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Developmental stage-specific variations in the physicochemical and nutritional profiles of oil extracted from *Hermetia illucens* larvae

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The growing need for sustainable, nutritionally valuable lipid sources has led to interest in exploring non-conventional oils. In the present study, oil extracted from three developmental stages of black soldier fly (*Hermetia illucens*) larvae (BSFL), namely 4th instar, 5th instar, and prepupa, was analysed for its physicochemical properties and fatty acid composition. The BSFL collected from the controlled biocomposting system was used in the experiment. Collected BSFL were sorted, dried, powdered, and subjected to Soxhlet extraction followed by solvent recovery using a rotary evaporator. Oil yield varied from 25.66 to 30.80%, depending on the developmental stage. Progressive larval development resulted in improved oxidative stability, while acid values remained within acceptable limits. The oil obtained from the 5th instar exhibited a high saponification value (196.35 mg KOH/g), showing its potential for various industrial and feed applications. Fatty acid analysis revealed stage-dependent variation in lipid composition. The 5th instar oil was dominated by omega-9 oleic acid (52.48%) and contained a moderate level of omega-3 docosahexaenoic acid (4.03%), highlighting its nutritional potential. In contrast, prepupa oil had more medium-chain fatty acids, decanoic acid (78.72%), indicating its suitability for industrial use. Oil from the 4th instar stage contained comparatively lower levels of oleic acid (1.16%) and linoleic acid (1.16%). Thus, these findings highlight that BSFL oil is a promising and suitable lipid source, especially the oil from the 5th instar stage, which shows considerable potential for animal feed, industrial applications, and as a dietary supplement.

Keywords: Alternative protein, Food security, Life on land, Stage-specific distinctions, Sustainability

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Introduction

Fatty acids are essential for human health, contributing to various physiological functions. They help reduce the risk of cardiovascular disease, possess anti-inflammatory properties, and are essential for critical processes such as embryonic development, brain growth, and overall physiological processes[1]. With increasing recognition of their importance in nutrition and health, there is a growing need for sustainable and alternative sources of fatty acids for both nutritional and industrial applications[2]. Currently, vegetable oils, particularly palm oil, dominate the global market due to their affordability and widespread availability. However, these oils rarely contain important amounts of polyunsaturated fatty acids (PUFAs), such as, omega-3, which are crucial for health and highly valued in the aquaculture, pharmaceuticals and cosmetics industries[3,4]. Fish oil, another prominent lipid source, is rich in omega-3 fatty acids but is associated with several challenges, including overfishing, fluctuating marine stock availability, rising costs, and variable quality[5]. These challenges, coupled with a growing emphasis on environmental sustainability, have driven the search for alternative lipid sources that meet global demands without exacerbating the strain on marine ecosystems.

Oil derived from black soldier fly (*Hermetia illucens)* larvae (BSFL) has gained attention as a viable alternative. With its sustainable production and rich nutritional composition, BSFL oil presents an opportunity to reduce dependency on marine-derived oils while maintaining a high-quality nutritional profile. Unlike fish oil, BSFL oil production does not deplete marine resources, making it an environmentally friendly option for addressing global fatty acid requirements[6]. Moreover, BSFL oil demonstrates versatility for use in both nutritional and industrial applications[7,8,9].

Analysing the physicochemical properties of oil is essential to understand its quality and possible uses. Factors like how easily the oil breaks down, its acid

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content and saponification value help to determine its stability and suitability for different applications. Examining the fatty acid composition, usually done using gas chromatography-mass spectrometry (GC-MS), gives a clear picture of its nutritional and functional value. Thus, this focused on assessing the fatty acid profile and key properties of oil extracted from BSFL at three different growth stages: 4th instar, 5th instar, and prepupa. The results highlight differences in oil composition at each stage, emphasising its potential as an eco-friendly and adaptable source of fat for food and industrial use.

