INNOVATIVE USE OF FERMENTED PUMPKIN AS AN ATTRACTANT AND FEED SUBSTRATE IN BLACK SOLDIER FLY PRODUCTION: EVALUATING ITS AGRICULTURAL APPLICATIONS

Pichet Praphawilai, Bajaree Chuttong, Ming-Cheng Wu, Sampat Ghosh, Chuleui Jung, Kanokwan Klaithin and Khanchai Danmek

SUMMARY

This study investigates the application of fermented pumpkin as a substrate for Black Soldier Fly (BSF) larvae production, offering a sustainable solution for waste recycling and protein generation. Pumpkin, fermented with Lactobacillus plantarum *and* Trichoderma viride *for 21 days, was tested in five northern provinces of Thailand: Phayao, Chiang Mai, Phrae, Lampang, and Lamphun. At each site, ten baskets of fermented pumpkin were placed in orchards, with an average of 7–8 baskets successfully supporting larval development. The average yield of BSF larvae per basket was 1.9 ± 0.27kg. Nutritional analysis of the harvested larvae revealed a high crude protein content of 38.99%, with leucine, lysine, and valine identified as the dominant essential amino acids. Furthermore, saturated fatty acids (SFAs) were found to be more abundant than unsaturated fatty acids (USFAs), with lauric acid and palmitic acid being the most prominent among the SFAs. The findings emphasize the potential of fermented pumpkin as a cost-effective and sustainable substrate for BSF larvae production, contributing to innovative approaches in organic waste management and the development of alternative protein sources.*

Introduction

The black soldier fly (BSF), *Hermetia illucens* L., is a true fly (Diptera) of the family Stratiomyidae. Originally from the Americas, it now thrives in diverse climates worldwide, from the tropics to temperate regions (Sheppard *et al*. 1994, Čičková *et al*. 2015). Black soldier fly larvae (BSFL) are highly efficient consumers of organic waste, demonstrating success in waste management programs (Sheppard, 1983; Yu GuoHui *et al*., 2009). Their ability to convert organic waste into a valuable protein source aligns with sustainability goals by reducing reliance on resource-intensive feed ingredients (Singh and Kumari, 2019; Raman *et al*., 2022). This makes them a promising solution for organic waste management and a valuable resource for animal feed due to their voracious appetite and efficient food conversion (Abd El-Hack *et al*., 2020). They can be raised and collected without specialized equipment and pose minimal pest risks. Their larvae are a valuable source of nutrition, containing 42% crude protein and 29% fat. While higher in saturated fats compared to some other insects, they do not accumulate harmful pesticides or mycotoxins. BSF are already utilized as animal feed in some regions, though regulations on their production and use may vary (Wang and Shelomi, 2017).

In agricultural settings, where incorporating these larvae into feed cycles can help close nutrient chains and promote more effective resource usage, this change is particularly important. BSFL's ability to process organic waste materials into valuable biomass addresses both environmental and economic concerns. This process minimizes waste that would otherwise contribute to environmental degradation, such as greenhouse gas emissions from landfills (Isibika *et al*., 2023; Siddiqui *et al*., 2024). Moreover, the larvae's conversion efficiency can help

KEYWORDS / Black Soldier Fly / BSF / BSF Attraction / Fermented Pumpkin / Nutrition /
Received: 05/06/2024. Modified: 01/23/2025. Accepted: 01/24/2025.

Pichet Praphawilai. M.Sc. in Applied Microbiology, Chiang Mai University, Thailand. Researcher, Chiang Mai University, Thailand.

Bajaree Chuttong (Corresponding author). PhD in Post-harvest Technology, Chiang Mai University, Thailand. Lecturer, Chiang Mai University, Thailand. Address: Meliponini and Apini Research Laboratory, Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand. e-mail: bajaree.c@cmu.ac.th

Sampat Ghosh. PhD in Zoology, Arunachal Pradesh, India. Research Professor, Agricultural Science and Technology Research Institute, Andong National University, Andong Republic of Korea.

Chuleui Jung. PhD in Entomology, Oregon State University, USA. Professor, Department of Plant Medicals, Andong National University, Republic of Korea.

Ming-Cheng Wu. PhD in Chemical Biology, The University of Manchester, Manchester, UK. Associate Professor, Department of Entomology, College of Agriculture and Natural Resources, National Chung Hsing University Taichung, Taiwan.

Kanokwan Klaithin. M.Sc. in Entomology, Chiang Mai University, Thailand. Scientist, Meliponini and Apini Research Laboratory, Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand.

Khanchai Danmek (Corresponding author). PhD in Biotechnology, Chulalongkorn University, Thailand. Lecturer, University of Phayao, Thailand. Address: School of Agriculture and Natural Resources, University of Phayao, Phayao 56000, Thailand. e-mail: khanchai.da@up.ac.th.

