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Research article

# Facile acid fermentation extraction of silkworm pupae oil and evaluation of its physical and chemical properties for utilization as edible oil

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ABSTRACT

Considering the increasing demand for edible oil in recent times, their price in the world market is becoming skyrocketing. In this research, we produced cost-effective edible oil from desilked silkworm pupae (*Bombyx mori*) applying a facile acid fermentation process, for the first time. The extraction was performed using two different types of organic acids, 3% of each acetic and citric acid. The yield of the extracted oil was 3.52 ± 0.23% from fresh silkworm pupae. The produced oil was then characterized physically and chemically to know its suitability to be used as edible oil. The oil was found with a low peroxide and acid value of 4.82 meq/kg and 1.35 mg KOH/g oil, respectively, and comprised of different fatty acids, in which palmitic acid (32.04%) and oleic acid (34.62%) were in large portions among the total fatty acids. Additionally, the extracted oil included linoleic, α-linolenic, and dihomo-gamma-linolenic acid which have health benefits. The oil was rich with minerals such as Iron, Sodium, Potassium, Calcium, Magnesium, Zinc, and Phosphorus with a negligible concentration of toxic elements such as Manganese, Cobalt, Nickel, Copper, Lead, Cadmium, Chromium, Arsenic, and Silver, indicating a good nutritive value of the extracted oil. Overall, the outcomes of all the characterizations showed that the extracted oil could be used as good edible oil and the corresponding acid fermentation extraction process has the potential to be used as an effective oil extraction method for silkworm pupae.

## 1. Introduction

Natural silk is renowned for its applications in the counting, textile, parachute, tire, electrical, medical, and cosmetics industries.

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Silk thread results from the bioconversion of plant materials and highly nutritive biomass through silkworms. In sericulture, the most common silkworm species adopted over the globe are the mulberry silkworm (*Bombyx mori* L.), oak silkworm (*Antheraea pernyi*), and eri silkworm (*Samia cynthia ricina*). Most silkworm pupae are a highly potential by-product of the agro-textile industrial supply exclusively devoted to silk production. After silk extraction, the desilked silkworm pupae consisting of 60% of dry cocoon weight are considered waste in the silk industry and are commonly dumped in local places or on agricultural lands to be utilized as a bio-fertilizer [1]. Dumping a massive amount of silkworm pupae in open areas can lead to environmental damage due to its ablative properties [2]. However, exploiting these valuable biological resources containing protein, oil, and fatty acids (α-linolenic acid) [3,4] in the feed and food industry may create a path to abate the environmental crisis of silk production.

In recent years, the demand for edible oils (e. g., soybean oil) is increased greatly and their price in the local markets is also increasing frequently [5]. For instance, the price of soybean and palm oil has increased considerably in the last few years [5]. From May 2021 to May 2022, the bottled soybean oil price in Bangladesh increased by around 47% per liter [6,7] and in June 2022, the price increased further by 3.5% per liter [8]. Other edible/plant oils such as peanut, sesame, and mustard oil are also available in the country, however, their prices are much higher than the commonly consumed soybean and palm oil. The total annual demand for edible oil and fat in Bangladesh is around 3 million tonnes [5]. The country imports around 90% of the total annual demand for edible oil from the countries such as Brazil, Argentina, Malaysia, Indonesia, and so on which is very costly [5]. Further, in addition to the recent Covid-19 pandemic, the Ukraine-Russia war exacerbates the worse of the global economy and assists the price increase of essential edible oil. Now, what should do developing countries like Bangladesh in this worse backdrop? The probable answer to this crucial question may be the turning of Bangladesh into a self-depended edible oil production country utilizing the existing natural resources. Bangladesh being an agricultural country has the ability to grow many oil-extracting crops or seeds such as mustard, sesame, coconut, soybean, olive, almond, and so on. In addition, most importantly, some insect species such as mulberry silkworm, oak silkworm, and eri silkworm may also be an important alternative for oil extraction.

Extracts of silkworm pupae are a source of high protein content, which lies between 45 and 80% on a dry matter (DM) basis [3]. In addition, it contains a high level of good lipids for human health, while omega-3 ranges from 35 to 40% of its total fatty acids (FAs) [4]. It has been studied that silkworm pupae oil is completely secure and nutritionally similar to some regularly consumed vegetable oils, for example, sunflower oil [9]. It is enriched with unsaturated fatty acids (60–70% of the total fatty acid content), especially α-linolenic and oleic acids [10]. A couple of α-linolenic and oleic acids are recognized for their health benefits and are utilized in the production of food, supplements, and feed [11,12,13,14]. However, the extraction of oil from silkworm pupae is challenging since the approach should be easy, cost-effective, and secure.