Materials and Methods

Larval collection and rearing

The BSFL were collected by field sampling by placing a container (35×51 cm) filled with fresh organic waste, such as vegetable and fruit waste, in an open environment under aerobic conditions. The container was covered with a muslin cloth to prevent contamination of other insects from breeding. This setup was made at the Parivarthana Bio-Compost Unit at Christ University, Bengaluru[10]. The compost was prepared by layering dry leaves and food waste in a 1:1 ratio, giving it an optimal environment rich in nutrients and moisture to support BSFL growth. The food waste, collected and segregated to remove certain items (e.g., cauliflower, pineapple, orange peels, watermelon), was chopped into small pieces and layered evenly on top of dry leaves. A bio-inoculum solution was prepared by mixing one spoon of bio-inoculum with one bucket of water, which was then sprinkled on the compost daily to facilitate microbial activity and waste breakdown. Once the compost tank was filled to three-quarters capacity, it was covered with a layer of dry leaves and sealed to provide a stable environment for the larvae. The BSFL, nourished by organic waste, is grown in this nutrient-rich medium, benefiting from the moisture, temperature, and oxygen levels maintained through daily monitoring and adjustments. BSFL were collected from this controlled biocompost environment for the study.

Drying and grinding of BSFL

Collected BSFL were cleaned and sacrificed by immersing them in boiling water for 40 seconds[11]. They were then separated by developmental stage. In this study, the 4th, 5th, and pre-pupa stages were taken. The mentioned stages were weighed individually. The selected BSFL were dried in a hot air oven for 3 days at 60ºC, as shown in Fig. 1. Their wet and dry weights were recorded, and the dry weight percentage was calculated. Once dried, the larvae were ground into a fine powder using a mortar and pestle for further analysis.

Oil extraction method

According to Wu *et al.*[12], oil extraction from various BSFL developmental stages was performed using a Soxhlet apparatus that included a hot plate, a condenser, a siphon tube, a stand, and a 250 mL round-bottom flask. N-hexane was used as the solvent in the extraction procedure. A total of 200 mL of n-hexane was added to a thimble containing 10 g of dried BSFL, and the extraction was conducted for four hours at a temperature of 60-65°C on a hot plate[13,14]. After extraction, the solvent was removed using a rotary evaporator at 40°C[15]. To ensure purity and remove any impurities, the extracted oil was filtered through a 0.22 µm nylon syringe filter.

Analysis of physicochemical properties

Yield determination

The extracted oil was weighed to determine the extraction yield using the equation below[16]:

$$\text{Extraction yield } (\%) = \frac{\text{Oil weight}}{\text{Sample Weight}} \times 100$$

Colour determination

The oil colour was determined using the Lovibond scale[17] (Fig. 2) for oil extraction, and the oil intensity was determined using a UV spectrophotometer (SHIMADZU UV-1800). For this, the sample was diluted 1:9 with hexane. Absorbance was recorded at 425 nm[16].

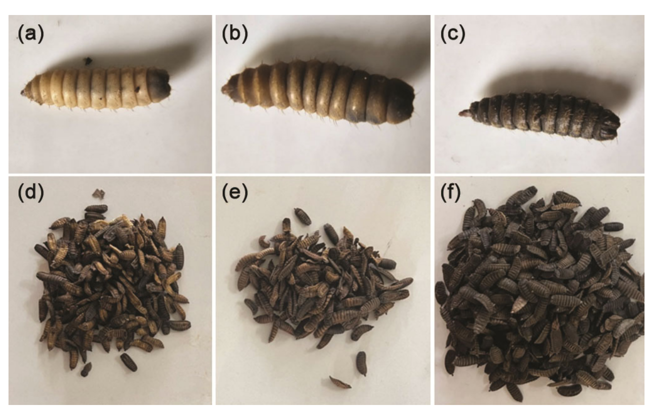

Fig. 1 — Different stages of BSFL used for the present study. (a-c) visual representation of freshly hatched (wet) BSFL in the 4th, 5th, and prepupal stages, respectively. (d-f) dry samples of the 4th, 5th, and prepupal phases, respectively, in a hot air oven (dried).

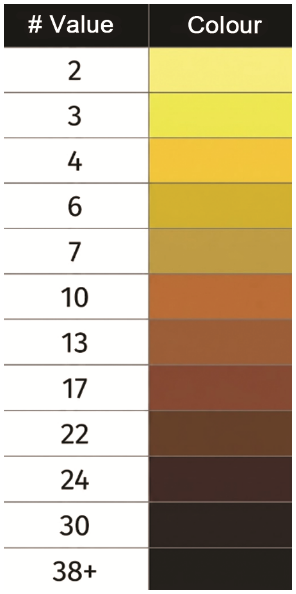

Fig. 2 — Lovibond scale for oil extraction colour.