USO INNOVADOR DE CALABAZA FERMENTADA COMO ATRACTANTE Y SUSTRATO ALIMENTICIO EN LA PRODUCCIÓN DE BSF: EVALUACIÓN DE SUS APLICACIONES AGRÍCOLAS

Pichet Praphawilai, Bajaree Chuttong, Ming-Cheng Wu, Sampat Ghosh, Chuleui Jung, Kanokwan Klaithin y Khanchai Danmek

RESUMEN

El estudio investiga la aplicación de calabaza fermentada como sustrato para la producción de larvas de Mosca Soldado Negra (BSF, por sus siglas en inglés), ofreciendo una solución sostenible para el reciclaje de residuos y la generación de proteínas. La calabaza, fermentada con Lactobacillus plantarum *y* Trichoderma viride *durante 21 días, fue probada en cinco provincias del norte de Tailandia: Phayao, Chiang Mai, Phrae, Lampang y Lamphun. En cada sitio, se colocaron diez cestas de calabaza fermentada en huertos, con un promedio de 7–8 cestas que apoyaron exitosamente el desarrollo larval. El rendimiento promedio de larvas de BSF por cesta fue de 1,9 ± 0.27kg. El análisis nutricional de las larvas cosechadas reveló un alto contenido de proteína cruda de 38.99%, con leucina, lisina y valina identificadas como los aminoácidos esenciales dominantes. Además, se encontró que los ácidos grasos saturados (AGS) eran más abundantes que los ácidos grasos insaturados (AGI), siendo el ácido laúrico y el ácido palmítico los más prominentes entre los AGS. Los resultados enfatizan el potencial de la calabaza fermentada como un sustrato rentable y sostenible para la producción de larvas de BSF, contribuyendo a enfoques innovadores en la gestión de residuos orgánicos y al desarrollo de fuentes alternativas de proteína.*

USO INOVADOR DE ABÓBORA FERMENTADA COMO ATRATIVO E SUBSTRATO ALIMENTAR NA PRODUÇÃO DE BSF: AVALIANDO SUAS APLICAÇÕES AGRÍCOLAS

Pichet Praphawilai, Bajaree Cuttong, Ming-Cheng Wu, Sampat Ghosh, Chuleui Jung, Kanokwan Klaithin e Khanchai Danmek

RESUMO

O estudo investiga a aplicação de abóbora fermentada como substrato para a produção de larvas da Mosca Soldado Negra (BSF), oferecendo uma solução sustentável para a reciclagem de resíduos e geração de proteínas. A abóbora, fermentada com Lactobacillus plantarum *e* Trichoderma viride *por 21 dias, foi testada em cinco províncias do norte da Tailândia: Phayao, Chiang Mai, Phrae, Lampang e Lamphun. Em cada local, foram colocadas dez cestas de abóbora fermentada em pomares, com uma média de 7–8 cestas que apoiaram com sucesso o desenvolvimento larval. O rendimento médio de larvas de BSF por cesta foi de 1,9 ± 0,27kg. A análise nutricional das larvas colhidas revelou um alto teor de proteína bruta de 38,99%, com leucina, lisina e valina identificados como os aminoácidos essenciais predominantes. Além disso, os ácidos graxos saturados (AGS) foram encontrados em maior quantidade do que os ácidos graxos insaturados (AGIs), com ácido láurico e ácido palmítico sendo os mais proeminentes entre os AGS. Os resultados destacam o potencial da abóbora fermentada como um substrato econômico e sustentável para a produção de larvas de BSF, contribuindo para abordagens inovadoras na gestão de resíduos orgânicos e no desenvolvimento de fontes alternativas de proteínas.*

mitigate feed shortages, especially in regions heavily dependent on imported feed ingredients for livestock, thus fostering food security (Rumpold and Schlüter, 2013). BSF functions as a sustainable feed substitute by effectively converting organic waste into proteins and lipids. Food waste can be turned into insect-based meals that promote global agriculture, save feed costs, and lessen reliance on imports of protein. It is possible to recycle over 2 billion tons of appropriate food waste by 2030 (Jensen *et al*., 2021).

Nevertheless, the effectiveness of BSF production is influenced by various factors, such as substrate type and the process parameters employed in the larval rearing process. Nutritional values in BSF generally mirror the nutritional content of the feeding substrates. Larvae reared on a nutritionally well-balanced feed such as chicken feed, brewery waste, and fruit waste had the greatest yield and highest bioconversion ratio, while cow feed and food waste had a good result in larval survival, larval weight, bioconversion ratio, and material reduction, although larval survival was highest in bread and vegetables and lowest in cow feed and food waste (Lopes *et al*., 2023; Adebayo *et al*., 2021). Moreover, rearing BSFL on organic waste yielded lower larval weight than well-balanced feed (Opoku *et al*., 2023). These reports suggest that there is a need to find nutritional interventions that can enrich the composition of the substrates for enhanced larval mass. Beyond the nutritional value in BSF feed issues, it is essential to ensure that BSF production is both sustainable and comprehensive. The type of feed substrate can significantly impact the quantity of the prepupae. All substrates allowed the successful development of larvae, but pig manure was more productive than the others (Boafo *et al*., 2023). This information is necessary for the effective and continuous production of BSFL in both small-scale and large-scale BSF farming. Our work distinguishes itself by exploring the substrate for BSFL production in Thailand. This approach leverages locally abundant agricultural byproducts, presenting a cost-effective and sustainable alternative for BSF cultivation that is tailored to regional conditions.

