THE EFFECTS OF KILLING PROCESSES AND DIFFERENT DRYING METHODS ON NUTRIENT CONTENT AND PHYSICOCHEMICAL CHARACTERISTICS OF BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE

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UNIVERSITI SAINS MALAYSIA

2025

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by

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Thesis submitted in fulfilment of the requirements
for the degree of
Master of Science

September 2025

ACKNOWLEDGEMENT

First and foremost, I would like to thank God for providing me with excellent health throughout my candidacy and the chance to complete this MSc thesis. Words cannot express my gratitude to my main supervisor, Dr Hadura Abu Hasan, for her unwavering support for my MSc studies. From the very beginning of my journey, Dr Hadura's expertise and commitment have been a constant source of inspiration and motivation. Her invaluable insights, constructive feedback, and thoughtful advice have significantly shaped my research, helping me navigate the challenges of the academic world. Her support extended far beyond the academic realm, offering me the confidence to persevere and the encouragement to pursue excellence. I could not have asked for a better supervisor for my studies.

Not only that, I would also like to thank my co-supervisor, Dr Siti Baidurah for her constructive feedback that has significantly contributed to the development of my thesis. I am deeply appreciative of her willingness to offer advice and share her knowledge whenever I encounter challenges. Furthermore, my research would not have been possible without financing from the Fundamental Research Grant Scheme (FRGS) FRGS/1/2021/STG03/USM/02/13 and financial assistance from the Graduate Fellowship Scheme by Universiti Sains Malaysia.

My sincere appreciation also goes to my lab members in Lab 128 (G08) (Norhakimah binti Soad, Mohamad Ibrahim bin Mohamad Isa and Ikram Ullah) for all the stimulating discussions. I would also like to thank all acquaintances in Lab 128 (G09) in the School of Biological Sciences as well as Lab 137 and Lab 255 in the School of Industrial Technology for the support and facilities provided. As well to all the people that I have crossed paths with, thank you for the warmth and support.

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°C	Degrees Celcius
%	Percentage
±	Plus or minus (indicates a range or margin of error)
<	Less than
>	Greater than
$p \leq 0.05$	Statistical significance threshold
mm/s	Millimeters per second (velocity)
mg/g	Milligrams per gram (concentration or ratio)
g/g	Grams per gram (ratio of mass)
CO_2	Carbon dioxide
O_2	Oxygen
N_2	Nitrogen
mBar	Millibar (pressure)
ml	Milliliter (volume)
cm	Centimeter (length)
N	Normality (concentrations)
H_2BO_3	Boric acid
NaOH	Sodium hydroxide
volts	Voltage (electric potential)
amps	Amperes (electric current)
rpm	Revolutions per minute (rotational speed)
kg	Kilogram (mass)
g-f	Gram-force (force)
pH	Power of hydrogen (acidity/alkalinity measure)

Ca	Calcium
Mg	Magnesium
Zn	Zinc
Fe	Iron
Cu	Copper

LIST OF ABBREVIATIONS

TPA	Texture profile analysis
LAB	Lactic acid bacteria
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
HCL	Hydrochloric acid
BSF	Black soldier fly
EE	Ether extract
AA	Amino acid
DM	Dry matter
PKE	Palm kennel expeller
EAA	Essential amino acid
NEAA	Non-essential amino acid
ANOVA	Analysis of variance
Tukey HSD	Tukey's honestly significant difference

LIST OF APPENDICES

KESAN PROSES MEMATIKAN DAN KAEDAH PENGERINGAN YANG BERBEZA TERHADAP KANDUNGAN NUTRIEN DAN CIRI FIZIKOKIMIA LARVA LALAT ASKAR HITAM (*Hermetia illucens*)

ABSTRAK

Kenaikan kos makanan ternakan telah menyerlahkan potensi larva lalat askar hitam (BSF) sebagai sumber alternatif makanan haiwan yang mampan dan kaya nutrien, dengan proses pengeringan diperlukan untuk memastikan kestabilan produk. Kajian ini menilai kesan dua kaedah mematikan larva (penceluran (100 °C selama 40 saat) dan pembekuan (-20 °C selama 24 jam)) dan tiga kaedah pengeringan (pengeringan matahari (25-33 °C selama 23-41 jam), pengeringan beku (-18 hingga -50 °C selama 72 jam diikuti pengeringan ketuhar pada 80 °C selama 24 jam) dan pengeringan elektrik (60 °C selama 24 jam)) terhadap kualiti nutrien dan sifat fizikokimia larva BSF. Larva seberat 0.08 ± 0.007 g dan sepanjang 1.31 ± 0.21 cm menunjukkan penurunan berat kepada 0.02-0.03 g selepas pengeringan, dengan panjang akhir antara 1.11-1.62 cm. Kaedah mematikan ($p < 0.001$) dan pengeringan ($p \leq 0.001$) menunjukkan kesan signifikan terhadap parameter ini. Hasil produk tertinggi diperoleh daripada larva yang dicelur dan dikering beku (33.67%) manakala terendah daripada larva dibekukan dan dikering matahari (24.00%). Hasil ini dipengaruhi oleh kaedah mematikan ($p < 0.001$) dan pengeringan ($p = 0.009$), namun bukan interaksinya ($p = 0.057$). Penceluran meningkatkan hasil dan rupa melalui penyahaktifan enzim serta penyingkiran kelembapan. Pengeringan beku mengekalkan nutrien tetapi kurang efisien. Pengeringan matahari paling kurang berkesan, manakala pengeringan elektrik lebih seimbang. Kandungan lembapan dipengaruhi oleh kaedah mematikan ($p = 0.002$) dan pengeringan ($p < 0.01$), dengan sampel kering beku mengekalkan lembapan lebih tinggi. Analisis proksimat menunjukkan kandungan protein (46.93-52.17%), lipid (8.70-17.44%), abu (9.67-12.11%) dan serat (2.09-13.97%). Larva dicelur dan dikering matahari mencatatkan lipid tertinggi (17.44%), manakala larva dibekukan dan dikering beku mencatatkan protein tertinggi (52.17%). Kandungan lipid dipengaruhi oleh proses mematikan ($p < 0.001$), pengeringan ($p = 0.032$) dan interaksi ($p < 0.001$). Serat dipengaruhi oleh pengeringan ($p < 0.001$) dan interaksi ($p = 0.004$), tetapi bukan kaedah mematikan ($p = 0.990$). Kandungan abu dipengaruhi oleh kaedah mematikan ($p = 0.002$) dan interaksi ($p = 0.002$), tetapi bukan pengeringan ($p = 0.268$). Protein menunjukkan perbezaan signifikan bagi ketiga-tiga faktor: mematikan ($p = 0.004$), pengeringan ($p < 0.001$) dan interaksi ($p = 0.003$). Sebanyak 16 asid amino telah dikenal pasti, dengan glutamat (3.30-4.90%), histidin (5.40-7.10%) dan leusin (2.40-3.10%) sebagai yang utama, manakala lisin (1.60-2.70%) dan metionin (0.60-0.80%) adalah rendah, kemungkinan disebabkan oleh substrat hampas isirung sawit. Nilai pH berkisar antara 5.10 hingga 6.41 ($p < 0.001$). Parameter warna (L^*, a^*, b^* dan intensiti warna) menunjukkan perbezaan signifikan ($p < 0.001$), manakala hue angle hanya dipengaruhi oleh kaedah mematikan dan interaksi ($p = 0.065$). Kekerasan (1956.18-7498.97 g-f) tidak dipengaruhi oleh proses mematikan ($p = 0.169$), tetapi dipengaruhi oleh pengeringan ($p = 0.101$) dan interaksi ($p = 0.002$). Kesimpulannya, penceluran diikuti pengeringan beku menghasilkan larva BSF dengan kualiti nutrien dan penampilan terbaik, manakala pengeringan elektrik merupakan kaedah yang lebih efisien untuk pengeluaran berskala sederhana.

