

# Fatty Acid Profiles of Extracted Black Soldier Fly Larvae (*Hermetia Illucens*) Lipid Either Enriched Into Saturated and Unsaturated Fatty Acids Fractions as Feed Additive

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## ABSTRACT

Black soldier fly larvae fat (BSFL fat) derived from *Hermetia illucens* has gained considerable attention as an alternative lipid source due to its distinctive fatty acid composition, particularly its high proportion of medium-chain saturated fatty acids. However, crude BSFL fat contains a heterogeneous mixture of fatty acids with different saturation levels, which may limit its application when a more defined lipid profile is required. This study aimed to fractionate BSFL fat into Extracted FFA, Enriched SFA, and Enriched UFA fractions and to characterize the fatty acid profile of each fraction using gas chromatography-mass spectrometry (GC-MS). Chemical hydrolysis using potassium hydroxide in an ethanol–water system, followed by acidification with hydrochloric acid, produced the Extracted FFA fraction. The FFA fraction was then dissolved in *n*-hexane and subjected to winterization at  $-30^{\circ}\text{C}$  for 48 h to separate the solid Enriched SFA fraction from the liquid Enriched UFA fraction. GC-MS analysis confirmed that chemical hydrolysis produced an FFA fraction with a fatty acid profile nearly identical to crude BSFL fat, indicating that hydrolysis mainly changed the lipid form without altering the relative fatty acid distribution. Winterization produced two contrasting fractions: the Enriched SFA fraction was dominated by lauric acid (C12:0, 46.10%), myristic acid (C14:0, 20.19%), and palmitic acid (C16:0, 16.91%), with a total SFA proportion of 86.70%. The Enriched UFA fraction, in contrast, contained the highest total UFA proportion (62.50%), driven by markedly elevated oleic acid (C18:1n-9, 47.24%) and linoleic acid (C18:2n-6, 11.66%). These findings demonstrate that chemical hydrolysis combined with winterization is a simple and practical approach to obtain BSFL fat fractions with targeted saturation characteristics, providing a compositional basis for future lipid-based applications in animal nutrition and feed technology.

**Keywords:** black soldier fly larvae fat; chemical hydrolysis; fatty acid profile; fractionation; lauric acid; winterization

## INTRODUCTION

*Hermetia illucens* (black soldier fly, BSF) has emerged as one of the most widely investigated insects in sustainable bioconversion and animal feed research. Its larvae are capable of converting various organic substrates into biomass rich in protein, lipid, chitin, and minerals, positioning them as a viable alternative

ingredient for animal feed, aquaculture, and other bioproduct applications (Wang & Shelomi, 2017; Barragan-Fonseca et al., 2017). The nutritional composition of black soldier fly larvae varies with substrate, larval age, and processing method, but the lipid fraction is consistently regarded as an important component of its potential value (Barragan-Fonseca et al., 2017; Spranghers et al., 2017).

BSFL fat differs from most conventional vegetable oils because of its high proportion of medium-chain saturated fatty acids. Lauric acid (C12:0) is frequently reported as the dominant fatty acid in BSFL fat, followed by myristic acid (C14:0), palmitic acid (C16:0), oleic acid (C18:1n-9), and linoleic acid (C18:2n-6) (Ewald et al., 2020; Srisuksai et al., 2024). This fatty acid pattern makes BSFL fat chemically distinct from highly unsaturated plant-derived oils such as soybean, sunflower, or canola oil, and is one of the primary reasons BSFL fat has attracted interest as a distinctive insect-derived lipid material.

The crude oil form, however, contains a complex mixture of fatty acids with different chain lengths, saturation levels, and physicochemical properties. Some downstream applications require fractions with more targeted lipid characteristics. For example, an Enriched SFA fraction may be useful in studies requiring a lauric acid-rich saturated lipid material, while an Enriched UFA fraction may be valuable for investigations requiring higher availability of oleic and linoleic acids. Fractionation therefore has the potential to increase the practical value of BSFL fat by separating it into lipid fractions with distinct chemical profiles.