Pumpkins belong to the Cucurbitaceae family, a widely cultivated vegetable with significant economic importance. Various parts of the pumpkin, including its seeds, peel, and flesh, are rich sources of essential nutrients, such as carbohydrates, fiber, amino acids, monounsaturated and polyunsaturated fatty acids, vitamin E, and carotenoids (Batool *et al*., 2022). Pumpkins (*Cucurbita moschata* Duch. ex. Poir.) are a staple crop in Thailand and are extensively cultivated in tropical and subtropical regions. They are among the most widely grown vegetables in Thailand (Pongjanta *et al*., 2006). Low-grade pumpkins, typically inexpensive and often overlooked by the market, can be effectively utilized in insect farming, particularly with BSFL. This approach not only reduces production costs due to the low input but also enhances the nutritional value of the resulting insect biomass, making it a more valuable product. This study investigates the efficacy of a novel methodology incorporating fermented pumpkin into BSF production. By fermenting pumpkins, we aim to enhance larval growth efficiency and nutritional value while providing a sustainable and cost-effective feed source. This simple and scalable fermentation process offers an environmentally friendly solution for BSF production, addressing the need for affordable and readily available feedstocks.

Materials and Methods

Fresh pumpkins were mechanically chopped into uniform pieces using a conventional forage harvester, ensuring consistent substrate size necessary for even fermentation. Subsequently, 50 kilograms of chopped pumpkin were transferred into a 150L plastic container, serving as the fermentation vessel for substrate preparation.

To initiate ensiling, a microbial consortium comprising *Lactobacillus plantarum* (lactic acid bacteria) and *Trichoderma viride* (known for its fibrolytic enzymes) at a 1:1 ratio was added at a concentration of 0.1% (w/w) to the chopped pumpkin, following the methodology of Thana *et al*. (2019). The container was securely sealed with a plastic lid to create anaerobic conditions and allowed to ensile for 21 days at an average ambient temperature of 30°C.

The chemical composition of the fermented pumpkin was determined according to standard methods outlined by the Association of Official Analytical Chemists (AOAC, 2016) for dry matter, crude protein, ether extract, ash, neutral detergent fiber, and acid detergent fiber. Organic acid concentrations (lactic acid, acetic acid, and butyric acid) and pH were analyzed following the method described by Thana *et al*. (2019). This comprehensive approach allowed for the assessment of key parameters related to the ensiling process, providing valuable insights into the fermentative and nutritional characteristics of the pumpkin substrate.

A field trial was conducted across five distinct locations in fruit orchards within the northern region of Thailand (Phayao, Chiang Mai, Phrae, Lampang, and Lamphun) to investigate the effectiveness of fermented pumpkin as an attractant and feed substrate for BSF. Ten containers of fermented pumpkin were strategically placed within each orchard to ensure comprehensive coverage and representation of diverse geographical conditions. Observations and data were collected over a 30-day period to monitor attraction and consumption.

For the nutritional assessment of BSFL production, using an innovative fermented pumpkin substrate method, the 21-day-old larvae (pre-pupa stage) were harvested. The larvae of BSF were subjected to an oven-drying process at 50°C for 48 hours. Subsequently, they were ground prior to oil extraction. The crude protein content was assessed using the Kjeldahl process, which involves determining the nitrogen (N) content and then multiplying it by the N-to-protein conversion factor of 6.25. Crude fat was determined using the Soxhlet apparatus with hexane as the solvent. Crude fiber content was estimated using the acid and alkali digestion method. Furthermore, the amino acid profile of the fermented pumpkin was analyzed, encompassing both essential and non-essential amino acids, utilizing High-Performance Liquid Chromatography (HPLC) technique as described by AOAC (2016). The fatty acid profile of oil extracted from BSF was analyzed using a Gas Chromatography machine (Agilent Technologies 6890N) equipped with a Flame Ionization Detector (FID) by AOAC (2012). For statistical analysis, a one-way ANOVA (Analysis of Variance) followed by Duncan's Multiple Range Test (DMRT) was used in this experiment.

Results and Discussion

The results revealed significant differences in both the chemical composition between fresh and fermented pumpkin samples, with and without microbial inoculation as detailed in Table I. Notably, the fermented pumpkin supplemented with microbial inoculum

TABLE I
COMPARATIVE ACCOUNT OF THE CHEMICAL COMPOSITION BETWEEN FRESH AND FERMENTED PUMPKIN SAMPLES, WITH AND WITHOUT MICROBIAL INOCULATION, ON DAY 21