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ABSTRACT

Rising feed costs highlight black soldier fly (BSF) larvae as a sustainable feed option, with drying essential for stability. This study evaluated the effects of two killing methods (blanching (100 °C for 40 seconds) and freezing (-20 °C for 24 hours)) and three drying methods (sun drying (25-33 °C for 23-41 h), freeze-drying (-18 to -50 °C for 72 h with oven drying at 80 °C for 24 h) and dehydrator drying (60 °C for 24 h)) on the nutritional and physicochemical qualities of BSF larvae. The initial wet weight and length of larvae were 0.08 ± 0.007 g and 1.31 ± 0.21 cm. After drying, weight reduced to 0.02-0.03 g, and length ranged between 1.11-1.62 cm. Killing processes ($p < 0.001$) and drying methods ($p \leq 0.001$) significantly affected these parameters. Product yield ranged from 24.00% (frozen-sun dried) to 33.67% (blanched-freeze dried), significantly influenced by both killing ($p < 0.001$) and drying ($p = 0.009$), but not their interaction ($p = 0.057$). Blanching improved yield and appearance due to enzyme inactivation and better moisture release, while freeze-drying offered better nutrient preservation but demanded high energy and time. Sun drying showed the longest drying duration and highest nutrient loss. Dehydrator drying balanced energy use, product quality, and nutrient retention. Moisture content was significantly affected by killing ($p = 0.002$) and drying ($p < 0.01$), freeze-dried samples retained more moisture. Proximate analysis revealed crude protein ranged from 46.93% to 52.17%, lipid from 8.70% to 17.44%, ash from 9.67% to 12.11% and fiber from 2.09% to 13.97%. The blanched-sun dried group had the highest lipid (17.44%), while frozen-freeze dried had the highest protein (52.17%). Lipid was significantly affected by killing ($p < 0.001$), drying ($p = 0.032$) and interaction ($p < 0.001$). Fiber varied with drying ($p < 0.001$) and interaction ($p = 0.004$), but not killing ($p = 0.990$). Ash was affected by killing ($p = 0.002$) and interaction ($p = 0.002$), but not drying ($p = 0.268$). Protein showed significant differences across killing ($p = 0.004$), drying ($p < 0.001$) and interaction ($p = 0.003$). Sixteen amino acids were identified, with glutamic acid (3.30-4.90%), histidine (5.40-7.10%) and leucine (2.40-3.10%) as major ones. Lysine (1.60-2.70%) and methionine (0.60-0.80%) were slightly low due to palm kernel expeller-based feed. pH ranged from 5.10-6.41, significantly affected by both factors ($p < 0.001$). Colour parameters (L*, a*, b*, colour intensity) showed significant differences ($p < 0.001$), while hue angle was only affected by killing and interaction ($p = 0.065$). Hardness (1956.18-7498.97 g-f) was unaffected by killing ($p = 0.169$), but influenced by drying ($p = 0.101$) and interaction ($p = 0.002$). In conclusion, blanching with freeze-drying provided the best nutrient retention and appearance, while dehydrator drying offered a more energy-efficient solution for medium-scale BSF larvae production.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The price of feed meal has suddenly risen due to a surge in the cost of raw materials. The situation has urged the industries to delve into alternative, sustainable feed meal options to cut down production costs. According to Vauterin et al., (2021), insects are gaining popularity as a viable alternative to traditional animal feed components in animal, bird and aquaculture production. Insects have been proposed as a promising candidate due to their high nutritional quality and ease of growing (Janssen et al., 2017; Osimani et al., 2017; Purschke et al., 2018).

Not only that, the need for space and water for insect rearing is also low compared with conventional feedstock production (Van Huis et al., 2013). Moreover, insects may be farmed on biodegradable waste, release less greenhouse gases and have a lower environmental impact (Van Huis & Oonincx, 2017). Furthermore, the biological properties of insects, including robust fertility rates, polyvoltine life cycles, remarkable feed utilization effectiveness, varied dietary preferences and low substrate demand, can enhance global food security through feed or direct consumption (EFSA Scientific Committee, 2015). Commonly used insects as feed are the domestic cricket (*Acheta domesticus*), the mealworm (*Tenebrio molitor*) and the black soldier fly (BSF) (*Hermetia illucens*) larvae.

Insect-based feed has been found in trials to revamp animal productivity and save operational expenses (Sogari et al., 2023). Makkar et al. (2014) have presented that rainbow trout fed mealworms had better development performance and greater protein efficiency ratios than those given fishmeal. Not only that, St-Hilaire et al.

