisture content, thereby prolonging product shelf life and preventing microorganism growth (Oonincx and de Boer, 2012). Various methods of drying insect meal exist, such as sun drying, oven drying, and freeze-drying. Sun drying involves exposing the insects to sunlight until they are dry, oven drying is faster, and uses specialized equipment such as convection ovens or fluidized bed dryers, while freeze-drying is the most expensive and involves removing moisture through sublimation (Makkar et al., 2014). The drying process has significant implications for the quality and nutritional value of insect meal. Excessive heat during drying can denature proteins, reducing the meal's nutritional value (Oonincx and de Boer, 2012). Additionally, improper drying can lead to mold growth, which produces toxic metabolites harmful to animals (Makkar et al., 2014). It is clear that both fish meal and insect meal are crucial protein sources in animal feed production. However,

proper drying techniques are even more critical in insect meal production due to microbial contamination and protein denaturation risks. Therefore, proper drying techniques must be employed to ensure the quality and safety of insect meal as a feed ingredient.

1.2 Scope of Study

The scope of this study consists of three phases. The first phase is the experimental design using various drying temperature and drying time of the Black Soldier Fly (BSF) harvested at prepupal stage. The second phase is to study the optimum condition via the Design Expert Response Surface Methodology (RSM) using Central Composite Design. The final phase studies the functional properties of the oil.

1.3 Problem Statement

As the global demand for protein-rich food and feed continues to grow, there is a pressing need to find sustainable sources of protein. Edible insects are known to be a rich source of nutrients, including lipids, which are a vital source of energy for humans and animals. In fact, some edible insects contain a higher lipid content than conventional protein sources like beef and pork. The preservation of nutritional quality in insects processed for consumption is paramount. One of the main factors that affect product quality and shelf-life is moisture. Various drying methods have been studied for edible insects, but there is limited research on the optimum drying process to maintain nutrient content for insect drying process.

1.4 Objectives

The goals of this study were defined as follows:

1. To examine the effect of drying time and temperatures of the oil properties.
2. To optimize the drying parameters using Response Surface Methodology (RSM).
3. To characterize the changes on the functional properties of oil.

CHAPTER 2

LITERATURE REVIEW

2.1 Edible Insects

The domestic cricket (*Acheta domesticus*), mealworm (*Tenebrio molitor*), and black soldier fly (BSF or *Hermetia illucens*) are the most often reared edible insect (Kierończyk et al., 2022). Provided that BSF has the quickest growth period, the lowest food conversion ratio (i.e., the amount of feed required to generate 1 kg of biomass), and provides excellent nutritional quality, as indicated in Table 2.1, the BSF appears to be particularly promising for large-scale rearing systems. Polyunsaturated fatty acids in human nutrition help prevent inflammatory-related diseases such arthritis, asthma, and coronary heart disease, among others, and should therefore be a component of the diet (McDaniel, Ickes, and Holloman, 2013). Additionally, feeding BSF larvae for only 3 hours while adding 40 % fish meal enables one to lower this ratio to 2.8 while also raising the overall amount of unsaturated fatty acids by 5 % (Barroso et al., 2017). Additionally, compared to mealworms and crickets, the BSF has two to four times higher mineral content. It potentially lowers the need for supplementation as they are a requirement in all animal feed (Barragan-Fonseca, Dicke and van Loon, 2017). Indeed, compared to crickets and mealworms, BSF larvae contain 26 times as much calcium, at least twice as little salt, and four times as much iron on a dry basis (Larouche et al., 2019). The BSF also contains high-quality protein since their essential amino acid profile matches the needs for fish meal (Barroso et al., 2014).

Table 2.1: The proximal composition, polyunsaturated fatty acids, minerals content and production characteristics of the black soldier fly (BSF) larvae and prepupae, mealworms (*Tenebrio molitor*) larvae and domestic cricket (*Acheta domestics*).

Species	***H. illucens***	***T. molitor***		***A. domesticus***
Stage	Larvae	Prepupae	Larvae	Adult
Proximal composition (%, dry basis)				
Crude protein (N x 6.25)	36.2	40.7	58.4	73.1
Crude lipid	18.0	15.6	30.1	15.9
Ash	9.3	19.7	3.5	5.6
Nitrogen-free extract	36.5	24.0	8.0	5.4
Polyunsaturated fatty acid				
Omega-3 (% lipid)	0.6 – 1.5	0.1 – 0.9		0.4 – 1.5
Omega-6 (% lipid)	4.2 – 17.3	13.6 – 25.6		23.6 – 34.9
Minerals content (mg/ 100 g; dry basis)				
Calcium	2,900	3,000	100	166
Phosphorus	350	620	760	860
Sodium	100	50	174	363
Iron	200	8	6	6
Zinc	61	3	11	14
Production characteristics				
Food conversion ratio	1.4 – 2.3	3.8 – 6.1		2.3 – 10.0
Development time (d)	21 – 37	116 – 227		48 – 167
References	Barroso et al. (2017)	Oonincx et al. (2019)		Bassett, Dunn, Pike and Jefferies (2021)

2.2 Life Cycle and Description of Black Soldier Fly

The multi-beneficial black soldier fly (BSF), *Hermetia illucens* (Linnaeus), like the rest of flies, has no stinger and just two wings (wasps have four). BSF is likely the most well-known member of the Dipteran family Stratiomyidae. Since most fly only have two wings, the word "diptera" (meaning "two") comes from the Greek, according to Hall and Gerhardt, (2002). Adult black soldier flies are not dangerous, even though the loud buzzing they make when flying is enough to worry a lot of people. BSF is one of the groups of true flies, whereby they reproduce in compost, dung, and outdoor toilets, and are mostly found in Australia and the tropical and subtropical regions of the Western Hemisphere.

The black soldier fly is considered as one of the most beneficial flies and is not considered as a pest. Unlike other flies, the mature black soldier fly does not consume waste or have mouthparts, making it incapable of biting humans. Furthermore, the larvae are the only ones that consume waste, reducing the likelihood of spreading infections and diseases (Rehman et al., 2022). The presence of black soldier flies can also decrease the attractiveness of breeding grounds for other flies. The adult black soldier flies are typically around 7 to 8 inches long and prefer to rest in and around livestock production facilities due to their limited flying ability. Based on the findings by Tomberlin et al. (2002), the first abdominal segment of BSF contains two clear regions near the second segment, which gives them a "wasp waist" appearance.

The female black soldier fly has a reddish abdomen, while the male is in bronze colour. The BSF forelegs, or tarsi, have white yellow colouring with black upper legs. This species has long antennae that project forward from their tapering, arista-free head, which functions as a sensory organ for touch. After mating, the female lays egg masses near decomposing organic materials, usually around 500 eggs to 650 eggs per female (Tomberlin et al. 2001). The eggs typically incubate for four days to three weeks before hatching. The oval eggs measure up to 0.039 inches in length and start off as cream-colored before darkening over time. Once hatched, the off-white larva measures 0.07 inches in length.

According to Rehman et al. (2022), the black soldier fly larvae (BSFL) have become a popular solution for waste management because of their remarkable ability

to consume various organic waste, including food waste, manure, and sewage sludge, and convert it into high-quality protein and fat for animal feed and other products (Diener et al., 2011). These larvae can reduce waste volume by up to 50-70% and significantly decrease the production of greenhouse gases like methane, which are typically produced during the decomposition of organic waste (Surendra et al., 2016). Additionally, BSFL can convert nitrogen and phosphorus in the waste into valuable fertilizer, making them a sustainable waste management option (Tomberlin et al., 2002). BSFL are particularly effective in managing food waste, which is a major contributor to the growing waste crisis. In a laboratory setting, Lalander et al. (2013) found that BSFL could consume 76 % of the food waste provided to them. Furthermore, the study demonstrated that BSFL could significantly reduce the amount of volatile fatty acids and ammonium produced during the anaerobic digestion of food waste. In summary, BSFL have proven to be a sustainable solution for waste management. They reduce organic waste, decrease greenhouse gas emissions, and produce valuable resources such as protein and fertilizer (Craig Sheppard et al., 1994). As a result, they are being increasingly recognized as a sustainable waste management solution for both industrial and household waste.

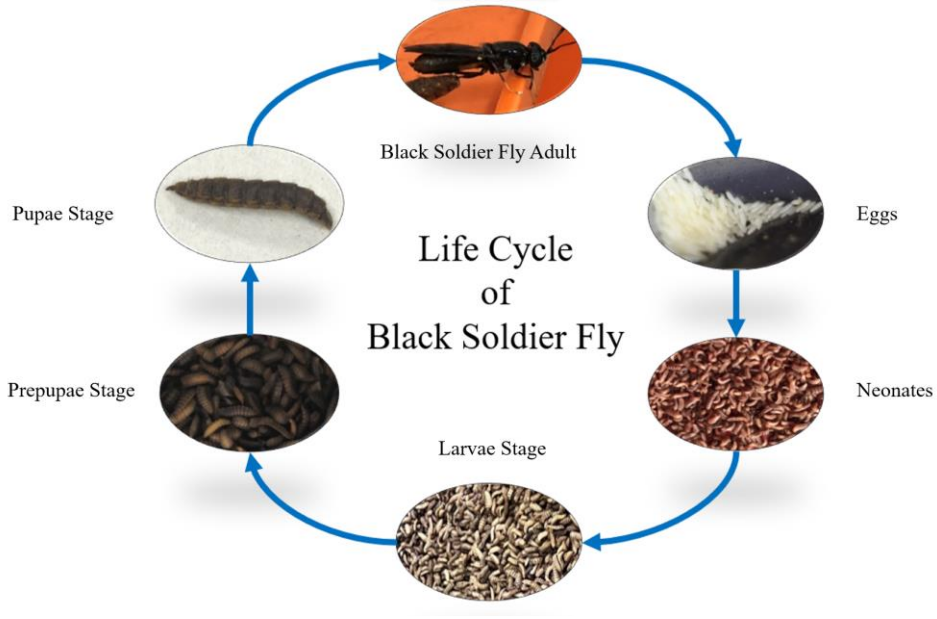

Figure 2.1: Life cycle of black soldier fly (BSF).

2.3 Insects Larvae Processing

Insect processing for industrial use is gaining momentum due to various reasons. By 2050, the population of world is projected to grow from 7.7 billion to around 10 billion people, leading to an increase in the demand for protein by 50 %, which equates to over 256 million tonnes annually, with the addition of 2.3 billion (Food and Agriculture Organization in United States (FAO), 2022). However, the amount of agricultural land available for producing traditional meat and plant-based proteins is limited. To bridge the protein gap, significant changes must be made to the food supply chains. One solution that could help address these issues is the use of insects. Insects provide a sustainable protein source and can help address the problems associated with organic waste disposal. Currently, farmed animals and crops are the primary sources of sustenance. However, there are vast amounts of organic waste, totalling billions of tons, that are generated during processing, purchasing, and consumption. Most of this waste is discarded and cannot be reused. However, it can be used as feed for insects in insect farms, providing ideal nutrition to promote insect growth when appropriately dosed. The larvae produced can be converted into valuable proteins and lipids, which can be fed to farm animals. Additionally, the remaining leftovers from insect farming can be used as fertilizer for crops consumed by humans, promoting a circular economy that transforms food waste into crop fertilizer and a source of protein for animal feed (van Huis, 2013).

Insect farming has emerged as a potential solution to address the world's protein and waste challenges, and this can be attributed to several factors. One of the key drivers is the anticipated population growth to approximately 10 billion people by 2050, which will result in a 50 % increase in the demand for protein, representing over 256 million tons of protein per year. However, limited agricultural land is available for traditional protein sources, making it necessary to explore alternative sources. Insects offer a sustainable and promising source of protein and can assist in organic waste disposal (van Huis et al., 2013). Furthermore, insects are already part of the natural diets of many animals and are widely used in the fish farming, poultry, and pet food industries. Insect farming is advantageous because insects can be raised locally, reducing the need for vast farming areas required for conventional protein sources such as cattle. The black soldier fly, for example, is highly adaptable and can consume a broad range of feedstock, including organic waste, which can be

transformed into high-quality protein and fat for animal feed (Diener et al., 2011). The larvae of the black soldier fly are rich in nutrients, including proteins, fats, and minerals, making them a highly nutritious food source for animals. Additionally, insect farming has a smaller environmental footprint compared to traditional protein sources (Oonincx and de Boer, 2012). Overall, the growing demand for protein and the need for sustainable waste management practices have prompted interest in insect farming, which offers a promising and sustainable source of protein that can help address these challenges.

The initial stage in insect processing is the harvesting, which must produce a safe and stable product in terms of microbiological and physicochemical properties. The quality of the final product is greatly impacted by processing, which can improve its colour and nutritional stability while reducing microbial contamination. Furthermore, processing can modify the functional properties of proteins and affect their nutritional value. Processing techniques may involve a variety of steps such as post-harvest processing, killing, extraction of macronutrients, decontamination, drying, and grinding, depending on the intended use of the insect product. In the food industry, each processing step should aim to enhance or maintain nutritional quality while reducing the microbial load.

2.4 Black Soldier Fly Larvae Harvesting

Harvesting, which involves separating the larvae from their feeding environment, can be accomplished using two methods: sieving or “autonomous migration behaviour” performed by BSFP. The growing stage of BSFL, the humidity, and the particle size of the feeding substrate all influence the approach employed (Cheng et al., 2017). Sieving the substrate to retain the larvae or prepupae inside the sieve is the most popular method used in the business world. A vibrating sieve or a rotating drum system can be used to collect the substrate, which can potentially serve as a biofertilizer (Cheng et al., 2017). However, the substrate must retain a maximum of 50 % humidity, undergo complete digestion, and have small enough particle sizes to prevent sieve blockage. Auto-collection, the second harvesting method, only works with prepupae, taking advantage of their migratory behaviour to a drier site before

they pupate, and it can tolerate higher humidity substrate (Craig Sheppard et al., 1994; Cheng et al., 2017). One possible way to harvest the BSFP is to utilize the migration behaviour by directing their movement on a ramp to a collecting bucket as they try to escape the substrate (Tomberlin, Sheppard, and Joyce, 2002). While this method ensures that only live organisms are collected, it can mobilize energy reserves, lowering the nutritional content of the food, and reduce harvesting efficiency due to the unexpected movement of some prepupae.

2.5 Post-Harvest Processing Steps

During large-scale manufacturing, post-harvest processing methods are used to create a stable insect product and enhance its quality. Although not all producers employ them, these techniques can enhance microbiological activity of the product (Larouche et al., 2019). The techniques involve withholding feed for a certain period and a washing procedure.

2.5.1 Feed Withdrawal Period

Due to the complexity of removing the insect gastrointestinal tract, residual insect meal often contains undigested feed, frass, and associated microbial burden. Various methods, such as starving or degutting the insects, have been proposed to address this issue. However, to reduce the insect microbial load, the Food and Agriculture Organization (FAO) suggests a feed withdrawal period (FWP) or post-harvest fasting, which involves purging the gut (van Huis et al., 2013). In addition, the egestion time for Diptera larvae, such as *Musca domestica* and *Lucilia sericata*, is about 100 minutes and between 60 and 90 minutes, respectively, and is associated with the gastrointestinal evacuation time (GET) (Espinoza-Fuentes and Terra, 1987; Barnes et al., 2010). An FWP of 12 to 48 hours is believed to be sufficient to empty most of the digestive tract, although the GET of insects is poorly understood (Tzompa-Sosa et al., 2014; Charlton et al., 2015).

Currently, only mealworms have been examined to assess the suitability of an FWP for reducing microbial burden, and it was found that an FWP of up to 48 hours did not affect the microbial load of mealworms, with or without faecal contact. However, frass may remain in the digestive system, resulting in a continued high microbial load, as the GET of mealworms is unclear (Wynants et al., 2017). Furthermore, fasting may lead to an increase in energy reserve metabolism, which is crucial to evaluate when determining the optimal FWP (McCue, 2010). Given that BSFL larvae consume contaminated leftover organic waste, it is essential to investigate the efficacy of an FWP in reducing microbial burden at a reasonable cost.

2.5.2 Washing

Several insect species, including Coleoptera (*T. molitor*) and Diptera (*M. domestica*, *Piophila casei*, and *H. illucens*) larvae, are washed with water before or after killing to remove any feed or frass that may remain on their cuticle, similar to many crustacean species. Washing is an efficient procedure used in animal production as it physically removes organic materials and any potential pathogens at a low cost, thus increasing product shelf-life. Water can be used for washing through dipping or spraying, and factors such as increased pressure, time, temperature, repeated dipping, and the addition of sanitizers like chlorine and organic acids can improve washing effectiveness (Hultberg et al., 2019).

An initial study on the effectiveness of washing *T. molitor* by immersing it in agitated water for one minute did not result in any significant microbial reduction. Therefore, future research should focus on streamlining the washing procedure for edible insects (Hultberg et al., 2019). Although BSFL are currently fed pre-consumption meals, washing them may enable them to process highly contaminated leftover organic waste. Hence, it is essential to investigate the potential benefits of washing BSF larvae as a means of transforming polluted organic waste (van Huis et al., 2013).

2.6 Inactivation Process

The stage of inactivation is crucial during the processing of insects because it can impact the nutritional value, microbiological safety, colour stability, and flavour of the final product. Quick and efficient inactivation methods are preferred to reduce microbial burden and retain the nutritional value of the product, making it an essential step in animal production (van Huis et al., 2013). Various methods, including cold, heat, asphyxiation, and mechanical disturbance, can be used to kill insects, but the technique needs to be adapted to meet the specific requirements. Blanching and freezing are the most used techniques in the industry, although other methods may be equally effective.

Table 2.2: Summary of inactivation techniques used on an industrial.

Individual or company	**Species**	**Killing method**	**References**
Tarique Arsiwalla, Protix Biosystems	*H. illucens*	Mechanically crushed by centrifugation	(De Goede et al., 2013)
Jagran B. V. Insect Rearing Concepts	*M. domestica*	Grinding and freezing, experimenting asphyxia with high N_2/low O_2.	(Larouche et al., 2019)
Leon Westerd, Wageningen University	Various insects	Small insects: stunned with CO_2 and sprayed with hot water. Large insects: freeze-drying	(Larouche et al., 2019)
Kreca	Orthoptera, Coleoptera and Diptera	Freeze or freeze-drying	(De Goede et al., 2013)
Van der Ven	*T. molitor*	Boiling or freeze-drying	(De Goede et al., 2013)

2.6.1 Non-Thermal Inactivation

Insects are commonly killed by freezing, which allows for product preservation while slowing down their metabolism and reducing the development of microorganisms and the action of rotting enzymes due to their poikilothermic nature (Makkar et al., 2014). This method can be done by freezing, freeze-drying, or immersion in cold water. However, the freezing process can affect the quality of the lipids and proteins as well as the colour stability of the insect. Research has shown that spoilage enzymes remain active even after freezing and cause lipid breakdown and polyphenol oxidation. In particular, the lipolytic enzyme in BSF larvae has been found to be extremely active even at freezing temperatures, leading to lipid degradation during storage at -20 °C (van Huis, 2017). Therefore, it is crucial to quickly stop these enzymes to maintain the nutritional quality of the insect.

2.6.2 Thermal Inactivation

Various invertebrates, such as insects, lobster, and shrimp, can be effectively killed through the application of heat. Blanching is one of the most used killing methods in insect production due to its rapid nature and ability to reduce microbial load (Larouche et al., 2019). While some insects may perish at temperatures as low as 50 °C, higher temperatures are generally required to inactivate microorganisms. Heat treatment can be administered via wet heat, such as steaming and blanching, or dry heat, such as in an oven. The denaturation of endogenous spoilage enzymes by heat treatment can help limit product degradation during storage and reduce the microbial burden of insects. Dry heat can allow for significant non-enzymatic browning via the Maillard process, unlike blanching which can reduce it due to the high-water content. The immersion technique of blanching has been reported to reduce the protein and water content of shrimp, which can speed up the drying process. Studies have explored the use of blanching to kill black soldier fly larvae and mealworms in insects. The pH of the treated material increased by 0.5 units for at least 48 hours, suggesting a more stable outcome than raw material. Blanching can also reduce water retention in insects and shorten the drying process, while preserving their colour and nutritional value (van Huis, 2017).

2.6.3 Asphyxia

In the production of insects, one of the most commonly used methods for anaesthetizing them is carbon dioxide (CO_2) exposure, which lasts from 3 to 60 minutes depending on the insect species (Nilson et al., 2006). Insects become immobile when CO_2 concentrations exceed 40 %, without affecting neuron conductance. However, prolonged exposure to CO_2 may lower the haemolymph pH and encourage spiracle opening, leading to dehydration and potential mortality (Jongema et al., 2017). Decreasing metabolic activity is another advantage of CO_2 exposure, which extends the duration of anaerobic conditions, but this can also cause oxygen depletion and decrease the chance of survival (Jongema et al., 2017). The time required to kill insects with CO_2 depends on various factors, including species, insect stage, temperature, CO_2 and O_2 concentration, humidity, and more. Pure CO_2 exposure is fatal to Drosophila melanogaster larvae in 30 minutes, while *Cadra cautella* and *Tribolium castaneum* larvae require 72 hours (Badre et al., 2005). CO_2 exposure can also anaesthetize insects, which speeds up the drying process resulting from spiracle opening (Jongema et al., 2017).

