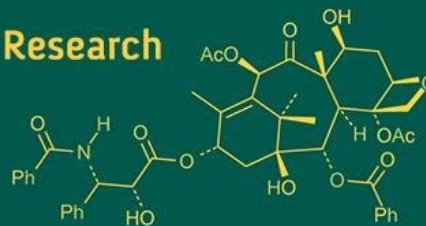


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Influence of extraction techniques on the quality characteristics of silkworm pupal oil: A comparison of Soxhlet and maceration method

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Abstract

Silkworm pupae, a valuable by-product of sericulture, represent a rich source of proteins, essential fatty acids, cholesterol, chitin and vitamins, with potential nutritional, cosmetic and pharmaceutical applications. The present study ascertained oil extraction from silkworm pupae using two methods: Soxhlet and maceration. The quality characteristics analyzed included physicochemical properties and compositional characteristics (pH, color, moisture, viscosity, carotenoids, cholesterol, unsaponifiable matter and tyrosinase inhibition activity) and oxidative stability indices (free fatty acid, acid value, peroxide value, p-aniside value, TOTOX value and iodine value). Results indicated that the quality characteristics have been significantly affected by different extraction methods. The comparative analysis revealed distinct variations between the two extraction techniques: Soxhlet showed pH (6.17), color (14Y + 1.5R), moisture (0.1%), viscosity (35.00 cP), carotenoids (10 µg/g), cholesterol (154.96 µg/mL), unsaponifiable matter (1.81 %), and tyrosinase inhibition activity (IC₅₀ = 25.04 mg/mL) and maceration showed pH (6.0), color (14Y + 1.2 R), moisture (0.2 %), viscosity (31.00 cP), carotenoids (12 µg/g), cholesterol (160.825 µg/mL), unsaponifiable matter (1.60 %) and tyrosinase inhibition activity (IC₅₀ = 6.60 mg/mL). The Soxhlet method showed free fatty acid (1.40 %), acid value (2.8 mg KOH/g), peroxide value (2.44 meq/Kg), P-aniside value (5.004), TOTOX (9.8) and iodine value (120 gI₂/100 g), while as the maceration method had showed free fatty acid (0.95 %), acid value (1.9 mg KOH/g), peroxide value (2.00 meq/Kg), P-aniside value (5.267), TOTOX (9.2) and iodine value (119 gI₂/100 g). These results affirm the influence of the extraction methods on the quality indices of the silkworm pupal oil.

Keywords: Extraction, silkworm pupal oil, quality characteristics, Soxhlet, maceration

1. Introduction

Insects are considered very important for the survival of humankind and deliver several kinds of ecological services for agriculture and nature. They play a crucial role in improving soil fertility through waste bio-degradation (Sarpong *et al.*, 2019) [52], controlling infestation of harmful pests as natural bio-control agent (Godfray & Godfray, 1994) [20] and facilitating plant reproduction as pollinators (Ingram *et al.*, 1996) [26]. Silkworm *Bombyx mori* L, is an economically important lepidopteran insect that has been domesticated for 5000 years now. Silkworms are reared under controlled environmental conditions for the production of cocoons which are then reeled into silk. Silk is one of the exquisite natural fibers that has immense properties like strength, luster, softness etc. as compared to other natural and man-made fibers. India produced 41,121 MT of raw silk during the year 2024-2025 (Anonymous, 2025) [4].

Like all other agro-industries, sericulture industry also generates a great deal of waste in the process of silk production. About 2 Kg of dried pupae is obtained as by-product during reeling process of producing one Kg raw silk (Sadat 2022) [51]. The Indian silk industry generates approximately 2.82 lakh metric tonnes of spent pupae annually (Arasakumar *et al.*, 2021) [5]. Spent silkworm pupae are highly degradable product of silk reeling sector and the unscientific disposal of large quantities of pupae can not only cause serious threat to environment but also to human health and ecology.

Recently, the studies on properties of de-silked silkworm pupae have attracted considerable attentions in the world and these are considered to be a good source of a large number of bioactive substances.