Formerly, the utilization of silkworm pupae for extracting oil was performed with differential techniques, for example, mechanical pressing extraction [15], solvent and supercritical fluid extraction [1,16,17,18], microwave-assisted gravimetric extraction [11], and aqueous saline oil extraction [19]. Some of the mentioned methods involve a lot of labor, time, and solvents, making the extraction process expensive. Initially, these procedures need to dry the silkworm pupae before the extraction and thus the protein by-product's characteristics may also be disrupted due to the extraction conditions. Additionally, the physicochemical parameters of oil can be changed by the consequential lipid oxidation due to sun or oven drying [20]. However, the yield of solvent extraction and enzymatic extraction was high with an expensive extraction cost, while the mechanical pressing extraction reached a low yield [20]. Furthermore, supercritical fluid extraction is rarely used for complex operating methods and is expensive for apparatus [21]. The yield of microwave-assisted extraction is high, but the apparatus price and the used solvent make the extraction expensive. On the other hand, the aqueous saline oil extraction method requires a frozen sample and repeated centrifugation for a long time [19]. In this regard, acid fermentation with organic acid can be used as a new feasible method with low cost and comparatively high yield for the wide utilization of available silkworm pupae. In acid fermentation, generally, raw samples were used to extract the protein concentrated with spoiled yogurt; it took a long time in the primary stage, while supernatant oil was a byproduct [22]. Next, inorganic and organic acids were added in a minimal amount to provoke the fermentation in a short time [22]. Recently, a low concentration of organic acids was also used to extract oil from fish waste [23].

However, there is no effective work on oil extraction from insects by acid fermentation. Consequently, the primary aim of this work was to develop a facile acid fermentation extraction process for separating oil from silkworm pupae, followed by its physical and chemical characterization to appraise its suitability to be used as edible oil. The outcome of this research work will introduce a new dimension in the oil extraction process from insects, and the extracted oil could find a potential application as an edible oil in the food industry based on the characterization results.

**Fig. 1.** Collected fresh desilked silkworm pupae (*Bombyx mori*).

## 2. Materials and methods

### 2.1. Raw materials collection, processing, and storing

Fresh desilked silkworm pupae (*Bombyx mori*) (Fig. 1) were collected from the Bangladesh Sericulture Research and Training Institute (BSRTI), Rajshahi, Bangladesh. After collection, the silkworm pupae were processed to remove visible impurities and unsuitable pupae for utilization. These were then stored at −20 °C in the refrigerator for further analysis at several laboratories of the Bangladesh Council of Scientific and Industrial Research (BCSIR), Rajshahi, Bangladesh.

### 2.2. Proximate composition analysis

The fresh desilked pupae were used to estimate the contents of moisture, crude protein, crude fat, crude fiber, total carbohydrate, and ash in the silkworm pupae following standard procedures [24,25,26]. The moisture content of the silkworm was determined by drying it in an oven at 105 °C, and data were obtained until a constant weight loss was achieved. The crude protein content was estimated by the measurement of the total nitrogen content of the samples using the Kjeldahl method (DKL 42/26 automatic digestion and UDK 129 distillation unit, Velp Scientifica, Italy) followed by the multiplication of the result of total nitrogen with a conversion factor of 6.25. Fat content was measured by a Soxhlet apparatus. The ash content was determined by incineration of precisely 5 g of samples (in the ceramic crucible) in a muffle furnace at 600 °C for 6 h. For the estimation of crude fiber content, approximately 5 g of the moisture- and fat-free samples were heated with a mixture of dilute $H_2SO_4$ (1.25%) and NaOH (1.25%) solutions. After heating, the samples were placed on the porcelain crucible and dehydrated overnight at 80 °C. The crucible with the sample was heated in the furnace at 600 °C for 1 h. Finally, the crude fiber content of samples was counted by the weight difference of the samples of post-drying (80 °C) and ashing (600 °C). The carbohydrate fraction was estimated by deducting the sum of the other contents from 100.