N_2 saturation can also cause asphyxiation, but the oxygen concentration must be below 30 %, and it is challenging to obtain air saturation on a large scale. Vacuum packing and drowning are other techniques that can be used, but insect survival varies depending on the species and developmental stage (Nakagaki et al., 2019). For example, *Manduca sexta* pupae can survive in water for 5 days, while the larvae can only survive for 4 hours. Some adult grasshopper species can live for 7.5 to 22 hours when submerged in water, while their nymphs can only last for 3 to 13 hours (Jongema et al., 2017). The Lepidoptera larvae *Ephestia cautella* takes between 96 to 144 hours to die in 98 % N_2, which is one to two days longer than in a 60 % CO_2 aerobic environment. Although the anoxic resistance BSFL has not been studied, it may be as effective as *D. melanogaster* exposed to 100 % CO_2 (Jongema et al., 2017). However, prolonged anaerobic conditions may promote the growth of anaerobic microorganisms, necessitating thorough cleaning procedures.

2.6.4 Mechanical Disruption

The mechanical disruption of the neural system has emerged as a promising method for insect death due to its speed and cost-effectiveness. Protix has reported that centrifugation or grinding can be used for this purpose (Liland et al., 2017). However, no studies have investigated the effect of mechanical disruption on the quality of the final insect product, even though it is commonly used in industry. Grinding has been observed to cause browning in several insect species due to the exposure of insect components to oxygen, which can also lead to lipid oxidation. Grinding can also increase surface evaporation, thus boosting the drying capacity of the insect. Furthermore, Jagran, a company specializing in housefly rearing, asserts that grinding is the most animal-friendly among alternative death methods (De Goede et al., 2013).

2.7 Decontamination

The final product is a wet, deceased larva, whose microbial load may have been reduced depending on the method employed to inactivate it (Lorenzo et al., 2018). Its pH level is nearly neutral, classifying it as a perishable product that requires the same treatment as low-acid food. To enhance the shelf life of the larvae, they may undergo processing. However, only a limited number of decontamination methods developed for the food industry have been tested on insects. A range of techniques, including blanching, desiccation, high hydrostatic pressures, direct and indirect cold plasma, and microwave have been examined for their potential to reduce the microbial load of insects (Larouche et al., 2019).

2.7.1 High Hydrostatic Pressure

According to the study, one effective non-thermal method for reducing the microbial load of a product without compromising its quality is the use of high hydrostatic pressure (HHP) technology (Balasubramaniam et al., 2016). In this process, the

product is placed in a chamber filled with a compressible fluid and the air is removed, resulting in immediate and uniform pressure on the product due to the compressed fluid. This pressure, which can be accompanied by a high temperature, helps to lower the microbial load while preserving the structure of the product. However, to optimize the effectiveness of the HHP therapy, the pressure (measured in MPa), the duration of the pressure holding, and the temperature must be carefully adjusted (Aouadhi et al., 2013).

Although HHP technology is expensive, it is widely used in food processing due to its numerous benefits compared to conventional methods. HHP can pressurize foods at low temperatures, reduce the load of certain bacteria, yeast, and moulds, inhibit enzyme activity, and even develop new functional foods. Gram-negative bacteria, yeast, and mould are notably reduced by HHP, while gram-positive and spore-forming bacteria are less affected. However, the presence of spore-forming bacteria resistant to the treatment can reduce HHP's effectiveness on total viable counts when applied to insects. Therefore, HHP must be tailored to the product's microbial population, which varies depending on the types of insects, stages of development, and facilities. HHP treatment has also shown the potential to prevent enzymatic activity and reduce water-holding capacity, such as in mealworms treated at 400 and 500 MPa for 3 min (Aouadhi et al., 2013). However, it can also have negative effects on product quality by promoting lipid peroxidation, as seen in gigantic tiger shrimp where lipid oxidation levels increased at 300 MPa. The benefits and drawbacks of HHP technology for insects, especially BSF larvae, must be further evaluated before implementation.

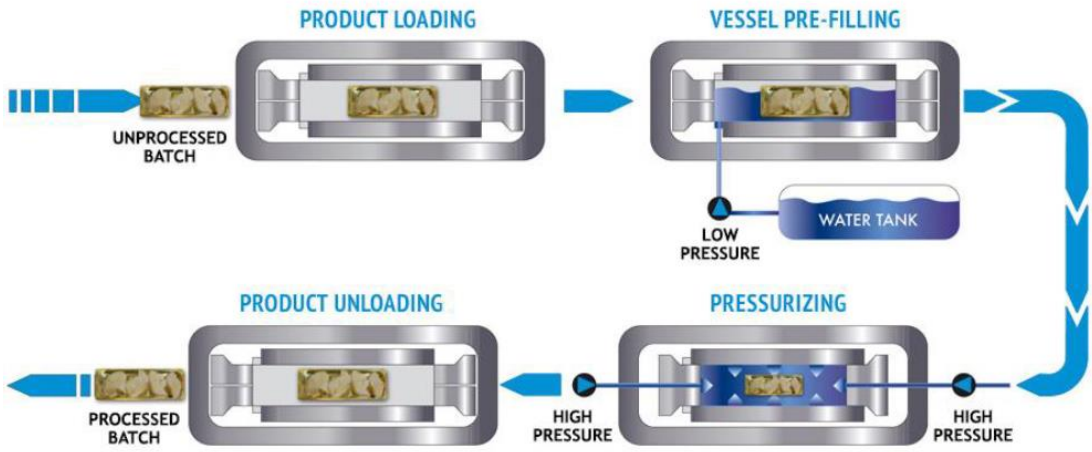

Figure 2.2: Batch system with high hydrostatic pressure (Marangoni Júnior et al., 2019).

2.7.2 Cold Atmospheric Pressure Plasma

Cold atmospheric pressure plasma is a non-thermal method that has the potential to reduce a variety of microorganisms, including spore-forming bacteria and viruses, in food processing, although its use can be expensive. The technique works by attacking bacterial cell membranes with neutral and sparsely charged particles (Rumpold et al., 2014). However, it is less effective against gram-positive bacteria and spores. As shown in Figure 2.3, the antimicrobial effects of applying plasma discharge to a product directly, semi-directly, or indirectly can vary. Direct and indirect application of cold plasma to fresh mealworms has little to no effect, but semi-direct application to powder may reduce overall viable numbers by up to 3 logs. Additional research is needed to determine the feasibility of using cold atmospheric pressure plasma for controlling microbial loads in insect powders.

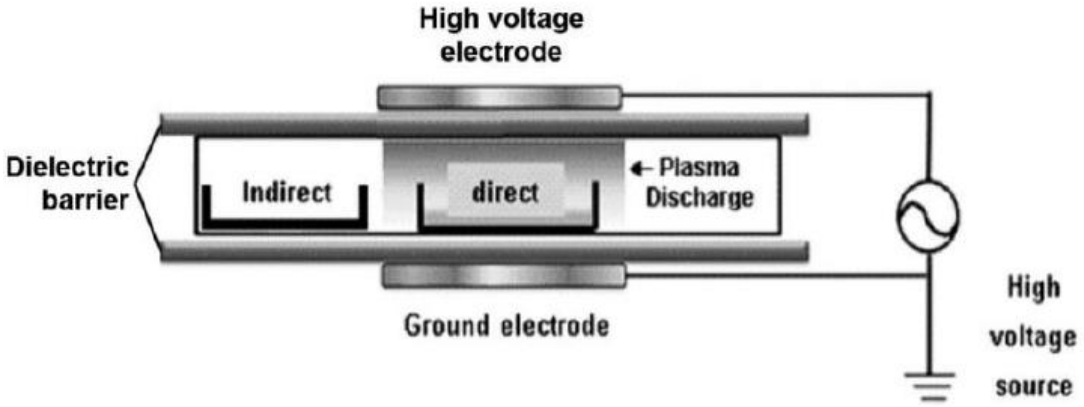

Figure 2.3: Direct and indirect cold plasma processes in the atmosphere (Almeida et al, 2015).

2.8 Drying Technologies in Insects Processing

Drying insect products has numerous benefits in terms of preserving them by reducing microbial growth and spoilage enzyme activity. However, the method of drying can have significant effects on the colour, water activity, proximate composition, lipid oxidation, and protein solubility of the final product. Various techniques such as sun-drying, oven-drying, freeze-drying, and microwave-drying can be employed for insect processing, each with its own unique impact on the product quality.

2.8.1 Oven Drying

Numerous studies have demonstrated the potential of oven drying as a highly effective insect drying method, whereby diverse systems such as vacuum and rotating ovens can be employed, each exerting a distinct influence on the resulting product, and a lower drying temperature is preferred to mitigate protein denaturation and minimize undesirable phenomena such as the Maillard reaction, shrinkage, and tissue collapse, often requiring a temperature range of 50 to 120 °C over a period of

several hours up to days, with optimal outcomes observed at a temperature of 60 °C, effectively minimizing these detriments and achieving expeditious drying.

2.8.2 Freeze Drying

According to research, freeze-drying is a popular non-thermal method used in insect processing, which involves drying mealworms for a minimum of 24 to 53 hours (Kröncke et al., 2018). This process does not trigger the Maillard reaction, producing the whitest powder, and is considered one of the best techniques for maintaining the colour of insects. However, it may cause a minor 10% reduction in protein solubility, and the most significant lipid oxidation compared to other drying methods, resulting in a substantial loss of quality. Nevertheless, studies have shown that blanching before freeze-drying reduces the oxidation by half (Kröncke et al., 2018). Freeze-drying can also make whole larvae appear expanded instead of shrunk, potentially increasing consumer acceptance of BSFL (Larouche et al., 2019).

2.8.3 Microwave Drying

Microwave drying is a quick drying technique, taking only 10 to 15 minutes, depending on the specifications of microwave. However, the use of microwave drying for insect processing is limited due to the potential for browning and inflated entire dry larvae (Larouche et al., 2019). Research has shown that microwave drying of mealworms can lead to a 40 % reduction in protein solubility. Furthermore, compared to oven drying at 60 °C, microwave drying of black soldier fly larvae stimulates protein polymerization, resulting in a powder with larger particle sizes and lower digestibility. As a result, the digestible amino acid score is also lower for microwave dried BSF larvae powder (Kröncke et al., 2018).

2.8.4 Other Drying Method Available

In addition to the previously mentioned techniques, insect drying can also be accomplished with fluidized beds and sun drying (Rumpold and Schlüter, 2013). Sun drying is a low-cost and traditional drying method that has been utilized for centuries to preserve various insect species, such as grasshoppers, caterpillars, termites, and maggots. On the other hand, fluidized bed drying is a high-temperature, short-time technique that can also be employed to dry insects, with minimal increase in lipid oxidation when conducted at 130 °C for 110 min (Kröncke et al., 2018). While numerous studies have investigated the effects of drying on the quality of edible insects, with *T. molitor* being the most studied, little research has been conducted on BSFL or BSFP. Therefore, a greater understanding of how drying techniques affect BSF larvae can aid in improving the quality of the final product.

2.9 Modelling of Drying Process

Drying is the process of removing moisture from a material through evaporation, which can be achieved through several techniques, including hot air drying, freeze drying, and spray drying. Modelling the drying process is essential for optimizing the process and ensuring that the final product meets the desired specifications (Van Impe et al., 2018).

The theory of drying is based on the principles of mass transfer, which refers to the movement of moisture from areas of high concentration to areas of low concentration. This movement occurs as a result of the driving force, which is the difference in moisture content between the material and the surrounding air. The drying process involves three stages: the constant-rate period, the falling-rate period, and the equilibrium period (Lambert, 2003). During the constant-rate period, the rate of drying is limited by the rate of moisture transfer from the surface of the material to the surrounding air. The moisture content of the material remains constant during this period. The falling-rate period occurs when the rate of moisture transfer decreases as the moisture content of the material decreases. Finally, in the equilibrium period, the rate of moisture transfer is equal to the rate of moisture

uptake, and the moisture content of the material reaches equilibrium with the surrounding air. Several factors can affect the drying process, including temperature, humidity, air velocity, and the surface area of the material. The drying rate increases with an increase in temperature, humidity, and air velocity, while the surface area of the material affects the rate of moisture transfer during the constant-rate period. The two processes involved in drying are the heat transfer process and the mass transfer process. The heat transfer process involves the transfer of heat from the surrounding air to the material, which increases the temperature of the material and causes moisture to evaporate. The mass transfer process involves the movement of moisture from the material to the surrounding air (Van Impe et al., 2018).

In conclusion, modelling the drying process is crucial for optimizing the process and ensuring that the final product meets the desired specifications. The theory of drying is based on the principles of mass transfer, and the drying process involves three stages: the constant-rate period, the falling-rate period, and the equilibrium period. Several factors can affect the drying process, and the two processes involved in drying are the heat transfer process and the mass transfer process.

2.10 Design of Experiment (DOE) by Central Composite Design (CCD)

In this study, the Central Composite Design presented by Box and Wilson is employed. The design consists of three parts which is, a full factorial or fractional factorial design, an additional design in which experimental points are set at a distance α from its centre, and a central point. Figure 2.4 illustrate the full central composite design for optimization of two variables (Bezerra et al., 2008).

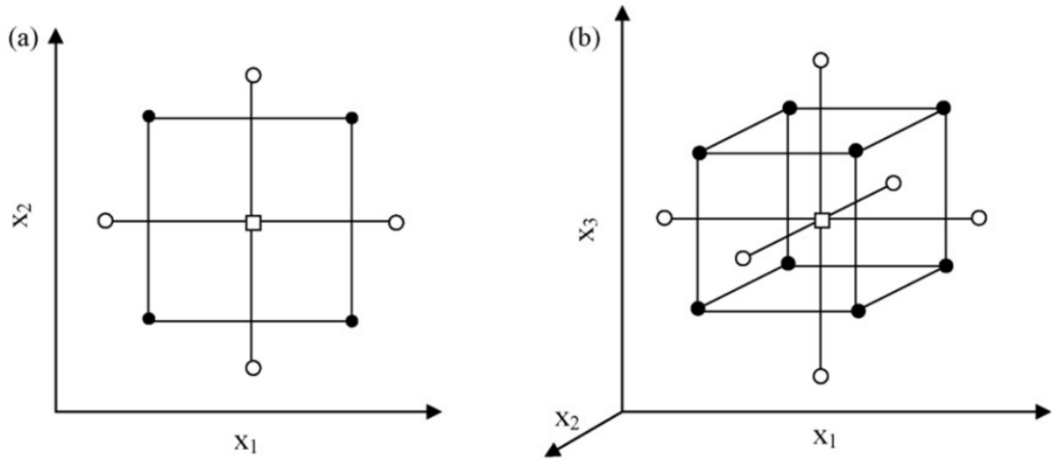

Figure 2.4: Central composite designs for optimization (Bezerra et al., 2008).

(a) two variables ($\alpha = 1.41$)

(b) three variables ($\alpha = 1.68$)

The model of the experiment design:

$$2^k + 2k + 1 + n_c$$

Whereby,

$k = Numeric\ factor\ (2\ varying\ variable\ for\ CCD)$

$n_c = Center\ points\ (5\ for\ CCD\ ranging\ from\ 2\ factor\ to\ 3\ factor)$

According to Bin (2013), claims that the above model offers 13 different parameter combinations, and that these combinations should result in an optimum response or system performance. The CCD has been used in certain BSFL-related experiments and has shown to be a useful tool for enhancing the system responsiveness. A five-level, four-factor central composite design was used in a recent study by Ishak et al. (2018) to ascertain the best biodiesel yield of Black Soldier Fly Larvae (BSFL) and examine the impact of variables A, B, C, and D of methanol: oil molar ratio (A), catalyst amount (B), time (C), and temperature (D). The statistical analysis of variance (ANOVA) and regression coefficient (R^2) were created based on the response received.

2.11 Impact of Various Drying Methods on the Chemical Properties and Nutritional Quality in Edible Insects

Studies have investigated the crude nutritional values of various insects, including Longhorn Grasshopper (LHG) adults, *Ruspolia differens*, Yellow Mealworm Larvae (YML), *Tenebrio Molitor*, and BSF larvae and prepupae, using different drying methods such as vacuum, freeze, microwave, and oven drying (Fombong et al., 2017; Kröncke et al., 2019; Cappellozza et al., 2019; Spranghers et al., 2016). Table 2.3 presents the impact of different drying techniques on the crude protein, ash, and lipid content of these insects.

The data in Table 2.3 show that the crude protein, ash, and lipid contents vary for all crude nutritive values. BSF prepupae had the highest crude lipid content at 40.99 % when subjected to oven drying at 103 °C until constant weight (Spranghers et al., n.d). YML had the highest crude protein concentration at 60.18 % in a 4 - steps microwave drying procedure (Lenaerts et al., 2018). The highest crude protein content for BSF was 43.1 % when BSF prepupae were freeze-dried at -20 °C until constant weight (Spranghers et al., 2016). On the other hand, the highest ash level of 7.08 % was found in BSFL when dried in an oven at 70 °C until it reached a consistent weight (Cappellozza et al., 2019). It can be concluded from the data in Table 2.3 that different drying techniques can affect the nutritional qualities of insects. Drying until constant weight resulted in higher crude lipid and crude protein content in BSF prepupae at 40.99 % and 43.10 %, respectively (Spranghers et al., n.d). Oven drying produced high crude lipid content and ash content for both BSF prepupae and BSFL (Spranghers et al., 2016; Cappellozza et al., 2019).

Table 2.3: Compositions of various type of insects under different drying method.

Drying Method	Type of Insect	Drying parameters	Composition (% dry matter)			References
			Crude Lipid	Crude Protein	Ash	
Oven Drying	LHG Adult	60 ℃ 24 hours	35.53	47.7	4.66	Fombong, Borght and Broeck (2017)
Microwave Drying	YML	120 ℃, 1 hour	27.27	56.30	3.43	Kröncke et al. (2019)
	BSF Prepupae	103 ℃ until constant weight	40.99	40.88	4.98	Spranghers et al. (2017)
	BSF Larvae	70 ℃ until constant weight	35.62	39.42	7.08	Cappellozza et al. (2019)
Freeze Drying	YML	1) 30s at 2 kW 2) 5 min at 3Kw 3) 10 min at 1 kW 4) 2 min at 0.5kW	28.61	60.18	4.25	Lenaerts et al. (2018)
	LHG Adult	1) -30 ℃, 20 minutes 2) vacuum main freeze drying at -50 ℃ (0.04 bars, 48 hours) 3) vacuum final drying at -55 ℃ (0.021 bars,48 hours)	35.56	46.41	4.79	Fombong et al. (2017)
	BSF Larvae	-20 ℃ until constant weight	33.80	40.00	5.10	Liland et al. (2017)

	vacuum appleid				
YML	-50 ℃ 24 hours	26.80	52.23	4.25	Kröncke et al. (2019)
	Vacuum applied				
BSF	-20 ℃ until	38.60	43.10	2.70	Spranghers
Larvae	constant weight				et al. (2016)
	applied				

The quality of crude oil lipids in insects may be influenced by various drying factors, including temperature and drying time (Mai et al., 2019; Zheng et al., 2011). Non-dried black soldier fly larvae (BSFL) showed higher peroxide and acid values compared to dried BSFL, indicating a faster oxidation rate and oil fouling (Karseno et al., 2017). Additionally, non-dried BSFL had lower iodine and unsaturated fatty acid (UFA) content due to accelerated PUFA oxidation (Mai et al., 2019). However, drying parameters may not be the only factor affecting lipid characteristics, as the use of raising substrates could also have an impact (Ewald et al., 2020).

Yellow Mealworm Larvae (YML) dried in a microwave at 850W for an hour had higher peroxide and acid values compared to YML dried in an oven at 200℃ for 15 minutes, likely due to the high temperature and prolonged drying time (Santhosh, 2019). This may have resulted in a lower iodine value and UFA content in the microwave dried YML, as Polyunsaturated Fatty Acids (PUFAs) are more likely to oxidize at high temperatures (Gunstone, 2008). Different stages of insect species may also exhibit varying lipid properties, as seen with Yellow Mealworm Beetle (YMB) dried at 60 ℃ for three days compared to YMW counterparts (Selaledi and Mabelebele, 2021).

Blanching or boiling insects before drying may also improve the quality of crude lipids, as shown by the enhanced characteristics of *Fabricius* Larvae (FL) crude oil after blanching (Lee et al., 2023). The blanching process reduced the peroxide and acid values, indicating a slower oxidation rate and reduced oil fouling.

In a nutshell, the lipid properties of insect crude oil can be influenced by various drying parameters and other factors such as raising substrates, insect species, and different stages of growth. Blanching or boiling before drying may also be a

feasible approach to improve crude lipid characteristics. (Mai et al., 2019; Zheng et al., 2011; Ewald et al., 2020; Santhosh, Binod and Ashish, 2019; Gunstone, 2008).

Table 2.4: The effects of various drying conditions/methods on the chemical composition of edible insect.

Method/ Drying Parameter	**Type of insects**	**Peroxide Value (mEq O_2 /kg)**	**Iodine Value (gI_2/ 100g)**	**Acid Value (mgKOH/g)**	**UFA (%)**	**References**
70 ℃ until constant weight	BSFL	0.04	89.70	7.10	51.00	Zheng et al. (2011)
60 ℃ for 2 days	BSFL	0.03	96.00	8.70	39.90	Li et al. (2011)
Non-dried	BSFL	176	73.40	11.88	37.20	Mai et al. (2019)
60 ℃ for 3 Days	YMB	0.27	96.00	7.60	46.90	Zheng et al. (2012)
Microwave Dried 850W for 1 hour	YML	21.03	87.79	1.51	N/A	Santhosh, Binod and Ashish (2019)
Oven Dried 200 ℃ for 15 minutes	YML	4.98	88.25	1.22	71.27	Jeon et al. (2016)
60 ℃ for 24 hours	FL	0.00	73.00	1.10	54.62	Li et al. (2012)
Blanched &	FL	0.08	86.3	1.9	50.34	

Oven Dried 60 °C, 12 hours						Lee et al. (2023)
Oven Dried 60 °C, 12 hours	FL	0.20	88.5	8.80	N/A	

2.11.1: Lipid

Larouche et al. (2019) conducted a study that utilized primary and secondary oxidation levels to evaluate the stability of lipids after drying. The study found that microwave drying is better than freeze-drying in reducing the denaturation of antioxidants. Lipid peroxidation can be caused by various factors such as light, heat, and oxygen (Bartosz, 2016). Oven drying, on the other hand, has been shown to result in significant levels of Thiobarbituric Acid Reactive Substances (TBARS) which indicate high peroxidation (Larouche et al., 2019).