(2007) reported that broiler chickens fed with BSF larvae had higher growth rates and feed-to-output ratio compared to classic feed. Another study by Cullere et al. (2017) and Bessa et al. (2020) demonstrated the utilisation of BSF larvae as a protein supplement and its positive impacts on growth performance, carcass characteristics and meat attributes in broiler quails.

Insect based meal production involves a series of steps starting from breeding, harvesting, killing, drying and post drying steps. Among the steps, drying is the most crucial step because it decreases moisture content extending shelf stability and inhibiting microbial growth (Oonincx & de Boer, 2012). Drying is particularly important in the event insects are to be kept at room temperature since it offers stability throughout the storage (Klunder et al., 2012). Kamau et al. (2018) reported that when cricket and BSF powders are dried to moisture content of 5g/100g, they can be stored for 7 months at 25°C.

Regularly used drying methods for edible insects are roasting, frying, sun drying, freeze-drying and microwave assisted drying (Melgar-Lalanne et al., 2019). The most favoured drying methods in edible insects are oven drying and freeze-drying (Melgar-Lalanne et al., 2019). Oven drying is the commonly used method since it is the cheapest among the more efficient drying process. When the quality of the dried product is necessary, freeze-drying is regularly used even though it is costly and requires batch processing (Ratti, 2001). High temperature while drying might denature proteins, lowering the nutritional content (Oonincx & de Boer, 2012). Moreover, inadequate drying might also promote mould formation, which creates harmful metabolites that are detrimental to the consumers (Makkar et al., 2014). Hence, to assure the quality and safety of insect meal as feed, appropriate killing process and drying method need to be used.

Given the importance of proper killing and drying methods in preserving the quality and nutritional value of insect-based feed, this study aims to assess how different processing techniques impact the physical and nutritional properties of black soldier fly larvae. The experimental design was structured to compare the effects of various killing processes and drying methods on the larvae. The larvae underwent different killing techniques, such as freezing and blanching, followed by drying methods including sun drying, freeze-drying and dehydrator drying. Measurements were taken before and after drying to evaluate weight, length and product yield, while nutritional composition was assessed through proximate and amino acid analysis. Additionally, physicochemical characteristics like colour, pH and texture were measured to determine the overall quality and stability of the dried larvae. This experimental design allows for a comprehensive evaluation of which drying method best maintains the larvae's quality, providing valuable insights for producing sustainable, high-quality insect-based feed.

1.2 Problem Statement

In Malaysia, the rising cost of imported animal feed has increased interest in locally produced insect-based feed meals, such as BSF larvae, which contain 399 to 431 g/kg of dry matter (Spranghers et al., 2017). Malaysia’s federal initiatives suggest that integrating BSF larvae into national feed chains could reduce import dependence (The Sun Daily, 2024). In February 2024, Science, Technology and Innovation Minister Chang Lih Kang announced a collaborative research project between the Federal Land Development Authority (FELDA) and Abeco Nature Science Sdn Bhd. This initiative aims to develop BSF larvae-based feed to replace imported soybean feed, enhancing food security and reducing antibiotic dependence in livestock farming. The research is part of the National Biotechnology Policy 2.0, focusing on advancing agricultural biotechnology and food security (The Sun Daily, 2024). However, the nutritional quality and physicochemical properties of BSF larvae are highly sensitive to post-harvest processing, necessitating systematic optimization of killing and drying methods.

Killing process is important to stop enzymatic degradation and lower microbial loads prior to drying. Blanching disrupts the insect cuticle, reducing drying time by up to sixfold and improving color lightness, yet may slightly diminish certain amino acid digestibility profiles (Liang et al., 2024; Son et al., 2023). Freezing, alternatively, preserves cellular integrity and enzymatic stability but has been less explored in combination with novel drying methods, leaving questions about its influence on subsequent moisture removal mechanisms and nutrient retention (Liang et al., 2024).

Following killing, drying techniques has shown profound effects on proximate composition, physicochemical attributes and in vitro digestibility. Hot-air drying at 65 °C for 24 h achieves rapid moisture reduction (~14 % residual moisture) and high protein digestibility (> 94 %) (Oh et al., 2024). Freeze-drying (-40 °C, 48 to 72 h) maintains original color and nutrient profiles but is energy-intensive, whereas microwave and infrared drying has shorter drying times. However, infrared drying has demonstrated comparable protein utilization to hot-air methods, with enhanced textural qualities in some cases (Butwong et al., 2025; Oh et al., 2024).

Several studies have explored methods to dry insects such as mealworm larvae, crickets, and BSF utilising hot air drying, microwave drying, freeze-drying, and the effect on product qualities (Huang et al., 2019; Purschke et al., 2018; Vandeweyer et al., 2018). Despite growing literature on killing methods and drying techniques, no study to date has systematically compared the combined effects of blanching vs. freezing pre-treatments with a range of modern (freeze-drying, dehydrator drying) and traditional (sun drying) drying methods on both the nutritional (proximate, amino acids) and physicochemical (pH, colour, hardness) characteristics of BSF larvae produced under Malaysian conditions. Most of the studies only focus on the drying method itself. Moreover, studies on established killing processes and drying methods that preserve BSF larvae with the highest nutritional quality are also limited.

1.3 Significance of Study

Black soldier fly larvae must be dried to ensure long-term use as livestock feed and to inhibit microbial growth. Even if the BSF larvae are dried, the nutritional properties of the larvae must not be reduced. Hence, in this study, different types of drying processes were carried out and the nutritional properties of the larvae were determined after each type of drying process. This study will be a significant contributor in determining the most suitable drying method to produce dried larvae as a feedstock supply with a longer shelf life while maintaining all its nutritional quality. Not only that but results from this study can help the farmers to switch to this alternative feed, which is low-cost, hygienic, sustainable and has longer shelf life.

Investigating the killing processes and drying methods on BSF larvae is significant because it focuses on understanding the impact of different drying methods on the physical characteristics of the BSF larvae. By measuring the weight and length of the larvae before and after drying, as well as determining the percentage of product yield, this study will provide valuable insights into the effectiveness of each drying method in terms of moisture removal and product shrinkage. The comparisons between different drying techniques will help identify the most efficient method, ensuring high-quality larvae with optimal product yield while minimizing loss during the drying process. These findings are crucial for scaling up production and improving the overall efficiency of BSF larvae drying processes for use as a sustainable feed source.

