A novel advanced technique for dual production of black soldier fly oil and protein

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Abstract. The increasing demand for sustainable feed ingredients has promoted the use of Black Soldier Fly Larvae (BSFL) as a source of protein and oil. This study evaluated the effects of Ultrasound-Assisted Extraction (UAE) and hot press extraction on oil yield, proximate composition, fatty acid profile (GC–FID), and amino acid composition (qualitative HPLC). Larvae were reared for 14 days on a palm kernel meal–pollard substrate (1:3), microwave-dried at 120 °C for 5 minutes and processed into flour. Both extraction methods were performed in triplicate, and data were analyzed using independent t-tests. UAE produced a significantly higher oil yield than hot press extraction. The defatted meal from UAE contained significantly higher ash, protein, and crude fiber, whereas hot press extraction resulted in higher residual lipid content. No significant differences were observed in fatty acid profiles, with lauric acid (~44%) remaining predominant in both methods. Amino acid profiles were also comparable, with glutamate and leucine as the most abundant non-essential and essential amino acids, respectively. In conclusion, extraction methods affected oil yield and proximate composition but did not alter fatty acid or amino acid profiles. UAE is recommended for higher oil recovery and improved defatted meal quality.

1 Introduction

Rapid population expansion accompanied by economic development has intensified livestock production worldwide [1]. Demographic projections estimate that the global population may approach 10 billion by 2050, resulting in a substantial rise in demand for animal-derived foods, including milk and meat, to ensure food security [2]. This expansion consequently elevates the requirement for nutritionally dense feed ingredients, particularly those supplying protein and

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energy, with total feed demand projected to exceed 1 billion tons of dry matter annually [3]. Traditionally, feed formulations have relied heavily on protein-rich ingredients such as soybean meal and fish meal, along with various vegetable oils. Nevertheless, volatility in global markets, reliance on imports, and increasing environmental pressures have raised concerns regarding the long-term sustainability of these conventional resources [4]. Therefore, the identification of alternative feed materials that can secure supply continuity while sustaining livestock productivity has become increasingly important.

The exploration of insects as alternative feed resources has gained increasing attention as a strategy to fulfill animal protein requirements. Among the species studied, black soldier fly (Hermetia illucens) larvae have emerged as a promising candidate due to their capacity to bioconvert organic waste into nutrient-dense biomass. The crude protein content of BSF larvae generally ranges between 32% and 53%, making them suitable for incorporation into animal diets [5]. Beyond their protein value, the larvae also provide a considerable lipid fraction characterized by a predominance of saturated fatty acids, particularly lauric acid (C12:0), which has been associated with antioxidant and antimicrobial activities [6]. Nevertheless, the quantitative distribution of fatty acids may vary according to the extraction technique applied, indicating that processing conditions can influence the resulting lipid profile.

Extraction is one of the crucial processing steps in the application and recovery of lipids and determines the nutritional characteristics of the extracted solids. Among conventional methods, the Hot Press method is widely used in industrial applications due to its practicality. However, the high temperatures in the extraction process can lead to sub-optimal oil recovery and high residual lipid content in the extracted solids, as well as potentially affecting heat-sensitive nutrients. These limitations have encouraged the development of better extraction technologies such as ultrasound-assisted extraction (UAE), with its ability to enhance solvent penetration and cellular disruption through acoustic cavitation. This mechanism results in faster and more efficient lipid release, while maintaining oil quality and significantly increasing extraction efficiency [7]. Although UAE shows better potential, information regarding the compositional quality of the extracted solids, including proximate nutrients, fatty acid profiles, and amino acid distribution, as well as a direct comparison between UAE and Hot Press, is still limited. Therefore, this study evaluates and compares UAE extraction and Hot Press extraction based on oil recovery and the associated nutritional properties of the defatted meal.

2 Materials and methods

Black soldier fly (*Hermetia illucens*) larval meal utilized in this research was obtained from PT Bio Cycle Indo. N-hexane served as the solvent for oil extraction. The experimental setup included an ultrasonic cell disruptor, a Phlinice CO2491SI mechanical oil press, a rotary evaporator, and standard laboratory glassware such as beakers and graduated cylinders. Whatman No. 41 filter paper, a blender, and an analytical balance were also employed during sample preparation and analysis.

2.1 Insect based

Black soldier fly larvae (BSFL, Hermetia illucens) were sourced from PT Bio Cycle Indo, Riau, Indonesia. The larvae were cultivated for 14 days. During the first six days, they were fed a mixture of palm kernel meal and pollard at a 3:7 ratio. From day 7 to day 14, palm kernel meal was provided as the sole substrate. After harvesting, the larvae were dried in a microwave oven at 120 °C for 5 minutes and subsequently milled using a Philips blender to produce a fine powder.