Fractionation of oils and fats is generally based on differences in physicochemical properties, particularly melting point, solubility, and crystallization behavior. Saturated fatty acids, having straight carbon chains, exhibit higher melting points and a greater tendency to crystallize during cooling. Unsaturated fatty acids, containing one or more double bonds that introduce structural bends, remain more concentrated in the liquid phase under low-temperature treatment. This principle underlies common fat processing strategies including dry fractionation, solvent fractionation, and winterization (Gunstone, 2011; Calliauw, 2019).

Chemical hydrolysis can be applied prior to winterization to convert triacylglycerols into free fatty acids. In alkaline hydrolysis, potassium hydroxide reacts with triacylglycerols to form soap stock and glycerol; subsequent acidification releases free fatty acids from the soap stock, allowing separation of the lipid phase from the aqueous phase. This approach is particularly relevant for lauric acid-rich fats, as the free fatty acid form facilitates subsequent concentration or processing steps (Vázquez et al., 2025).

Previous studies have demonstrated the feasibility of fractionating BSFL fat. Bogevik et al. (2022) reported that BSFL fat can be fractionated into lipid products with distinct physicochemical characteristics, while Vázquez et al. (2024) evaluated scalable fractionation methodologies to produce concentrated lauric acid from BSFL fat. These findings suggest that BSFL fat is not only useful in its crude form but can also be processed into targeted lipid fractions with defined properties. However, additional laboratory-scale characterization data remain valuable, particularly for applied research settings where simple methods and accessible equipment are needed.

Therefore, this study aimed to produce FFA-, SFA-, and Enriched UFA fractions from BSFL fat (*Hermetia illucens*) using chemical hydrolysis followed by winterization and to characterize the fatty acid profile of each fraction by GC-MS analysis. The working hypothesis was that chemical hydrolysis would produce an Extracted FFA fraction with a fatty acid profile similar to crude BSFL fat, whereas winterization would separate the FFA fraction into SFA- and Enriched UFA fractions with clearly distinct saturation profiles.

## MATERIALS AND METHODS

### Raw Material

Crude BSFL fat (*Hermetia illucens*) was obtained from PT Biocycleindo, Riau, Indonesia. The fat was stored in amber glass vials, protected from light, prior to fractionation to minimize oxidation.

### Preparation of the Extracted FFA Fraction

The Extracted FFA fraction was prepared by chemical hydrolysis following the procedure described by Vázquez et al. (2025), scaled from 50 g to 350 g of crude BSFL fat. Crude BSFL fat (350 g) was melted at 50°C until

fully liquid and visually homogeneous, then dissolved into 560 mL of 3.7 N potassium hydroxide prepared in a 1:1 (v/v) mixture of 96% ethanol and distilled water. The mixture was maintained at 50°C under continuous magnetic stirring for 20 min to promote saponification and soap stock formation.

After saponification, 280 mL of distilled water was added to facilitate phase separation, followed by gradual acidification with 455 mL of 4 M hydrochloric acid until  $\text{pH} \approx 2$  to release the free fatty acids from the soap stock. The FFA-containing lipid phase was separated by decantation, then washed with 140 mL of distilled water to remove residual salts and ionic impurities. The washed FFA phase was dried over anhydrous sodium sulphate, then clarified by centrifugation at 4,000 rpm for 10 min to remove residual particulates. The supernatant was collected as the Extracted FFA fraction. The procedure was replicated to obtain sufficient material for subsequent winterization. From 350 g of crude BSFL fat, a total of 218.75 g of Extracted FFA was recovered, corresponding to a yield of 62.50%.