There are many technical processes involved in the extraction of oils from the same origin, making the final products different in physicochemical properties and nutritional values (Issaoui *et al.*, 2019) [27]. Solvent extraction is one of the cheapest and most efficient techniques for producing edible oils (Luque de Castro and Priego-Capote 2010) [32]. In the solvent extraction method, oil seeds are grinded and then placed in a suitable solvent to extract the oil from the solid matrix to the liquid phase. Solvent extraction techniques, such as maceration (cold solvent extraction) and Soxhlet (hot solvent extraction), utilize organic solvents to extract oil (Suo *et al.*, 2018) [56]. However, limited information is available about qualitative comparison of silkworm pupal oil obtained by Soxhlet and maceration. The objective of this study was to determine the effect of Soxhlet and maceration extraction on quality characteristics of silkworm pupal oil.

2. Materials and Methods

2.1 Collection of silkworm pupae

Spent silkworm pupae were collected from the local cocoon reeling unit. The collected fresh spent pupae were subjected to drying in the hot air oven at 85-90 °C until the moisture content reached below 10% level. The dried pupae were ground to powder and oil extraction was done using hexane as organic solvent.

2.2 Oil extraction

2.2.1 Soxhlet extraction

Dried silkworm pupal powder (5 g) was packed in a thimble, placed in Soxhlet apparatus (Fig.1) then extracted with hexane 79.52 mL and refluxed for 8.49 hours at 83.08 °C. The recovered oil was then used to determine the oil quality parameters.

2.2.2 Maceration extraction

150g of silkworm pupal powder sample was taken into a flask and 150.71 mL of hexane was added. This was agitated and sealed (Fig.2). The sample was shaken intermittently for 7.11 days and then filtered. The filtered oil was separated from solvent by a rotary vacuum evaporator. The recovered oil was then used to determine the oil quality parameter.



Fig 1: Soxhlet extraction of silkworm pupal oil



Fig 2: Maceration extraction of the silkworm pupal oil

2.3 Determination of physicochemical properties and compositional characteristics

2.3.1 pH

An amount of 0.1 g of oil sample was dissolved in a mixture of ethanol and water. The pH of the solution was measured by a pH meter. After calibrating with phosphate-buffer saline, the electrode was dipped in silkworm pupal oil and the pH was recorded.

2.3.2 Colour

Colour was determined by Lovibond Tintometer. The colour of the oil was reported in terms of Lovibond units as follows:

$$\text{Colour reading} = a Y + 5 b R$$

Where,

a = sum total of the various yellow slides (Y) used

b = sum total of the various red (R) slides used

Y + 5 R = mode of expressing the colour of the light-coloured oils

2.3.3 Moisture (%)

Silkworm pupal oil was weighed in a previously dried and tared dish. Then it was heated in an oven at 105 ± 1 °C for 1 hour. Then the dish was removed from the oven. Cooled in a desiccator containing phosphorus pentoxide and weighed. Heated in the oven for a further period of 1 hour, cooled and weighed again. This process was repeated until change in weight between two successive observations did not exceed 1 mg. It was calculated by using the formula

$$W1/W \times 100$$

Where,

W1 = loss in weight (gm) of the material on drying

W = weight in gm of the material taken for test

2.3.4 Viscosity (cP)

The viscosity was measured by using a viscometer (Anton Paar/Visco QC 300), 20 rpm and spindle No.2 at 30 °C.

2.3.5 Carotenoids (µg/g)

The carotenoid content was estimated by following the protocol given by Borello *et al.*, (2019) [7]. The carotenoid estimation was carried out by the preparation of a 1% solution of silkworm pupal oil in cyclohexane followed by measuring the absorbance at 470 nm. Carotenoid content was calculated using the following formula:

$$\frac{A_{470} \times 10^6}{2000 \times 100 \times d}$$

where

A_{470} corresponds to absorbance at 470 nm

d corresponds to optical path length of the cell (1 cm).

2.3.6 Cholesterol ($\mu\text{g/mL}$)

The Bligh and Dyer (1959) [6] method was used to estimate the total fat content in the sample. The stock solution of each sample was taken and considered 100% and then prepared three different dilutions from the stock solution (1%, 10% and 100%) in respective solvents as per their solubility. 50 μL of sample was centrifuged for 5 minutes to get separate layers. The lower layer was collected in a separate tube. Again 1 ml of chloroform was added to the mixture and mixed and centrifuged for 5 minutes and the lower layer was collected in preweighed tube. The above procedure was repeated two more times to collect lower layer in separate tube. 100 μL of concentrated sulphuric acid was added to each tube of sample and incubated for 20 minutes at 90 °C and after incubating, 10 μL from each tube was transferred to 96 well plate and 100 μL Vanillin-Phosphoric acid reagent (SRL Chem-20563) was added to each well as per experimental setup. 50 μL of Perilla oil (Salvia Cosmeceuticals) was used as Standard. Finally, Absorbance was measured using ELISA Microplate reader (iMark Biorad-USA) at 540 nm after 10 minutes of color development.