Parameters (g/100g substrate)	Fresh pumpkin	Fermented pumpkin		p-value*
		Without microbial inoculation	With microbial inoculation	
Dry matter	13.68±0.97	13.03±0.87	13.12±0.34	0.567
Crude Protein	12.91±0.17^{b}	12.54±1.50^{b}	15.95±1.33^{a}	0.022
Ether extract	4.21±0.96	4.03±0.62	4.11±0.41	0.955
Ash	1.53±0.50	0.97±0.21	0.89±0.22	0.112
Neutral detergent fiber	223.33±6.11^{a}	212.67±4.73^{b}	209.0±04.36^{b}	0.033
Acid detergent fiber	263.00±10.39^{a}	245.67±13.58^{ab}	232.67±4.04^{b}	0.029
Lactic acid	0.00^{c}	60.00±3.00^{b}	68.00±3.00^{a}	0.000
Acetic acid	0.00^{b}	14.33±3.21^{a}	14.00±3.61^{a}	0.001
Butyric acid	0.00^{b}	0.70±0.20^{a}	0.73±0.06^{a}	0.000
pH	6.77±0.40^{a}	4.70±0.66^{b}	4.43±0.21^{b}	0.002

The results are presented as mean values ± standard deviation of triplicate *Significant differences among groups were determined by one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) ($p < 0.05$). Different letters indicate significant differences between groups.

exhibited the highest crude protein (CP) content, alongside significantly diminished values of Acid detergent fiber (ADF) and Neutral detergent fiber (NDF) in comparison to the other treatments. This finding suggests a plausible synergistic or additive effect of the microbial consortium in enhancing lactic acid fermentation and facilitating cell wall degradation, thereby reducing ADF and NDF in the samples, as previously observed by Irawan *et al*. (2021). Consistent with theoretical expectations and corroborating earlier research outcomes (Thana *et al*. 2019), the pH values of the fermented pumpkin samples were consistently below 5.0, indicating well-preserved fermentation. Both variants of fermented pumpkin (with or without microbial inoculum) exhibited significantly lower pH values than the control group, likely attributable to the heightened concentration of lactic acid bacteria (LAB) and subsequent amplification in acid production. However, the presence of an elevated concentration of acetic acid in the fermented pumpkin alludes to the proliferation of natural heterofermentative LAB, as elucidated by Bureenok *et al*. (2012). Intriguingly, no statistically significant differences were discerned in the concentrations of acetic acid and butyric acid between the two fermented pumpkin samples, highlighting the uniformity in this facet of fermentation across the experimental conditions. These findings underscore the pivotal role of the fermentation process, not only in enhancing the palatability of pumpkin but also bolstering its nutrient availability, thereby enriching the dietary substrate for BSFL.

We propose utilizing pumpkins for the fermentation process due to their affordability. In the northern region of Thailand, which emerges as a major pumpkin seed production area, pumpkin flesh is considered an agricultural byproduct. Additionally, low-grade pumpkins, which are inexpensive, provide another feasible option. Typically, both flesh pumpkin and low-grade pumpkin are priced at an average of 0.071 USD per kilogram. As a result, the production cost of fermented pumpkin substrate using 50kg of flesh pumpkin, adding microbial agents, and fermenting in a 150L plastic container, amounts to 4.25 USD. Consequently, the cost of fermented pumpkin substrate is 0.085 USD/kg. This economic approach renders fermented pumpkin a viable option for feeding BSFL. By utilizing readily available and low-cost pumpkins for fermentation, this method offers a practical solution to reduce expenses associated with BSFL production, thereby improving the feasibility and accessibility of utilizing fermented pumpkin as an animal feed source in agricultural settings.

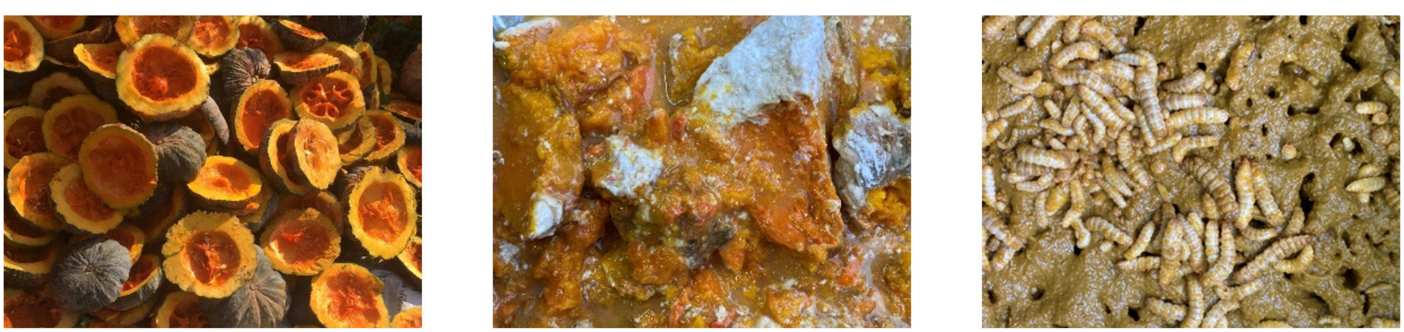
This field trial aimed to provide valuable insights into the potential viability of fermented pumpkin as a sustainable feed source for BSFL, particularly within agricultural contexts.