Specific gravity

Specific gravity, an important physical parameter of oil quality, is a dimensionless measure representing the ratio of a substance's density to that of water at a given temperature. It helps determine the oil's purity[18,19]. The specific gravity was measured using a gravimetric technique with a specific gravity bottle method and calculated using equation below[20]:

$$\text{Specific gravity at } \frac{30°\text{C}}{30°\text{C}} = \frac{(\text{A} - \text{B})}{(\text{C} - \text{B})}$$

where A = the weight (g) of the oil-filled specific gravity bottle at 30°C, B = the specific gravity bottle's weight (g), and C = the specific gravity bottle's weight (g) at 30°C with water.

Saponification value

The measure of the oxidation during the storage of oil and its indication of deterioration is identified by the saponification value. It enhances the quality of the oil[19]. A high saponification value suggests the presence of fatty acid with a molecular weight[15]. To determine this value, approximately 0.5 g of the oil sample was placed in a 100 mL Erlenmeyer flask. Then, 10 mL of 0.5N ethanolic potassium hydroxide solution was added and thoroughly mixed. The flask was heated in a water bath at 80-85°C for 30 minutes. After heating, the solution was cooled to 30-40°C before being titrated with a 0.5N HCl standard solution, using 2-3 drops of phenolphthalein as an indicator. A blank test was also conducted and calculated using equation below[20,21]:

$$\text{Saponification value (mg KOH/g)} = \frac{28.05 \times (\text{A} - \text{B}) \times \text{F}}{\text{S}}$$

where S = sample weight, A = titration volume of blank (mL), B = titration volume of sample (mL), F = Factor of 0.5N HCl standard solution, and 28.05 = Half of the molecular weight of KOH.

Peroxide value

The peroxide value analysis can determine the amount of peroxide in the oil, as triglycerides in the oil oxidise in the presence of moisture to form peroxide[22]. It is an indicator of lipid oxidation or rancidity in the oil[19]. A 250 mL stoppered flask was used to hold a filtered oil sample weighing approximately 0.1 g. To dissolve the fats, 10 mL of a chloroform-acetic acid mixture (2:3, v/v) was added, and the flask was gently shaken. Then, 1 mL of saturated potassium iodide solution was added, the mixture was immediately stoppered, and it was left in the dark for 5 minutes. Following this, 20 mL of distilled water was added, and the mixture was shaken thoroughly. The released iodine was titrated using a 0.01N sodium thiosulfate ($Na_2S_2O_3$) solution until the solution turned pale yellow. At this stage, 1 mL of a 1.5% starch solution was added as an indicator, and titration continued until the solution became colourless[20,21,23]. A blank titration was also conducted, and the peroxide value was calculated using the formula below:

$$\text{Peroxide Value (mEq per 1000g)} = \frac{(\text{Vs} - \text{Vb}) \times \text{F} \times 10}{\text{W}}$$

where, Vs = titration volume of sample (mL), Vb = titration volume of blank (mL), F = factor of 0.01N $Na_2S_2O_3$ solution, W = weight of fat in the volume of extract used (g), and N = normality of $Na_2S_2O_3$ solution (in this case N/100).

$$\text{Peroxide Value (millimoles per 1000g)} = \frac{0.5 \times (\text{Vs} - \text{Vb}) \times \text{N} \times 1000}{\text{W}}$$

Acid value

As per the revised FSSAI Manual of Methods of Analysis of Foods (2021), the acid value represents the amount of potassium hydroxide (mg) needed to neutralise the fatty acids present in one gram of fat. A lower acid value indicates better oil stability over time, reducing the risk of rancidity and peroxidation[19]. To determine the acid value, the oil sample was dissolved in a 1:1 mixture of ethanol and diethyl ether in a conical flask. A few drops of phenolphthalein were added, and titration was carried out using a 0.5 M ethanolic KOH solution, slowly added from a 25 mL pipette while shaking the flask. The endpoint was identified when a persistent pink colour appeared and lasted for at least 10 seconds. The volume of KOH used was recorded, and the acid value was determined using Equation below[6]:

$$\text{Acid Value} = \frac{56.1 \times T \times V}{m}$$

where: V = mL of the KOH solution needed, T = the molarity of the KOH solution , m = mass (g) of the test portion, and 56.1 = molecular weight of potassium hydroxide (KOH).