### 2.3. Extraction of oil from silkworm pupae

Silkworm pupae oil was extracted by acid fermentation following Salih et al. [23] with slight modification and the entire process is shown as a flow diagram in Fig. 2. First, 100 mL of 3% acetic acid and citric acid was added to a weight of 500 g of desilked fresh pupae. After that, these samples were blended with a grinder (Jaipan, India) and placed in a 1000 mL glass container sealed tightly with a lid for fermentation. Then, this glass container containing the sample was sonicated in an ultrasound cleaner (Skymen Cleaning

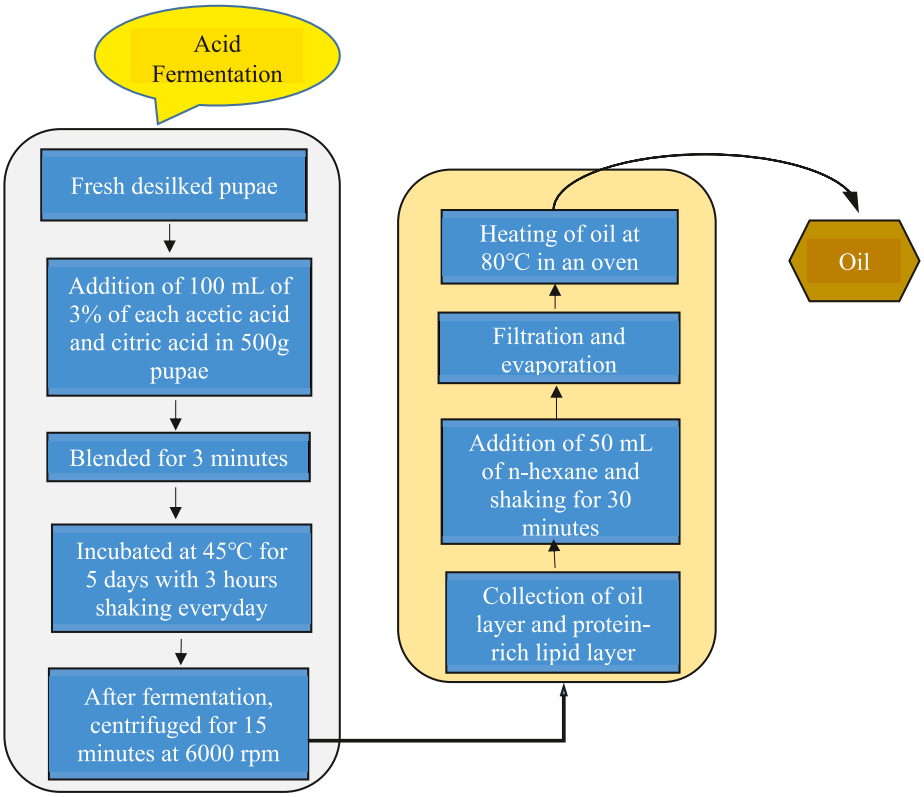


**Fig. 2.** Acid fermentation of oil extraction from silkworm pupae.

Equipment Shenzhen Co., Ltd, China) for 1 h, shaken in an orbital shaker for 3 h, and kept at 40–45 °C in an incubator for 5 days. After complete fermentation, the mixture was centrifuged for 15 min at 6000 rpm and obtained as three fractions, including the top (lipid layer), middle (protein-rich lipid layer), and bottom (aqueous residues) layers. The oil or lipid layer and protein-rich lipid layers were pipetted out and mixed with 50 mL of n-hexane. This n-hexane mixture was then shaken for 30 min and filtered with Whatman filter paper. After filtration, the residue solvent was volatilized by a rotary evaporator, and clear oil was heated at 80 °C in an oven. The different stages of oil extraction from silkworm pupae are visualized in Fig. 3. Finally, oil was collected, and the percentage of oil yield was determined by the following equation (1).

$$\text{Yield of oil } (\%) = \frac{\text{Weight of extracted oil} \times 100}{\text{Weight of sample}} \quad (1)$$

### 2.3.1. Analysis of physicochemical properties

Physical properties such as moisture content, refractive index, density, specific gravity, and viscosity of the silkworm oil were investigated using standard methods [27,28,29]. Acid value, saponification value, iodine value, peroxide value, unsaponifiable matter, and free fatty acid were determined following the AOAC [30] and official methods of the American Oil Chemists Society (AOCS) [31]. The viscosity of oil was estimated by the Fungilab digital rotational viscometer.

### 2.3.2. Fatty acid profiling

The fatty acid content of the silkworm oil was determined by following Ferdousi et al. [26]. In brief, 3.5 mL of 0.5 M Sodium methoxide was added to 200 mg of oil sample in a 10 mL test tube. After heating on a burner to remove the bubbles, 1.5 mL of n-hexane was mixed with the mixture and homogenized well using a vortex. Then, 5 mL of deionized water was slowly flowed into the test tube and waited for precipitation. After precipitation, the top organic layer was collected for fatty acid analysis.