The experiment yielded several noteworthy findings. Firstly, BSFL production was observed in all five locations across northern Thailand, indicating the widespread presence of these insects within agricultural environments. The use of fermented pumpkin proved effective in attracting BSF to lay eggs within the fermented pumpkin substrate. On average, approximately 7 to 8 out of every 10 plastic containers with a capacity of 150L per area were found to contain BSFL, underscoring the attractiveness and practicality of fermented pumpkin as a suitable substrate for egg-laying by BSF (Figure 1). Additionally, the BSFL harvested from the fermented pumpkin after the 30-day period displayed promising growth rates, with an average fresh yield of 1.9±0.27kg per container. This finding is consistent with research by Ribeiro *et al*. (2022), which suggested that BSFL exhibit superior bioconversion rates and optimal growth when fed pumpkin compared to other substrates such as red cabbage and red onion. Furthermore, the substrates were found to sustain BSFL growth for up to 6 months. Our observations, conducted from June to November 2023, indicated continuous attraction of BSF as long as the fermented pumpkin substrate remained available.

The nutritional content of BSFL derived from different substrates generally reflects the nutrient composition of those substrates. The proximate composition, including crude protein, fat, and fiber content, of BSFL fell within the range reported for other edible insects in previously published literature (Rumpold and Schlüter, 2013; Ghosh *et al*., 2017; Meyer-Rochow *et al*., 2021). The rearing of BSFL can be optimized on identified organic substrates to edible biomass suitable for use as an alternative protein source. BSFL sourced from fermented pumpkin exhibited significantly higher crude protein content, accounting for 38.99% compared to larvae from other substrates (food remain and fruit waste), as previously reported in the literature by Adebayo *et al*., (2021), illustrated in Figure 2.

Therefore, optimization in the production of larvae with high-quality protein content within the rearing system might directly impact the quality of BSF output.

This aligns with Shao *et al*. (2024), which indicates that even when using low-quality rearing substrates (pig manure and pineapple waste), the results showed that after rearing substrates were pre-treated



(a) (b) (c)

Figure 1. The flesh of low-grade pumpkins was used as a substrate for producing BSF larvae feed (a) through a fermentation process with a microbial consortium consisting of *L. plantarum* and *T. viride* (b), and the presence of BSF larvae growing on the fermented pumpkin substrate was observed (c).

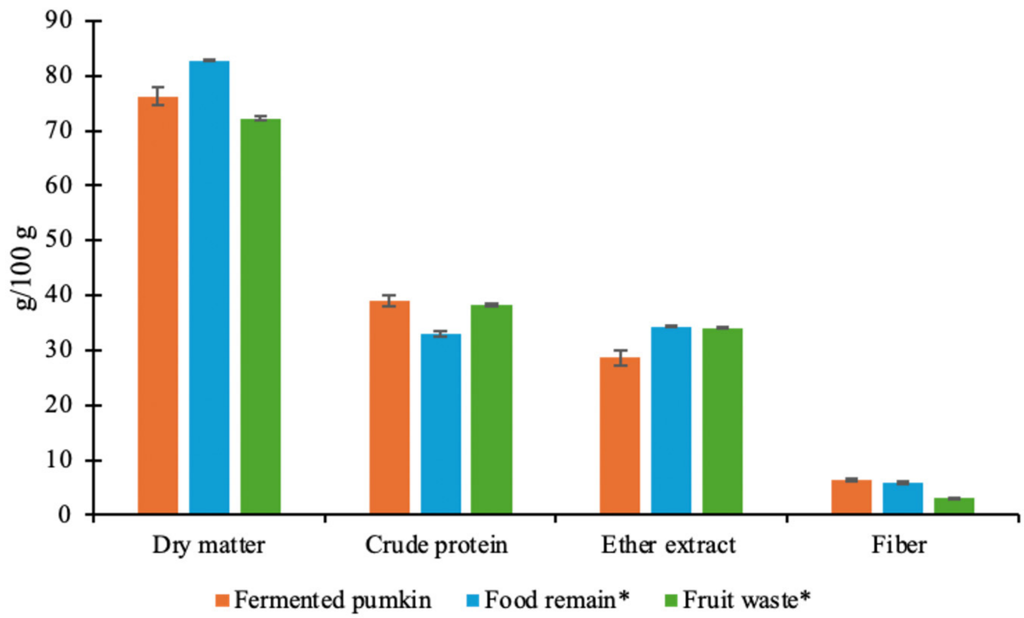


Figure 2. Proximate compositions of the BSF larvae reared on fermented pumpkin (This study), Food remain* and fruit waste* (*Adebayo et al., 2021).