2.3.7 Unsaponifiable matter (%)

5 g of oil sample was taken in a 250 mL conical flask and 50 ml of potassium hydroxide solution was added to it. Then the content was boiled for one hour until the saponification was complete. Then 50 ml of petroleum ether was added to the flask and shaken vigorously, and the layers were allowed to separate. Transferred the lower soap layer into another separating funnel and repeated the ether extraction for another 3 times using 50 ml portions of petroleum ether. Then evaporated the ether. When all ether was removed, 2-3 mL acetone was added and while heating in water bath, the solvent was completely removed under a gentle air. Then the residue was dissolved in 50 ml of warm ethanol and titrated with 0.02N NaOH. The Unsaponifiable matter was calculated by using the formula

$$100(A-B)/W$$

Where,

A = weight in g of the residue

B = weight in g of the free fatty acids in the extract as oleic acid = 0.282 VN

V = volume in ml of standard sodium hydroxide

N = normality of standard Sodium hydroxide

W = weight in g of the sample

2.3.8 Tyrosinase inhibition activity

Oil samples at concentrations of 100, 50, 25, 12.5, 6, and 3 mg/mL, along with the standard antioxidant's vitamin C and kojic acid, were prepared in 5% (v/v) DMSO and evaluated for tyrosinase inhibitory activity using the modified dopachrome method with L-tyrosine as the substrate, as described by Piao *et al.* (2002) [44]. Briefly, 40 μL of each sample or standard solution was mixed with 40 μL of 0.1

mg/mL L-tyrosine, 50 μL of 0.1 mg/mL mushroom tyrosinase, and 80 μL of 0.1 M phosphate buffer in a 96-well microplate. A solution of 5% (v/v) DMSO served as the negative control. The reaction mixtures were incubated at 37 °C for 60 min. Before and after incubation, the amount of dopachrome produced in the reaction mixture was measured at 450 nm. It was calculated by using the formula $[(A-B) (C-D)]/(A-B) \times 100$

Where

A = the absorbance of the blank after incubation

B = the absorbance of the blank before incubation

C = the absorbance of the sample after incubation

D = the absorbance of the sample before incubation.

Sample concentrations providing 50% inhibition (IC_{50}) were calculated from the graph plotted between tyrosinase inhibition activity percentages and the concentrations.

2.4 Determination of Oxidative stability indices

2.4.1 Free fatty acids (%)

Free fatty acids of oil samples were determined by following the procedures of Ravi *et al.* (2015) [49]. The sample solution was prepared by dissolving 10 g of sample in 50 mL chloroform. The solution was placed in an orbital shaker for 1 hour followed by filtration through a filter paper. 20 mL of the filtrate was pipetted out into an iodine flask to which 50 mL of benzene alcohol mixture was added followed by few drops of indicator solution. The solution was then titrated against standardized alcoholic alkali (potassium hydroxide) solution to a pale pink end point. Free fatty acid (%) was estimated according to the equation given below

$$\text{Titre} \times N \times 28.4 / \text{weight of the sample}$$

where

Titre = mL of potassium hydroxide used

N = normality of potassium hydroxide

2.4.2 Acid value (mg KOH/g)

5.0 g of silkworm pupal oil was taken in a dried conical flask. Then 25 ml of absolute ethanol alcohol and 2-3 drops of phenolphthalein were added. Then it was heated in water bath for 10 minutes, and then cooled and titrated the solution against 0.1 N KOH until pink color appeared. The acid value (AV) was calculated by using formula

$$\text{ml of KOH} \times N \times 56 / \text{Weight of Sample}$$

where

N = normality of potassium hydroxide

2.4.3 Peroxide value (meq/kg)

5 g of silkworm pupal oil sample was put into a 250 ml stoppered conical flask and 30 ml acetic acid chloroform solvent mixture was added to it and swirl to dissolve. Then 0.5 ml of saturated potassium iodide solution was added with a Mohr pipette and kept for 1min in dark with occasional shaking, and then 30 ml of water was added to it. Then the liberated iodine was titrated with 0.1 N sodium thiosulphate solution, with vigorous shaking until yellow colour was almost gone. 0.5 mL starch solution was used as indicator until blue colour disappeared. It was calculated by using the formula:

Titre $\times N \times 100 / \text{Weight of the sample}$

Where

Titre = mL of sodium thiosulphate used

N = normality of sodium thiosulphate solution.