2.2 Extraction test

2.2.1 Ultrasound-assisted extraction (UAE)

Oil extraction from BSFL flour was performed using the ultrasound-assisted extraction (UAE) method. A total of 30 grams of BSFL and 270 ml of n-hexane (1:9 w/v) were used in the extraction process, which was conducted at a frequency of 40 kHz for 20 minutes at a temperature of 50 °C. The extract was then filtered using Whatman filter paper. The solvent was evaporated using a rotary evaporator at 40 °C, and the obtained oil was weighed and transferred to a glass bottle for further analysis. The defatted BSFL flour solids were also weighed and stored for subsequent evaluation. The extraction capacity of the ultrasonic system was limited to a maximum of 30 g per cycle.

2.2.2 Hot press extraction

Hot press was carried out using BSFL meal. The oil extraction was performed using an oil press machine. The temperature was kept at 280 °C after preheating the machine for 10 minutes. The extraction temperature was determined through preliminary trials, as mechanical pressing at lower temperatures did not result in effective oil release. Therefore, 280 °C was selected as the minimum temperature that enabled consistent oil separation. For each replication, 200 g of BSFL meal was used. The extracted oil and the resulting solid residue were collected, weighed, and transferred into designated containers for further analysis. Preliminary trials indicated that 30 g of sample did not produce measurable oil under hot press conditions. Therefore, 200 g was used to obtain sufficient extract for yield calculation and further analyses.

2.3 Determination of oil and defatted BSF meal yield (%)

Oil recovery (%) was calculated by expressing the mass of extracted oil relative to the initial sample weight and converting the value into percentage form. Likewise, the percentage of defatted BSFL meal was obtained by comparing the weight of the residual solid after extraction with the starting material weight. All extraction procedures were conducted in three independent replicates, and the data are presented as mean ± standard deviation. To ensure comparability between extraction methods, oil recovery was expressed as a percentage of the initial sample weight.

2.4 Proximate analysis

The chemical composition of the defatted BSFL meal was evaluated by determining moisture, ash, crude lipid, crude protein, and crude fiber contents following the standardized analytical protocol described in reference [8].

2.5 Fatty acid composition

Fatty acid profiling was performed using gas chromatography after transforming the lipid fraction into fatty acid methyl esters (FAME). Transesterification was carried out with methanolic reagents in the presence of either acidic or alkaline catalysts. The resulting methyl esters were extracted with n-hexane, dried over anhydrous sodium sulfate, and filtered prior to analysis. Separation was achieved on a capillary GC column under helium as carrier gas, and detection employed a flame ionization detector (FID). Fatty acids were identified by comparison with authenticated standards based on retention time, while relative composition was determined from peak area normalization according to method [9].

2.6 Amino acid analysis

Amino acid composition was analyzed at the Biotechnology Center, IPB University. Samples were subjected to hydrolysis using 6 N hydrochloric acid at 110 °C for 24 h to liberate bound amino acids. After neutralization and concentration under reduced pressure, the residues were dissolved in 0.01 N HCl and filtered. Pre-column derivatization was conducted with ortho-phthalaldehyde (OPA) in borate buffer (pH 10.4). A 5 µL aliquot was injected into a reverse-phase C18 HPLC column. Elution was performed using a sodium acetate–methanol mobile phase at a flow rate of 1 mL/min, and detection was achieved fluorometrically at 254 nm. Identification and quantification were based on comparison with external standards following method [8].

2.7 Data analysis

Statistical comparisons of oil yield, proximate composition, and fatty acid profiles between extraction treatments were conducted using an independent samples t-test in IBM SPSS Statistics version 26. Each treatment consisted of three replicates. Differences were considered statistically significant at $p < 0.05$.

3 Result and discussion

3.1 Oil and defatted BSF meal yield

The comparison between the UAE and hot press methods showed significant differences in the yields of oil and defatted BSFL (Table 1). UAE significantly produced a higher oil yield compared to hot press, with values of 20.58% and 19.06%, respectively. The high extraction yield of UAE is due to ultrasonic waves generating vibrations that can release lipids rapidly and increase yield. The ultrasonic cavitation produced by the transducer directly influences the ability to achieve an optimal oil extraction process with appropriate time, temperature, ultrasonic power, and solvent-to-material ratio [10]. Ultrasonic cavitation plays a crucial role in oil release from BSFL because it has a structurally dense tissue and is rich in medium-chain fatty acids.