The intensity of the Maillard reaction and the antioxidant activity of sugars can be increased by raising temperature, which is directly related to the browning of a sample (Karseno et al., 2017). The presence of tocopherol in BSFL and the inactivation of the enzymes responsible for decreased oxidation state of heat-dried larvae (Liland et al., 2017) also play a role in preventing lipid oxidation reactions during drying. According to Zamora and Hidalgo (2011), drying procedures in general produce components of the Maillard reaction that prevent lipid oxidation reactions from occurring. The Maillard reaction reduces lipid oxidation due to the antioxidative qualities of the Maillard products, as suggested by Zamora and Hidalgo with the research in 2011. The drying temperature, regardless of technique, affects lipid oxidation, with higher temperatures resulting in lower levels of oxidation due to the Maillard reaction products.

As temperature rises during the drying process, lipids become more susceptible to oxidative degradation. The solubility of oils in oxygen increases as temperature rises, leading to autoxidation, which is a radical chain reaction. The initiation, propagation, and termination processes are the first steps in the radical

chain process. In the drying process, higher temperatures lead to an increase in the initiation step, which boosts the rate of autoxidation. High SFA concentration oils, which contain high melting-point triacyclglycerols (TAGs) that readily solidify at low temperatures, may require specialized storage conditions (Gunstone, 2008). BSFL prepupae contain a high saturated fatty acid level of 76.47 % while BSF prepupae have a content of 64.7 % (Leong et al., 2015). For oils with a high content of saturated fatty acids, low temperature drying is not recommended. Even in the absence of oxygen, high drying temperatures can affect polyunsaturated fatty acids. These changes, which can take the form of polymerization, geometrical isomerism, or cyclization, are usually undesirable. If the quality of the polyunsaturated fatty acids is critical, high temperature drying may not be appropriate since these changes impair the retention of the ideal all-cis pattern of unsaturation (Gunstone, 2008).

2.11.2 Protein

Huang et al. (2018) conducted a study on the use of microwave and oven drying methods for drying black soldier fly larvae (BSFL) protein (BSFLP). The BSFLP was found to contain several major amino acids. Aspartic acid and glutamic acid were the two most abundant amino acids in the BSFLP that was microwave dried. Histidine and aromatic amino acids were produced in substantially greater quantities by both drying methods than those recommended by the Food and Agriculture Organization (FAO).

The Digestible Indispensable Amino Acid Score was better for the oven dried BSFLP, indicating that protein particles become denser and more difficult to digest during microwave drying at high temperatures. Scanning electron microscopy was used to examine the dried BSFL samples (Huang et al., 2018). The compactness of the samples and their larger diameters suggested polymerization had occurred. The peak temperature (540 °C) of the microwave-dried BSFLP in Differential Scanning Calorimetry (DSC) was higher than that of the oven-dried BSFLP (520 °C), indicating that the microwave-dried sample had a better degree of stability.

The drying process and the composition of the proteins in BSF prepupae may impact the lipid extraction from the biomass. Lipids bind to proteins and carbohydrates of the lipid-saturated biomass at high drying temperatures, which could reduce the effectiveness of hexane solvent in lipid extraction (Dzanis, 1994). Additionally, the lipid binding proteins may interact with lipids in a reversible and non-covalent manner, which could make them less constrained in cellular soluble cytoplasm lipid transit (Glatz, 2015).

2.11.3 Moisture Content

Kröncke et al. (2019) conducted a study to determine the most effective method for drying yellow mealworm larvae, specifically *Tenebrio molitor*. It was found that oven drying was more effective than microwave drying in reducing moisture content, with the oven-dried sample having a significantly lower moisture content than the microwave-dried sample. Even when the thickness of the larvae was changed, oven drying was still more effective at removing moisture. This is consistent with the research by Durrani et al. (2003), which found that water activity increases as moisture content increases. Kröncke et al. (2019) also found that the oven-dried sample had a water activity below 0.3, while the microwave-dried sample had a water activity over 0.3, indicating that drying in the oven was more effective in reducing water activity and moisture content. However, low moisture content and water activity can increase lipid oxidation, while reducing enzymatic and non-enzymatic browning processes.

Da Silva et al. (2019) demonstrated that hot air drying of black soldier fly (BSF) larvae at low temperatures of 80 °C caused moisture gradients to form, as the moisture content of the larvae was forced towards the outer surface when equilibrium was attained (Salim et al., 2016). Moisture gradients indicating diffusion were not visible until three hours had passed.

2.12 Application of Edible Insects

2.12.1 Animal Feed

Edible insects are a promising alternative source of protein in animal feed. Insects have high protein content, ranging from 35% to 75%, and are rich in essential amino acids and minerals such as calcium, iron, and zinc (van Huis et al., 2013). Insects such as black soldier fly larvae, mealworms, and crickets have been studied for their potential use in animal feed.

Black soldier fly (BSF) larvae have been shown to be an effective source of protein and fat in animal feed. The larvae have a high protein content of up to 43%, and their fat content ranges from 25% to 35% (St-Hilaire et al., 2007). BSF larvae can be reared on organic waste, making them a sustainable and environmentally friendly source of protein (van Huis et al., 2013).

Mealworms are another insect that has potential as a protein source in animal feed. Mealworms have a high protein content of up to 50% and are rich in amino acids such as lysine, methionine, and tryptophan (Rumpold and Schlüter, 2013). Mealworms can be reared on a variety of organic waste, making them a versatile and sustainable protein source (van Huis et al., 2013).

Crickets have also been studied for their potential use in animal feed. Crickets have a high protein content of up to 70 % and are rich in essential amino acids such as lysine and threonine (Perera et al., 2022). Crickets can be reared on a variety of feed sources, including plant-based diets, making them a sustainable and environmentally friendly protein source. The use of insects as a protein source in animal feed has several benefits. Insects have a low feed conversion rate, meaning they require less feed to produce the same amount of protein as traditional livestock such as cattle and pigs (van Huis et al., 2013). Insects can also be reared on organic waste, reducing the environmental impact of animal feed production (Oonincx and De Boer, 2012).

2.12.2 Black Soldier Fly Larvae as Biodiesel

For the purpose of the application at hand, BSFL are lipid-rich insects that are easily adaptable to various rearing circumstances. The result of this process is FAMEs,

often known as biodiesel, which is produced from the transesterification of the lipids. The capacity of BSFL to create high-quality biodiesel was demonstrated in research by Zheng et al. (2011). Biodiesel was made from the crude lipid that was removed from the larvae while they were being raised on Solid Residual Fractions (SRF) from restaurant garbage. The FAME production was very high, with the ability to synthesise 93.1 % of FAME for every gramme of crude lipid. When it comes to oxidative stability and cold flow, biodiesel frequently runs into problems. Methyl oleate (also known as oleic acid) and methyl palmitoleate (also known as palmitoleate acid) were discovered in the fatty acid profile in the investigation by Zheng et al. (2011). These fatty acids provide the biodiesel improved qualities, such as resolving issues with cold flow. According to Zheng et al. (2011), the high saturated fatty acid content of BSFL also aids in oxidative stability, which extends the shelf life of the biodiesel.

The BSFL exceptional capacity to retain lipids and ability to transform organic waste into nutrients have demonstrated its promise in the manufacture of biodiesel (Zheng et al., 2011). The potential to manufacture high-quality biodiesel has also been demonstrated by the Yellow Mealworm Beetle (YMB), *Tenebrio molitor*, Flesh Fly (FFL), *Boettcherisca peregrine*, and Oriental Latrine Fly Larvae (OLFL), *Chrysomya megacephala*. The ability of the insects to convert a variety of organic waste, including manure, food waste, rotten meat, and a variety of spoiled vegetables, has led to their designation as a viable feedstock for the production of biodiesel (Zheng et al., 2012; Yang et al., 2012). The fuel characteristics of BSFL biodiesel and other insect biodiesels are compared in Table 2.5.

The study by Zheng et al. (2011) showed the greatest crude lipid production at 39.2 percent and the highest biodiesel conversion at 93.10 %, according to Table 2.5. The sustainability of manufacturing biodiesel from BSFL is improved by a greater crude lipid production and biodiesel conversion yield. The insect biodiesels' densities were within the parameters of EN14214, with BSFL and YMB biodiesel having the lowest densities at 860 kg/m^3. When compared to YMB and FFL biodiesel, the BSFL with 4.9 % biodiesel viscosity was within the EN14214 biodiesel standard permitted range. The BSFL biodiesel reduced viscosity can help lessen engine deposits brought on by the atomization of fuel in the combustion chamber (Santhosh Kumar et al., 2017). Furthermore, not all insect biodiesel

contained sulphur. The BSFL biodiesel had the greatest ester concentration, indicating a greater rate of oil conversion to biodiesel. The BSFL biodiesel's water content showed the lowest percentage and was well within the EN14214 standard's parameters. According to Yang et al. (2012), the OLFL biodiesel had the highest flash point, measuring 170 °C, making it safer to store and handle. Despite having a lower flash point of 128 °C, BSFL nonetheless complied with standard EN14214, which calls for a flash point of at least 120 °C. The greatest cetane numbers were found in BSFL and YMB biodiesel, indicating that these fuels will ignite more easily in biodiesel-powered engines (Santhosh Kumar et al., 2017). Except for OLFL biodiesel, all the insects examined in Table 2.1 showed high acid values that exceeded the EN14214 limitations (0.35 mgKOH/g). By adding a pre-treatment step, such as an acid-catalysed esterification process for the insect crude lipids, the high acid value can be reduced. Most of the BSFL biodiesel characteristics, including viscosity, ester content, water content, and cetane number, are better than those of other insect-based biodiesels.

Table 2.5: Comparison of the fuel characteristics of insect biodiesel with BSFL biodiesel.

Rearing Substrate	**N/A**	**Restaurant Waste Solid Residual Fraction**	**Decayed Vegetables**	**Restaurant Waste Solid Residual Fraction**	**Restaurant Garbage**
Properties	**EN14214**	**BSFL Biodiesel**	**YMB Biodiesel**	**FFL Biodiesel**	**OLFL Biodiesel**
Crude Lipid Yield (%)	N/A	39.2	17.7	31.1	26.29
Biodiesel Yield (%)	N/A	93.10	82.21	92.3	87.71
Density (kg/m^3)	860 - 900	860	860	884.2	874.3

Viscosity at 40 °C (mm^2 /s)	3.5 – 5.0	4.9	5.9	5.6	4.0
Sulphur content (wt.%)	0.02 maximum	N/A	N/A	N/A	N/A
Ester content (%)	96.5 minimum	96.9	96.8	98.6	N/A
Water content (mg/ kg)	500 maximum	200	300	< 300	< 300
Flash point (°C)	120 minimum	128	127	146	170
Cetane Index	51 minutes	58	58	52	54.8
Acid number (mgKOH/g)	0.5 maximum	0.6	0.9	0.62	0.35
Methanol or ethanol (m/m)	0.2 %	N/A	0.2 %	N/A	N/A
Distillation (°C)	N/A	360 °C	360 °C	323 °C	337 °C
References	**Zheng et al. (2011)**		**Zheng et al. (2012)**	**Yang et al. (2012)**	**Li et al. (2012)**

2.12.3 Application of Edible Insects in Green Material Application

Edible insects can be utilized in green material application in several ways. One of the primary ways is through the use of insect frass, which is the excrement of insects. Insect frass is an excellent natural fertilizer that is rich in nutrients such as nitrogen,

phosphorus, and potassium, which are essential for plant growth (Fowles and Nansen, 2019). In addition to being a rich source of nutrients, insect frass also contains beneficial microorganisms that help to improve soil health (Menino and Murta, 2022).

Another way that edible insects can be used in green material application is through the production of insect-based compost. Composting is the process of breaking down organic materials into a nutrient-rich soil amendment. Insect-based compost can be produced by combining insect frass with other organic materials such as food waste and yard trimmings (Menino and Murta, 2022). The resulting compost is rich in nutrients and beneficial microorganisms, making it an excellent soil amendment for plant growth. Edible insects can also be used in green material application through the production of insect-based bioplastics. Bioplastics are plastics that are made from renewable materials such as plants or animal products. Insect-based bioplastics are made from the chitin and protein found in insect exoskeletons. These bioplastics are biodegradable and have the potential to replace petroleum-based plastics, which are non-biodegradable and harmful to the environment (Palencia et al., 2021). Insect-based oils can also be used in green material applications such as biodiesel production. Insect oils are high in fat and can be extracted through a process called cold pressing. The resulting oil can be used as a feedstock for biodiesel production, providing a renewable and sustainable alternative to traditional fossil fuels.

One of the challenges to using edible insects in green material applications is the need for large-scale insect rearing operations. However, recent advancements in insect rearing technology have made it possible to rear insects on a large scale, making their use in green material applications more feasible.

2.13 Circular Economy of Black Soldier Fly

Circular economy, also known as closed-loop economy, it simply means that waste in the material and energy flows of industrial and commercial systems is minimised. The adoption of multidisciplinary scientific approaches results in the closed looping system (Wynants et al., 2017). The circular economy system involves narrowing, shutting, and slowing down material and energy loops to minimise overall resource and energy input. In a circular economy, biological materials that are already existing or that are produced as a consequence of a process, or a model are managed such that they may be recycled back into the biosphere without having an adverse effect on the environment. Ecosystems are employed as a model for the circular economy in this material flow, which is a type of biomimicry (limitation of the living) (Wynants et al., 2017). Contrasting the conventional linear economy, the linear economy produces the final product and discards the waste. The guiding principles of circular economy place a strong emphasis on recycling and reusing waste materials to create goods with higher perceived value. The biotransformation of black soldier fly into beneficial nutrients and uses in agriculture are illustrated in Figure 2.5.

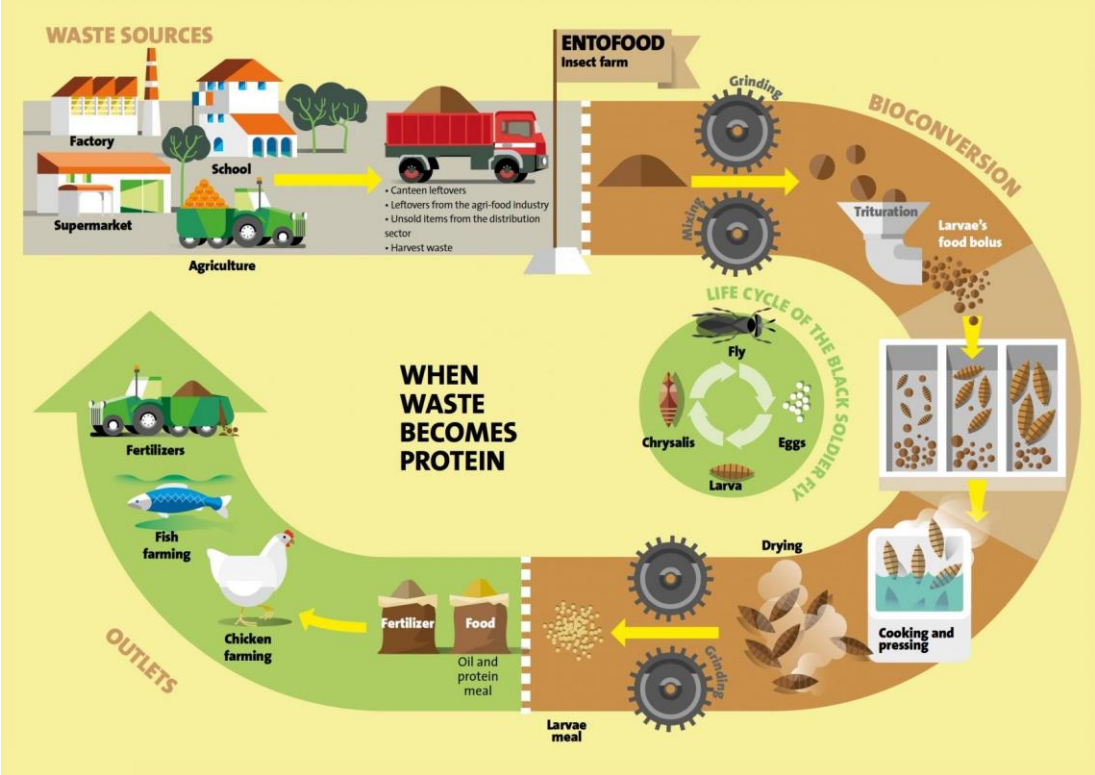

Figure 2.5: Biotransformation of black soldier fly into beneficial nutrients and uses in agriculture applications (Verma, 2022).

Nevertheless, the anthropogenic flow of energy is integrated by the circular economy (Dréau, 2014). This energy flow also demonstrated by BSF, where the raising of BSFL from agricultural fruit waste represented the mining of primary energy resources. Solvent extraction was used to get the main energy source in the form of crude lipids. To generate biodiesel, FAME, the crude lipids, which are a precursor for the fuel, are then transformed into secondary energy. In addition, for biodiesel-powered machinery or fuel-electric products to provide electricity and mechanical energy for their respective applications, the secondary energy, biodiesel, will be transformed to mechanical, heat, and electrical energy. These energy sources might eventually lead to the primary energy extraction process, where electrical energy could be used to provide heat energy for solvent extraction. However, not all energy can be stored and regenerated; as a result, some energy may be transferred to the environment. Figure 2.6 shows the energy flow that humans are responsible for.

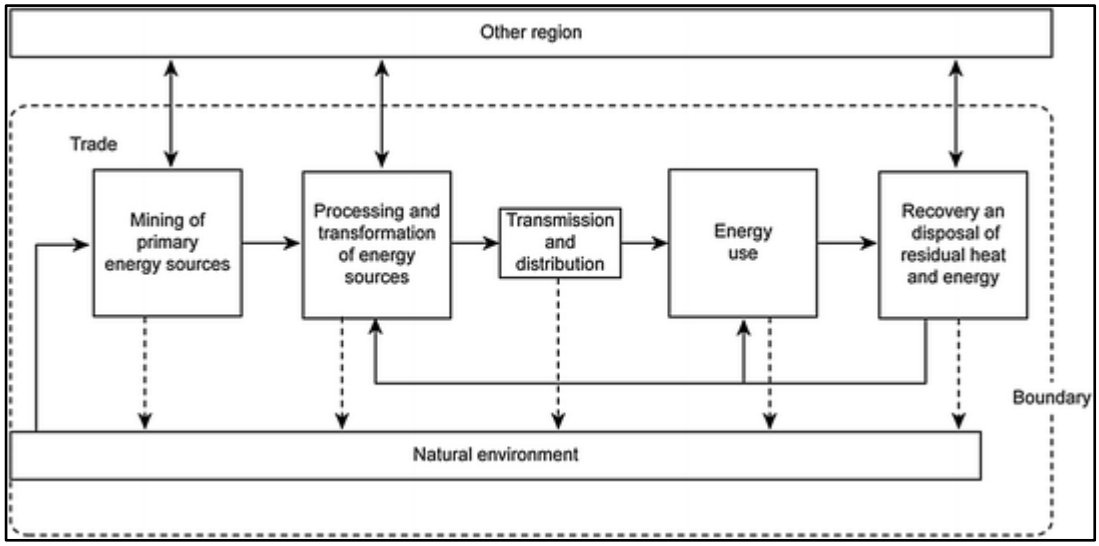

Figure 2.6: Frame model of the anthropogenic energy (Dréau, 2014).

CHAPTER 3

RESEARCH METHODOLOGY

Phase 1, Phase II, and Phase III of the strategy are visualized in Figure 3.1. Firstly, BSFL larvae farming, and harvesting comprise Phase I. Phase II of the investigation studies at optimization based on various drying parameters by using Response Surface Methodology (RSM). Subsequently, Phase III explained the characterization of changes on the functional properties of oil based on BSFP.