The fatty acid profiling of the oil was performed by GC-MS (GC-2010, Shimadzu, Japan). The GC was assembled with an auto-sampler (AOC-20s), auto-injector (AOC-20i), and SH Rxi 5MS Sill capillary column with 30 m × 0.25mm × 0.25 μm film. The flow rate of helium gas was 2.0 mL/min. The initial oven temperature of GC was 40 °C for 10 min, then hiked up at 70 °C/min and reached 250 °C to continue for 10 min. The injector was injected at a 1.0 μL volume with a 75:1 split ratio at 250 °C. The total running time was 35.71 min while the solvent cutting time was 3.40 min. The detector (Shimadzu GCMS-QP-2020, Japan) was operated at 25–50 °C. Identification and quantification of fatty acids in the samples were performed by differentiating retention times for the samples with authenticated standards.

### 2.3.3. Fourier transform infrared (FTIR) spectroscopic analysis

FTIR is an essential analytical tool that can be used to identify the functional groups of the organic compounds present in substances. In this work, the functional groups of chemical biomolecules in the oil sample were determined by performing FTIR analysis with a PerkinElmer Spectrum IR Version 10.6.2 (UK). The absorption frequency spectra were organized as transmittance versus wavenumber in a plot.

## 2.4. Analysis of chemical elements in the fresh silkworm pupae and extracted oil

To determine the elemental content in the fresh silkworm pupae and extracted oil, the samples were prepared according to our published works [32,33]. Briefly, about 6 g of the fresh silkworm pupae were incinerated in an electrical muffle furnace at 600 °C for 6

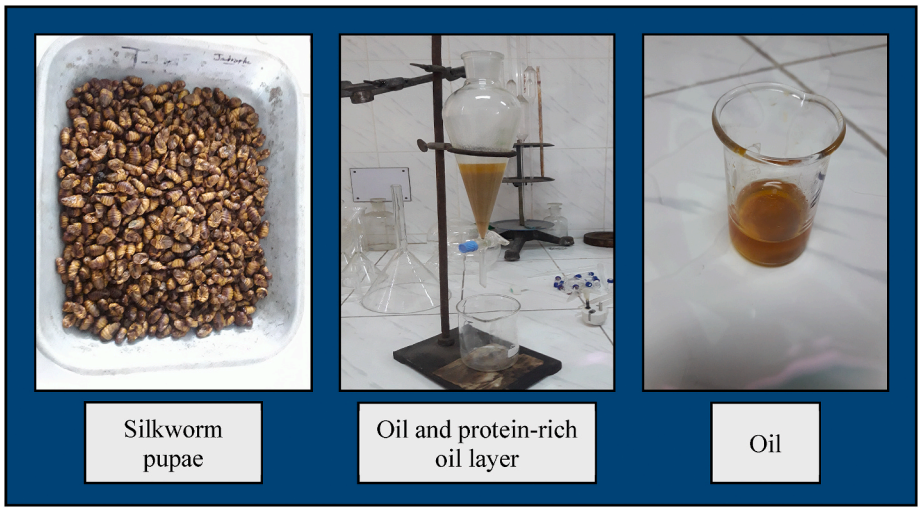


**Fig. 3.** Different stages of oil extraction from silkworm pupae.

h to make an ash of the sample. The obtained ash was then treated with a mixture of concentrated nitric acid and perchloric acid in a ratio of 2:1 and heated on a hot plate for complete digestion of the sample. For the preparation of the oil sample, around 6 g of the sample was digested with a mixture of nitric acid, perchloric acid, and hydrogen peroxide in the ratio of (2:1:0.5) in a Kjeldahl flask under reflux. The final volume of both silkworm pupae and oil samples was made to 100 mL with deionized water. The samples were prepared in triplicates and blank samples were also prepared following the same approaches.

The concentrations of 16 chemical elements *viz.,* Fe, Mn, Na, K, Ca, Mg, Zn, P, Co, Ni, Cu, Pb, Cd, Cr, As, and Ag in the samples were determined using spectrophotometric techniques at the ISO/IEC 17025:2017 accredited laboratory of the Institute of National Analytical Research and Service (INARS), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka Bangladesh. All elements were measured using an Atomic Absorption Spectrophotometer (Model: AA240FS, Varian, Australia), except for P, which was analyzed using an Ultraviolet–Visible Spectrophotometer (Model: UV-1650PC, Shimadzu, Japan) in the colorimetric method. The details of the experimental parameters are provided in Supplementary Table S1. To produce highly reliable data, the scheme of quality assurance and quality control during the analysis of samples was almost the same as those of previous works [33,34,35]. All samples were measured in triplicates (relative standard deviation was less than 8%) and the mean results were reported for each chemical element.