Fatty acid profile analysis

The filtered oils were analysed under GC-MS to investigate the fatty-acid profile in their oil[10]. The sample was loaded and analysed using a SHIMADZU GC-MS-QP2010 SE for a detailed biochemical analysis. The instrument's m/z 1.5-1000 mass range enabled the detection of a variety of analytes with an R=2M (FWHM) resolution for accurate peak separation. The analysis scan range was set to m/z 200-300 with an interval of 0.5 seconds, optimising the data obtained for target analytes. The column oven temperature was set to 60°C, and the injection temperature was maintained at 280°C in splitless mode with a sampling time of 1 minute. The flow control mode operated in linear velocity with a pressure of 76.2 kPa, a total flow of 4.3 mL/min, a column flow of 1.25 mL/min, and a purge flow of 3.0 mL/min. The linear velocity was set at 40.8 cm/sec. The oven temperature program involved an initial hold at 60°C for 3 minutes, followed by a rise of 12°C/min to 200°C, held for 2 minutes, and then a ramp of 25°C/min to a final temperature of 280°C, held for 16 minutes. The ion source and interface temperatures were set to 200°C and 290°C, respectively. The acquisition mode was set to scan with an event time of 0.30 seconds and a scan speed of 1666. The mass range analysed spanned from 35.00 to 500.00 m/z. These parameters ensured optimal resolution and compound identification within the sample.

Results and Discussion

Quantitative assessment of extracted oil

The oil yield from BSFL was evaluated across three developmental stages: 4th instar, 5th instar, and prepupa. For a 10 g sample, the oil yields were 59.8, 55.8, and 43.39%, respectively. The extracted oil volumes were consistent with the oil yield percentages, yielding 2.50±0.02, 2.80±0.05, and 3.08±0.16 mL for the 4th instar, 5th instar, and prepupal stages, respectively, for 10g of sample (Fig. 3). The progressive increase in oil extraction highlights its potential for large-scale production, particularly in the prepupal stage.

Physicochemical analysis

The physicochemical properties of BSFL oil from the 4th instar, 5th instar, and prepupa stages were evaluated. The results showed a stage-specific distribution among BSFL developmental stages. The comparison with fish and other vegetable oils, based on previously published studies, shows similarities in fatty acids in the oils[24,25,26].

Colour intensity

The oil extracted from the three larval stages exhibited a yellow hue on the Lovibond scale value: 7 (Table 1, Fig. 2). Upon dilution with hexane (1:9), the absorbance values at 475 nm of the oils were measured as 0.758 AU, 0.638 AU, and 0.674 AU for the 4th instar, 5th instar, and prepupa stages, respectively. The previously studied literature on mixed stages gave an absorbance of 2.03±0.0023 AU for the crude and 0.365±0.014 AU for the filtered[27]. Variations in absorbance may be attributed to pigments and antioxidant compounds in the oil. The 4th instar oil showed the highest absorbance, indicating a potentially higher concentration of these compounds.

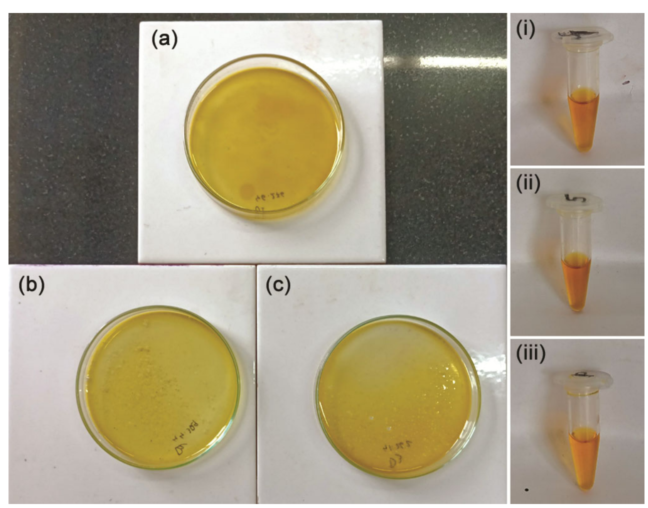

Fig. 3 — Oil extracted from different stages of BSFL. (a) Crude oil extracted after rotary evaporation from the 4th instar (top), 5th instar (left), and prepupa (right) stages, respectively. (b-d) filtered oil of the 4th instar, 5th instar, and prepupa stage, respectively.