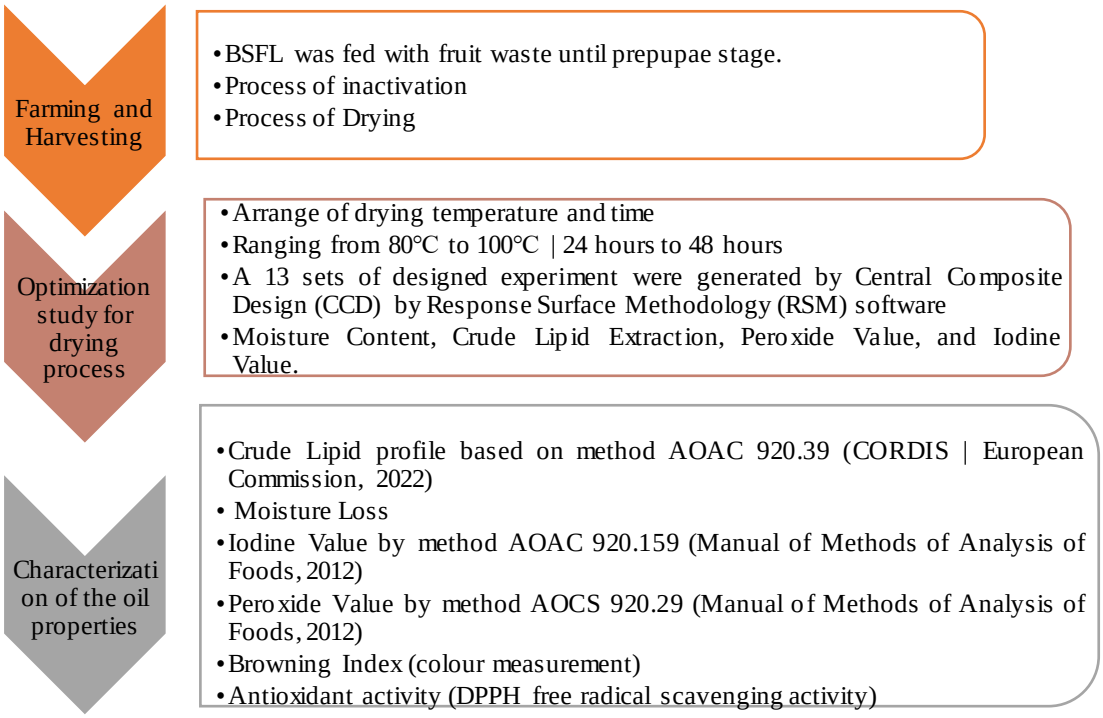

Figure 3.1: Flowchart for overview of study.

The BSF young larvae were reared in Block J, Engineering Workshop, University Tunku Abdul Rahman (UTAR). The BSFL were fed with Argo waste (fruit waste) collected from the fruit suppliers in Ipoh, Perak, Malaysia.

3.1 Phase I: Farming and Harvesting

The BSFL were raised from larvae stage until prepupae stage. The process of harvesting started when there was an obvious change in colour of BSFL from beige into dark brown.

3.1.1 Feed Preparation

To prevent microbial growth and prolong the shelf life of the fruit waste obtained from the fruit stalls, it was stored in a refrigerator at a temperature of -16 °C. Prior to feeding, the fruit waste was thawed in a fume hood a day in advance. To ensure appropriate feeding, a specific amount of fruit waste was provided to each tray containing individual larvae. The fruit waste obtained from the fruit providers is shown in Figure 3.2.

Figure 3.2: Fresh fruit waste collected from fruits suppliers and prepared into slices for the larvae.

3.1.2 Harvesting

During the prepupae stage, the BSF was confined in a cage (Figure 3.3) where they would remain until they reached adulthood. This area within the cage would serve as their designated mating center after the successful hatching of the prepupae. Additionally, some trays were placed inside the cage, containing fruit peels and waste, which provided an excellent medium for the adult black soldier flies to lay their eggs after mating process.

Figure 3.3: Cage served as activity centre for BSF adult for mating process.

Figure 3.4: Trays containing fruit peels and fruit waste as a medium for deposition of BSF eggs.

According to Figure 3.5, BSFP were placed inside an ice cream container before placed into the cage for the prepupae to hatch into adult. In addition, a pot of plant serves not only as a mating site for BSF, but also a place for the adult BSF to rest. After the hatching of the adult BSF, the mating would start soon. It can be observed that the BSF adult roaming around the cage for mating process whereby the

male adult impregnates the female adult to lay eggs. Figure 3.6 shows the mating process of BSF adult.

Figure 3.5: BSFP undergoing the stage of transformation into adult fly.

Figure 3.6: Mating process of male and female BSF adult.

The potted plant was well-cared by watering daily and the activity of BSF inside the cage is monitored. The cage is visited from time to time to observed if there are new eggs deposited on the fruit peels. To elaborate more, the fruit waste

inside the trays were to be fed to all neonates or the young larvae which is not discovered to avoid them from dying.

As for the rearing of larvae, fruit waste was used as feed for the young larvae (beige colour) as well as the prepupae (dark brown colour) in the tray placing on the four-tiers metal rack. The portions of fruit waste fed were based on the feed integrity. Taking 300 grams of BSF larvae as instance, 1 gram of BSFL able to consume 2 times of its weight per day. Therefore, approximately of 600 grams of fruit waste was measured by electronic weighing scale before the feeding for the larvae.

Figure 3.7: Process of weighing the fruit waste prior to feeding.

The trays of larvae containing fruit waste were cleaned daily to avoid disturbance from the rodents (i.e., birds, mice, cockroaches). This is because encroachment of rodents will eventually contaminate the BSF larvae subsequently affect the following results analysis. Apart from that, laundry bags were used to cover all the tray containing the BSFL not only to avoid disturbance from other creatures but allowing the BSFP to escape from the moist environment and ease for collection. The escaped prepupae were collected underneath the trays of the laundry bag and were harvested for lab experimentation.

After the harvesting process of prepupae, the BSF prepupae then undergo washing over a sieve and running tape water to get rid of any fruit waste (feed) adhered on the body of the prepupae. The prepupae were then blot dry and cleaned

thoroughly. The cleaned prepupae were kept inside a plastic container for freezing in freezer before inactivation process, while leaving some portion of them into the cage for hatching into adult for new cycle of life. The life cycle was continued until enough prepupae raw sample was collected for lab experimentation.

3.2 Phase II: Optimization Study for Drying Process

In this study, the Central Composite Design (CCD), which generates the parameters of drying before feed processing, was used to create the experimental design. The next sections provide a detailed explanation of the procedure.

3.2.1 Experimental Design

The Response Surface Methodology (RSM), served as the basis for this study experimental design. The Central Composite Design was the one that was utilised (CCD). The experiment design was based on two variables: the oven drying temperature (°C) and drying time (hr).

The model of the experiment design:

$$2^k + 2k + 1 + n_c \tag{3.1}$$

Whereby,

$k = Numeric\ factor\ (2\ varying\ variable\ for\ CCD)$

$n_c = Center\ points\ (5\ for\ CCD\ ranging\ from\ 2\ factor\ to\ 3\ factor)$

There are total of 13 combinations of the experiment based on the CCD. The parameters inserted into the Design-Expert Software Version 13 were tabulated in Table 3.1.

Table 3.1: Input parameters of Central Composite Design (CCD).

Drying Method	Drying time (hour)	Drying Temperature (°C)
Oven Drying	24 – 48	80 - 100

The BSFP was defrosted from freezer, and weighted ranging 50g to 100g (depending on daily harvesting basis) wet weight per sample. The defrosted prepupae were dried in laboratory drying oven for parameters based on CCD. Prior to drying, the prepupae were distributed evenly in rectangular folded aluminium sheet just before drying to ensure sample drying process run smoothly. Figure 3.8 shows the drying oven.

Figure 3.8: Drying Oven.

For Oven Drying, the 13 experimental sets in the Response Surface Methodology (RSM) tool were set at ranging from temperature of 80 °C to 100 °C, with drying time ranging from 24 hours to 48 hours. The experimental combinations generated from the RSM tool are shown in Table 3.2.

Table 3.2: Oven Drying experimental set parameters generated based on central composite design.

Drying Method: Oven Drying			
Std	Run	Drying Temperature (°C)	Drying Time (hour)
11	1	90.00	36.00
2	2	100.00	24.00
8	3	90.00	52.97
5	4	75.86	36.00
10	5	90.00	36.00
1	6	80.00	24.00
9	7	90.00	36.00
7	8	90.00	19.03
13	9	90.00	36.00
6	10	101.14	36.00
4	11	100.00	48.00
12	12	90.00	36.00
3	13	80.00	48.00

The prepupae biomass was sent to the oven (Figure 3.9) according to the experimental set parameters generated based on central composite design.

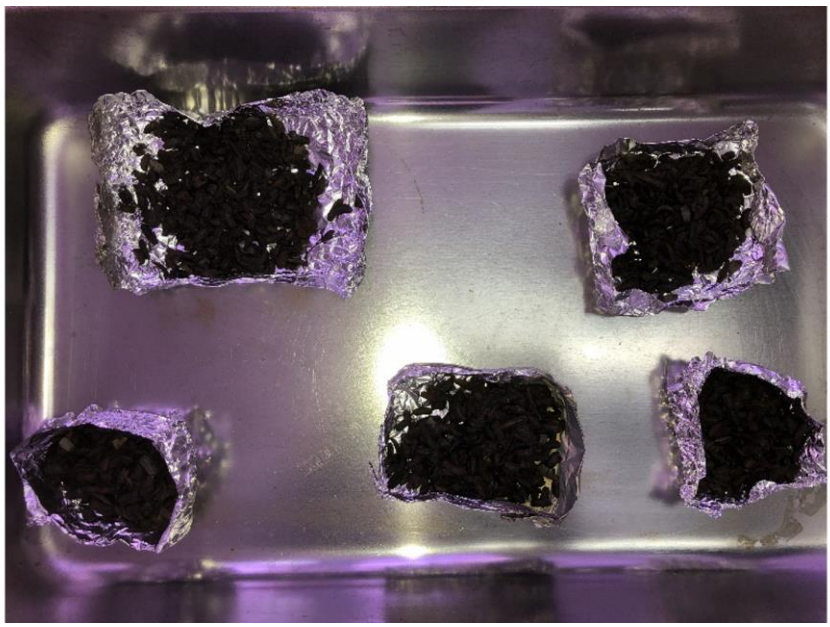

Figure 3.9: Oven dried prepupae biomass.

To minimize the dried BSFP prepupae biomass (DPB) free volume and enhance the surface area during crude lipid extraction, the biomass will be deposited in the blender and grinded into a fine powder or paste. The moisture content of the DPB will alter because of the different drying temperatures and drying times. Thus, it was hypothesized that the macerated DPB would have a texture ranging from a fine powder to a paste. Figure 3.10 shows the DPB in fine powder form.

Figure 3.10: Macerated DPB powder.

3.3 Phase III: Characterization Changes on the Functional Properties of Oil

The crude lipid of BSFP was extracted by using Soxhlet Extraction method. The dried prepupae biomass (DPB) was macerated by using a blender prior to the extraction. The methods listed below were used to characterise the characteristics of crude lipid. All the chemicals used in this study were purchased from Merck with a purity assay of ≥ 95 %.

Table 3.3: Method used in characterization of crude lipid.

Type of Characterization	Method
Crude Lipid	AOAC 920.39 (CORDIS | European Commission, 2022)
Iodine Value (IV)	AOAC 920.159 (Manual of Methods of Analysis of Foods, 2012)
Peroxide Value (PV)	AOCS 920.29 (Manual of Methods of Analysis of Foods, 2012)
Browning Index (BI)	Spectrophotometer (CIELAB colour space) CM-600d
Antioxidant Activity	2, 2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant assay

3.3.1 Crude Lipid Yield Determination

According to AOAC Technique 920.39, the Soxhlet method was applied to determine the crude lipid content of dried BSF prepupae biomass (DPB). DPB weighing in at around 5.0 g was recorded as W_1. In a thimble, the DPB was poured and then put in the extraction chamber. The weight of a dry, clean boiling flask was measured and recorded as W_2. The round bottom flask was filled with about 200 mL of n-hexane, and the DPB was allowed to reflux for four hours. Figure 3.11 shows the setup for the Soxhlet crude lipid extraction experiment. As seen in Figure 3.12, the crude lipid will be extracted using the n-hexane solvent.

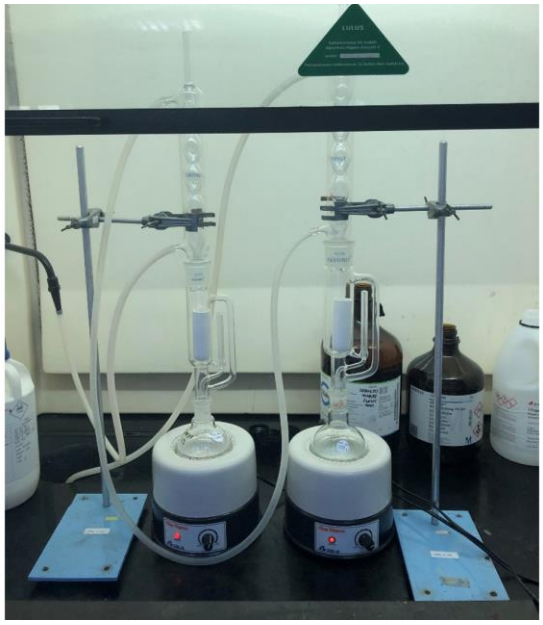

Figure 3.11: Apparatus set-up for Soxhlet Extraction.

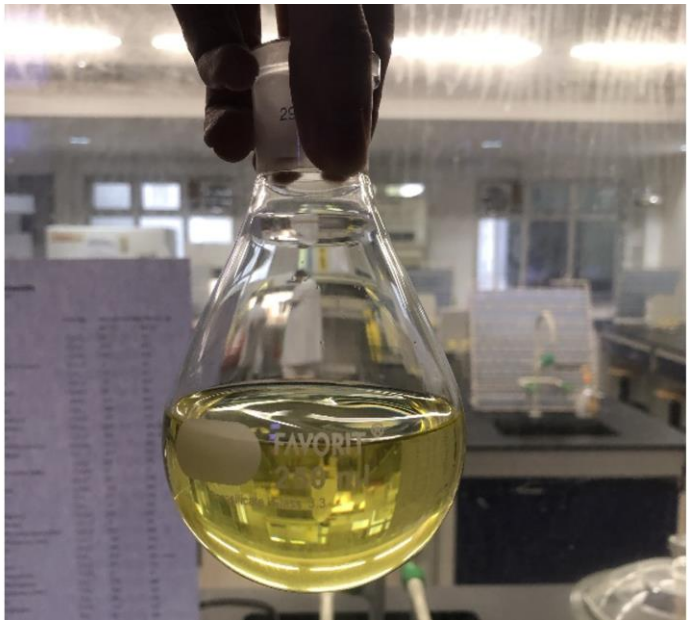

Figure 3.12: Extraction of crude lipid by n-hexane.

After the Soxhlet Extraction, the n-hexane containing dissolved crude lipid in the round bottom flask was taken to rotary evaporator (Figure 3.13) for removing the n-hexane from the dissolved crude lipids. The pure extracted crude lipid which is free of n-hexane is shown in Figure 3.14.

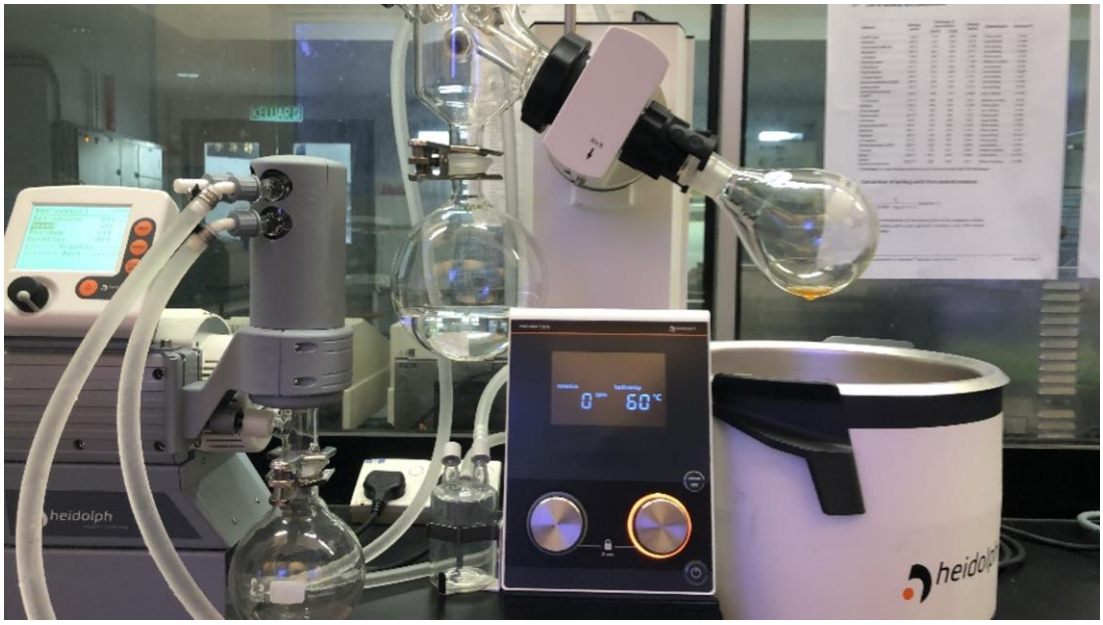

Figure 3.13: Rotary evaporator is used for the removal of n-hexane from crude lipid.

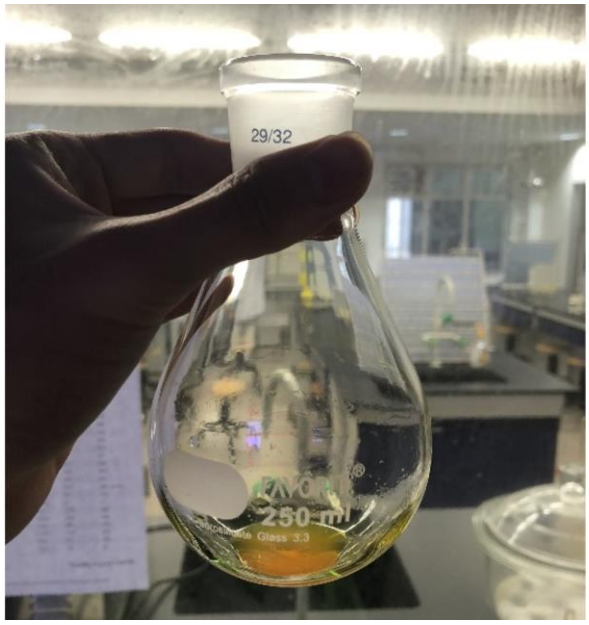

Figure 3.14: Extracted crude lipid.

The round bottom flask containing lipid extracts was recorded as W_3. The following equation was used to obtain the crude lipid percentage.

$$Crude\ Lipid\ Content\ (\%) = \frac{W_3 - W_2}{W_1} \times 100\% \qquad (3.2)$$

Whereby,

W_1 =Weight of DPB (g)

W_2 = Weight of boiling flask (g)

W_3 = Weight of the extract and boiling flask (g)

3.3.2 Iodine Value (IV)

The iodine value was calculated with the AOAC 920.159 standard. A measurement of the unsaturation of fats and oils is the iodine value expressed in terms of the percentage weight of iodine absorbed (number of centigrammes of iodine absorbed per gramme of sample).

The starch solution was obtained by dissolving 1 g of soluble starch (ACS Reagent Grade) in 100 mL of distilled water. The labels "BLANK" and "SAMPLE" were placed on two 250 mL iodine flasks, respectively. Each flask is pipetted with 20 mL of chloroform. A 1 mL micro-beaker was filled with 0.5 g of crude oil. Chloroform was dissolved in the micro-beaker and put into the "SAMPLE"-labeled iodine flask. Iodine monochloride (Wijs) solution of around 25 mL was pipetted into the "BLANK" and "SAMPLE" iodine flasks. For 30 minutes, both flasks were left in the dark. The burette was filled with standard 0.1 N sodium thiosulfate solution. The "BLANK" flask was taken out of the shadows. The flask was filled with 20 mL of a 10% potassium iodide solution, then 100 mL of distilled water, and the mixture was quickly shaken while being titrated to a yellow hue with the standardised sodium solution. The flask then received around 2 mL of starch solution. A dark purple colour served as the solution's indicator. The titration process was repeated with sodium thiosulfate, which was added drop by drop while being forcefully shaken into a clear and colourless solution. It was recorded exactly how much of the 0.1 N standardised sodium thiosulfate solution was used in the titration. The "SAMPLE" iodine flask underwent the identical procedures, beginning with the filling of the burette with standardised 0.1 N sodium thiosulfate solutions. Figure 3.15 shows the apparatus set up for iodine value experiment.

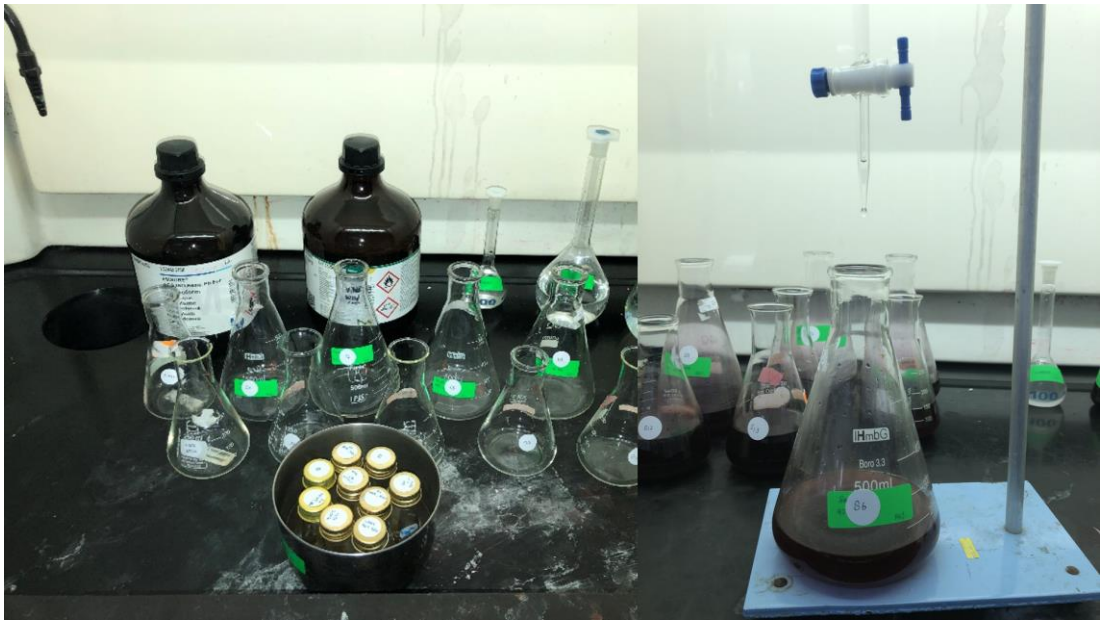

Figure 3.15: Experimental setup of iodine value.