Evaluating the effect of killing processes and drying methods on the nutritional quality of BSF larvae is important because it will determine how different processing techniques affect key nutrients such as proteins, lipids and amino acids. Identifying the drying and killing conditions that preserve or enhance the larvae's nutritional profile is essential for supporting the use of BSF larvae as a sustainable and efficient protein source. This will contribute to food security and reduce reliance on traditional livestock feed ingredients.

Assessing the physicochemical characteristics of BSF larvae, including colour, pH and texture, is vital for determining their quality and suitability for use as feed. Colour influences the visual acceptability of the larvae, pH affects microbial stability and shelf life and texture plays a role in handling, storage, and palatability for livestock. By evaluating these properties, this study ensures that the larvae maintain high quality and safety standards while being acceptable for use in animal feed. These findings will help optimize drying and killing methods to preserve the larvae's overall integrity, making them a viable, long-lasting feed alternative.

1.4 Objectives

The general aim of this study was to investigate the impact of killing and drying processes on the nutritional quality and physicochemical characteristics of black soldier fly larvae, providing insights for optimizing the processes. Therefore, the specific objectives were:

1. To investigate the killing processes and drying methods on black soldier fly larvae.
2. To evaluate the effect of killing processes and drying methods on the nutritional quality of black soldier fly larvae.
3. To evaluate the effect of killing processes and drying methods on the physicochemical characteristics of black soldier fly larvae.

CHAPTER 2

LITERATURE REVIEW

2.1 Insects as Feed

Insects are the most diverse creatures in nature. They serve several niches and offer numerous ecological functions. Many animals rely on insects as a source of food. Animals including bats, fishes, frogs, lizards, moles, shrews, boars and large apes, are insectivorous (Deblauwe et al., 2003; Schley & Roper, 2003; McGrew, 2014; Henry et al., 2015). Farmed animals, such as chickens, pigs, and fish, readily eat insects. Flies, crickets, mealworms and silkworms are examples of insects that can be used as feed. The extensive use of insects in ecosystems and by diverse species underscores their potential in both natural food chains and sustainable farming operations.

Livestock can be fed with the larvae of the black soldier fly (*Hermetia illucens*), housefly (*Musca domestica*), and yellow mealworm (*Tenebrio molitor*). Fly species ingest decaying plant and animal remains, including fungus and animal excrement. Most of these readily available materials are inappropriate for inclusion in diets or feed ingredients hence fly larvae transform these waste substrates into high-quality protein, making them appropriate for human consumption and cattle feed (Čičková et al., 2015; Barragan-Fonseca et al., 2017). Among these, BSF larvae are particularly noteworthy for their exceptional protein content and adaptability across various feed applications

For instance, BSF larvae have protein levels ranging from 37 to 63% of dry matter, depending on the materials they were raised (Makkar et al., 2014; Barragan-Fonseca et al., 2017). Not only that, substituting fish meal and fish oil with BSF pupae raised on swine manure and housefly pupae raised on cow manure, while maintaining crude protein and crude fat levels, resulted in rainbow trout with comparable feed conversion ratio but lower levels of EPA and DHA (St-Hilaire et al., 2007).

Besides BSF larvae, housefly larvae have also shown considerable promise in improving cattle growth and production. According to Hwangbo et al. (2009), the addition of 10-15% housefly larvae to chick feed increased both growth performance and carcass quality in broiler chickens. The chicks' breast muscles showed no change in overall protein content, but there was an increase in lysine and tryptophan levels. Diets containing 10-15% maggots (which are formed as houseflies decompose chicken manure) enhanced broiler chicken carcass characteristics and growth progression (Hwangbo et al., 2009). Furthermore, dried fly larvae compared favourably to soybean meal as a protein supplement for turkey poults (Zuidhof et al., 2003).

Likewise, yellow mealworms and silkworm pupae have demonstrated efficacy as alternate protein sources in various animal diets. Yellow mealworm has also been used as a protein source for African catfish (*Clarias gariepinus*) and for broiler chickens (Ng et al., 2001; Ramos-Elorduy et al., 2002). Silkworm (*Bombyx mori*) pupae meal has been effectively employed as a substitute for fish meal in poultry diets, promoting growth and egg production simultaneously. Comparable outcomes were attained using silkworm (*Anaphe infracta*) caterpillar food (Ijaiya & Eko, 2009). In Italy, the preimaginal stages of *Spodoptera littoralis* have been tested as prospective fish meal alternatives in the rainbow trout diet (Danieli et al., 2011).

Other insects, including termites and acridids, have also been investigated as potential nutrition sources. Despite playing an important role in wood degradation, termites being the insect with the second highest consumption rate in the world have also been incorporated as feed due to their high fat and protein content (Rumpold & Schlüter, 2012). Acridids (grasshoppers) have also been proposed as viable feed components for chicken due to their greater protein content compared to soybean meal and fish meal, as well as their high micronutrient content (Ca, Mg, Zn, Fe, and Cu) (Anand et al., 2008). Not only that, vertebrates such as birds, lizards, snakes, amphibians, and fish feed on acridids (Van Huis, 2013).

2.2 Black Soldier Fly

Hermetia illucens widely recognised as the black soldier fly (BSF) is a Stratiomyidae family true fly (Diptera). Despite its origins in America, it now flourishes in the tropics and temperate climes across the world (Čičková et al., 2015). Adults only require water to survive, avoid human contact, do not sting or bite and do not spread illness (Čičková et al., 2015). Its complete absence of cold adaptability precludes it from establishing itself in non-indigenous areas such as Northern Europe (Spranghers et al., 2017). They are typically regarded as non-pest insects that provide benefits. They only consume food when in the larval stage, hence they are not involved in the spread of diseases.