2.4.4 p-anisidine value (p-AV)

The p-anisidine value of the silkworm pupal oil samples was measured using the methodology by Delfanian *et al.*, (2018) [13]. 4 g of the oil sample was diluted in 25 mL of volumetric flask with isooctane. After dissolution, 5 mL of the solution was transferred to a test tube, covered in aluminium foil, followed by the addition of 1 mL of p-anisidine and kept at a dark place for 8 min. 4 ml of the solution was mixed in a volumetric flask with 1 ml glacial acetic acid and kept in a dark place for 8 min. Both the samples were examined at 350 nm using a spectrophotometer. p-anisidine value was calculated by using the formula:

$$25 \times [1.2 \times (A_s - A_b)] / W$$

Where,

A_s = the absorbance of the fat solution after reaction with the p-anisidine reagent

A_b = the absorbance of the fat solution

W = weight of oil used, in grams.

2.4.5 TOTOX value

TOTOX is an empirical parameter representing the oxidation state of the fat and its potential to evolve into additional oxidation products (Myat *et al.*, 2009) [37]. It was calculated by using the formula:

$$\text{TOTOX} = 2\text{PV} + \text{p-AnV}$$

Where,

PV = peroxide value

p-AnV = p-anisidine value

2.4.6 Iodine value (gI₂/100 g)

0.25 g of silkworm pupal oil was taken in a 250 mL conical flask and then 10 ml of chloroform, 30 ml of Hanus solution was added to it and the flask was completely closed by para film, and then the solution was left for 30 minutes with shaking continuously on shaker. Then 10 mL of 15% potassium iodide solution was added and then shaken. 100 mL of distilled water (DW) was also added. The iodine solution was titrated against 0.1 N Sodium thiosulfate solution till appearance of yellow colour, then 2-3 drops of starch solution were added till the appearance of blue solution and then continued with the titration till the blue colour disappeared. Iodine value was calculated by using the formula:

$$(B-S) \times N \text{ of Na}_2\text{S}_2\text{O}_3 \times 0.127 \text{ g/meq} \times 100 / \text{weight of the sample (g)}$$

Where,

B = ml of $\text{Na}_2\text{S}_2\text{O}_3$ volume for blank

S = ml of $\text{Na}_2\text{S}_2\text{O}_3$ volume for sample

4 Results and Discussion

4.1 Physicochemical properties and compositional characteristics

Silkworm pupal oil extracted by Soxhlet and maceration methods in the present study was analysed for different physicochemical and compositional characteristics and the results are given in Table 1.

Table 1: Physicochemical properties and compositional characteristics of silkworm pupal oil extracted by Soxhlet and maceration

Parameter	Silkworm pupal oil (Soxhlet method)	Silkworm pupal oil (Maceration method)	t Stat	P value
pH	6.17	6.0	4.075	<0.01
Color (Lovibond units; Y+5R)	14Y+1.5R (21.5)	14Y+1.2R (20)	3.353	<0.05
Moisture (%)	0.1	0.2	50.00	<0.01
Viscosity(cP)	35.00	31.0	80.903	<0.01
Carotenoids ($\mu\text{g/g}$)	10	12	8.728	<0.01
Cholesterol ($\mu\text{g/ml}$)	154.96	160.825	11.080	<0.01
Unsaponifiable matter (%)	1.81	1.60	2.003	<0.05
Tyrosinase inhibition activity (IC ₅₀)	25.04 (mg/mL)	6.60 (mg/mL)	296.15	<0.01

4.1.1 pH

The pH of silkworm pupal oil extracted by the Soxhlet was found significantly ($p < 0.01$) higher (6.17) compared to the maceration (6.0). Srivastava *et al.*, (2023) [55] also observed similar pH value of silkworm pupal oil extracted by Soxhlet. Similar observation was demonstrated by Adegbanke and Bada (2024) [2] while comparing the oil extraction from clove and ginger using maceration and Soxhlet methods. Soxhlet operates at elevated temperature with continuous solvent reflux, which can enhance the removal of acidic impurities and degradation of certain acidic components, leading to a slightly higher pH in the extracted oil, while as maceration is done at room temperature with milder conditions, which may leave more acidic compounds intact in the oil, resulting in a comparatively lower pH. pH makes silkworm pupal oil slightly acidic but well within the neutral range for edible oils, supporting its stability and commercial potential.