### Winterization of the Extracted FFA Fraction

A total of 200 g of the Extracted FFA fraction was used for winterization. The FFA was dissolved in *n*-hexane at a ratio of 1:15 (v/v), producing 3,000 mL of solution, and stirred until homogeneous. The solution was then stored at  $-30^{\circ}\text{C}$  for approximately 48 h to induce preferential crystallization of saturated fatty acid components. Following the cooling period, the liquid phase was carefully decanted as the Enriched UFA fraction, and the solid crystallized phase was collected as the Enriched SFA fraction. Solvent was removed from each fraction using a rotary evaporator until complete removal of *n*-hexane was confirmed. Both fractions were transferred to sealed amber vials and stored at  $4^{\circ}\text{C}$  until analysis. From 200 g of Extracted FFA, 147.60 g of Enriched SFA (73.80% yield) and 49.48 g of Enriched UFA (24.74% yield) were recovered, with a total mass recovery of 98.54%.

### Fatty Acid Profile Analysis by GC-MS

The fatty acid profiles of all four fractions (crude BSFL fat, Extracted FFA, Enriched SFA, and Enriched UFA) were determined by gas chromatography-mass spectrometry (GC-MS) analysis conducted at the Integrated Research and Testing Laboratory (*Laboratorium Penelitian dan Pengujian Terpadu/LPPT*), Universitas Gadjah Mada, Indonesia. Prior to GC-MS injection, fatty acids were converted into fatty acid methyl esters (FAME) through methylation using standard transesterification procedures. Results were expressed as the relative percentage of each identified fatty acid methyl ester to the total identified fatty acids. Fatty acids are reported using their systematic nomenclature alongside common names where applicable.

### Fatty Acid Class Calculation

Detected fatty acids were grouped into saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). Total unsaturated fatty acids (UFA) were calculated as the sum of MUFA and PUFA. Values below the reporting limit ( $<0.1\%$ ) were treated as not detected for class-level summarization. Data were interpreted descriptively, as the study focused on characterization of fraction profiles rather than statistical comparison of replicated treatment effects.

## RESULTS AND DISCUSSION

### Fractionation Yield and Fatty Acid Profile Overview

Chemical hydrolysis of 350 g of crude BSFL fat yielded 218.75 g of Extracted FFA (62.50% yield). Subsequent winterization of 200 g of the Extracted FFA at  $-30^{\circ}\text{C}$  produced 147.60 g of Enriched SFA (73.80% yield) and 49.48 g of Enriched UFA (24.74% yield), with a total mass recovery of 98.54%. The high recovery from winterization confirms that mechanical losses during phase separation and solvent evaporation were minimal. The fatty acid profiles of all four fractions are presented in Table 1, and the corresponding fatty acid class distributions are summarized in Table 2. GC-MS analysis confirmed distinct differences in fatty acid composition across fractions, validating the effectiveness of the fractionation procedure.

Table 1. Major fatty acids in crude BSFL fat (*H. illucens*) and its fractions (% of total identified fatty acids)

Fatty Acid	Crude Oil	Extracted FFA	Enriched SFA	Enriched UFA
C10:0	1.13	1.14	0.72	2.35
C12:0	39.60	39.55	46.10	20.39
C14:0	17.04	16.98	20.19	6.76
C16:0	13.83	13.90	16.91	3.41
C18:0	1.88	1.92	2.34	0.59
C16:1n-7	1.38	1.40	0.81	1.89
C18:1n-9	19.20	19.11	9.84	47.24
C18:2n-6	4.64	4.71	2.40	11.66
C18:3n-3	0.16	0.16	<0.1	0.40

Notes: FFA = Extracted FFA fraction; SFA = Enriched SFA fraction; UFA = Enriched UFA fraction. Values expressed as percentage of total identified fatty acids. <0.1 indicates values below the reporting limit.

Table 2. Fatty acid class distribution in crude BSFL fat (*H. illucens*) and its fractions (% of total identified fatty acids)

Fatty Acid Class	Crude Oil	Extracted FFA	Enriched SFA	Enriched UFA
SFA (%)	73.99	74.00	86.70	37.48
MUFA (%)	21.05	20.99	10.90	50.12
PUFA (%)	4.96	5.02	2.40	12.38
Total UFA (%)	26.01	26.01	13.30	62.50

Notes: SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; UFA = total unsaturated fatty acids (MUFA + PUFA). Values below reporting limit (<0.1%) were excluded from class totals.