## 3. Results and discussion

### 3.1. Proximate composition of silkworm pupae

The proximate composition of the silkworm pupae is displayed in Table 1. In this work, the protein content of fresh silkworm pupae was found to be 10.77%, which is slightly lower than the protein content (11.70%) of the previously published report for silkworm pupae [19], though it was noticed that the protein percent (10.30%) of the edible insect *Acheta domesticus* was a little bit lower than our finding [36]. However, it was investigated that the protein content of the eri silkworm was 16 and 24% in pupae cultured on two different types of leaves tapioca and castor, respectively [37]. In general, most of the edible insect's pupae and adults are enriched with high protein (more than 10 g proteins/100 g edible portion of fresh weight basis) [38]. On a dry weight basis, the protein content (49.43%), in this work, was slightly less than previous findings (approximate range: 51–53%) [12,19,37].

In this study, the fat content of the fresh silkworm pupae was found to be around 6.25%, which is the same as the precedent study [19], on the other hand, lower than the fat content (8.0–8.6%) in eri silkworm pupae mentioned by Longvah et al. [37]. Studies observed that the different sexes of the silkworm *Bombyx mori* pupae are responsible for a diverse range of fat content [15]. On a dry weight basis, the fat content (27.82%) in this work was found to be higher than in the former investigations [12,19,37]. The total carbohydrate (3.95%) and crude fiber (1.05%) contents of this work were largely consistent with the findings of the earlier research [19,37]. However, these values greatly vary on the insect's diet, species, and life stage [15]. According to the former research, silkworm pupae contain 30–55% protein, 10–15% carbohydrates, and 25–30% oil on a dry weight basis, which is enriched with several saturated and unsaturated fatty acids [39,40,41].

### 3.2. Oil yield of silkworm pupae by the acid fermentation process

In our work, the acid fermentation process was replicated three times and the oil yield was 3.52 ± 0.23% on fresh pupae, slightly higher than the extracted oil by saline aqueous process [19]. Earlier studies reported that the yield of the extracted oil from silkworm pupae significantly depends on the applied methods, time, and species [19]. Hence, the few diversifications in the overall findings of oil yield of silkworm pupae are observed in this work compared to the former studies, which may be accomplished by utilizing the varieties of the applied methodology, nourished food sources, and geographical locations.

**Table 1**
Proximate compositions of silkworm pupae, yield of oil by acid fermentation, and physicochemical characteristics of silkworm pupae oil.

| Proximate compositions | | | Physicochemical characteristics | |
|---|---|---|---|---|
| Parameters | Content (g per 100 g) | | Characteristics | Results |
| | Fresh Pupae | Dry Basis | Moisture content (%) | 0.06 ± 0.00 |
| Moisture | 78.26 ± 0.21 | – | Density (g/ml) at 25 °C | 0.912 ± 0.03 |
| Protein | 10.77 ± 0.08 | 49.43 ± 0.75 | Specific gravity (g/ml) at 25 °C | 0.919 ± 0.05 |
| Crude fat | 6.25 ± 0.21 | 27.82 ± 0.85 | Refractive index at 25 °C | 1.43 ± 0.01 |
| Total carbohydrate | 3.96 ± 0.07 | 16.75 ± 0.32 | Viscosity, cst | 30.34 ± 1.25 |
| Crude fiber | 1.05 ± 0.03 | 4.85 ± 0.81 | Free Fatty Acid (as oleic %) | 2.14 ± 0.13 |
| Ash | 0.89 ± 0.02 | 3.96 ± 0.15 | Acid value (mg KOH/g) | 1.35 ± 0.01 |
| | | | Peroxide value (meq/kg) | 4.82 ± 0.21 |
| Yield of oil (acid fermentation) | 3.52 ± 0.23% | – | Iodine value (g/100 g) | 129.23 ± 1.53 |
| | | | Unsaponifiable matter (%) | 3.1 ± 0.45 |

Note: Values are means ± standard deviations of triplicate analyses.

### 3.3. Physicochemical properties of silkworm pupae oil

The physicochemical properties of silkworm pupae oil are summarized in Table 1. In this research, the moisture content is found to be 0.06% which is lower than the value of moisture content (0.2%) in edible oils [42]. The higher moisture content (more than 0.2%) is responsible for the growth of the fungus species including, *Aspergillus niger* and Mucor species on the material surface [43]. In this regard, our extracted silkworm pupae oil seems good. In our investigation, the density (0.91 g/mL at 25 °C) of silkworm oil was similar to the density value of eri silkworm pupae oil reported by Ravinder et al. [44]. Furthermore, the specific gravity (0.92 g/mL) and refractive index (1.43) of the oil extracted by this acid fermentation process were largely consistent with the previous studies [11,45]. The oil's viscosity refers to a property that resists oil flow. In the current work, the viscosity of the extracted oil (Table 1) was slightly less than the eri silkworm pupae oil noticed by earlier research [44].