4.1.2 Color

The colour of the silkworm pupal oil extracted by Soxhlet appeared as orange which corresponds to the values 14Y + 1.5 R (21.5 Lovibond units). The colour of the silkworm pupal oil extracted by maceration appeared as Yellow-orange which corresponds to the values 12Y + 1.2 R (20 Lovibond units). The colour of the silkworm pupal oil extracted by Soxhlet was significantly ($p < 0.05$) darker as compared to the silkworm pupal oil extracted by maceration. Similar results related to the effect of extraction methods on the color intensity were observed by Adegbanke and Bada (2024) [2] while studying comparative analysis of oil extraction from clove and ginger using maceration and Soxhlet extraction methods. The Soxhlet performs at higher temperature and prolonged heating which leads to the oxidation, thermal degradation of pigments, Maillard reactions between proteins and sugars present in the sample thereby making the oil darker as compared to maceration. Color in oil is an important measurement to predict quality and acceptability of edible oil (Muzzio *et al.*, 2014) [36].

Color is not related to chemical or physical properties only (Pedro and Ferreira, 2005) ^[43] but also to the consumer's perception of the product quality (Francis, 1995) ^[18]. It can affect the subjective qualities like the flavor, odor, or sweetness (Morrot *et al.*, 2001^[35], Wadhvani and McMahon, 2012^[63]).

4.1.3 Moisture (%)

In the macerated silkworm pupal oil, the moisture was significantly ($p < 0.01$) higher (0.2%) and in the Soxhlet extracted silkworm pupal oil it was significantly lower (0.1%). Similar moisture percentage was reported by Arasakumar *et al.*, (2021) ^[5] for Soxhlet extracted silkworm pupal oil. Kouyate *et al.*, (2025) ^[28] also reported higher moisture content in maceration extraction method as compared to Soxhlet extraction. Soxhlet uses hot, refluxing solvent, repeated washing and evaporation cycles that removes water and volatile residues more effectively. Maceration is typically room-temperature soaking, solvent can retain more water in the oil. Moisture is among key factors determining the oils rancidity or deterioration time (Negash *et al.*, 2019) ^[38]. The presence of sufficient amount of moisture favors microbial growth (Okechalu *et al.*, 2011) ^[40]. In both the methods the moisture content was found to be at the optimal level. The maximum allowed moisture content in edible oils is 0.2 % (Yeasmin *et al.*, 2021) ^[65].

4.1.4 Viscosity (cP)

The viscosity was found to be significantly ($p < 0.01$) higher in Soxhlet (35.00 cP) compared to maceration (31.00 cP). Similar Viscosity in silkworm pupal oil extracted by maceration was reported by Dev (2018) ^[14]. These results are in conformity with Raknam *et al.*, (2018) ^[48] who demonstrated that viscosity of para-rubber (*Hevea Brasiliense*) seed oil extracted by Soxhlet was more as compared to maceration. Soxhlet extraction led to a higher viscosity compared to maceration because the extraction in this method occurs at the solvent's boiling point, it is far more effective at solubilizing high-molecular weight compounds which act as natural thickening agents thereby increasing the viscosity of the oil.

4.1.5 Carotenoid (µg/g)

The carotenoid content in silkworm pupal oil was found to be significantly ($p < 0.01$) higher in maceration (12 µg/g) and lower in Soxhlet (10 µg/g). Fajriati *et al.*, (2022) ^[16] also reported lower carotenoid content in Soxhlet extraction method as compared to maceration method. The maceration allows for enough contact between β -carotene and n-hexane solvent thereby increasing the yield of carotenoids in the maceration method (Gul *et al.*, 2015) ^[21]. Adequate carotenoid supplementation significantly reduces the risk of several non-transmissible chronic diseases due to its potential role as an antioxidant (Fiedor and Burda, 2014) ^[17]. Carotenoids are very efficient physical as well as chemical quenchers (Nour *et al.*, 2018) ^[39]. The carotenoid content in silkworm pupal oil was found higher than the carotenoid content of cold-pressed hemp oil, flax oil, and canola seed oil (Teh and Birch 2013) ^[59], crude rapeseed and sunflower oils (Kreps 2014) ^[29].