Table 1 — Physicochemical properties of oil extracted from various developmental stages of BSFL

BSFL stage	Colour	Yield (%)	Specific gravity	Saponification Value (mgKOH/g)	Peroxide Value (mEq/1000)	Acid Value (mgKOH/g)
4th instar	Yellow Scale 7	25.66±0.21	1.05±0.006	193.54±6.272	4.89±0.141	1.79±0.614
5th instar	Yellow Scale 7	28.35±0.29	1.11±0.010	196.35±11.451	2.65±0.087	2.91±0.614
Prepupa	Yellow Scale 7	30.80±1.6	1.22±0.009	162.69±8.097	2.88±0.113	4.71±0.501

Oil yield percentage

The oil yield increased progressively from the 4th instar (25.66%) to the prepupal stage (30.80%), as shown in Table 1. Previous studies reported a yield of 29.60% with mixed stages using Soxhlet extraction methods[12]. The higher yield in BSFL oil compared to fish oil (19.395%)[28], soybean oil (14.51%), and groundnut oil (10.54%)[29] underscores its potential as a sustainable source for various commercial uses- food industry, animal feed, industrial and cosmetics.

Specific gravity

The specific gravity values increased with larval development, recorded at 1.05, 1.11, and 1.22 for the 4th instar, 5th instar, and prepupa stages, respectively (Table 1). These values were higher than those of fish oil (0.892), soybean oil (0.920), and groundnut oil (0.891)[28,29]. The elevated specific gravity indicates higher oil density, making BSFL oil suitable for industrial applications, such as biodiesel and biofuels[23].

Saponification value

The saponification values of BSFL oil were 193.54±6.27 mgKOH/g (4th instar), 196.35±11.45 mgKOH/g (5th instar), and 162.690±8.10 mgKOH/g (prepupa) (Table 1). These values suggest stage-specific variations in fatty acid chain length. Oils with saponification values above 200 mgKOH/g typically contain shorter-chain fatty acids, which are ideal for soap production[30]. The 5th instar oil's value is comparable to soybean oil (228.19 mgKOH/g) and groundnut oil (227.49 mgKOH/g)[29], indicating its suitability for soap and detergent applications[31]. Oils with typically lower saponification values (<195 mg KOH/g) contain longer-chain fatty acids, which are common in unprocessed vegetable oils[30]. The previously studied Saponification value for BSFL mixed-stage oil was 213.22±0.023 mg KOH/g for the crude, and the purified BSFL oil gave 215.78 mg KOH/g[27]. This shows that oils extracted from earlier larval stages, particularly the 4th and 5th instars, have shorter fatty acid chains compared to the prepupa stage, making them more suitable for producing soluble soaps. However, no stages reached a saponification value of over 200 mg KOH/g, indicating that it is associated with hydrogenated or modified oils containing even shorter-chain fatty acids. Then, compared to fish oil (53.298 mgKOH/g)[28] and groundnut oil (227.49 mgKOH/g)[29], BSFL oil in the 5th instar exhibited a saponification value comparable to that of soyabean oil (228.19 mgKOH/g)[29]. This places BSFL oil in the 5th instar, closer in saponification value to unprocessed vegetable oils such as groundnut and soybean, suggesting it has similar potential for applications such as soap production.

Peroxide value

As shown in Table 1, the highest peroxide value was observed in the 4th instar oil (4.89 mEq/1000), decreasing to 2.65 and 2.88 mEq/1000 in the 5th instar and prepupal stages, respectively. These values demonstrate improved oxidative stability as larvae progress to the prepupal stage. Previous studies show the peroxide value of BSFL mixed-stage oil was 2.26 mEq/kg ± 0.44[5] and 6.272 mEq/kg[12]. A general rule is that peroxide value should not exceed 10-20 mEq/1000 to avoid rancidity (Jacob 1973). A high peroxide value indicates high oxidative rancidity and also suggests no or low amounts of antioxidants[32]. This shows that the results align with the range of studies. These values are below the threshold (10-20 mEq/1000), showing oxidative stability and less rancidity. Compared to fish oil (6.735 mEq/1000)[28] and groundnut oil (5.58 mEq/1000)[25]. The BSFL oil in the later stages exhibited higher oxidative stability. The lower peroxide value of soyabean oil (1.5 mEq/1000)[32] reflects its higher resistance to rancidity. The peroxide values of BSFL oil make it a stable alternative to certain traditional oils.