with thermophilic cellulose-degrading bacteria, it could result in significantly higher survival rates, fresh weight, and larval conversion rate compared to the non-treatment control group. The synergy between microflora and the microbial inoculum has the potential to improve co-conversion processes, resulting in increased utilization of low-grade rearing substrates by BSFL (De Smet *et al.*, 2018; Norgren *et al.*, 2023). There is a report that, after inoculating the probiotic consortium (*L. plantarum* E2 and *L. fermentum* F5) in the waste substrate, it resulted in produced larvae biomass with the best performance, particularly weight and waste reduction index (Witriana *et al.*, 2023). Moreover, the balance of gut microbiota in BSFL, which is dominated by *Bacteroidetes*, *Proteobacteria*, and *Firmicutes*, significantly contributes to amino acid synthesis and nutrient absorption, thereby enhancing larval protein accumulation (De Smet *et al.*, 2018). Notably, Leucine (Leu), Lysine (Lys), and Valine (Val) emerged as the predominant essential amino acids in the sample, highlighting their pivotal role in nutrition. Additionally, among the non-essential amino acids, Glutamic acid (Glu), Aspartic acid (Asp), and Alanine (Ala) exhibited elevated nutritive values (Table II). The pattern of amino acid composition agreed with earlier studies (Zulkifli *et al.*, 2022; Zamri *et al.*, 2023). The saturated fatty acid (SFA) concentration in BSF was higher compared to the unsaturated fatty acids (USFA). Lauric acid and palmitic acid concentrations were notably higher among SFAs (Table III). Similarly, Kim *et al.* (2020) and Zulkifli *et al.* (2022) also reported elevated levels of lauric acid and palmitic acids in BSF. Therefore, the approach to improving the quality of materials using microorganisms has great potential to be used to develop BSF production.

Conclusion

The findings of the experiment corroborate the hypothesis that fermented pumpkin effectively attracts BSF to lay eggs on the substrates and serves as a nutrient-rich medium for their growth and development. These results underscore the potential of fermented pumpkin as a cost-effective and sustainable solution for BSFL production within agricultural contexts, thereby offering implications for organic waste management and protein supplementation in animal feed. Furthermore, composting BSFL contributes value to organic waste by converting it into BSF feed and then to BSFL production. BSFL are potentially suitable for various applications such as animal feed ingredients and soil

TABLE II
AMINO ACID COMPOSITION (G/100G) OF THE BSF REARING BY FERMENTED PUMPKIN

Group	Amino acid profile	Amino acid (g/100g)	
		This study	Zulkifli *et al.*, (2022)
	Arginine	2.26±0.16	1.80
	Histidine	1.47±0.16	2.08
Essential	Isoleucine	1.79±0.05	1.76
	Leucine	2.78±0.06	2.67
	Lysine	2.44±0.18	2.44
	Methionine	0.80±0.07	0.61
	Phenylalanine	1.79±0.14	1.35
	Threonine	1.62±0.15	1.42
	Valine	2.43±0.20	2.29
	Alanine	2.35±0.12	3.13
Non-essential	Aspartic acid	3.63±0.11	3.30
	Glutamic acid	4.23±0.28	4.59
	Glycine	2.31±0.10	0.12
	Proline	2.31±0.08	2.30
	Serine	1.92±0.05	1.55
	Tyrosine	2.57±0.09	1.71

TABLE III
FATTY ACID COMPOSITION (G/100G) OF OIL EXTRACTED FROM THE BLACK SOLDIER FLY LARVAE REARING BY FERMENTED PUMPKIN

Fatty acid	This study	Kim *et al*., (2020)	Loho and Lo (2023)
Caprylic acid (C8:0)	0.01 ± 0.01	-	0.003 ± 0.000
Capric acid (C10:0)	1.40 ± 0.20	-	0.201 ± 0.001
Undecanoic acid (C11:0)	0.02 ± 0.01	-	0.004 ± 0.000
Lauric acid (C12:0)	42.84 ± 1.98	37.55	8.567 ± 0.103
Tridecanoic acid (C13:0)	0.03 ± 0.01		0.009 ± 0.000
Myristic acid (C14:0)	8.39 ± 0.40	6.73	2.488 ± 0.025
Pentadecanoic acid (C15:0)	0.09 ± 0.00		0.048 ± 0.000
Palmitic acid (C16:0)	10.19 ± 0.79	15.60	8.870 ± 0.060
Heptadecanoic acid (C17:0)	0.31 ± 0.43		0.053 ± 0.000
Stearic acid (C18:0)	1.26 ± 0.05	3.90	1.194 ± 0.004
Arachidic acid (C20:0)	0.07 ± 0.01	-	0.041 ± 0.000
Behenic acid (C22:0)	0.04 ± 0.01	-	-
Myristoleic acid (C14:1)	0.25± 0.01	-	0.042 ± 0.000
Palmitoleic acid (C16:1n7)	2.99 ± 0.13	2.53	0.924 ± 0.014
trans-9-Elaidic acid (C18:1n9t)	0.06 ± 0.04	3.48	-
cis-9-Oleic acid (C18:1n9c)	12.52 ± 1.32	14.40	8.869 ± 0.035
cis-9,12-Linolenic acid (C18:2n6)	6.46 ± 0.55	12.72	3.815 ± 0.016
α- Linolenic acid (C18:3n3)	0.45 ± 0.08	1.51	0.400 ± 0.001
Arachidonic acid (C20:4n6)	0.04 ± 0.00	0.07	0.118 ± 0.000
cis-11-Eicosenoic acid (C20:1n11)	0.04 ± 0.00	0.08	-

bioremediation. An intriguing aspect of this methodology lies in its utilization of fermented pumpkin as a substrate for BSFL production. This innovative approach not only streamlines the BSF rearing process but also provides a cost-effective and environmentally sustainable solution. Moreover, by capitalizing on the inherent attractiveness of fermented pumpkin to adult BSF, this method presents a practical and accessible means of enhancing larvae production efficiency in agricultural settings.