Free fatty acid (FFA) is considered edible oil's most important quality parameter. In this study, the FFA value (Table 1) was similar to the value of eri silkworm pupae oil reported in the literature [44], but higher than avocado oil (0.29–0.38 as oleic %) [46]. The acid value (AV) (1.35 mg KOH/g) and peroxide value (PV) (4.82 meq/kg) of oil extracted by the acid fermentation process in the present investigation were slightly lower than oil extracted by the microwave-assisted extraction method reported by Hu et al. [11] but higher than oil extracted by saline aqueous process [19]. From this result, it can be summarized that the silkworm pupae oil with low AV and PV could be considered a good quality oil according to Codex Standards [47]. Additionally, the iodine value (IV) of oil (129.2 g I/100 g oil) in this work was comparably higher than oil extracted by microwave-assisted extraction and Soxhlet extraction reported by previous research [11]. Comparison with the previous works on plant oils of almost similar profiles shows that our extracted silkworm pupae oil has higher IV than the avocado (80–85 g I/100 g) [11] and papaya (66–69 g I/100 g) [48] seed oils, but almost similar IV with sunflower (120–127 g I/100 g) [49] and Kalahari melon (125–141 g I/100 g) [50] seed oils. Furthermore, it was found that the unsaponifiable matter for oil was 3.1% which is mostly consistent with the documented result for palm oil [51]. Hence, the overall results of physicochemical parameters for the silkworm pupae oil revealed that the oil obtained by our acid fermentation process fulfilled almost all qualities of oils and could be used as a good source of edible oil.

### 3.4. Fatty acid composition of silkworm pupae oil

Table 2 presented the fatty acid composition of the silkworm pupae oil extracted from the acid fermentation process. In this experiment, the vital fatty acid constituents were myristic acid (C14:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and linoleic acid (18:2). The saturated fatty acids (SFA) were approximately 54.82% among total fatty acids which dominated among the total fatty acids, but this result was significantly higher than previous studies [11,19]. Palmitic (32.04%) and stearic (C18:0, 10.95%) acids were the main acids found in SFA which corresponded with previously published data [12,19], though in avocado oil, palmitic acid (17%) was the main SFA like this extracted oil [46]. The total unsaturated fatty acids (USFA) were about 45% including, polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) which were significantly less than the previously reported data [11,17,19]. However, PUFA in extracted oil was lower than PUFA in avocado oil [46]. Furthermore, oleic acid (18:1) in MUFA and linoleic acid (C18:2, n-6) in PUFA were the main components in the extracted oil, which are mostly in agreement with the findings of Tangsanthatkun et al. [19] and Tan [52]. Furthermore, a literature comparison exhibits the oleic acid (18:1) of the extracted oil is in agreement with avocado oil's oleic acid (30–61%) [46,52] but, lower than papaya seed oil (70–78%) [53]. However, the different ratios of the fatty acid composition may be due to the variations in extraction processes and factors, such as the origin of species, maturation stage, sex, season, and geographical regions, as observed in previous studies [11,14].

**Table 2**
Fatty acid compositions the silkworm pupae oil and concentrations of chemical elements in the fresh silkworm pupae and silkworm pupae oil obtained from acid fermentation extraction.

| Fatty acid compositions | | Concentrations (mg/kg) of chemical elements | | |
|---|---|---|---|---|
| Fatty acids | (% Total fatty acids) | Elements | Fresh silkworm pupae | Silkworm pupae oil |
| **Saturated fatty acids (SFA)** | **54.82 ± 1.98** | Fe | 29.2 ± 0.4 | 9.42 ± 1.6 |
| Lauric acid (C12:0) | 2.84 ± 0.12 | Mn | 9.15 ± 0.4 | 0.72 ± 5.7 |
| Myristic acid (C14:0) | 7.26 ± 0.09 | Na | 218.1 ± 1.5 | 106.9 ± 0.7 |
| Palmitic acid (C16:0) | 32.04 ± 1.44 | K | 15589.2 ± 0.4 | 479.5 ± 0.3 |
| Behenic acid (C22:0) | 1.73 ± 0.07 | Ca | 2176.7 ± 1.2 | 159.0 ± 0.2 |
| Stearic acid (C18:0) | 10.95 ± 0.53 | Mg | 3856.7 ± 1.5 | 71.1 ± 0.3 |
| | | Zn | 247.7 ± 0.2 | 2.91 ± 0.2 |
| **Monounsaturated fatty acids (MUFA)** | **35.16 ± 1.06** | P | 9520.8 ± 0.1 | 979.2 ± 0.1 |
| Palmitoleic acid (C16:1) | 0.34 ± 0.01 | Co | 0.35 ± 2.6 | 0.32 ± 6.5 |
| Oleic acid (C18:1) | 34.82 ± 1.01 | Ni | 1.13 ± 0.6 | 1.00 ± 6.8 |
| | | Cu | 1.35 ± 2.3 | 0.20 ± 5.6 |
| **Polyunsaturated fatty acids (PUFA)** | **10.41 ± 0.34** | Pb | 0.37 ± 0.8 | 0.17 ± 1.5 |
| Linoleic acid (C18:2, n-6) | 6.70 ± 0.29 | Cd | 0.18 ± 3.9 | 0.14 ± 1.3 |
| Linolenic acid (C18:3, n-3) | 1.14 ± 0.12 | Cr | 0.38 ± 7.8 | 0.22 ± 6.1 |
| Dihomo-Gamma-linolenic acid (C20:3) | 2.57 ± 0.08 | As | 0.09 ± 0.9 | 0.03 ± 4.9 |
| | | Ag | 0.38 ± 7.3 | 0.27 ± 0.2 |