Acid value

The acid value determines the quality of fatty acids in oil[32]. The acid values of BSFL oil increased across the developmental stages, from 1.79 mgKOH/g (4th instar) to 2.91±0.61 mgKOH/g (5th instar) and 4.71 mgKOH/g (prepupa) (Table 1). The stability and quality of oil can be determined by the acid value. Lower acid values, as observed in the 5th instar,

Table 2 — Fatty acid profile (%) of oil extracted from different stages of BSFL

Fatty acid	4th instar	5th instar	Prepupa
Octanoic acid (C6:0)	7.04	-	-
Nonanoic acid/ Pelargonic acid (C9:0)	-	0.23	10.55
Pentanoic Acid/ Valeric Acid (C5:0)	1.17	-	-
Decanoic acid (C10:0)	7.04	25.30	78.72
Undecanoic acid (C11:0)	7.04	0.44	-
Dodecanoic Acid/ Lauric Acid (C12:0)	-	0.23	-
Tridecanoic acid (C13:0)	7.04	0.23	-
Myristic acid/Tetradecanoic acid (C14:0)	1.16	0.43	-
Pentadecanoic acid (C15:0)	7.04	-	-
Hexadecanoic acid (C16:0)	7.04	-	-
Nonadecanoic Acid (C19:0)	-	4.16	-
Eicosanoic acid/ Arachidic acid (C20:0)	-	4.16	-
Behenic acid (C22:0)	1.16	-	-
Docosanoic Acid (C22:1)	-	-	-
Oleic Acid (C18:1n-9)	1.16	52.48	-
Docosahexaenoic acid (DHA) (C22:6n-3)	-	4.03	-
Linoleic acid (C18:2n-6)	1.16	-	-

correlate with greater stability and suitability for prolonged storage[19]. Higher acid values in the prepupal stage may limit its use in food applications but support its potential for industrial uses, such as in soap and paint manufacturing[19]. According to a previous study, BSFL at mixed stages fed on an orange diet showed 14.59 mg KOH/g, lime diet- 12.34 mg KOH/g, potato diet- 11.22 mg KOH/g, and beetroot diet- 16.04 mg KOH/g[14]. The higher values may indicate the suitability for non-food applications such as the paint and soap industries. Another study reports an acid value of 3.00±0.81 mg KOH/g in BSFL oil across mixed developmental stages[9]. While the acid value of the 5th instar oil (2.91 mg KOH/g) was slightly higher than that of fish oil (2.305 mg KOH/g), it remained significantly lower than that of soybean oil (1.1 mg KOH/g).

Fatty acid analysis

The fatty acid profiles of BSFL oil were analysed across three developmental stages, 4th instar, 5th instar, and prepupal stages, revealing stage-specific variations in lipid composition (Table 2). These variations reflect metabolic activity and lipid storage dynamics as the larvae mature. The results were compared with published studies on fish and vegetable oils, demonstrating compositional similarities in certain fatty acids[24,29,33]. The chromatographic peaks of the fatty acids were analysed, and the results are presented in Fig. 4.

The fatty acid composition of BSFL oil varied significantly across the 4th instar, 5th instar, and pre-pupa stages, reflecting various metabolic activities and lipid storage as the larvae mature. The composition was analysed through the different peaks from the chromatogram, as shown in Fig. 4. In the 4th instar stage, fatty acids include decanoic acid (C10:0), hexadecanoic acid (C16:0), and octanoic acid (C6:0), all of which are present at 7.04%. Medium-chain fatty acids like undecanoic acid (C11:0) and tridecanoic acid (C13:0) were also detected, each contributing 7.04%, indicating a great concentration of saturated fatty acids in this developmental stage. Oleic acid (1.16%) and Linoleic acid (1.16%) show the presence of omega-6 fatty acids and omega-9 fatty acids, respectively[35,36]. In the 5th instar stage, there is an increase in decanoic acid (C10:0) reaching 25.30%, while oleic acid (C18:1n-9) becomes the predominant fatty acid at 52.48%, highlighting increased accumulation of omega-9 fatty acids. Fatty acids such as nonanoic acid (C9:0), undecanoic acid (C11:0), and nonadecanoic acid (C19:0) are detected in small quantities. Docosahexaenoic acid (DHA, C22:6n-3) at 4.03% in this stage suggests the potential for omega-3 enrichment. The prepupa stage shows an increase in decanoic acid (C10:0), which reaches 78.72%. Pelargonic acid (C9:0) reaches 10.55%, an increase compared to earlier stages. This composition highlights the larvae's dependence on medium-chain fatty acids as they prepare for pupation. Fatty acids such as oleic acid and DHA are absent or negligible in this stage, focusing mainly on lipid storage for the metabolism of the next developmental stages. Most of the omega-3, omega-6, and omega-9 fatty acids are predominantly present in the 5th instar stage, with smaller amounts observed in the 4th instar stage and