Table 1. Comparison of oil yield and defatted BSFL meal obtained from different extraction methods

Variables	UAE	Hot Press	P Value
Oil Yield (%)	20.58 ± 0.43^a	19.06 ± 0.03^b	0.004
Deffated BSFL Meal (%)	78.61 ± 0.44^b	80.12 ± 0.03^a	0.004

Values in the same row with different superscript letters differ significantly ($p < 0.05$). UAE=Ultrasonic assisted-extraction, BSFL=Black soldier fly larvae

In contrast, Hot Press extraction produced a higher proportion of residue compared to UAE, with values of 80.12% and 78.61%, respectively. This is because the mechanical pressure process is not optimal in breaking down the BSFL tissue, thus failing to release the lipids entirely. Previous literature reviews have reported that extraction processes using high pressure and temperature are not sufficiently optimal because much of the oil remains trapped within the compressed biomass [11]. These results indicate that UAE not only improves oil recovery but also maximizes lipid release in BSFL and is more efficient in extracting oil.

3.2 Proximate composition of defatted BSFL meal

The proximate analysis presented in Table 2 shows that the extraction method has a significant effect ($p < 0.05$) on the chemical composition of the BSFL meal. The dry matter and ash content in UAE are significantly higher than in Hot Press, indicating the release of lipids and moisture as well as higher mineral concentration during the sonication process. The fat content in the defatted BSFL meal from Hot Press is higher than from UAE, which is consistent with the yield results. Mechanical pressure extraction in Hot Press leaves a larger amount of fat (7.38%), whereas UAE (5.81%) is more optimal in reducing the fat fraction in BSFL meal.

Table 2. Proximate composition of BSFL meal before and after oil extraction using different extraction method

Component	UAE	Hot Press	P Value
Dry Matter (%)	2.94 ± 0.33^{a}	1.86 ± 0.45^{b}	0.015
Ash (%)	11.73 ± 0.08^{a}	11.50 ± 0.02^{b}	0.006
Fat (%)	5.81 ± 0.50^{b}	7.38 ± 0.13^{a}	0.006
Protein (%)	56.05 ± 0.36^{a}	54.94 ± 0.09^{b}	0.006
Crude Fiber (%)	19.21 ± 0.12^{a}	18.83 ± 0.03^{b}	0.007
NFE (%)	5.56 ± 0.04^{a}	5.45 ± 0.01^{b}	0.006

Values in the same row with different superscript letters differ significantly ($p < 0.05$). UAE= Ultrasonic assisted-extraction, NFE=Nitrogen-free extract

Protein, crude fiber, and nitrogen-free extract (NFE) also showed statistically significant differences between the extraction methods. The protein content was higher in the UAE meal (56.05%) than in the hot-pressed residue (54.94%), largely due to the more efficient lipid removal which concentrated the protein by percentage. This is consistent with previous results indicating that after extraction, most lipids are removed from the BSFL, resulting in a low-fat BSFL meal with increased protein [12]. A similar pattern was observed in the crude fiber fraction, where UAE produced a slightly higher fiber value (19.21%) than hot pressing (18.83%). Although the numerical differences appear small, they reflect structural changes caused by the disruption of the larval matrix due to ultrasonic induction. NFE values also differed significantly, with UAE showing a slightly higher proportion (5.56%) compared to hot press extraction (5.45%).

3.3 Fatty acid profile

The fatty acid profiles of BSFL oil produced by UAE and Hot Press extraction methods are presented in Table 3. Lauric acid (C12:0), myristic acid (C14:0), oleic acid (C18:1), and linoleic acid (C18:2) show higher values in Hot Press extraction. Meanwhile, palmitic acid (C16:0) shows a higher value in the UAE extraction method. These results are similar to the report by [12], stating that there are variations in fatty acid profile values between mechanical extraction and solvent extraction, yet they still reflect fatty acid stability across various extraction conditions. Furthermore, these results prove that ultrasonic technology works faster at lower temperatures, thus maintaining the quality of sensitive fatty acids and preventing damage from excessive heat or prolonged processes [13].