Note: Values are means ± standard deviations of triplicate analyses.

### 3.5. Chemical analysis of the extracted silkworm pupae oil using FTIR spectroscopy

Fig. 4 presents the FTIR spectra of the produced oil by acid fermentation extraction. From Fig. 4, the band at 3010.41 $cm^{-1}$ is ascribed to the = C–H stretching vibration of the alkene group [54]. The robust absorbance between 2853.3 and 2923.1 $cm^{-1}$ was similar to the aliphatic C–H stretching vibration and displayed a high quantity of methyl and methylene groups [55,56]. The absorption peak near 1743.7 $cm^{-1}$ showed the C═O group stretching vibration in ketones or carboxylic acids, which was in agreement with a large number of ketones in the oil. The low-intensity peak lining at~1600 $cm^{-1}$ was consistent with the presence of alkenes (-C═C- stretch) groups. The absorbance peaks between 1376.8 and 1462.8 $cm^{-1}$ represented the X–H stretching vibrations (X = C, N). These peaks proved the presence of triglyceride functional groups in the silkworm oil. The presence of aromatic amine (C–N stretch) was confirmed by the absorption peak at 1160 $cm^{-1}$. The band at 1097.9 $cm^{-1}$ indicated the ester group (C–O stretch or C–H bend). The FTIR bands reported in this investigation are mostly similar to the previously published works for the extraction of fat content from other feedstocks and insects [56,57,58,59]. The band at 721.46 $cm^{-1}$ was referred to as the rocking vibration of the $–CH_2–$ group.

### 3.6. Elemental contents in the fresh silkworm pupae and extracted oil

Table 2 showed the concentrations (mg/kg) of the analyzed chemical elements in the fresh silkworm pupae and silkworm pupae oil samples. In these findings, the concentration of K (15589.2 mg/kg) was the highest among other mineral contents in silkworm pupae powder which is, however, two times less than the K content (34000 mg/kg) of silkworm *Antheraea pernyi* [60]. Though P content (979.2 mg/kg) among other elements was highest in silkworm pupae oil, this was similar to the range of olive oil (900–1145 mg/kg) [61]. In the present study, the ratio of Na/K is significantly lower (0.01) in silkworm pupae than the Na/K ratio (0.08) of silkworm *Antheraea pernyi*, which is good for health because of reducing the risk of stroke, hypertension, and cardiovascular disease, etc. [62,63], but higher in silkworm pupae oil (0.22) which is still in the safe limit (<0.5) [62]. Other minerals such as Mg, Zn, P, and Ca concentrations in silkworm pupae are at a good level but slightly lower than previously observed data [64]. In addition, Fe and Na concentrations in the extracted oil are found higher than in sunflower oil [65]. Furthermore, Mg, Zn, P, and Ca concentrations in extracted silkworm pupae oil were lower than earlier mentioned mineral concentrations in olive [61] but higher than in rice bran and sunflower oils [65,66]. Nevertheless, the concentrations of elements, Pb and As in both silkworm pupae and extracted oil were less than the acceptable level (Pb: 0.5 mg/kg, As: 0.1 mg/kg) for food and feed according to FAO [67] and Codex Standards [47].