ACKNOWLEDGEMENTS

This research has received financial support from the National Research Council of Thailand (NRCT 2567) grant no. N71A670517 and the Thailand Science Research and Innovation Fund and the University of Phayao (260/2567). Additionally, it was partially supported by Chiang Mai University. We thanks to Mr. Chanakorn Niyom of University of Phayao for his assistance in filed study.

ETHICAL STATEMENT

Not Applicable

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

AOAC (Association of Official Analytical Chemist) (2012) In; Horwitz W and Latimer GW (Ed.), *Official methods of analysis of the AOAC, 19th ed*. Washington D., USA: Association of Official Analytical Chemists.

AOAC (Association of Official Analytical Chemist) (2016) In: George W and Latimer Jr (Ed.), *Official methods of analysis of the AOAC, 20th ed.* Washington D., USA: Association of Official Analytical Chemists.

Abd El-Hack ME, Shafi ME, Alghamdi WY, Abdelnour SA, Shehata AM, Noreldin AE, Ashour EA, Swelum AA, Al-Sagan AA, Alkhateeb M, Taha, AE, Abdel-Moneim AME, Tufarelli V, Ragni M (2020) Black soldier fly (*Hermetia illucens*) meal as a promising feed ingredient for poultry: A comprehensive review. *Agriculture 10*: 339. https://doi.org/10.3390/agriculture10080339

Adebayo HA, Kemabonta KA, Ogbogu SS, Elechi MC, Obe MT (2021) Comparative assessment of developmental parameters, proximate analysis and mineral compositions of black soldier fly (*Hermetia illucens*) prepupae reared on organic waste substrates. *International Journal of Tropical Insect Science 41*: 1953-1959. https://doi.org/10.1007/s42690-020-00404-4

Batool M, Ranjha MMAN, Roobab U, Manzoor MF, Farooq U, Nadeem HR, Nadeem M, Kanwal, R, AbdElgawad H, Al Jaouni SK (2022) Nutritional value, phytochemical potential, and therapeutic benefits of pumpkin (*Cucurbita* sp.) *Plants 11*: 1394. https://doi.org/10.3390/plants11111394.

Boafo HA, Gbemavo DSJC, Timpong-Jones EC, Eziah V, Billah M, Chia SY, Aidoo OF, Clottey VA, Kenis M. (2023) Substrates most preferred for black soldier fly *Hermetia illucens* (L.) oviposition are not the most suitable for their larval development. *Journal of Insects as Food and Feed 9*: 183–192. https://doi.org/10.3920/JIFF2022.0034

Bureenok S, Yuangklang C, Vasupen K, Schonewille JT, Kawamoto Y (2012) The effects of additives in napier grass silages on chemical composition, feed intake, nutrient digestibility and rumen fermentation. *Asian-Australasian Journal of Animal Sciences 25*: 1248. https://doi.org/10.5713%2Fajas.2012.12081

Čičková H., Newton GL, Lacy RC, Kozánek M (2015) The use of fly larvae for organic waste treatment. *Waste Management 35*: 68–80.

De Smet J, Wynants E, Cos P, Van Campenhout L (2018) Microbial community dynamics during rearing of black soldier fly larvae (*Hermetia illucens*) and impact on exploitation potential. *Applied and Environmental Microbiology 84*: e02722–e02717. https://doi.org/10.1128/AEM.02722-17

Ghosh S, Lee SM, Jung C, Meyer-Rochow VB (2017) Nutritional composition of five commercial edible insects in South Korea.

Journal of Asia-Pacific Entomology 20: 686–694. https://doi.org/10.1016/j.aspen.2017.04.003

Irawan A, Sofyan A, Ridwan R, Hassim HA, Respati AN, Wardani WW, Sadarman, Astuti WD, Jayanegara A (2021) Effects of different lactic acid bacteria groups and fibrolytic enzymes as additives on silage quality: A meta-analysis. *Bioresource Technology Reports 14*: 100654. https://doi.org/10.1016/j.biteb.2021.100654

Isibika A, Simha P, Vinnerås B, Zurbrügg C, Kibazohi O, Lalander C (2023) Food industry waste-An opportunity for black soldier fly larvae protein production in Tanzania. *Science of The Total Environment 858*: 159985. https://doi.org/10.1016/j.scitotenv.2022.159985

Jensen H, Elleby C, Domínguez IP, Chatzopoulos T, Charlebois P. (2021) Insect-based protein feed: From fork to farm. *Journal of Insects as Food and Feed 7*: 1219–1233. https://doi.org/10.3920/JIFF2021.0007.