The iodine number was calculated using the formula below:

$$Iodine\ Value\ (IV) = \frac{(B - S) \times N \times 12.69}{SW} \tag{3.3}$$

where IV = Iodine Value (gI/100g)

B = Volume of sodium thiosulphate used in titration of "BLANK" sample (mL)

S = Volume of sodium thiosulphate used in titration of "SAMPLE" sample (mL)

N = Normality of sodium thiosulphate solution used in titration of "BLANK" and "SAMPLE" samples (mEq/L)

SW = Weight of oil acid sample used (g)

3.3.3 Peroxide Value (PV)

To determine the peroxide value, the AOAC 920.29 method was utilized, which involves measuring the amount of peroxide oxygen per 1 kilogram of oil or fat. A glass stoppered Erlenmeyer flask was used to hold approximately 0.5 g of the oil sample, and the weight was noted. Next, a solvent mixture of acetic acid to

chloroform in a 3:2 ratio was added to the flask, and the contents were swirled until the sample was completely dissolved. Then, a 1 mL Mohr pipette was used to add about 0.5 mL of saturated potassium iodide solution to the flask, which was then swirled for precisely one minute. Graduated cylinders were used to add about 30 mL of distilled water to the flask, and the resulting mixture was shaken vigorously to release the iodine from the chloroform layer. A burette was filled with 0.01 N sodium thiosulfate, and titration began when the solution's initial deep red-orange colour was noted. The sodium thiosulfate was added until the colour lightened. If the solution was initially light amber in colour, 5 to 7 drops of 1 % starch indicator were added using a pipette, and titration was continued until the blue-grey colour disappeared in the upper aqueous layer. The volume of titrant used was recorded, and the setup of the experiment is shown in Figure 3.16.

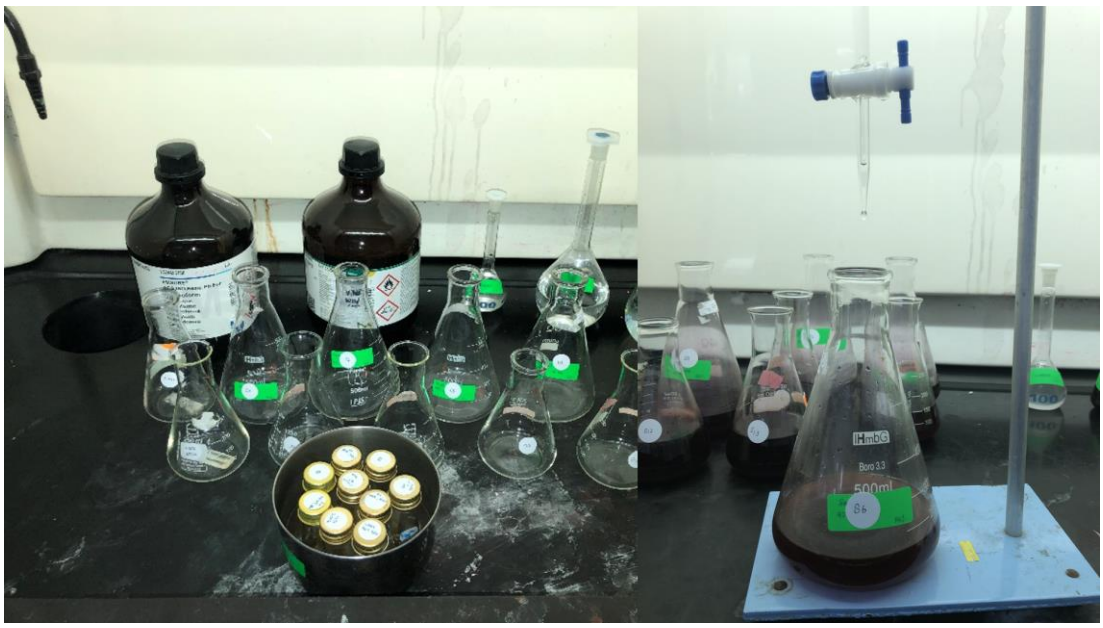

Figure 3.16: Experimental setup of peroxide value.

The formula for peroxide value is as follows:

$$Peroxide\ Value\left(\frac{mEq\ O_2}{kg}\right) = \frac{(S-B) \times N\ Thiosulfate\ \times\ 1000}{weight\ of\ sample} \quad (3.4)$$

Where,

PV = Peroxide Value

S= Titration of sample (mL)

B=titration of blank (mL)

N= Normality of sodium thiosulfate (mEq/L)

3.3.4 Browning Index (BI)

The spectrophotometer CM-600d in the CIELAB colour space was used to determine the browning index of the oven-dried BSFP. Approximately 2 grams of the sample were evenly spread in a sample dish, and the color was measured at three different points. The LAB values were recorded immediately following the drying process, and the browning index was calculated using a specific equation.

$$x = \frac{(a* + 1.75L*)a*)}{5.645L* + a* - 3.012b*} \tag{3.5}$$

$$Browning\ Index = 100 \times \frac{x - 0.31}{0.17} \times 100\% \tag{3.6}$$

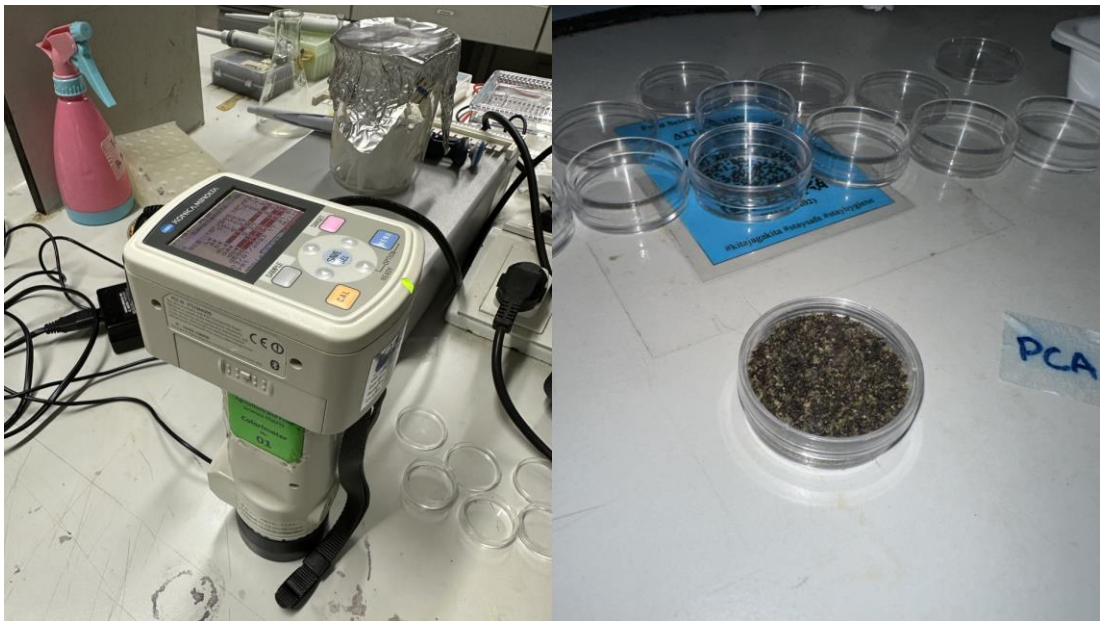

Figure 3.17: Experimental set-up for browning index determination.

3.3.5 DPPH Free radical Scavenging Activity

To determine the antioxidant properties of oven dried, the DPPH free radical assay was employed as described in reference. Alcoholic extraction was carried out on approximately 5 g of raw material for oven dried BSFP. The sample was soaked in 100 mL of 99 % methanol at a ratio of 1:20 and incubated at 25 °C for 60 minutes. The methanol extract (ME) was obtained by vacuum-filtering the solution to separate it from the residue and then centrifuging the filtrate at 6800 rpm for 5 minutes to obtain a supernatant with a concentration of 50 mg DM/mL. The ME was stored at -20 °C until the DPPH assay was conducted.

For the DPPH assay, 0.10 mM DPPH reagent was freshly prepared by mixing 1.97 mg of DPPH powder with 50 mL of methanol. Then, 500 µL of ME was added to 700 µL of the DPPH reagent in a 1.5 mL micro-centrifuge tube and vortexed for 10 seconds. The ME mixture was incubated at room temperature and in the dark for 30 minutes, while a negative control was prepared by reacting 500 µL of methanol with 700 µL of DPPH reagent in a 1.5 mL micro-centrifuge tube and vortexed for 10 seconds. A blank control was also prepared by 1.2 mL of methanol in a 1.5 mL micro-centrifuge tube. The negative control and blank control were treated similarly to the ME mixture. After the incubation period, 200 µL of each of the samples, blank control and negative control were transferred to a 96-well microtiter plate and their absorption was measured at 517 nm using a BMG Labtech microplate reader. The DPPH free radical scavenging activity was calculated using a specific equation.

$$DPPH\ FR\ Scavenging\ Actvity\ (\%) = \frac{(An - Ab) - Ame}{(An - Ab)} \times 100\% \quad (3.7)$$

Where

An = The absorbance of DPPH solution with methanol as negative control

Ab = The absorbance of methanol as blank control

Ame = The absorbance of DPPH solution with methanol extract of sample.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Process Optimization using Response Surface Methodology

The process optimization study is carried out using RSM with Design Expert optimization tool. The effect of different drying parameters (24 – 48 hours, 80 °C – 100 °C) towards moisture loss, crude lipid yield, iodine value, peroxide value, browning index, and antioxidant activity were discussed in this chapter.

4.2 Optimization of Moisture Loss using Response Surface Methodology

The Design Expert software with response surface methodology generated 13 experimental sets from central composite design to optimize the parameters for oven drying on the moisture loss of Black Soldier Fly Prepupae (BSFP). The results, including predicted and experimental data for moisture loss, a summary table of model fit summary, analysis of variance (ANOVA), and a 3D surface plot for oven drying, were presented on Table 4.1, Table 4.2, Table 4.3, and Figure 4.1.

Table 4.1: The central composite design and moisture loss results for oven dried BSFP.

Experimental Set	Drying Temperature (°C)	Drying Duration (hour)	Predicted Moisture Loss (%)	Experimental Moisture Loss (%)
D19.03T90	90	19.0294	62.3843	62.7956
D24T80	80	24	60.044	59.7228
D24T100	100	24	65.4279	65.1771
D36T90A	90	36	64.9015	64.5316
D36T90B	90	36	64.7526	64.5316
D36T90C	90	36	64.629	64.5316
D36T104.14	104.142	36	65.1568	65.0906
D36T90D	90	36	64.3693	64.5316
D36T90E	90	36	64.0056	64.5316
D36T75.86	75.8579	36	60.2389	60.2722
D48T100	100	48	64.7463	65.1003
D48T80	80	48	63.4567	63.7404
D52.97T90	90	52.9706	66.0263	65.5822

The fit summary in Design Expert is a statistical report that provides an overview of how well the fitted model represents the experimental data. It includes various statistical measures that describe the goodness of fit of the model, such as the coefficient of determination (R-squared), adjusted R-squared, root mean square error (RMSE), lack of fit, and p-values.

Table 4.2: The model fit summary for moisture loss of oven dried BSFP.

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0014	0.0108	0.6771	0.4738	
2FI	0.0480	0.0194	0.7731	0.6012	
Quadratic	**0.0021**	**0.2577**	**0.9498**	**0.8569**	Suggested
Cubic	0.1030	0.9016	0.9717	0.9784	Aliased

According to Table 4.2, there were 4 model presented in the model fit summary. Choosing the right model in Response Surface Methodology (RSM) involves defining the response variable, identifying the factors that affect it, designing the experiment, fitting the model, optimizing the response, validating the model, and refining the model as needed.

Based on Table 4.2, quadratic model was chosen out of the 4 sources. The R-Squared value obtained is 0.9707 which is the highest compared to other sources of model. The greater the R-Squared value, the stronger the correlation of the effect variables. Besides, the Adjusted R-Squared value obtained is 0.9498 which is higher than other models available.

Table 4.3: Analysis of Variance (ANOVA) for the model terms in moisture loss of BSFP.

Source	Sum of Squares	Degree of Freedom	Mean Square	F-value	p-value	
Model	41.14	5	8.23	46.38	< 0.0001	significant
A-Temperature	23.22	1	23.22	130.86	< 0.0001	
B-Time	7.77	1	7.77	43.77	0.0003	
AB	4.19	1	4.19	23.62	0.0018	
A^2	5.95	1	5.95	33.55	0.0007	
B^2	0.2043	1	0.2043	1.15	0.3189	
Residual	1.24	7	0.1774			
Lack of Fit	0.7438	3	0.2479	1.99	0.2577	not significant
Pure Error	0.4982	4	0.1245			
Cor Total	42.39	12				
R^2	0.9707	**Adjusted R^2**	0.9498	**Adequate Precision**	20.4758	

The model is significant, as indicated by the F-value of 46.38. The model terms are significant if the model P-value is less than 0.0500. A, B, AB, and A^2 are significant model terms. If the P-value is greater than 0.1, the model is deemed not significant. In this case, B^2 is an insignificant model term. The obtained R-squared and Adjusted R-squared values were, respectively, 0.9707 and 0.9498, and both are in reasonable agreement (Majdi and Esfahani, 2018). The Adequate Precision measures the signal to noise ratio. Adequate Precision compares the range of predicted values at design points to the average prediction error. The ratio of 20.4758 in this study is greater than 4 which indicates an adequate signal to noise ratio. Therefore, the model can be used to navigate the design space.

The moisture loss equation presented in terms of coded factors can be utilized to estimate the response for specific levels of each factor. The coded equation is

valuable for evaluating the impact of the factors by analysing the factor coefficients, which was calculated based on equation 4.1.

$$Moisture\ Loss = 64.53 + 1.70A + 0.9852B - 1.02AB - 0.9251A^2 - 0.1714B^2 \quad (4.1)$$

Where,

$A = Drying\ Temperature\ (°C)$

$B = Drying\ Duration\ (hours)$

4.2.1 Effect of Drying Temperature and Time on Moisture Loss

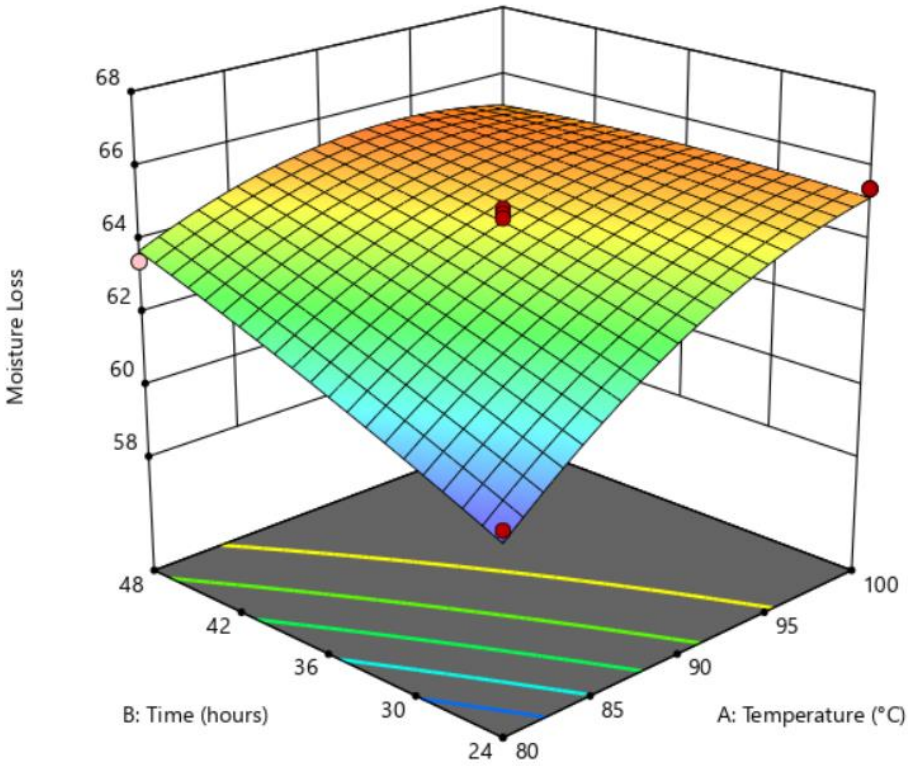

Figure 4.1: 3D surface plot between different drying duration and temperature for moisture loss of BSFP.

The Figure 4.1 illustrates the 3D response interaction of moisture loss between drying duration and drying temperature. Based on the response interaction (Figure 4.1), as the temperature increases from 80 °C, the moisture loss of oven dried BSFP increases gradually until a nearly constant rate of 65.4279 % at 100 °C. The drying curves demonstrate that as drying time increases, the moisture loss increases until a plateau surface. According to Zhang et. al (2019), the temperature of the drying air affects the moisture content, with higher temperatures resulting in greater moisture loss and shorter drying times since the evaporation rate increases. This is likely due to increased heat transfer between the BSFP and the air temperature inside the oven, leading to faster moisture removal.

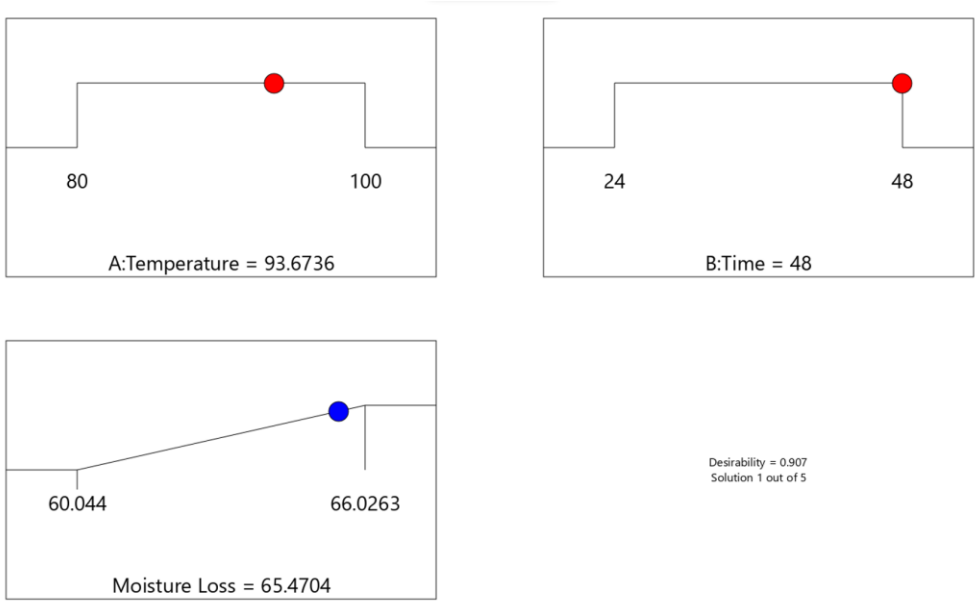

Figure 4.2: Selected optimization result for moisture loss.

Referring to Figure 4.2, a predicted optimum result of moisture loss is generated with drying parameters of drying temperature at 93.67 °C and drying duration of 48 hours. As the surface area exposed during oven drying can also results in lesser moisture loss. The heat transfer during the drying process may not be efficient as the surface area exposed to drying is lesser compared to other drying parameters and it can be explained by the evaporation phenomenon. Evaporation refers to the process in which a substance, moisture in BSFP in this case, transforms from a liquid to a gas through the application of heat (Zhang et al., 2019). The rate of evaporation rises as the surface area expands. This is because the more extensive the

surface area that comes into contact with the air, the greater the number of molecules that will break free into the air (Poós and Varju, 2020).

In addition to the drying conditions in the oven, it is important to consider the surrounding environment, which can also impact the experimental data obtained. To ensure the most accurate moisture loss results, immediate weighing of the dried BSFP biomass is necessary. However, changes in room temperature in the laboratory can affect these results, as the dried samples may re-absorb moisture from the surrounding environment when removed from the oven at a high temperature. This can lead to moisture absorption or rebound, also known as moisture regain, is a phenomenon that occurs when a dried material absorbs moisture from the surrounding environment (Zhang et al., 2019). In the context of drying biomass, moisture rebound can happen when the dried sample is exposed to humidity in the laboratory or ambient air after being removed from the drying oven. This will eventually cause an increase in moisture content and a subsequent increase in weight of the sample, leading to experimental data that differs from the predicted moisture loss. As mentioned by Zhang et al. (2019) earlier, Moisture rebound is an important consideration when performing moisture analysis on dried materials, as it can impact the accuracy of the results. resulting in slight differences between the experimental data and predicted moisture loss.