4.1.6 Cholesterol (µg/ml)

In the silkworm pupal oil, the cholesterol level was found significantly ($p < 0.01$) higher (154.96 µg/mL) in the Soxhlet and lower in the maceration (160.825 µg/mL). These results coincide well with the results of Winitchai *et al.*, (2011) ^[64].

This may be because Soxhlet's higher temperatures and prolonged solvent cycling can degrade or remove more cholesterol from the oil, whereas the gentler maceration (Elouafy *et al.*, 2022) ^[15] preserves more cholesterol in the oil. Elevated cholesterol intake, particularly from dietary sources, is strongly associated with an increased risk of cardiovascular diseases (CVDs), including coronary artery disease, stroke, and peripheral artery disease (Aboagye *et al.*, 2024) ^[1]. The total cholesterol in silkworm pupal oil is less than that of common sausage as reported by Rowe *et al.*, (1991) ^[50].

4.1.7 Unsaponifiable matter (%)

The unsaponifiable matter (%) in Soxhlet extraction was significantly ($p < 0.05$) higher in Soxhlet (1.81 %) and lower in the maceration (1.60%). Similar results related to the unsaponifiable matter (%) in silkworm pupal oil extracted by Soxhlet were reported by Shanker *et al.*, (2006) ^[54]. The unsaponifiable matter (%) was found to be significantly higher in Soxhlet extraction process as compared to the maceration extraction process. Similar results were observed by Ahmed *et al.*, (2019) ^[3] and Ozcan *et al.*, 2019^[41]. Soxhlet extraction uses continuous hot solvent reflux (Zhang *et al.*, 2018) ^[67] which more effectively transfers unsaponifiable compounds from matrix into the oil. In contrast, maceration involves soaking the material in solvent at room temperature, which is less efficient at extracting these components. The unsaponifiable matter generally constitutes about 0.5-2% of oil (Malecka 2002) ^[33]. The unsaponifiable matter of silkworm pupal oil extracted by both the methods was within the range of edible oils. A higher unsaponifiable content might indicate adulteration with substances like mineral oils.

4.1.8 Tyrosinase inhibition activity

The tyrosinase inhibition activity of silkworm pupal oil was found significantly ($p < 0.01$) higher in maceration (IC_{50} = 6.60 mg/ml) compared to Soxhlet (IC_{50} = 25.04 mg/ml). Winitchai *et al.*, (2011) ^[64] also reported similar observations regarding tyrosinase inhibition activity of silkworm pupal oil extracted by Soxhlet and maceration. Similar observations were also demonstrated by Chutoprapat *et al.*, (2020) ^[9] while comparing tyrosinase inhibition activity of bambara groundnut (*Vigna subterranea*) extracted by Soxhlet and maceration extraction methods. Heat involved in Soxhlet extraction may degrade heat-sensitive compounds (De and Garcia-Ayuso 1998) ^[10] responsible for tyrosinase inhibition, resulting in reduced activity in the Soxhlet extraction method. Tyrosinase inhibitors have been a great concern solely due to the key role of tyrosinase in both mammalian melanogenesis and fruit or fungi enzymatic browning (Chang 2009) ^[8]. Tyrosinase inhibitors with high efficacy and less adverse side effects, have huge demand in cosmetic and medicinal industries due to their preventive effect on pigmentation disorders as well as skin-whitening effect (Masum *et al.*, 2019) ^[34]. Silkworm pupal oil owing to its potential tyrosinase-inhibitory activity may be useful in skin-brightening and anti-hyperpigmentation products.

4.2 Oxidative stability indices

Silkworm pupal oil extracted by Soxhlet and maceration methods in the present study was analysed for different oxidative stability indices and the results are given in Table 2.