Adult black soldier flies are black or blue and resemble wasps in appearance (Larouche et al., 2019). On the first abdominal segment, it possesses membranous wings that are folded horizontally and overlapped while at rest. The adults are about 15 to 20 mm long (Sheppard et al., 2002). The adult's legs feature white pigmentation near the end of each leg, and its antennae are lengthy with three segments. The possibility of mating exists two days after the adult emerges from the pupal case. A male BSF intercepts a female flying by, and they land while mating (Tomberlin & Sheppard, 2001). Female BSF lays a cluster of around 500 eggs in crevices and gaps adjacent to or amidst organic waste such as dung, carrion, rubbish, and other waste that is in the process of decomposing (Tomberlin & Sheppard, 2001).

According to Larouche et al. (2019), in around four days, the eggs develop into larvae. Each egg is in an oval shape and is around 1 mm long. Its hue is either creamy white or pale yellow. BSF larvae may grow to a length of 27 mm and 6 mm in width. A tiny, protruding head with chewing mouthparts and a dull, pale tint characterise them. Larvae experience six instars and need around a fortnight to reach full growth (Barros-Cordeiro et al., 2014; Kim et al., 2015). The beige larva with photoreceptors permits them to avoid being exposed to light (Oonincx et al., 2016). Animal dung, fruits, vegetables, food wastes, and fish offal are among the organic things consumed by the larvae (St-Hilaire et al., 2007; Lalander et al., 2019).

The pre-pupa tends to move to arid places to metamorphose into a pupa during this seven to 10-day period. It subsequently stops feeding and begins melanisation, which results in a darker cuticle colouration a few moments before it moults to become a pre-pupa (Kim et al., 2015). The adult emergence follows the pupal stage, which endures for a minimum of eight days and during this stage, the pupae do not move or consume (Diener et al., 2011). During its eight to nine days of life, the fly mates and deposits its eggs (Tomberlin et al., 2002).

Throughout the BSF lifecycle as shown in Figure 2.1, larvae and pre-pupae stages are of most concern due to their nutrition values (Liu et al., 2017). As the protein composition is higher relative to the cattle, BSF larvae might be a viable protein-rich diet (Tschirner & Simon, 2015). Relative to the larval stage, the pupae contain about 20% less lipid, reducing their suitability as a feed source (Liu et al., 2017). With respect to nutrition, BSF larvae are fascinating insects, as they are packed with a diverse range of micro- and macronutrients. Accordingly, it is frequently utilised for feeding animals like chickens, fish, and pigs (Veldkamp & Bosch, 2015; Huyben et al., 2019; Pieterse et al., 2019; Zarantoniello et al., 2020).

Figure 2.1 Lifecycle of black soldier fly (1: Egg, 2: Larvae, 3: Pre-pupae, 4: Pupae, 5: Adult)

2.3 Nutritional Content of Insects

Insects' nutritional contents differ greatly across and within species, depending on their metamorphic stage, environment, and food (Shah et al., 2022). Furthermore, they are high in proteins (e.g., AA, such as cysteine, lysine, methionine, and threonine), carbohydrates, lipids, and many minerals (e.g., calcium, iron, zinc, and phosphorus), as well as vital vitamins (e.g., vitamin A, B complex, and vitamin C). The essential amino acids in insect protein are also of excellent quality (Jantzen da Silva Lucas et al., 2020). Table 2.1 shows the proximate analysis and chemical composition of commonly used insects as feed. Among the listed insects' larvae in Table 2.1, black soldier fly larvae had the highest crude protein, crude fat and crude ash content compared to other insects.

Table 2.1 Proximate analysis and chemical composition of commonly used insects as feed

Parameter	Insect species		
	Black soldier fly larvae (St-Hilaire et al., 2007; Moreki et al., 2012; Cullere et al., 2017; Jayanegara et al., 2017; Schiavone et al., 2017; Makkar, 2018; Campbell et al., 2020)	Housefly larvae (Rumpold & Schlüter, 2013; Hussein et al., 2017; Campbell et al., 2020)	Mealworm larvae (Marono et al., 2015; Gugołek et al., 2020)
Dry matter (%)	27.40	83.47	94.60
Crude protein (%)	56.10	33.29	55.83
Crude fat (%)	23.20	6.20	25.19
Crude ash (%)	9.85	6.25	4.84
Calcium (%)	2.14	0.49	0.21
Phosphorus (%)	1.15	1.09	1.06
Magnesium (%)	0.39	0.23	0.30
Potassium (%)	1.35	1.27	1.12
Sodium (%)	0.13	0.54	0.21

However, the nutritional content of BSF larvae varies greatly depending on the feeding substrate and developmental stages as shown in Table 2.2 and Table 2.3. The larvae and prepupae are the two crucial stages for the potential use of BSF as food or feed. When raised in the same circumstances, the larvae have higher lipid and nitrogen-free extract levels but lower ash and protein levels than the prepupae and pupae (Barros-Cordeiro et al., 2014; Liu et al., 2017). Similarly, Rachmawati et al. (2010) reported a decline in protein content during the larval stage, from 61% (DM) at 5 days to 44% at 15 days and 42% at 20 days. The author also reported that the fat content rose from 13% (DM) at 5 days to 19% at 15 days and 23% at 20 days.

Another study by Wang et al. (2020) found that the chitin content of BSF larvae decreased from the larvae (3.6 g/100 g) to prepupae (3.1 g/100 g) stage, and reached its peak during the pupae stage (14.1 g/100 g). Prepupae contains 20% more water than larvae, hence requiring a longer drying time (Oonincx et al., 2015; Spranghers et al., 2017). Hence, larvae stages are commonly used. By applying this knowledge, it could be possible to harvest the larvae at specific stages of their growth and obtain the nutritional profiles needed for specific applications. Nevertheless, it is important to note that harvesting larvae from waste feed before pupation is a problem, since they naturally move away from their nutrition source (Wang & Shelomi, 2017).

Table 2.2 Proximate composition of black soldier fly larvae reared on different substrates.