### Fatty Acid Profile of Crude BSFL Fat and the Extracted FFA Fraction

The crude BSFL fat used in this study was dominated by saturated fatty acids, with lauric acid (C12:0) as the most abundant fatty acid at 39.60% of total identified fatty acids. Other major components were oleic acid (C18:1n-9; 19.20%), myristic acid (C14:0; 17.04%), and palmitic acid (C16:0; 13.83%). Total SFA accounted for 73.99% of the crude fat, while total UFA represented 26.01%. This profile is consistent with previous reports confirming the lauric acid-rich and medium-chain saturated character of BSFL fat (Ewald et al., 2020; Srisuksai et al., 2024; Mai et al., 2019).

The Extracted FFA fraction produced by chemical hydrolysis exhibited a fatty acid profile nearly identical to the crude fat. Lauric acid remained the dominant fatty acid at 39.55%, followed by oleic acid (19.11%), myristic acid (16.98%), and palmitic acid (13.90%). Total SFA and UFA proportions were 74.00% and 26.01%, respectively values virtually unchanged from the crude fat. This similarity indicates that alkaline hydrolysis

followed by acidification effectively converted triacylglycerols into their free fatty acid form without selectively concentrating or removing particular fatty acids. The hydrolysis step therefore altered the lipid chemical form rather than its relative fatty acid distribution.

This finding is important from a methodological standpoint, as it confirms that the FFA fraction is a chemically appropriate intermediate material for winterization. Had hydrolysis substantially altered the fatty acid profile, it would have been difficult to attribute observed post-winterization differences to selective crystallization alone. The near-identical profiles of the crude fat and FFA fraction also support the validity of comparing fraction compositions against the crude fat as a reference.

### **Enrichment of Saturated Fatty Acids in the Enriched SFA Fraction**

Winterization at  $-30^{\circ}\text{C}$  successfully increased the proportion of saturated fatty acids in the solid crystallized phase. The Enriched SFA fraction contained 86.70% total SFA, considerably higher than the crude fat (73.99%) and the FFA fraction (74.00%). This enrichment was driven primarily by increases in lauric acid (46.10%), myristic acid (20.19%), and palmitic acid (16.91%), while unsaturated fatty acids particularly oleic acid (9.84%) and linoleic acid (2.40%) were markedly reduced compared to crude fat.

The selective crystallization of saturated fatty acids during winterization is attributable to their straight-chain molecular structure, which enables closer intermolecular packing and confers higher melting points relative to unsaturated fatty acids [6], [7]. When the FFA fraction was dissolved in *n*-hexane and cooled to  $-30^{\circ}\text{C}$ , saturated components preferentially crystallized and were recovered in the solid phase. The resulting Enriched SFA fraction was particularly concentrated in lauric acid, making it a promising material for future investigations requiring a defined, lauric acid-rich lipid substrate derived from an insect source. The increase in total SFA in the solid phase is consistent with previous observations on BSFL fat fractionation (Bogevik et al., 2022; Vázquez et al., 2025).

### **Enrichment of Unsaturated Fatty Acids in the Enriched UFA Fraction**

The Enriched UFA fraction, recovered as the liquid phase after winterization, showed a markedly different fatty acid composition. Total UFA increased to 62.50%, compared to 26.01% in both crude fat and the FFA fraction. This shift was driven primarily by a substantial increase in oleic acid (C18:1n-9, 47.24%) and linoleic acid (C18:2n-6, 11.66%), while total SFA decreased to 37.48%. Lauric acid (20.39%), myristic acid (6.76%), and palmitic acid (3.41%) were all reduced relative to the crude fat.