The overall results of the elemental contents in the fresh silkworm pupae and silkworm pupae oil revealed that the concentrations of all the measured elements are lower in the extracted oil than in the fresh silkworm pupae. This is plausible since most of the elements will be removed after treating silkworm pupae with acetic acid and citric acid in the oil extraction process, forming the soluble acetate and citrate salts. However, it is interesting to note that even after acid treatments of silkworm pupae, the extracted oil still contains a significant amount of some minerals such as Fe, Na, K, Ca, Mg, Zn, and P, with a negligible concentration of toxic elements such as Mn, Co, Ni, Cu, Pb, Cd, Cr, As, and Ag. The sources of the minerals in the extracted oil may be attributed to the strong bindings of the metals within the core structure of the biomolecules present in the silkworm pupae or to the presence of some inorganic materials, which are not removable by the simple acid treatment of the silkworm pupae with the weak organic acids (acetic acid and citric acid). The considerable presence of the minerals in the extracted silkworm pupae oil enhanced the nutritive value of the oil extracted from the silkworm pupae through the adopted acid fermentation process.

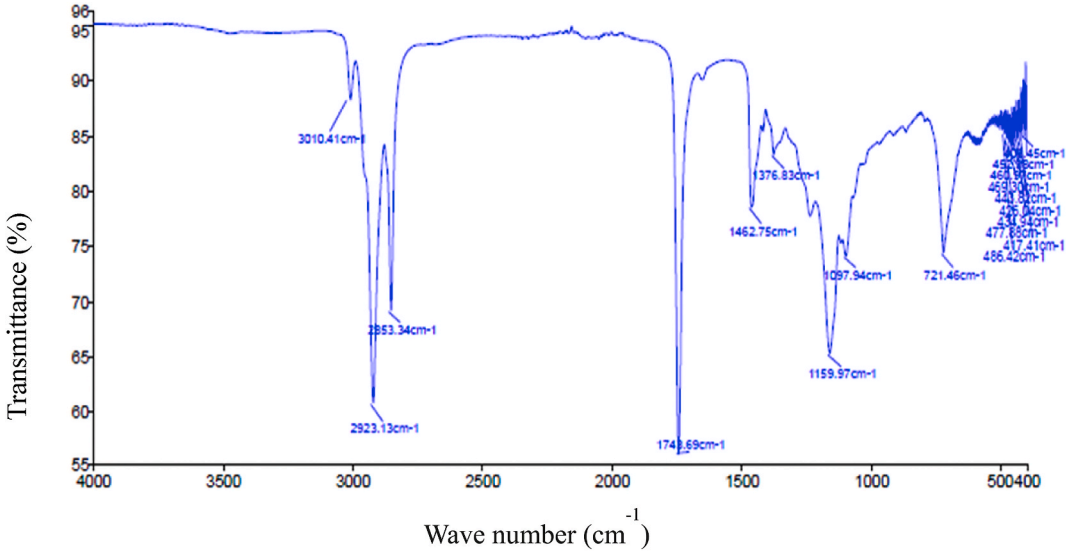


**Fig. 4.** FTIR spectra of the extracted oil by acid fermentation.

## 4. Conclusions

For the first time in this work, an acid fermentation process was applied for the extraction of oil from the fresh desilked silkworm pupae (*Bombyx mori*). The yield of the oil was 3.52 ± 0.23% in fresh pupae. The produced oil has a low peroxide value (4.82 meq/kg) and an acid value (1.35 mg KOH/g) indicating its good quality. The proximate composition of the silkworm pupae demonstrated that it is a nutrient resource, including protein, fat, and carbohydrates. The extracted oil contains negligible moisture content, considerably less viscosity, and comprises a wide variety of fatty acids, covering a large percentage of palmitic acid (32.04%) and oleic acid (34.62%). In addition, the oil is comprised of health beneficiary α-linolenic acid, linoleic acid, and dihomo-gamma-linolenic acid. FTIR analysis of the oil revealed the presence of several functional groups of organic molecules such as ketones, carboxylic acids, alkenes, triglycerides, aromatic amines, and esters. The oil contains considerable amounts of minerals such as Fe, Na, K, Ca, Mg, Zn, and P but with only a shallow content of toxic elements such as Mn, Co, Ni, Cu, Pb, Cd, Cr, As, and Ag, indicating its good nutritive value. Overall, the tested findings of several physical and chemical parameters suggested that the silkworm oil attained from this facile extraction process could be a potential candidate to be utilized as edible oil. However, the percentage of oil yield is still not at a very high level restricting its use to a small scale only. Further, the method also takes a long time for oil extraction. Thus, to be utilized this method extensively on a large or industrial scale, further fruitful research should be conducted to increase the percentage of oil yield in a short time through the probable apt modifications in the extraction steps as well as adopting suitable chemicals and reagents.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2023.e12815.

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