4.3 Optimization of Crude Lipid Yield using Response Surface Methodology

To optimise the oven drying conditions for (BSFP) and corresponding crude lipid yield, 13 sets of experiments have been generated by Design Expert software with response surface methodology. Table 4.4 shows the experimental and predicted crude lipid yield. Based on the results, the crude lipid yield obtained was consistent throughout various drying parameters.

Table 4.4: The central composite design and crude lipid yield results for oven dried BSFP.

Experimental Set	Drying Duration (hour)	Drying Temperature (℃)	Experimental Crude Lipid Yield (%)	Predicted Crude Lipid Yield (%)
D19.03T90	90	19.0294	49.6275	49.9078
D24T80	80	24	48.0881	48.8539
D24T100	100	24	47.2542	47.6612
D36T75.86	75.8579	36	47.9077	47.3893
D36T90A	90	36	49.4633	49.5228
D36T90B	90	36	49.9557	49.5228
D36T90C	90	36	49.0902	49.5228
D36T90D	90	36	49.7518	49.5228
D36T90E	90	36	49.3529	49.5228
D36T104.14	104.142	36	46.7321	46.7212
D48T80	80	48	46.1265	46.2489
D48T100	100	48	46.7332	46.4967
D52.97T90	90	52.9706	46.0521	46.2424

An overview of the degree to which the fitted model refers to the experimental data is presented in the fit summary (Table 4.5). The coefficient of determination (R-squared), adjusted R-squared, root mean square error (RMSE), lack of fit, and p-values are several types of statistical measurements that suggest the goodness of fit of the model.

Table 4.5: The model fit summary for crude lipid yield of oven dried BSFP.

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0014	0.0108	0.6771	0.4738	
2FI	0.0480	0.0194	0.7731	0.6012	
Quadratic	**0.0021**	**0.2577**	**0.9498**	**0.8569**	**Suggested**
Cubic	0.1030	0.9016	0.9717	0.9784	**Aliased**

Based on Table 4.5, quadratic model was chosen out of the 4. The R-Squared value is 0.9172 which is the highest obtained compared to other sources of model. The greater the R-Squared value, the stronger the correlation of the variables in effect. Besides, the Adjusted R-Squared value of 0.8581 is also higher than other models available.

Table 4.6: Analysis of Variance (ANOVA) for the model terms in crude lipid yield for BSFP.

Source	Sum of Squares	Degree of Freedom	Mean Square	F-value	p-value	
Model	23.34	5	4.67	15.52	0.0011	significant
A-Temperature	0.4464	1	0.4464	1.48	0.2626	
B-Time	7.10	1	7.10	23.62	0.0018	
AB	0.5189	1	0.5189	1.73	0.2304	
A^2	10.59	1	10.59	35.21	0.0006	
B^2	6.60	1	6.60	21.93	0.0023	
Residual	2.11	7	0.3008			
Lack of Fit	1.65	3	0.5487	4.78	0.0825	not significant
Pure Error	0.4594	4	0.1148			
Cor Total	25.44	12				

R^2	0.9172	**Adjusted R^2**	0.8581	**Adequate Precision**	8.8044

The model is significant with a model F-value of 15.52 and P-value of less than 0.0500, respectively. B, A^2, and B^2 are significant model terms (Table 4.6). The model is not significant if the P-value is greater than 0.1. In this case, A and AB are both insignificant model terms. The obtained R-squared and Adjusted R-squared values were, respectively, 0.9172 and 0.8581, and both are in reasonable agreement (Majdi and Esfahani, 2018). The signal to noise ratio must be determined by the adequate precision. The range of predicted values at design points, and the average prediction error are compared to identify adequate precision. In such case, the ratio of 8.8044 is greater than 4, which denotes a sufficient signal-to-noise ratio. The model can therefore be used for navigating the design space.

The crude lipid yield equation presented in terms of coded factors can be utilized to determine the response for specific levels of each factor. This coded

equation is valuable for evaluating the impact of the factors by analysing the factor coefficients, which was calculated based on equation 4.2.

$$Crude\ Lipid\ Yield(\%) = 49.52 - 0.2362A - 0.9424B + 0.3602AB - 1.23A^2 - 0.9738B^2 \quad (4.2)$$

Where,

$A = Drying\ temperature$

$B = Drying\ Duration$

4.3.1 Effect of Drying Temperature and Time on Crude Lipid Yield

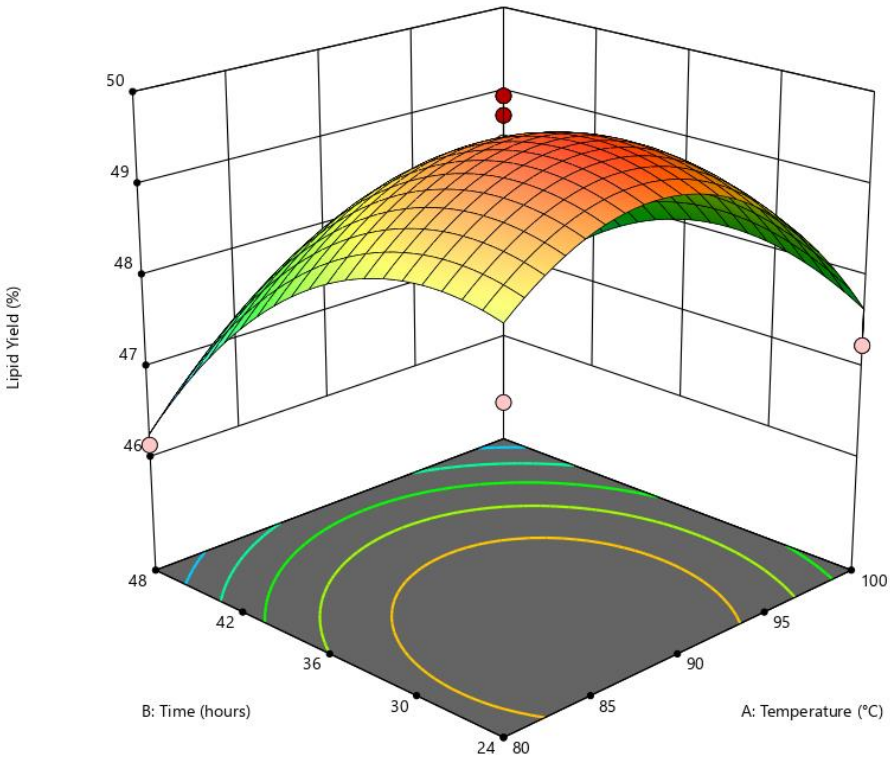

Figure 4.3: 3D surface plot between different drying duration and temperature for crude lipid yield of BSFP.

The 3D surface interaction revealed a quadratic relationship among the factors impacting crude lipid yield during oven drying of BSF prepupae. Additionally, the plot illustrated saddle points, as shown in Figure 4.3, indicating that the maximum crude lipid yield achievable was between 48% to 50% with a drying period of roughly 36 hours and a temperature range of 85 ℃ to 90 ℃. As the temperature rises from 80 ℃ to 100 ℃, a noticeable increase in crude lipid yield until a peak point, following with a decreased in crude lipid curve was observed. This can be attributed to the corresponding increase in moisture loss, as the dry weight of biomass is influenced by the drying temperature before crude lipid extraction. Higher drying temperatures lead to greater moisture loss, resulting in a lower contribution of moisture to the fixed weight of biomass sample. For instance, comparing a biomass sample dried at 80 ℃ for 24 hours with one dried at 100 ℃ for 24 hours, the former will have lower moisture loss and similar weight. Consequently, the higher moisture loss leads to a higher lipid composition in the biomass, ultimately resulting in increased crude lipid yield with rising drying temperature.

The crude lipid yield was observed to decrease after reaching approximately 90 ℃, which could be attributed to the loss of fat and moisture evaporation during the oven drying process. According to (Zhuang et al., 2022), high temperatures may cause the breakdown of lipid molecules through oxidation and hydrolysis reactions, leading to the formation of volatile compounds and loss of lipid content. At a higher temperature, rapid evaporation of the solvent used for lipid extraction, will result in lower lipid yields. Thermal decomposition of lipids may occur at high temperatures, leading to the formation of by-products that may not be extractable. Meanwhile, coagulation of proteins may occur at high temperatures, which can trap lipids within the protein matrix, making it difficult to extract them. This is consistent with (Okwakpam et al., 2023) study on preserving tilapia fish by drying at 110 ℃, which resulted in increased losses of lipids and fatty acids. However, Fombong et al. (2017) found that *Ruspolia Differens* did not exhibit a decrease in fat when dried at a lower temperature of 60 ℃, in contrast to Okwakpam et al. (2023) findings. As the decrease in crude lipid yield occurs at a temperature higher than that used in Fombong et al. (2017) study, it is possible that the fat may be lost along with the moisture evaporation. Additionally, high temperature drying of the oven can increase

the binding of lipids to prepupae biomass proteins and carbohydrates (AAFCO, 2014). Lipid-binding proteins can bind reversibly and non-covalently to lipids, enhancing their aqueous solubility and making them less constrained in transporting cellular soluble cytoplasm lipids (Glatz, 2015). The lipids were extracted using hexane, an organic solvent.

The efficiency of hexane organic solvent in extracting lipids from prepupae biomass may be compromised at a temperature of 75 °C, which aligns with AAFCO's (2014) findings that high temperature drying increases the binding of lipids to proteins and carbohydrates. Since there is limited research on the effect of temperature on insect lipid yield, microalgae studies are used as a reference. (Widjaja et al., 2009) found that higher drying temperatures reduce the recovery of triacylglycerol (TAGs) in microalgal biodiesel. TAGs are the main constituent of crude oil, and their reduction may result from high temperature oxidation that transforms TAGs into aldehydes and ketones, as seen in microalgae between 60 °C and 80 °C (Widjaja et al., 2009). According to Shen et al. (2016), triacylglycerol is responsible for the transesterification of Fatty Acid Methyl Esters (FAME), implying a direct correlation between the decrease in TAGs and the reduction in crude lipid yield.

On the account of that, according to Leong et al. (2016), BSF larvae fed with fruit waste had a higher crude lipid content compared to larvae fed with other wastes, except for fermented coconut waste endosperm. This is because the carbohydrates in the fruit waste were transformed into energy for growth, and any excess or unused energy was stored as lipid. However, Mohd-Noor et al. (2017) found that the highest crude lipid content was achieved at 57.95 % in larvae fed with fermented coconut endosperm, which is consistent with the study by Leong et al. (2022). Moreover, it is congruent with the findings from (Chun et al., 2019) which mentioned that the crude lipid (% in dried mass) between BSFP fed with fruit waste and palm decanter, sewage sludge, and fermented waste coconut endosperm has a significant difference in gap which are 46.83±1.37, 36.51±1.85, 29.85±1.45, and finally 57.95±2.05.

A potential explanation for the higher lipid yield in BSFL fed with fruit waste could be the indirect effect of environmental factors on their rearing conditions. According to (Harnden and Tomberlin, 2016), the metabolism of BSFL increases with environmental temperature, resulting in higher food intake and growth rates.

This is consistent with Hoefnagels (2021) findings that metabolism is a function of temperature due to the increase in kinetic energy of cells. Additionally, Rumpold and Schlüter (2013) have established that different rearing conditions and feed composition can directly affect the nutritional composition of mealworms. Since the rearing period of BSFL is approximately 4 months long, it is expected that weather, humidity, environmental temperature, and other factors may fluctuate throughout the period. Hot weather is more common in Malaysia, which may result in some batches of worms ingesting more food and storing more lipids. Conversely, some batches of worms may respond more to rainy weather, resulting in lower metabolic rates and food intake. These fluctuations may explain why the optimized parameter for crude lipid yield was lower than the predicted value. Furthermore, the new source of palm decanter waste used in the study may differ in dietary components, which could result in unbalanced nutrients and negatively impact the metabolism of the insects (Nation, 2008).

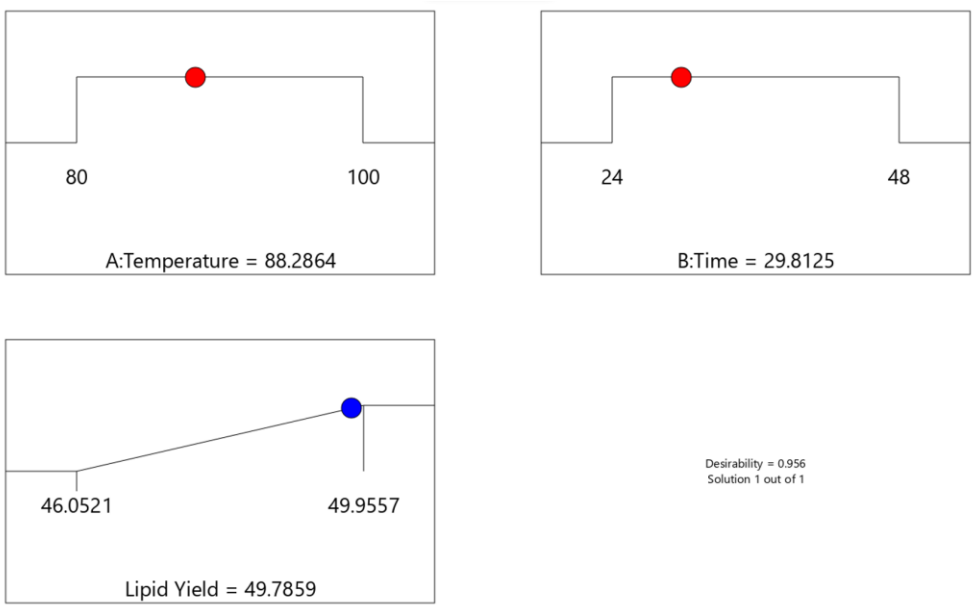

Figure 4.4: Selected predicted optimum result for crude lipid yield.

The Figure 4.4 shows the predicted optimum result for crude lipid yield. At drying temperature and time of 88.29 °C and 29.81 hours, a solution with desirability of 0.956 produces a lipid yield of 49.79 %. It can be further explained at drying parameters of 88.29 °C and 29.81 hours, the predicted optimum lipid yield is at 49.79 %, which can be prove that the BSFP fed with fruit wastes produces higher

lipid yield as compared to other feed compared to other studies which it is presented in Table 4.7.

Table 4.7: Comparison of crude lipid yield based on various type of feed on BSFP.

Types of feed fed on BSFP	Crude lipid yield (%)	References
Fruit waste	49.79	In this study
Fruit waste	46.83 ± 1.37	Chun et al. (2019)
Fruit waste	44.46 ± 0.79	Leong et al. (2016)
Restaurant waste with rice straw (Ratio of 7:3)	39.6 ± 1.2	Zheng et al. (2012)
Fermented waste coconut endosperm	57.95 ± 2.05	Mohd-Noor et al. (2017)
Food waste	31.8 ± 0.3	Surendra et al. (2016)
Food waste	43.7 ± 0.6	Salomone et al. (2017)

Based on Table 4.7, it can be observed the crude lipid yield obtained from BSFP fed with fruit waste does not differ much where the highest value obtained in this study is 49.79 %. The slight difference in the obtained lipid yield may be due to the different types of fruits fed on BSFP, meanwhile other types of feed on BSFP obtained lesser crude lipid as shown in Table 4.7. BSFP fed with self-fermented waste coconut endosperm by Mohd-Noor et al. (2017), showing crude lipid yield of 57.95 ± 2.05 %. This can be explained by the peak nutrients that was extruded from the self-fermented waste coconut endosperm which left for 4 weeks, allowing the BSFL to gain more weight at the same time promote the physiological growth of BSFL until reaches prepupae stage, resulting a highest crude lipid yield that can be observed from Table 4.7.

4.4 Optimization of Iodine Value using Response Surface Methodology

The utilization of Design Expert software along with response surface methodology led to the creation of 13 sets of experiments aimed at optimizing the parameters for oven drying of Black Soldier Fly Prepupae (BSFP) and its corresponding iodine value. The outcomes, which include a summary table of model fit summary, analysis of variance (ANOVA) and a 3D surface plot for oven drying, have been presented Table 4.8, Table 4.9, Table 4.10, and Figure 4.5.

Table 4.8: The central composite design and iodine value results for oven dried BSFP.

Experimental Set	Drying Duration (hour)	Drying Temperature (°C)	Experimental Iodine Value ($gI_2/100g$)	Predicted Iodine Value ($gI_2/100$)
D19.03T90	90	19.0294	13.0608	15.0608
D24T80	80	24	15.5795	13.0211
D24T100	100	24	16.4218	15.2761
D36T75.86	75.8579	36	9.3034	10.9639
D36T90A	90	36	23.4596	23.6143
D36T90B	90	36	22.8473	23.6143
D36T90C	90	36	23.4239	23.6143
D36T90D	90	36	24.6117	23.6143
D36T90E	90	36	23.7291	23.6143
D36T104.14	104.142	36	14.0698	13.7325
D48T80	80	48	11.6422	11.4647
D48T100	100	48	11.8899	13.1251
D52.97T90	90	52.9706	13.8064	12.7845

The fit summary in is a statistical report that provides an overview of how well the fitted model represents the experimental data. It includes various statistical measures that describe the goodness of fit of the model, such as the coefficient of

determination (R-squared), adjusted R-squared, root mean square error (RMSE), lack of fit, and p-values.

Table 4.9: The model fit summary for iodine value of oven dried BSFP.

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.8186	0.0001	-0.1529	-0.5455	
2FI	0.9633	< 0.0001	-0.2807	-1.0208	
Quadratic	**< 0.0001**	**0.0119**	**0.9052**	**0.6316**	**Suggested**
Cubic	0.0318	0.0438	0.9666	0.3881	**Aliased**

Based on Table 4.9, quadratic model was chosen. The R-Squared value is 0.9447 which is the highest obtained compared to other sources of model. The greater the R-Squared value, the stronger the correlation of the variables in effect. Besides, the Adjusted R-Squared value of 0.9052 is also higher than other models available.

Table 4.10: Analysis of Variance (ANOVA) for the model terms in iodine value for BSFP.

Source	Sum of Squares	Degree of Freedom	Mean Square	F-value	p-value	
Model	350.00	5	70.00	23.92	0.0003	significant
A-temperature	7.66	1	7.66	2.62	0.1496	
B-time	6.87	1	6.87	2.35	0.1693	
AB	0.0884	1	0.0884	0.0302	0.8670	
A^2	220.74	1	220.74	75.42	< 0.0001	
B^2	157.59	1	157.59	53.84	0.0002	
Residual	20.49	7	2.93			
Lack of Fit	18.83	3	6.28	15.16	0.0119	significant
Pure Error	1.66	4	0.4141			
Cor Total	370.49	12				

R^2	0.9447	**Adjusted R^2**	0.9052	**Adequate Precision**	10.8845

The model is significant, as indicated by the F-value of 23.92. The model terms are significant if the model P-value is less than 0.0500. A^2 and B^2 are both significant, respectively. A, B, and AB are insignificant model term, as the P-value is greater than 0.1. The obtained R-squared and Adjusted R-squared values were, respectively, 0.9447 and 0.9052, and both are in reasonable agreement (Majdi and Esfahani, 2018). In this case, the Adequate Precision metric is used to assess the signal to noise ratio. It determines the adequacy of the model by comparing the range of predicted values at design points with the average prediction error. The ratio of 20.4758 obtained in this study exceeds the threshold of 4, indicating a satisfactory signal to noise ratio. Consequently, the model is deemed suitable for navigating the design space.

The iodine value equation shown in terms of coded factors can be used to determine the response for specific levels of each factor. The coded equation generated by the model is calculated based on equation 4.3:

$$Iodine\ Value\ (IV) = 23.61 + 0.9778A - 0.9268B - 0.1486AB - 5.63A^2 - 4.76B^2 \quad (4.3)$$

Where,

$A = Drying\ Temperature$

$B = Drying\ Time$

4.4.1 Effect of Drying Temperature and Time on Iodine Value

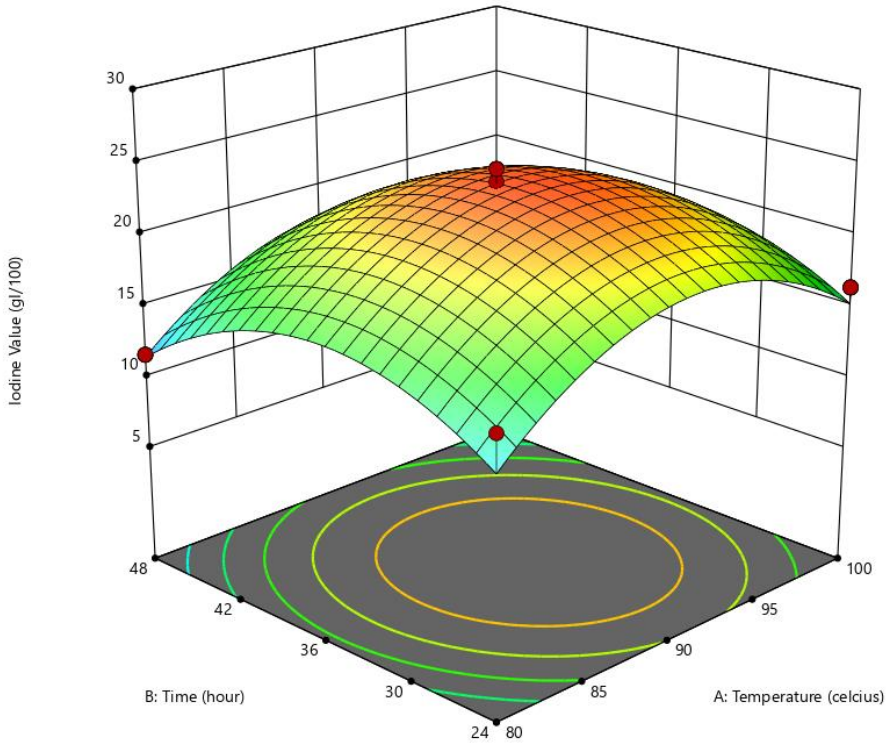

Figure 4.5: 3D surface plot between different drying duration and temperature for iodine value of BSFP.