Kim B, Bang HT, Kim KH, Kim MJ, Jeong JY, Chun JL, Ji SY (2020) Evaluation of black soldier fly larvae oil as a dietary fat source in broiler chicken diets. *Journal of Animal Science and Technology 62*: 187. https://doi.org/10.5187%2Fjast.2020.62.2.187.

Lopes IG, Wiklicky, Ermolaev E, Lalander C (2023) Dynamics of black soldier fly larvae composting–Impact of substrate properties and rearing conditions on process efficiency. *Waste Management 172*: 25–32. https://doi.org/10.1016/j.wasman.2023.08.045

Meyer-Rochow VB, Gahukar RT, Ghosh S, Jung C (2021) Chemical composition, nutrient quality and acceptability of edible insects are affected by species, developmental stage, gender, diet, and processing method. *Foods 10*: 1036. https://doi.org/10.3390/foods10051036

Norgren R, Jonsson A, Björkqvist O (2023) Fermented pulp and paper bio-sludge as feed for black soldier fly larvae. *Biomass Conversion and Biorefinery 13*: 5625–5632. https://doi.org/10.1007/s13399-021-01564-0.

Opoku O, Hamidu JA, Adjei-Mensah B, Quaye B, Sasu P, Benante V, Adu Amankrah M, Donkoh A, Atuahene, CC. (2023) Alternative organic wastes as substrates and its impact on growth, proximate chemical and mineral composition of black soldier fly (*Hermetia illucens*) larvae for subsistent animal feed. *Journal of Applied Animal Research 51*: 564–572. https://doi.org/10.1080/09712119.2023.2249545.

Pongjanta J, Naulbunrang A, Kawngdang S, Manon T, Thepjaikat T. (2006) Utilization of pumpkin powder in bakery products. *Songklanakarin Journal of Science and Technology 28*: 71–79.

Raman SS, Stringer LC, Bruce NC, Chong CS (2022) Opportunities, challenges and solutions for black soldier fly larvae-based animal feed production. *Journal of Cleaner Production 1*: 133802. https://doi.org/10.1016/j.jclepro.2022.133802

Rumpold BA, Schlüter OK (2013) Nutritional composition and safety aspects of edible insects. *Molecular Nutrition & Food Research 57*: 802–823. https://doi.org/10.1002/mnfr.201200735.

Sheppard C (1983) House fly and lesser fly control utilizing the black soldier fly in manure management systems for caged laying hens. *Environmental Entomology 12*: 1439–1442.

Sheppard DC, Newton GL, Thompson SA, Savage S (1994) A value added manure management system using the black soldier fly. *Bioresource Technology 50*: 275–279.

Siddiqui SA, Süfer Ö, Çalışkan Koç G, Lutuf H, Rahayu T, Castro-Muñoz R, Fernando I (2024) Enhancing the bioconversion rate and end products of black soldier fly (BSF) treatment–A comprehensive review. *Environment, Development and Sustainability 4*: 1–69. https://doi.org/10.1007/s10668-023-04306-6

Singh A and Kumari K (2019) An inclusive approach for organic waste treatment and valorisation using Black Soldier Fly larvae: A review. *Journal of Environmental Management 251*: 109569. https://doi.org/10.54083/ResBio/3.2.2021.128-132

Thana S, Anorach T, Sorachakula C, Danmek D (2019) Nutritional composition of maize husk silage generated from solid state fermentation by *Trichoderma viride* UP01. *Pakistan Journal of Botany 51*: 2255–2260. http://dx.doi.org/10.30848/PJB2019-6(28)

Wang YS, Shelomi M (2017) Review of Black Soldier Fly (*Hermetia illucens*) as Animal Feed and Human Food. *Foods 6*: 91. https://www.mdpi.com/2304-8158/6/10/91.

Witriana NI, Ardyati T, Jatmiko YD. (2023) The Use of probiotics in fermenting food wastes for production of black soldier fly larvae (*Hermetia illucens* L.; Diptera: Stratiomyidae). *Berkala Penelitian Hayati Journal of Biological Researches 29*: 99–105. https://doi.org/10.23869/bphjbr.29.3.20234

Yu GuoHui YG, Chen YanHong CY, Yu ZiNiu YZ, Cheng Ping CP (2009) Research progress on the larvae and prepupae of black soldier fly *Hermetia illucens* used as animal feedstuff. *Chinese Bulletin of Entomology 46*: 41–45

Zamri MZA, Ramiah SK, Jamein ES, Zulkifli I, Lokman IH, Amirul FMA, Fadzlin, SAA, Zamri SM, Jayanegara A, Jayanegara A (2023) Potential use of black soldier fly, *Hermetia illucens* larvae in chicken feed as a protein replacer: a review. *Journal of Animal and Feed Science 32*: 341–353. https://doi.org/10.22358/jafs/162066/2023.

Zulkifli NFNM, Seok-Kian AY, Seng LL, Mustafa S, Kim YS, Shapawi R (2022) Nutritional value of black soldier fly (*Hermetia illucens*) larvae processed by different methods. *PloS One 17*: e0263924. https://doi.org/10.1371/journal.pone.0263924.