Feeding substrate / Analysis	Palm kernel meal (Caruso et al., 2013)	Vegetable waste (Spranghers et al., 2017)	Restaurant waste (vegetables) (Spranghers et al., 2017)	Poultry manure (Newton et al., 2005)
Crude lipid (%)	38.0 ± 4.1	21.8	38.6	34.8
Crude fibre (%)	9.8 ± 1.6	-	-	-
Crude ash (%)	19.5 ± 4.9	9.6	2.7	-
Crude protein (%)	39.2 ± 4.1	39.9	43.1	42.1

Table 2.3 Proximate composition of black soldier fly in different developmental stages (Aizah et al., 2022)

Developmental stages / Analysis	Larvae	Pupae	Adult
Moisture content (%)	6.9	37.4	25.6
Crude lipid (%)	26.7	4.2	15.6
Crude fibre (%)	10.1	8.6	13.2
Crude ash (%)	9.0	10.6	7.5
Crude protein (%)	32.3	27.0	52.6

In addition to proximate analysis, amino acid profiling is an essential technique for assessing nutritional content, especially the quality of proteins. BSF larvae offer a rich array of amino acid profile resembling to conventional soybean meal and fish meal as in Table 2.4. BSF larvae had the maximum quantities of isoleucine, leucine, lycine, threonine and histidine among the insect species, conventional soybean meal and fishmeal compared. Not only that, BSF larvae has even higher valine content than conventional soybean meal and fishmeal.

Unlike some other nutritional components, the amino acid content of the larvae is less susceptible to external factors. Nevertheless, research has indicated that the amino acid content does change over the BSF larvae's life span, with younger larvae (typically those between 4 and 6 days old) having higher levels of essential amino acids (Liu et al., 2017). Additionally, processing methods such as the killing technique also have an impact on the amino acid composition. While blanching is thought to have no detrimental effects on the amino acid profile, freezing causes the activation of metabolic pathways that lead to the loss of cysteine and lysine (Leni et al., 2019).

Table 2.4 Amino acid content of black soldier fly larvae, mealworm, conventional soybean meal and fishmeal (mg/g protein) (National Research Council, 2012; Ravzanaadii et al., 2012; Yi et al, 2013; Bosch et al., 2014; Liland et al., 2017; Caligiani et al., 2018; Bessa et al., 2020)

Species / Amino acids	BSF larvae (*Hermetia illucens*)	Mealworm (*Tenebrio molitor*)	Conventional soybean meal	Fishmeal
Isoleucine	40.0 - 41.0	29.0 - 35.5	22.1	27.3
Leucine	61.0 - 75.0	34.0 - 73.0	38.6	47.7
Lysine	54.0 - 65.0	29.0 - 54.0	31.1	48.7
Threonine	36.0 - 42.0	18.0 - 39.0	19.8	27.5
Tryptophan	6.0	12.0	6.6	6.7
Valine	39.0 - 55.0	24.3 - 61.0	21.7	32.7
Histidine	28.0 - 33.0	15.2 - 29.0	14.2	15.4

2.4 Killing Methods of Insects

Prior to killing the insects, washing is done to remove the frass from the insect's cuticle (Stadtlander et al., 2017; Mutungi et al., 2019). Washing can be performed using either dipping or spraying. Parameters such as increased pressure, time, temperature, repeated dipping, and the addition of sanitizers such as chlorine and other organic acids can improve the effectiveness of the killing methods of insects (Hultberg et al., 2019).

The next step after washing is the killing method. Killing is a crucial stage in the processing of insects since it can affect the end product's nutritional value, microbiological safety, colour stability, and flavour (Farina, 2017).

Several factors, including cold, heat, asphyxiation, and mechanical disturbance, can kill invertebrates. Therefore, it is important to adjust the killing technique to the species requirements. Blanching and freezing are the common approaches, but there are numerous others that could be just as successful (Kouřimská & Adámková, 2016; Van Huis & Tomberlin, 2017). Freezing is widely utilised because it preserves the product while gradually terminating the insect. As poikilothermic organisms, insects experience a slowdown in metabolic activity when exposed to cold, which can minimize the potential for pain or distress during the killing process (Larouche et al., 2019). Additionally, cold treatment offers the advantage of reducing microbial growth and the activity of spoilage-related enzymes (Larouche et al., 2019). These effects can be achieved through methods such as freezing, freeze-drying, and submersion in ice water (Fu et al., 2014).

In contrast, the thermal tolerance of insects varies across species and life stages. Some species may begin to experience thermal stress or mortality at temperatures as low as 50°C. However, higher temperatures are often required to achieve simultaneous inactivation of associated microorganisms, including heat-resistant bacteria (Armstrong et al., 2009). Heat based methods are using either dry heat, like in an oven, or moist heat, such as steaming and blanching food. Heat treatment can prevent product degradation during storage by inducing endogenous spoilage-enzyme denaturation in addition to lowering the insects' microbiological burden (Manheem et al., 2012).

Blanching is one of the most used heat-based methods for killing insects since it is quick and allows for microbial reduction (Piyasena et al., 2003). Blanching has been reported as the killing method for BSF larvae and mealworm. Additionally, blanching decreases an insect's ability to retain water, speeds up drying, and inactivates spoilage enzymes while preserving colour and nutritional (Caligiani et al., 2019; Leni et al., 2019; Tonneijck-Srpová et al., 2019). Blanching may improve the amino acid profile of *Hermetia illucens* larvae by inactivating proteolytic enzymes, reducing microbial degradation, and enhancing protein solubility. These effects help preserve essential amino acids during subsequent drying processes (Lee et al., 2023).

The use of carbon dioxide as an anesthetic for invertebrates is also a common method. Insects cease movement at CO_2 levels higher than 40% because CO_2 causes neuron depolarization without changing conductance (Wong-Corral et al., 2013). The duration needed to efficiently eliminate insects with carbon dioxide differs considerably based on species, life stage, temperature, CO_2 and O_2 concentrations, as well as humidity (Husain et al., 2017). A study reported that for *Drosophila melanogaster* (Diptera) larvae, carbon dioxide in its pure form can cause fatality in only 30 minutes, but it takes larvae of *Cadra cautella* (Lepidoptera) and *Tribolium castaneum* (Coleoptera) 72 hours to die (Badre et al., 2005; Husain et al., 2017).

Asphyxiation can occur with N_2 saturation also, but oxygen concentrations must be below 30%. However, large-scale air saturation is quite difficult to achieve (Hashem et al., 2014). Other techniques such as vacuum packing and drowning can be used too, but the survival rate differs according to insect species and developmental stages (Fernandez-Cassi et al., 2019). Arthur Woods and Lane (2016) observed that *Manduca sexta* pupae can last five days when submerged in water, whereas the larvae can only survive for four hours. The death rate of *Ephestia cautella* larvae in 98% N_2

is very variable taking between 96 and 144 hours, which exceeds by one to two days than in an aerobic environment with 60% CO_2.