The properties of the lipid extracted from the BSFP that was fed with fruit waste were scrutinized. Iodine value is used to measure unsaturation or the average number of double bonds in fats and oils. Decrease in iodine value shows decrease in the number of double bonds and it indicates oxidation of the oil. As shown in Figure 4.5, it can be observed the iodine value of BSFP increases gradually from 80 °C until It reaches peaked about 90 °C and experiences a decreased from 80 °C to 100 °C. Similarly, as drying time increases from 24 to about 36 hours, the curve increases until a peak point and decreases from 35 to 48 hours. The predicted optimum result (Figure 4.6) obtained from RSM in this study shows that, when the drying temperature and duration is 90.88 °C and 34.82 hours, the optimum iodine value was 23.70 gI_2/100g.

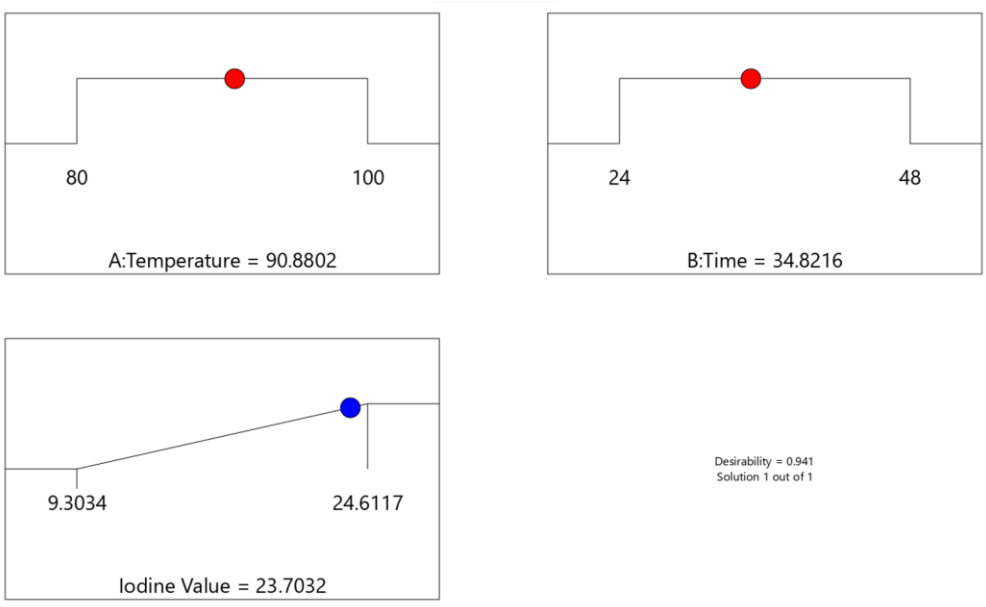

Figure 4.6: Selected predicted optimum result on iodine value.

This finding showed that the lipid contained a greater number of double bonds and a higher level of unsaturation. According to Chun et al. (2019), it was mentioned that BSFP with iodine value of 23.76 gI_2/100g had more double bonds and higher unsaturation as compared to the larvae with the value obtained at 16.94 gI_2/100g. This in line with the findings mentioned by Matthäus et al. (2019) as a higher level of unsaturation would lead to an increase in iodine values. In general, a lower iodine value indicates that the lipid is less susceptible to oxidation because of having fewer carbon double bonds and thus, lower unsaturation. The results for the low iodine value observed in this study may be attributed to the use of prepupae

instead of larval stages for experimentation. This finding is consistent with the research conducted by Liu et al. (2017), which demonstrated that later developmental stages of the larvae have a higher accumulation of diacylglycerol (DAG) lauric acid content, resulting in increased saturated fatty acid (SFA) content and decreased unsaturated fatty acid (UFA) content. As a result, the lipid composition contains lower levels of MUFA and PUFA, resulting in lower lipid unsaturation and a correspondingly lower iodine value. According to a published journal by Wanasundara et al. (2008), it mentioned that the heat will cause the double bonds to break, leading to formation of free radicals. The free radicals will react with oxygen in the air and cause oxidation of the crude lipid, resulting in an increase in iodine value. However, at even higher temperature the free radicals will become too unstable and break down further, resulting a decrease in iodine value which can be observed in this study (Figure 4.5).

4.5 Optimization of Peroxide Value using Response Surface Methodology

The utilization of Design Expert software along with response surface methodology led to the creation of 13 sets of experiments aimed at optimizing the parameters for oven drying of Black Soldier Fly Prepupae (BSFP) and its corresponding peroxide value. The outcomes, which include a summary table of model fit summary, analysis of variance (ANOVA) and a 3D surface plot for oven drying, have been presented in Table 4.11, Table 4.12, Table 4.13, and Figure 4.7.

Table 4.11: The central composite design and peroxide value results for oven dried BSFP.

Experimental Set	Drying Duration (hour)	Drying Temperature (°C)	Experimental Peroxide Value (mEq O_2/ kg)	Predicted Peroxide Value (mEq O_2/ kg)
D19.03T90	90	19.0294	3.6987	3.89631
D24T80	80	24	3.9684	3.17391
D24T100	100	24	5.8975	5.66251
D36T75.86	75.8579	36	3.4583	3.92502
D36T90A	90	36	5.4365	5.68473
D36T90B	90	36	5.3843	5.68473
D36T90C	90	36	5.1069	5.68473
D36T90D	90	36	5.2803	5.68473
D36T90E	90	36	5.3698	5.68473
D36T104.14	104.142	36	8.3256	7.44443
D48T80	80	48	6.4896	5.70694
D48T100	100	48	7.6315	8.19554
D52.97T90	90	52.9706	7.8543	7.47585

The fit summary is a statistical report that provides an overview of how well the fitted model represents the experimental data. It includes various statistical measures that describe the goodness of fit of the model, such as the coefficient of

determination (R-squared), adjusted R-squared, root mean square error (RMSE), lack of fit, and p-values.

Table 4.12: The model fit summary of peroxide value for BSFP between different drying parameters.

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**	**0.0021**	**0.8522**	**0.7551**	**Suggested**
2FI	0.5371	0.0017	0.8430	0.5952	
Quadratic	0.2449	0.0016	0.8650	0.4528	
Cubic	0.0006	0.1506	0.9900	0.8788	**Aliased**

Based on Table 4.12, linear model was chosen out of the 4 sources. The R-Squared value is 0.8769 which is the highest obtained compared to other sources of model. The greater the R-Squared value, the stronger the correlation of the variables in effect. Besides, the Adjusted R-Squared value of 0.8552 is also higher than other models available.

Table 4.13: Analysis of Variance (ANOVA) for the model terms in peroxide value for BSFP.

Source	Sum of Squares	Degree of Freedom	Mean Square	F-value	p-value	
Model	25.22	2	12.61	35.61	< 0.0001	significant
A-temperature	12.39	1	12.39	34.98	0.0001	
B-Time	12.83	1	12.83	36.24	0.0001	
Residual	3.54	10	0.3541			
Lack of Fit	3.47	6	0.5790	34.53	0.0021	significant
Pure Error	0.0671	4	0.0168			
Cor Total	28.76	12				

R^2	0.8769	**Adjusted R^2**	0.8522	**Adequate Precision**	17.5660

The analysis of variance is shown in Table 4.12, with Model-F value (35.61) proposes that the model is in the acceptable range or significant. The Model P-value is < 0.0001 presented that the model relations are significant. In this case, A, B are significant model. P-value greater than 0.1000 indicate the model terms are not significant, however it is not present in this model. The lack of fit is significant in this study is due to the experiment conducted was biological experiment where the samples (BSFP) was fed with different fruit waste which led to the results obtained to have slight difference. The adequate precision dealings with the signal to noise ratio, where it is considered acceptable when the ratio is more than 4. The value in this study is 17.5560 indicate an acceptable signal to noise ratio. Thus, linear model is chosen, and the developed model can be used to pilot the design. The coded equation for drying is calculated based on equation 4.4.

$$Peroxide\ value\ (PV) = 5.68 + 1.24A + 1.27B \qquad (4.4)$$

4.5.1 Effect of Drying Temperature and Time on Peroxide Value

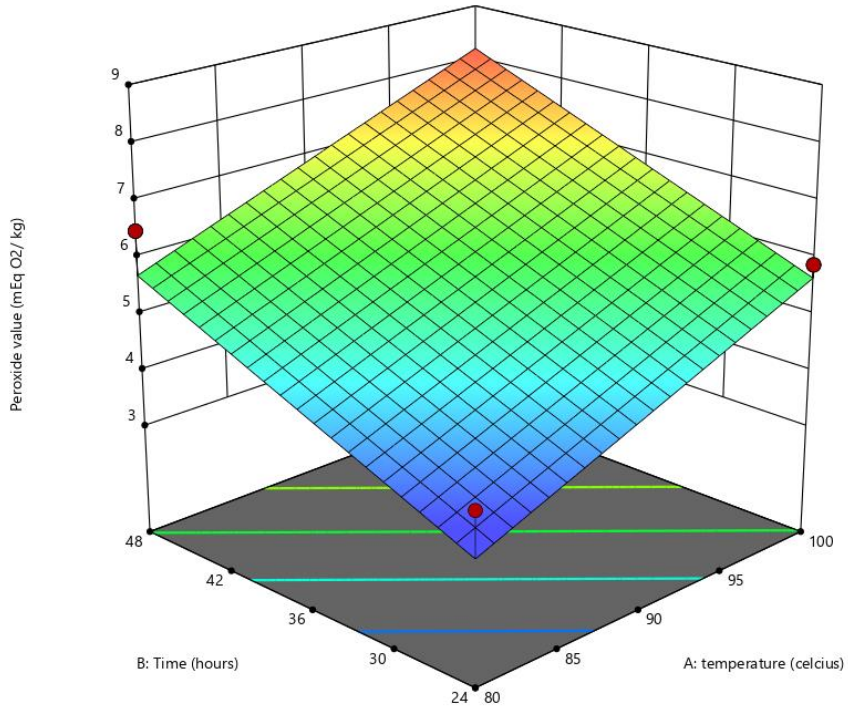

Figure 4.7: 3D surface plot between different drying duration and temperature for peroxide value of BSFP.

The oxidative rancidity of the BSFP was discovered when the hydroperoxides formed during the lipid oxidation. In this study, the peroxides values of oven dried BSFP with different parameters was less than 10 mEq O_2/kg which indicates a negligible rancidity Matthäus et al. (2019). Different drying temperature and drying duration will cause the peroxide value to be differ. During drying process, BSFP crude lipid undergoes thermal oxidation, which increases the concentration of peroxides in the oil. The extent of this increase depends on various factors such as the heating temperature, heating time, and initial peroxide value of the oil. At low drying temperatures, heating of BSFP can cause a slight increase in peroxide value due to the activation of enzymes that promote oxidation. However, as the temperature increases, the rate of oxidation accelerates, leading to a more significant increase in rancidity. Additionally, prolonged heating can also lead to an increase in

peroxide value due to the continuous formation of peroxides. Figure 4.8 shows predicted optimum result for peroxide value in this study is 8.956 mEq O_2/kg with drying temperature and drying time of 100 °C and 48 hours.

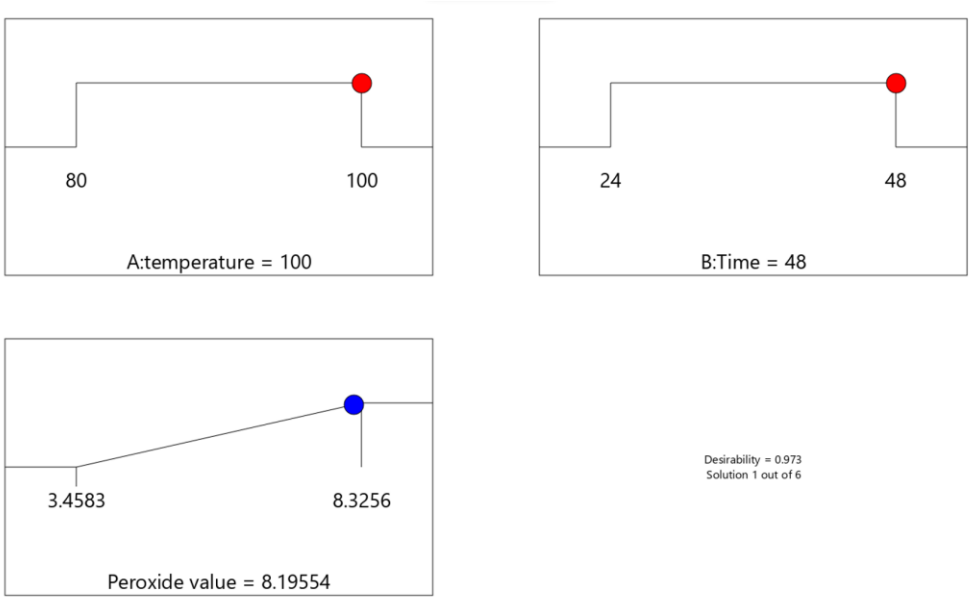

Figure 4.8: Selected predicted optimum result for peroxide value.

However, there was a study conducted by Mai et al. (2019) showing that a recorded peroxide value of 176 mEq O_2/kg. It was further discussed by Mai et al. that high value of peroxide value obtained may be due to the unsaturated fatty acids present in the crude lipid of BSF larvae, with high iodine value obtained which results in polyunsaturated fatty acids (PUFA) of black soldier fly larvae oil being susceptible to oxidation and polymerization during drying process. Takeungwongtrakul and Benjakul (2016) found that, a prolonged storage period of shrimp oil leads to a higher peroxide value, likely due to the lipoxygenase-catalysed oxidation of unsaturated fatty acids to produce lipid hydroperoxides via various reaction pathways. In this study, a lower peroxides value obtained in the experiment can be explained as the experiment was conducted a few days after the crude lipid extraction. Chen et al. (2011) noted that oxygen in the air is the primary reactant for lipid oxidation and fatty acid decomposition, ultimately leading to lipid rancidity. However, the tight fitting of the cap on the oil sample bottle can help to prevent rancidity by limiting the amount of oxygen that comes into contact with the oil. The high temperature drying process used in both oven (in this study) and microwave drying of black soldier fly prepupae may lead to the formation of Maillard reaction,

which it will enhance the antioxidative properties of the lipids in dried BSF prepupae and prevent lipid oxidation, thereby reducing the likelihood of rancidity in crude lipids (Gunstone, 2008; Zamora and Hidalgo, 2005). Additionally, the exceptionally high levels of tocotrienols in palm oil and high tocopherols in BSF may also contribute to the high antioxidative properties of these lipids (Liland et al., 2017).

4.6 Optimization of Browning Index using Response Surface Methodology

The utilization of Design Expert software along with response surface methodology led to the creation of 13 sets of experiments aimed at optimizing the parameters for oven drying of Black Soldier Fly Prepupae (BSFP) and its browning index value. The outcomes, which include a summary table of model fit summary, analysis of variance (ANOVA) and a 3D surface plot for oven drying, have been presented in Table 4.14, Table 4.15, Table 4.16, and Figure 4.9.

Table 4.14: The central composite design and crude lipid yield results for oven dried BSFP.

Experimental Set	Drying Duration (hour)	Drying Temperature (°C)	Experimental Browning Index	Predicted Browning Index
D19.03T90	90	19.0294	83.2179	69.7956
D24T80	80	24	42.7132	70.9447
D24T100	100	24	128.013	116.941
D36T75.86	75.8579	36	61.3872	36.7063
D36T90A	90	36	36.3606	35.4156
D36T90B	90	36	37.8771	35.4156
D36T90C	90	36	33.7678	35.4156
D36T90D	90	36	35.0000	35.4156
D36T90E	90	36	34.0726	35.4156
D36T104.14	104.142	36	213.581	244.483
D48T80	80	48	42.0052	46.8554
D48T100	100	48	329.152	294.699
D52.97T90	90	52.9706	158.812	178.456

The fit summary in Design Expert is a statistical report that provides an overview of how well the fitted model represents the experimental data. It includes various statistical measures that describe the goodness of fit to the model, such as the

coefficient of determination (R-squared), adjusted R-squared, root mean square error (RMSE), lack of fit, and p-values.

Table 4.15: The model fit summary of browning index of BSFP between different drying parameters.

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0170	< 0.0001	0.4689	0.2098	
2FI	0.1323	< 0.0001	0.5476	0.1424	
Quadratic	**0.0007**	**< 0.0001**	**0.9257**	**0.6926**	**Suggested**
Cubic	< 0.0001	0.0067	0.9978	0.9496	**Aliased**

Based on Table 4.15, quadratic model was chosen. The R-Squared value is 0.9567 which is the highest obtained compared to other sources of model. The greater the R-Squared value, the stronger the correlation of the variables in effect. Besides, the Adjusted R-Squared value of 0.9257 is also higher than other models available.

Table 4.16: Analysis of Variance (ANOVA) for the model terms in browning index for BSFP.

Source	Sum of Squares	Degree of Freedom	Mean Square	F-value	p-value	
Model	94352.68	5	18870.54	30.92	0.0001	significant
A-temperature	43171.11	1	43171.11	70.74	< 0.0001	
B-time	11807.06	1	11807.06	19.35	0.0032	
AB	10185.59	1	10185.59	16.69	0.0047	
A^2	19239.36	1	19239.36	31.53	0.0008	
B^2	13686.06	1	13686.06	22.43	0.0021	
Residual	4271.90	7	610.27			
Lack of Fit	4260.25	3	1420.08	487.86	< 0.0001	significant
Pure Error	11.64	4	2.91			
Cor Total	98624.58	12				
R^2	0.9567	**Adjusted R^2**	0.9257	**Adequate Precision**	15.4493	

According to Table 4.16, the Model-F value (30.92) indicates that the model is significant. The Model P-value of 0.0001 presented that the model relations are significant. In this case, A, B, AB, A^2, B^2 are significant model terms. The lack of fit obtained may be due to the big range between the measured browning index. As it can be observed from Table 4.14, the lowest and highest browning index obtained were 33.7678 and 329.152 due to the increase in drying temperature and time. As a result, the lack of fit in this case is significant. The adequate precision dealings with the signal to noise ratio, where it is considered acceptable when the ratio is more than 4. The value in present study is 15.4493 indicate an acceptable signal to noise ratio. Thus, quadratic model is chosen. The coded equation for drying was calculated based on equation 4.5:

$$Browning\ Index = 35.42 + 73.46A + 38.42B + 50.46AB + 52.59A^2 + 44.36B^2 \quad (4.5)$$

4.6.1 Effect of Drying Temperature and Time on Browning Index

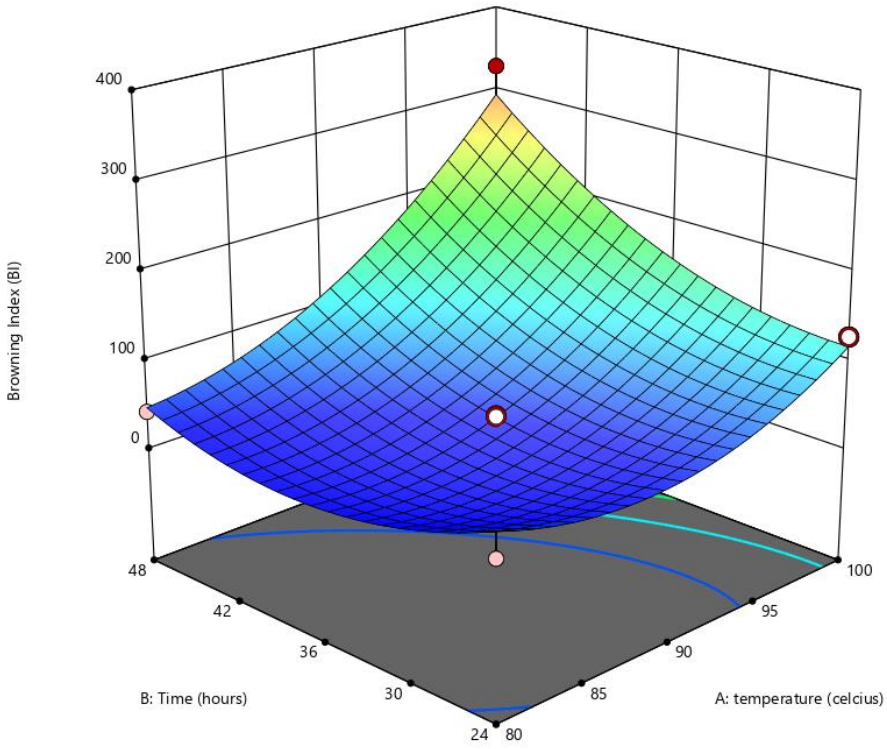

Figure 4.9: 3D surface plot between different drying duration and temperature for browning index of BSFP.