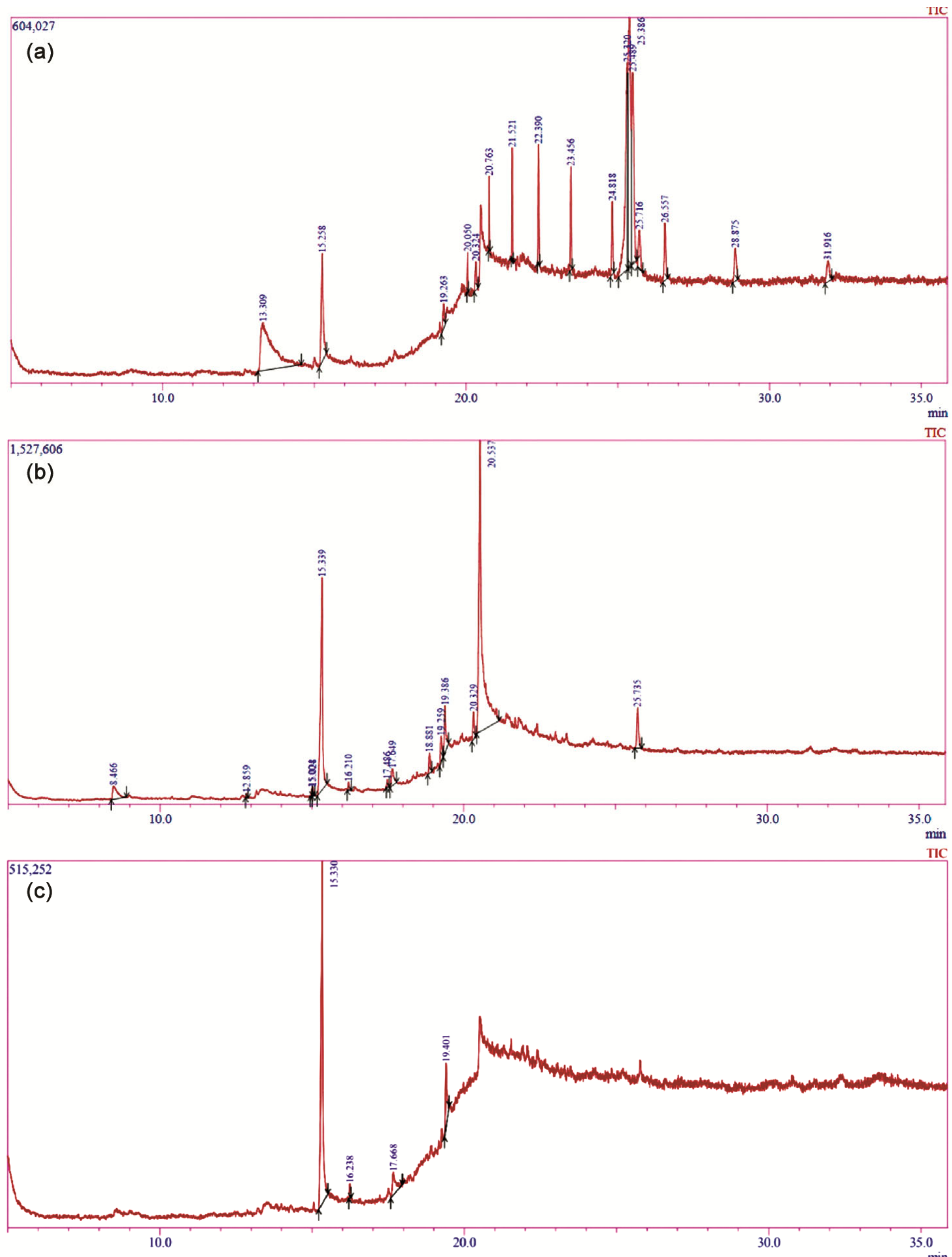

Fig. 4 — Chromatogram of fatty acids present in different stages of BSFL oil. (a-c) shows the chromatogram of the 4th instar, 5th instar, and prepupa stages.

negligible levels in the prepupal stage. Triglycerides, such as omega-3 fatty acids, which consist of fatty acids and glycerol, constitute the majority of fat content in insects, making them a valuable source of energy-rich nutrients similar to those found in fish and other vegetable oils[15,26,28,33,35].