The concentration of unsaturated fatty acids in the liquid phase during winterization is explained by the presence of *cis*-double bonds in their carbon chains, which impair molecular packing and reduce crystallization tendency at low temperatures [6], [7]. Consequently, oleic and linoleic acids remained preferentially in the liquid phase during solvent-assisted cooling. The Enriched UFA fraction obtained in this study represents a BSFL fat-derived lipid material with an unsaturation profile approaching that of common plant-based oils, while retaining its insect-source origin. This fraction may be useful for studies requiring a higher proportion of oleic and linoleic acids from a non-plant lipid source. It is noted, however, that unsaturated fatty acids are inherently more susceptible to oxidation; therefore, stringent storage conditions and oxidative stability analysis should be considered in future studies (Christie, 2003).

### **Effectiveness of Fractionation and Comparison with Previous Studies**

Taken together, the fractionation approach combining chemical hydrolysis and winterization successfully produced four fractions with clearly differentiated fatty acid compositions. The gradient from crude fat and FFA fraction (balanced SFA/UFA profiles) to the Enriched SFA fraction (high SFA, lauric acid-rich) and Enriched UFA fraction (high UFA, oleic acid-rich) demonstrates that simple, laboratory-accessible methods can meaningfully reshape the lipid profile of BSFL fat.

These findings are consistent with previous work. Bogevik et al. (2022) demonstrated that BSFL fat can be fractionated into products with distinct physicochemical and compositional characteristics through fractionation

techniques. Vázquez et al. (2025) similarly reported the successful concentration of lauric acid from BSFL fat using scalable fractionation strategies. The fatty acid profile of crude BSFL fat in the present study is also consistent with previously published data showing lauric acid as the predominant fatty acid in BSFL fat, with substantial contributions from myristic, palmitic, and oleic acids (Ewald et al., 2020; Srisuksai et al., 2024).

### Limitations

This study provides yield, recovery, and fatty acid compositional data for crude BSFL fat and three fractions, but has several limitations that should be acknowledged. First, fatty acid results are expressed as relative percentages; absolute fatty acid content per unit mass of fraction should be determined in subsequent studies for more precise quantitative comparison. Second, physicochemical quality parameters including acid value, iodine value, peroxide value, saponification value, and oxidative stability were not assessed. These parameters are important for a complete characterization of lipid quality and for evaluating the practical applicability of each fraction. Third, detailed GC-MS instrument parameters and chromatographic conditions were not fully documented in this study, which may limit method reproducibility; future studies should report complete analytical parameters. Fourth, no biological experiments were conducted; therefore, claims regarding antimicrobial activity, rumen fermentation effects, or animal performance cannot be made based on the present compositional data alone.

### CONCLUSION

Chemical hydrolysis followed by winterization successfully produced BSFL fat (*H. illucens*) fractions with distinct fatty acid profiles and quantified fractionation yields. Chemical hydrolysis of 350 g of crude BSFL fat produced 218.75 g of Extracted FFA (62.50% yield), retaining a fatty acid composition nearly identical to crude BSFL fat and confirming that hydrolysis altered the lipid chemical form without substantially changing the relative fatty acid distribution. Subsequent winterization of 200 g of Extracted FFA at  $-30^{\circ}\text{C}$  for 48 h produced 147.60 g of Enriched SFA (73.80% yield) characterized by elevated lauric acid (46.10%), myristic acid (20.19%), and palmitic acid (16.91%), with a total SFA proportion of 86.70% and 49.48 g of Enriched UFA (24.74% yield), dominated by oleic acid (47.24%) and linoleic acid (11.66%), with a total UFA proportion of 62.50%, at an overall mass recovery of 98.54%. These results demonstrate that chemical hydrolysis combined with winterization is a simple and practical approach to produce BSFL fat fractions with targeted saturation characteristics and acceptable recovery. Future studies should include physicochemical quality assessment and oxidative stability evaluation to strengthen the application potential of these fractions in animal nutrition and feed technology.

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### Ethical Approval

This study did not involve human subjects or animal experiments. No ethical approval was required.

### Conflict Of Interest

The authors declare no conflict of interest.

### Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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