Mechanical disruption of insects is also one of the promising methods to kill insects because of its rapidity and low cost. It may be done by either grinding or centrifugation. Additionally, electrocution has also been used in killing invertebrates like crustaceans (Fregin & Bickmeyer, 2016). According to reports, electrocuting crustaceans for one second at 120 volts and 20 amps would successfully kill them, but it would take five seconds to kill the lobster and 10 seconds to kill the crab (Yue, 2008).

In BSF larvae, various killing methods have been documented as shown in Table 2.5. From Table 2.5, blanching and freezing are the most used killing methods of BSF larvae. Other methods have been investigated as well as shown below but the outcomes were not as satisfying as the blanching and freezing methods. Hence, those methods were chosen as killing methods. Freezing was selected as a non-thermal killing method for its ability to preserve the nutritional and structural integrity of BSF larvae by minimizing heat-induced degradation. Blanching, in contrast, was chosen as a thermal method due to its effectiveness in inactivating browning enzymes and reducing microbial load on the larvae surface. These two methods represent contrasting pre-treatment approaches, cold versus heat, offering insight into their respective impacts on larvae quality post-drying. Their widespread use in previous studies and insect processing further supports their relevance in both research and industrial settings.

Table 2.5 Killing methods of black soldier fly larvae.

Killing methods	Conditions	Efficiency	References
Blanching	Temperature: 100°C Duration: 40 seconds	Efficient	(Larouche et al., 2019; Saucier et

		(Maintained the chemical composition and lowest microbial counts)	al., 2020; Rostika et al., 2022)
Blanching	Temperature: 90℃ Duration: 40 seconds	Efficient	(Hurtado-Ribeira et al., 2023)
Scalding	Temperature: 100℃ Duration: 2, 4, 6, 8 minutes	Not efficient	(Saucier et al., 2020)
Freezing	Temperature: -20℃ Duration: 24 hours	Efficient	(Huang et al., 2019; Hurtado-Ribeira et al., 2023)
Freezing	Temperature: -20℃/-40℃ Duration: 1 hour	Less efficiency compared to blanching (Can induce microbial load but not an effective method)	(Larouche et al., 2019)
Grinding	Homogenizing for 2 minutes at 15000 rpm	Not efficient (High microbial load and colour change)	(Larouche et al., 2019)
High hydrostatic pressure	larvae packed under a 95% vacuum followed by a pressure treatment at 600 MPa for 3 min	Not efficient (High LAB, *Clostridia* and other anaerobic bacteria counts)	(Larouche et al., 2019)
Desiccation (air oven)	Temperature: 60℃ Duration: 30 minutes	Not efficient (High microbial load and colour intensity)	(Larouche et al., 2019)
Liquid nitrogen	Immersing larvae in vacuum packaged liquid nitrogen for 40 seconds	Lesser efficiency than freezing	(Larouche et al., 2019)
Asphyxiation	Vacuum packaging the larvae with 100% CO_2 or N_2 using a syringe through a silicone stopper; stored at 27℃ in the dark for 120 hours and 144 hours, respectively	Not efficient (High microbial counts)	(Larouche et al., 2019)

2.5 Drying Methods of Insects

Once insects have been properly killed, the next step, which is drying becomes an important step in assuring their preservation and usage. According to Calín-Sánchez et al. (2020), drying involves extracting moisture from solid and liquid substances, usually leading to the formation of dry, solid products. This is commonly achieved with the intervention of heat. The final product's quality is influenced by the type of drying procedure utilized. Although drying has an impact on the colour, density, size, hardness, and nutritional qualities of the insects, it is still the method of choice. Frequently used drying methods in insect processing are sun, oven, freeze, and microwave drying (Van Huis & Tomberlin, 2017). Few research has examined the impact of drying on the quality of BSF larvae or prepupae, with most studies on drying edible insects focusing on *Tenebrio molitor*.

Sun drying is a classic low-cost drying process that has been used to treat a variety of insect species, including grasshoppers, caterpillars, termites, and maggots, at low temperatures and for long periods (Melis et al., 2018; Mutungi et al., 2019). In tropical and subtropical nations, where solar radiation is advantageous, open sun drying is commonly used to preserve agricultural produce. However, open sun drying has drawbacks, including difficulty adjusting the drying conditions and factors, unpredictability of the weather, high labour expenses, the necessity for a large drying area, potential insect infestations, and cross-contamination with dust and other foreign components. Caruso et al. (2014) documented the use of bamboo baskets for sun drying BSF larvae in Indonesia, highlighting its role in low-cost and community-level feed processing (Figure 2.2).

Figure 2.2 Sun drying of black soldier fly larvae using bamboo baskets in Indonesia (Caruso et al., 2014)

Despite the use of sun drying, recent investigations have highlighted the potential of oven drying as an alternative insect drying technique (Fombong et al., 2017; Huang et al., 2019). Lower temperatures are preferred to maintain protein solubility and reduce the Maillard reaction, shrinkage, and tissue degradation (Kröncke et al., 2018; Melis et al., 2018). Temperatures varying from 50°C to 120°C are generally applied for time spans ranging from one hour to multiple days. To prevent these issues and speed up the drying process, 60°C is the ideal temperature (Azzollini et al., 2016).

Microwave drying is also another type of drying method. Despite being costly, microwave drying takes just 10 to 15 minutes, depending on the microwave's settings (Lenaerts et al., 2018). Although browning is permitted, it also creates inflated entire dry larvae (Huang et al., 2019). Mealworm protein solubility was observed to be 40% lower after microwave drying (Kröncke et al., 2018). In comparison to oven drying at 60°C, microwave drying on BSF larvae increases protein polymerization, which results in a powder with larger particle sizes, a lower digestible amino acid score, and digestibility. In Malaysia, microwave drying technology has been adopted in community-scale initiatives to optimize the post-harvest processing of BSF larvae, contributing to sustainable feed production (Figure 2.3).