Lauric acid (C12:0) was detected only in trace amounts in the 5th instar (0.23%) and prepupa stages. It is highlighted as a dominant fatty acid, especially in larvae fed with bread or fresh mussels (up to 52%). It was lower in diets with higher mussel content at around 35%[36,37]. Palmitic acid was uniformly detected at 7.04% in the 4th instar stage and was absent in later stages. Previous studies reported higher concentrations depending on feedstock (e.g., rotten mussels). Oleic acid peaks at 52.48% in the 5th instar and is negligible in other stages. Minimal presence in the 4th instar (1.16%) of linoleic acid, but absent in later stages. DHA was detected only in the 5th instar. In previous studies, oleic acid levels were higher in fish-fed larvae

(25.1%) and food-waste-fed larvae (19.1%). Diets with increasing mussel levels showed slight variations, peaking at around 14%[36], indicating a high concentration of oleic acid in the 5th instar of oil. PUFAs, such as DHA, were observed predominantly in larvae fed mussel-inclusive diets (up to 8.2%).

The GC-MS analysis shows how fatty acid profiles shift with developmental stages, particularly during the transition to pupation. Also, studies[36,37] focus on dietary influences, noting higher PUFA and MUFA levels in omega-rich feeds like mussels or bread. Fish oil is characterised by a high content of Hexadecanoic acid (C16:0) at 8.532%, along with significant levels of Eicosanoic acid (C20:0) at 5.993% and Linoleic acid (C18:2n-6) at 6.283%. It also contains moderate amounts of Docosahexaenoic acid (DHA) (C22:6n-3) at 4.362% and traces of oleic acid (C18:1n-9) at 3.551%[14]. In soybean oil, linoleic acid dominates at 44.40%, followed by oleic acid at 26.80%. Hexadecanoic acid appears at 14.69%, while behenic acid (C22:0) was present in minor amounts (0.33%)[29,33]. Groundnut oil also demonstrates a high unsaturated fatty acid profile, with linoleic acid (C18:2n-6) at 5.50%, oleic acid (C18:1n-9) at 26.20%, and a small proportion of DHA at 1.25%[38]. The results indicate that fish oil contains a more diverse range of fatty acids, including long-chain polyunsaturated fatty acids, which are essential for various metabolic processes. Soybean oil, rich in linoleic acid, is well suited for dietary applications that require polyunsaturated fatty acids. Groundnut oil has a simpler profile with a balance of oleic and linoleic acids, making it suitable for general cooking and dietary use. These differences highlight the varied applications of oils in nutrition and industry, driven by their specific fatty acid compositions. The lipid profile of fish oil includes a key omega-3 fatty acid, i.e., DHA (4.362±0.03%), a long-chain polyunsaturated fatty acid important for brain and cardiovascular health. The 5th instar oil contains DHA (4.03%) at lower levels than fish oil, but can be a promising alternative for omega-3 fatty acid enrichment. Similarly, linoleic acid (C18:2n-6), which contains omega-6 fatty acids seen mostly in soybean oil, other vegetable oils, and fish oil, is also present in lower quantities in the BSFL oil.

Conclusion

This study highlights the potential of BSFL oil as a sustainable, alternative lipid source, focusing on its fatty acid composition and physicochemical properties across three developmental stages: 4th instar, 5th instar, and prepupa. The 5th instar stage demonstrated a nutritionally favourable fatty acid profile, with a predominance of omega-9 fatty acids (oleic acid) and omega-3 fatty acids, such as DHA, making it particularly suitable for dietary applications. Physicochemical analysis revealed an increase in oil yield and specific gravity with larval development, with the prepupa stage producing the highest oil yield (30.80%), emphasising its commercial viability. The stability of BSFL oil, as evidenced by its peroxide and acid values, further establishes it as a durable, high-quality lipid source. These findings underscore its versatility for applications in nutrition, animal feed, and industrial products, particularly from the 5th instar stage, owing to its superior nutritional profile and physicochemical properties. Future studies should aim to enhance the yield and functionality of key fatty acids in BSFL oil to maximise its utility in food and nutritional formulations.

Conflict of interest

The authors declare that they have no conflicts of interest.

AI use disclosure

The authors declare that the AI tool was used only for language improvement and grammar correction of the manuscript, and not used for any data generation, data analysis, interpretation of results, or other scientific purposes.

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