Table 3. Effect of extraction method on the fatty acid composition of the oil

Fatty acids	Fatty acid composition (%)		P Value
	UAE	Hot Press	
Capric acid (C10:0)	1.08 ± 0.04	1.10 ± 0.05	0.624
Lauric acid (C12:0)	44.08 ± 0.03^b	44.14 ± 0.02^a	0.039
Myristic acid (C14:0)	14.23 ± 0.10^b	15.00 ± 0.14^a	0.001
Palmitic acid (C16:0)	16.54 ± 0.24^a	16.04 ± 0.11^b	0.033
Stearic acid (C18:0)	2.21 ± 0.06	2.28 ± 0.02	0.093
Oleic acid (18:1)	16.30 ± 0.03^b	16.37 ± 0.03^a	0.040
Linoleic acid (C18:2)	2.97 ± 0.10^b	3.35 ± 0.09^a	0.008
Linolenic acid (C18:3)	0.08 ± 0.02	0.06 ± 0.03	0.398
SFA	78.13 ± 0.33	78.57 ± 0.20	0.121
MUFA	16.30 ± 0.03^b	16.37 ± 0.03^a	0.040
PUFA	3.05 ± 0.11^b	3.41 ± 0.12^a	0.012
UFA	19.34 ± 0.11^b	19.78 ± 0.15^a	0.014
MUFA/SFA	0.21 ± 0.00	0.21 ± 0.00	0.000
PUFA/SFA	0.04 ± 0.00	0.04 ± 0.00	0.000
UFA/SFA	0.25 ± 0.00	0.25 ± 0.00	0.000

Values in the same row with different superscript letters differ significantly ($p < 0.05$). UAE= Ultrasonic assisted-extraction, SFA=Saturated fatty acid, MUFA=Monounsaturated fatty acid, PUFA=Polyunsaturated fatty acid, UFA=Unsaturated fatty acid.

Overall, the fatty acid profile remains dominated by saturated fatty acids (SFA), which account for approximately 78% of the total FAME in both treatments, with unsaturated fatty acids (UFA) representing around 19-20%. The resulting UFA/SFA ratio is 0.25, indicating that both UAE and Hot Press maintain the fundamental lipid characteristics of BSF oil. Collectively, these results demonstrate that extraction using different methods will influence the proportions of specific fatty acids.

3.3 Amino acid

The extraction method turns out to affect not only the protein content but also the types of amino acids contained in the BSFL meal. Although the overall essential amino acid levels are slightly higher in the hot press method because more fat is removed, the UAE method is actually superior in maintaining protein quality. In the UAE method, important amino acids such as leucine, phenylalanine, and lysine are better preserved because the process does not use extreme heat. This is consistent with previous research stating that non-aggressive extraction conditions help maintain the original protein profile [14]. Overall, the UAE method is much better at maintaining the original nutritional balance of the larvae due to lower heat exposure.

Table 4. Amino acid composition (g/100 g protein) of the samples before and after extraction using different methods

Amino Acid	Category	UAE	Hot Press	Δ Difference
Histidine	Esensial	1.69	3.58	1.89
Threonine	Esensial	2.71	2.69	0.02
Valine	Esensial	5.45	3.07	2.38
Methionine	Esensial	1.94	3.68	1.74
Isoleucine	Esensial	2.86	3.33	0.47
Leucine (standard)	Esensial	13.92	9.12	4.80
Phenylalanine	Esensial	6.95	4.42	2.53
Lysine	Esensial	11.91	7.90	4.01
IEAA		**47.43**	**37.79**	**9.64**
Asparatic Acid	Non esensial	15.15	12.10	3.05
Glutamic Acid	Non esensial	18.20	18.08	0.12
Serine	Non esensial	3.22	5.34	2.12
Glycine	Non esensial	4.96	5.95	0.99
Arginine	Non esensial	2.35	3.74	1.39
Alanine	Non esensial	2.23	5.66	3.43
Proline	Non esensial	2.59	4.00	1.41
Tyrosine	Non esensial	2.08	4.58	2.50
Cysteine	Non esensial	1.79	2.75	0.96

UAE= Ultrasonic assisted-extraction, IEAA= Indispensable essential amino acid

The non-essential amino acid content also varies depending on the extraction technique used. The UAE method is better at preserving aspartic acid because its low temperature prevents damage to the protein structure. Meanwhile, in the hot press method, amino acids such as serine, proline, and cysteine appear higher due to the protein concentration effect after lipids are mechanically removed. This proves that heat and physical pressure can alter the form and composition of proteins differently, consistent with literature noting that processing conditions significantly influence amino acid recovery [14]. Overall, the UAE method is considered superior because its gentler process is better able to maintain the natural nutritional balance of BSFL.

4 Conclusion

The conclusion is that the ultrasound-assisted extraction (UAE) method proved to be superior and more efficient in producing BSF oil and protein meal compared to hot pressing, as indicated by the higher oil yield, the proximate composition of the residue, and its lower fat content.

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