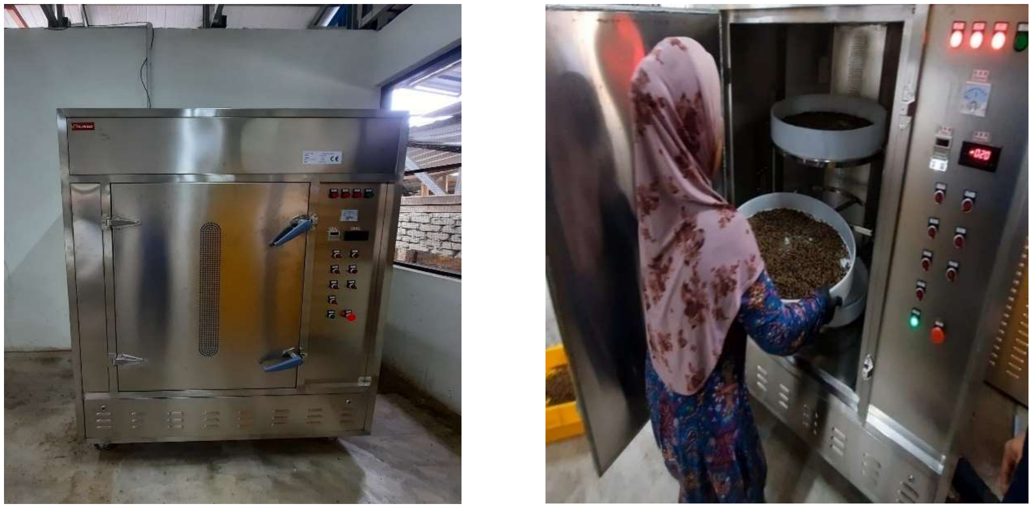

Figure 2.3 Microwave drying of black soldier fly larvae as a part of the Future Animal Feed Innovation Project in Tasek Gelugor, Penang, Malaysia

Although expensive and taking at least 24-53 hours to dry mealworms, freeze-drying is a procedure that does not use heat, extensively applied in insect processing (Kröncke et al., 2018; Lenaerts et al., 2018). It's one of the greatest ways to keep insect colour since it doesn't cause the Maillard reaction, which results in the whitest powder (Lenaerts et al., 2018). It does, however, cause a ten percent drop in protein solubility (Kröncke et al., 2018). In addition, freeze-drying causes the highest lipid oxidation, eventuating in considerable quality deterioration, but blanching before freeze-drying decreased oxidation by half (Kröncke et al., 2018; Lenaerts et al., 2018).

Using a dehydrator to dry larvae is still new to the field hence not much information regarding the drying process is available. According to Badenhop (2018), dehydrators use low-temperature air circulation to dry food. Dehydrators consist of ventilators, covers, thermostats, and timers. Dehydrators draw moisture out of the food instead of cooking it with heat. Not only that, removing water enhances the taste of the food. The advantages of using a dehydrator are it can be done at any time, the products are cleaner as they are free from pollution, the products have longer shelf time and they retain the original vitamins, minerals, and enzymes (WebMD Editorial Contributors, 2020).

Fluidized bed drying is another type of drying process which is fast and requires a shorter duration has been reported in insects since it induces a little lipid oxidation increase when applied at 130°C for 110 minutes (Larouche et al., 2019). The drying method that has been reported in the larvae of BSF includes sun drying, microwave drying, sand roasting (pan), sand roasting (drum), oven heating, sun drying and stone oven drying as shown in Table 2.6. From the table, microwave drying is the fastest drying method requiring only 15 minutes compared to other drying methods. Also, oven drying method is the most efficient method to dry large batch sizes of larvae as shown in the table.

Table 2.6 Drying methods of black soldier fly larvae.

Drying method	Drying condition	Results	Reference
Sun drying (Indonesia)	Temperature: 38 ± 4°C Humidity: 47 ± 6% Light intensity: >20 000 lux Duration: 17 hours	The study estimated a loss of 65% of their body weight.	(Caruso et al., 2013)
Microwave drying	Weight of larvae: 0.25 kg Power: 1000 watts Duration: 15 minutes	The research showed that microwave drying produces a high-quality product compared to other conventional drying method.	(Dortmans et al., 2017)
Microwave drying	Weight of larvae: 0.3 kg Power: 800 watts Duration: 25 minutes	The study resulted in golden yellow colour and crispy texture larvae.	(Purnamasari et al., 2022)
Oven drying	Weight of larvae: 30 kg Temperature: 65°C Duration: 24 hours	It yields hard, stiff and dark brown-coloured larvae.	(Dortmans et al., 2017)
Stone oven drying	Weight of larvae: 0.3 kg Duration: 75 minutes	It resulted in darker colour of	(Purnamasari et al., 2022)

		BSF larvae with firm texture.	
Sand roasting (pan)	Weight of larvae: 0.7 kg Maximum temperature: 180°C Duration: 15 minutes	It produced crispy, puffed and yellowish-brown larvae.	(Dortmans et al., 2017)
Sand roasting (drum)	Weight of larvae: 4 kg Maximum temperature: 150°C Duration: 30 minutes	It produced crispy, puffed and yellowish-brown larvae.	(Dortmans et al., 2017)

In this study, sun drying was selected as a representative of traditional methods due to its low cost and historical relevance, especially in low-resource settings. In contrast, freeze-drying and dehydrator drying were chosen as modern techniques, given their potential to better preserve the nutritional and physical qualities of BSF larvae. This selection enables a comparative evaluation between traditional and modern drying approaches, addressing a gap in the current literature concerning their effects on BSF larvae quality.

2.6 Effect of Killing Processes and Drying Methods on Insect Quality

The nutritional and physicochemical qualities of the product are greatly impacted by the processing techniques used, especially pre-treatment and drying methods. These processing techniques influence protein, lipid, and amino acid retention, as well as pH, colour and texture, which are vital for feed application.

Regarding nutritional value, killing process such as blanching have been found to decrease protein levels due to leaching but enhance lipid stability by deactivating enzymes (Lee et al., 2023). Studies indicate that freeze-drying better preserves protein and amino acid content compared to oven-drying, which may degrade heat-sensitive components (Dridi et al., 2021; Gachici et al., 2023). Differences in moisture levels and nutrient retention can result from varying drying methods; for example, oven