According to Table 4.16, at a significance level of $p < 0.05$, the oven dried BSFP exhibited an optimum browning index of 294.70, which is significantly higher compared to other drying parameters. The browning index is a crucial parameter for measuring the extent of fat oxidation, as highlighted by Lenaerts (2018), indicating that a higher browning index may result from the occurrence of fat oxidation, leading to a darker colour. Figure 4.9 illustrates as drying temperature and drying duration increases, the browning index of BSFP increases which in line with the peroxide value in this study. A significant increase in the browning index was observed with an increase in both drying time and temperature. The colour development in this study is likely attributed to the formation of browning substances during the oven drying process, which is consistent with other thermally processed oils, such as sesame oil (Ji et al., 2019). The production of browning substances is primarily due to the Maillard reaction, caramelization, and phospholipid degradation. Microwave

heating is another thermal process that has been reported to lead to an increase in colour intensity due to phospholipid degradation.

Moreover, an increase in temperature can cause the Maillard reaction to occur, which is a non-enzymatic browning reaction between reducing sugars and amino acids. This reaction can lead to the formation of brown pigments and the development of characteristic flavors and aromas. As temperature increases, the rate of the Maillard reaction also increases, resulting in a higher browning index. However, if the temperature becomes too high, it can lead to the degradation of these brown pigments and a decrease in the browning index (Nursten, 2005). Figure 4.10 shows the predicted optimum result obtained for browning index.

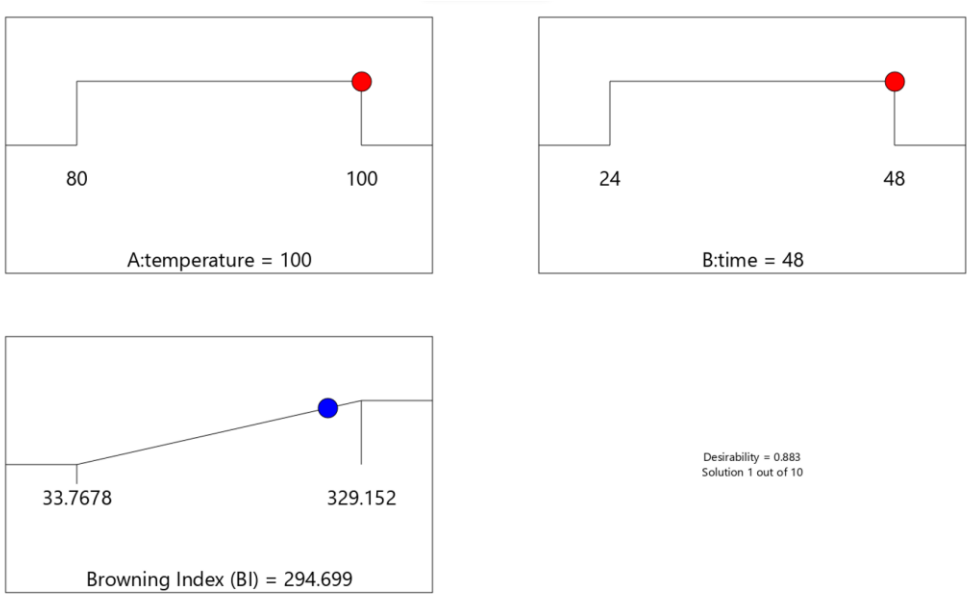

Figure 4.10: Selected predicted optimum result for browning index.

4.7 Optimization of Antioxidant Activity using Response Surface Methodology

The utilization of Design Expert software along with response surface methodology led to the creation of 13 sets of experiments aimed at optimizing the parameters for oven drying of Black Soldier Fly Prepupae (BSFP) and its antioxidant activity. The outcomes, which include a summary table of model fit summary, analysis of variance (ANOVA) and a 3D surface plot for oven drying, have been presented in Table 4.17, Table 4.18, Table 4.19, and Figure 4.11.

Table 4.17: The central composite design and antioxidant activity results for oven dried BSFP.

Experimental Set	Drying Duration (hour)	Drying Temperature (°C)	Experimental Browning Index	Predicted Browning Index
D19.03T90	90	19.0294	75.8127	74.1796
D24T80	80	24	57.4772	58.9427
D24T100	100	24	50.4551	52.2991
D36T75.86	75.8579	36	24.4473	23.0082
D36T90A	90	36	43.0528	46.8222
D36T90B	90	36	44.6034	46.8222
D36T90C	90	36	47.0741	46.8222
D36T90D	90	36	54.3563	46.8222
D36T90E	90	36	45.0243	46.8222
D36T104.14	104.142	36	52.2757	50.3013
D48T80	80	48	30.039	31.6086
D48T100	100	48	74.9025	76.8508
D52.97T90	90	52.9706	73.9922	72.2119

The fit summary generated by Design Expert serves as a statistical evaluation, offering a comprehensive assessment of the extent to which the fitted model accurately represents the experimental data. This summary encompasses a range of

statistical indicators that effectively gauge the goodness of fit of the model. These measures include the coefficient of determination (R-squared), adjusted R-squared, root mean square error (RMSE), lack of fit, and p-values, among others.

Table 4.18: The model fit summary of antioxidant activity on BSFP between different drying parameters.

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.2461	0.0067	0.0935	-0.5988	
2FI	0.0868	0.0097	0.2858	-0.1226	
Quadratic	**< 0.0001**	**0.7627**	**0.9424**	**0.9047**	**Suggested**
Cubic	0.9925	0.3390	0.9197	0.4725	**Aliased**

According on Table 4.18, quadratic model was chosen. The R-Squared value is 0.9664 which is the highest obtained compared to other sources of model. The greater the R-Squared value, the stronger the correlation of the variables in effect. Besides, the Adjusted R-Squared value of 0.9424 is also higher than other models available.

Table 4.19: Analysis of Variance (ANOVA) for the model terms in antioxidant activity for BSFP.

Source	Sum of Squares	Degree of Freedom	Mean Square	F-value	p-value	
Model	2959.08	5	591.82	40.30	< 0.0001	significant
A-temp	744.91	1	744.91	50.72	0.0002	
B-time	3.87	1	3.87	0.2636	0.6234	
AB	673.03	1	673.03	45.83	0.0003	
A^2	179.79	1	179.79	12.24	0.0100	
B^2	1209.67	1	1209.67	82.37	< 0.0001	
Residual	102.80	7	14.69			
Lack of Fit	23.61	3	7.87	0.3976	0.7627	not significant
Pure Error	79.19	4	19.80			
Cor Total	3061.89	12				
Predicted R^2	0.9664	**Adjusted R^2**	0.9424	**Adequate Precision**	20.6808	

The analysis of variance is shown in Table 4.19. The Model-F value (40.30) indicates that the model is significant. The Model P-value of < 0.0001 presented that the model relations are significant. A, AB, A^2, and B^2 are significant model terms, whereas B^2 is not significant model term. The R-squared value (0.9664) and adjusted R-squared value (0.9424) are inequitable with each other. The adequate precision dealings with the signal to noise ratio, where it is considered acceptable when the ratio is more than 4. The value in present study is 20.6808 indicate an acceptable signal to noise ratio. Thus, quadratic model is chosen. Moreover, the present developed model can be used to pilot the design. The coded equation for drying was calculated based on equation 4.6:

$$DPPH\ FR\ Scavenging\ activity\ (\%) = 46.82 + 9.65A - 0.6957B + 12.97AB - 5.08A^2 + 13.19B^2 \quad (4.6)$$

4.7.1 Effect of Drying Temperature and Time on Antioxidant Activity

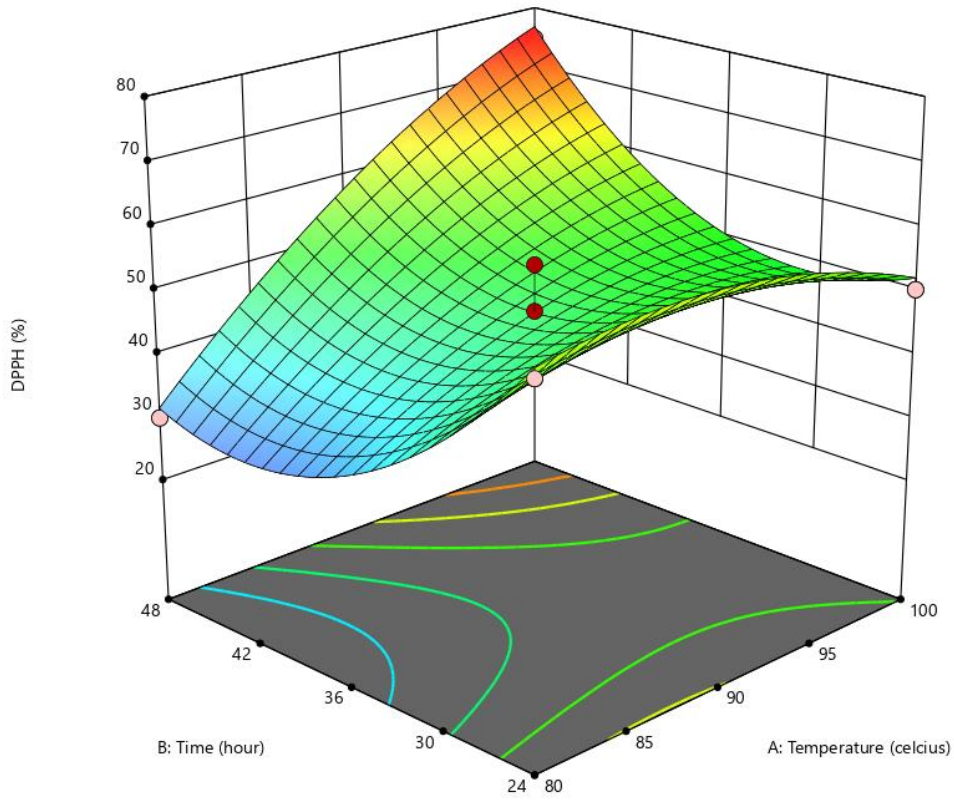

Figure 4.11: 3D surface plot between different drying duration and temperature for antioxidant activity of BSFP.

The results generated in Figure 4.11 demonstrated a significant impact of different drying temperatures and durations on the antioxidant activity of BSFP crude lipid. Oven drying yielded a predicted optimum DPPH value of 76.14 %. DPPH is used to monitor the total antioxidant activity by measuring the decrease in absorbance value of DPPH free radicals in the presence of antioxidants. According to Laurence et al. (2019), drying in ambient temperatures causes metabolically active plants to release phenolic compounds due to slow moisture loss, which is consistent with the finding of (Salih et al., 2014). Heating flaxseed hull oil, as reported by Herchi et al. (2014), resulted in a loss of phenolic acids and flavonoid contents. The loss of phenolic acids was lower than that of flavonoids, indicating that thermal treatment may cause oxidation and polymerization of phenolic compounds. Phenolic compounds not only exhibit antioxidant activity but also possess significant biological activity in vivo,

making them useful in combating diseases associated with excessive oxygen radical formation (Siger et al., 2012). The heating process also caused the degradation of carotenoids, possibly due to changes in β-carotene content, which undergoes dominant side-reactions of degradation and polymerization when heated and exposed to air (Qiu et al., 2009). Moreover, the heating process resulted in the loss of chlorophyll pigments, which are responsible for oil coloration, and this could be attributed to oxidative phenomena, polyphenol polymerization, or microbial degradation (Fernández-Arroyo et al., 2012).

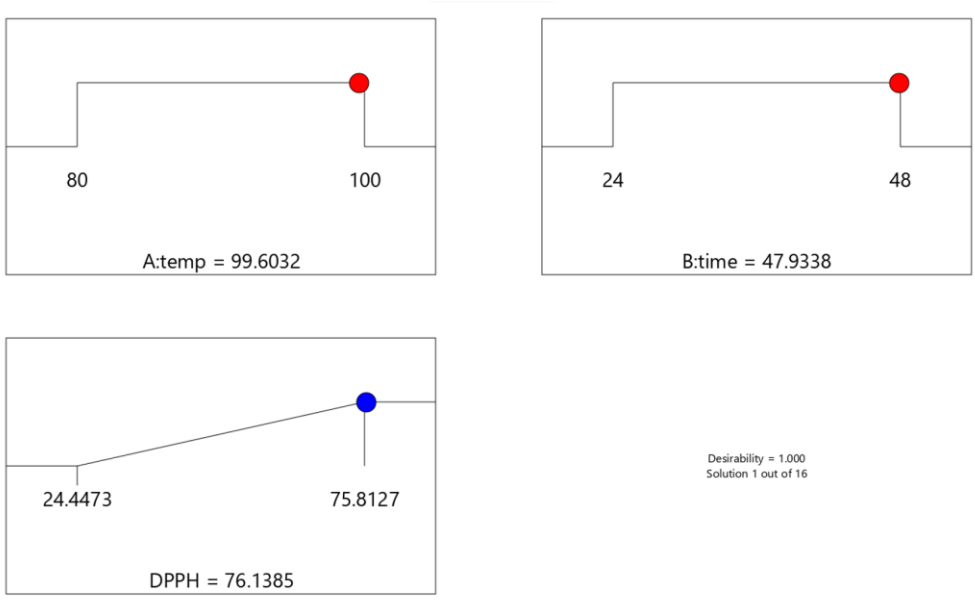

Figure 4.12: Selected predicted optimum result for antioxidant activity.

4.8 Predicted Optimization Result of Different Drying Parameters on the Physical and Chemical Characterization of Black Soldier Fly Prepupae

The predicted optimum results for various drying parameters for moisture loss, crude lipid yield, iodine value, peroxide value, browning index, and antioxidant activity has been conducted. The RSM tool generated the predicted optimum results in the form of solutions. The purpose of conducting process optimization is to determine the optimum value of the drying parameters for all the responses stated to achieve the desired value at once and to determine how temperature difference will affects the crude lipid yield and chemical composition presence in BSFP.

The Design Expert software produces several numbers of solution for each optimization responses which includes the respective drying temperature and drying duration together with the optimized response value. As mentioned earlier, the drying duration and drying temperature were between 24 hours – 48 hours and 80 ºC – 100 ºC. Meanwhile in the numerical optimization, the goal is to maximize the each of the response. Based on the desirability of processed by the RSM software, each experiment was carried out on the respective temperature and duration to see whether it achieve the optimized response.

The crude lipid yield was chosen for evaluation purpose as the drying parameters shown the lowest compared to other type of characterization in this study. At 88.29 ºC and 29.82hours, the predicted optimum crude lipid yield obtained was 49.79 %. By taking the mentioned drying temperature (88.29 ºC) and time (29.82 hours) for moisture loss, the predicted optimum was 64.657 %. When comparing with the predicted optimum parameters at 100 ºC, 34.71 hours, the value shows an insignificant change in moisture loss (65.31 %).

Meanwhile, the drying parameters at 88.29 ºC and 29.82 hours, the iodine value and peroxide value showed the predicted optimum of 22.4841 gI_2/ 100g and 4.82 mEq O_2/kg, respectively. In contrast with drying parameters of iodine and peroxide value at 90.89 °C 34.82 hours, and 100 °C, 48 hours, the predicted optimum results obtained were 23.704841 gI_2/100g and 8.20 mEq O_2/kg, respectively. A lower iodine value tends to have a higher proportion of saturated fatty acid, which are generally more stable and less susceptible to oxidation (Matthäus et al. 2019). Oils with a lower iodine value can be beneficial because they are less likely to undergo oxidative rancidity, which can result in the formation of harmful compounds and unpleasant flavours that will bring harm to the health of consumers. While oil with lower peroxide values indicate that they have undergone less oxidative damage. Higher peroxide values indicates that the oil has been exposed to air, heat, light, or other factors that can accelerate the oxidation process.

As for browning index, the value at 88.29 ºC and 29.82 hours was 20.8148. A lower browning index is observed at this parameter than that of 100 ºC for 48 hours, where the value attained was 294.70. A lower browning index indicates that the prepupae have a lighter colour and less melanisation (Lenaerts, 2018). Lighter coloured prepupae are typically preferred because they are associated with freshness

and high nutritional quality. Higher browning index values can indicate that the prepupae have been exposed to prolonged storage, high temperatures, or other factors that can lead to melanisation and potential degradation of nutritional value. Additionally, excessively dark prepupae may be less visually appealing to consumers. Therefore, in the case of black soldier fly prepupae, a lower browning index is generally considered desirable as it suggests fresher, higher quality, and visually appealing prepupae.

Whereas for antioxidant activity, the value obtained at 88.29 ºC and 29.82 hours was 50.0215 %. High antioxidant activity in black soldier fly oil indicates that it has a greater ability to neutralize free radicals and maintain its stability. This can contribute to a longer shelf life, better flavour, and potentially enhanced health benefits when consumed.

As for commercial application, it is recommended to operate at the drying parameters of 88.29 ºC and 29.82 hours. This is due to the lower drying temperature and time will lead to energy conservation, which save the cost of the production line for BSFP related products such as crude lipid yield, and others. According to Table 4.20, it is noted there is no need for increasing the drying temperature and time more than 88.29 ºC and 29.82 hours as it does not show a significant difference in results obtained.

Table 4.20: The Summary of predicted optimum results for different type of characterization for BSFP undergo oven drying.

Optimization Result						
Type of characterization	Drying Parameters				Optimized Result	
Moisture Loss	100	ºC	34.70	hours	65.31	%
Crude Lipid Yield	88.29	ºC	29.82	hours	49.79	%
Browning Index	100	ºC	48	hours	294.70	
Iodine Value	90.89	ºC	34.82	hours	23.70	$gI_2/100g$
Peroxide Value	100	ºC	48	hours	8.20	mEq O_2/kg
DPPH	99.60	ºC	47.93	hours	76.14	%

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.0 Conclusion

In this study, a quadratic model for the oven dried BSFP crude lipid yield experiment was generated using the Response Surface Methodology (RSM) optimization tool, resulting in an R-squared value of 0.9172, indicating a good regression analysis. Additionally, the RSM optimization tool was used to generate a quadratic model for oven dried BSFP in moisture loss experimentation, with an R-squared value of 0.9707. The optimized extracted crude lipid yield for oven dried BSFP was obtained at 49.79 % under specific drying parameters, revealing that higher moisture loss in BSFP led to higher crude lipid yield. The experimental loss of moisture was found to closely match the predicted loss of moisture obtained through the RSM optimization tool, indicating that loss of moisture significantly impacted the yield of crude lipid.

Furthermore, the RSM optimization tool was also used to generate an optimized result for the iodine value of oven dried BSFP, resulting in 23.70 $gI_2/100g$ at specific drying parameters. A low iodine value in oil suggests a high degree of saturation, indicating a higher proportion of saturated fatty acids and a lower proportion of unsaturated fatty acids. The peroxide value obtained from the study was 8.2 mEq O_2/kg at specific drying parameters, indicating a low level of rancidity in the extracted oil.

The antioxidant activity (DPPH) assay and browning index (BI) were measured for the oven dried BSFP, both of which were found to be substantially associated with fat oxidation. The antioxidant activity for the oven dried BSFP was 76.14 % at $P < 0.05$, with significant differences observed between different drying parameters. The study found that fat oxidation in oven dried BSFP is highly sensitive to heat and oxygen, and the presence of antioxidants. As the drying temperature

increases, BSFP absorbs more heat, resulting in increased fat oxidation. Additionally, the antioxidant components in BSFP may be destroyed under high heat conditions.

5.1 Recommendations

Black soldier fly (*Hermetia illucens*) larvae have shown great potential as a sustainable source of protein and lipid-rich biomass for various applications, including animal feed, biofuel production, and waste management. To maximize the potential of black soldier fly, several improvements can be made in terms of crude lipid yield, nutritional profile, rearing methods, and biodiesel quality.

Firstly, to increase the crude lipid yield of black soldier fly, optimization of rearing conditions and processing techniques can be done. According to a study by Huang et al. (2017), a significant impact of moisture loss on crude lipid yield was observed. Therefore, it is recommended to maintain the optimum moisture level in the rearing substrate to ensure maximum lipid accumulation. Additionally, Huang et al. (2017) also suggested that drying parameters such as temperature and time can affect the lipid yield and optimizing these parameters through the Response Surface Methodology (RSM) can further increase the yield.

Secondly, to improve the nutritional profile of black soldier fly, the rearing substrate can be modified to include nutrient-rich ingredients such as legumes and vegetables. Studies have shown that the nutrient composition of black soldier fly larvae can be enhanced by incorporating these ingredients into the rearing substrate (Surendra et al., 2016). This will not only improve the nutritional value of black soldier fly as a feed ingredient but also increase the economic value of the larvae.

Thirdly, to enhance the rearing methods of black soldier fly, the use of automated systems can be considered. The traditional manual methods of rearing black soldier fly larvae can be labour-intensive and time-consuming. Therefore, automated systems such as the Black Soldier Fly Automated Rearing Technology (BSF-ART) can improve efficiency and reduce labour costs (Tomberlin et al., 2009). Additionally, the use of artificial lighting can enhance the mating and egg-laying behaviour of black soldier fly, which can increase the yield of larvae (Sheppard et al., 2002).

Lastly, to improve the biodiesel quality of black soldier fly, the lipid profile of the larvae can be modified through diet manipulation. According to a study by Park et al. (2021), the fatty acid composition of the lipids extracted from black soldier fly larvae can be modified through dietary manipulation. Increasing the proportion of unsaturated fatty acids such as oleic acid and linoleic acid can improve the cold flow properties and oxidative stability of the biodiesel produced from the lipids (Park et al., 2021).

In conclusion, the potential of black soldier fly as a sustainable source of protein and lipid-rich biomass can be maximized through various improvements in crude lipid yield, nutritional profile, rearing methods, and biodiesel quality. These improvements can be achieved through optimization of rearing conditions, modification of the rearing substrate, use of automated systems, and dietary manipulation. By implementing these recommendations, black soldier fly can become a valuable and sustainable resource for various industries.

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