Table 2: Oxidative stability indices of silkworm pupal oil extracted by Soxhlet and maceration

Parameter	Silkworm pupal oil (Soxhlet method)	Silkworm pupal oil (Maceration method)	t Stat	P value
Free fatty acids (%)	1.40	0.95	42.988	<0.01
Acid value (mg KOH/g)	2.8	1.9	2.071	<0.05
Peroxide value (meq/kg)	2.44	2.00	2.690	<0.05
p-aniside value (p-AnV)	5.004	5.267	7.921	<0.01
TOTOX	9.8	9.2	6.678	<0.01
Iodine value (gI ₂ /100 g)	120	119	13.821	<0.01

4.2.1 Free fatty acids (%)

The free fatty acid percentage in silkworm pupal oil was significantly ($p < 0.01$) higher in Soxhlet extraction method (1.40%) than that of maceration method (0.95%). Shanker *et al.*, (2006) [54] also reported similar free fatty acid percentage of silkworm pupal oil when extracted by Soxhlet method. Uahomo *et al.*, (2023) [62] while assessing the essential oil from lime (*Citrus aurantifolia*) exocarp using maceration and Soxhlet extraction also reported higher free fatty acid (%) in Soxhlet method as compared to maceration method. This could be because the Soxhlet extraction is a more aggressive technique with repeated cycles of boiling and condensation, which can degrade heat-sensitive compounds and increase the free fatty acid content of the oil. In contrast, maceration is a gentler, room-temperature method without heat, minimizing lipid hydrolysis and preserving oil quality, resulting in lower free fatty acid levels. Free fatty acid percentage in silkworm pupal oil extracted by both the methods was found to be less than three percent and it can be regarded as good quality oil (Arasakumar *et al.*, 2021 and Hu *et al.*, 2017) [5, 24]. FFA are not desirable in edible oils because when oils with high FFA content are used in foods, they lower the oxidative stability of the product (Frega *et al.*, 1999) [19], increase acidity and lead to off-flavour formation.

4.2.2 Acid value (mg KOH/g)

In the Soxhlet extraction method the acid value was significantly ($p < 0.05$) higher (2.8 mg KOH/g) compared to the maceration (1.9 mg KOH/g). Patil *et al.*, (2022) [42] also demonstrated similar acid value of silkworm pupal oil when extracted by Soxhlet method. Eloufy *et al.*, (2022) [15] and Piravi-Vanak *et al.*, 2024 [45] also reported higher acid value in Soxhlet method as compared to maceration method. This difference is primarily due to the thermal stress and prolonged heat exposure inherent in the Soxhlet method which accelerates the hydrolytic cleavage of ester bonds in triglycerides, leading to the release of free fatty acids. Acid value is a key parameter that effects the quality of oil (Negash *et al.*, 2019) [38]. Consuming rancid edible oil is unlikely to cause immediate health impact, but can reduce significantly the nutritional value of foods by degrading the essential fatty-acids and nutrients (De *et al.*, 2015 and Li *et al.*, 2015) [11, 30].

4.2.3 Peroxide value (meq/kg)

The peroxide value in silkworm pupal oil was significantly ($p < 0.05$) higher in Soxhlet (2.44 meq/kg) and lower in the maceration (2.00 meq/kg). These results coincide well with the results of Arasakumar *et al.*, (2021) [5]. Putri *et al.*, (2020) [47] also demonstrated higher peroxide value in Soxhlet extraction method as compared to maceration method while determining the physiochemical characteristics of patin fish oil. The lower peroxide value found in maceration-extracted oil could be due to its gentler

extraction conditions, which reduce oxidative stress and thermal degradation compared to the Soxhlet method. The peroxide values of both the extraction methods of silkworm pupal oil were at the desirable level (Thirupathaiah *et al.*, 2016) [60].

4.2.4 p-anisidine value (p-AnV)

In the Soxhlet extraction method the p-AnV was significantly ($p < 0.01$) lower (5.00) and in the maceration extraction method it was significantly higher (5.267). Senphan *et al.*, (2025) [53] also reported that the extraction techniques effect the p-anisidine value of the oil. The p-AnV of the Soxhlet extracted oil was lower as compared to macerated oil. Similar observations were reported by Idrissi *et al.*, (2022) [25]. The continuous reflux and vigorous boiling associated with Soxhlet extraction may facilitate the volatilization (Delaunay-Bertoncini *et al.*, 2001) [12] and partial removal of low-molecular-weight aldehydes, which constitute the primary analytes detected by the p-anisidine assay and thereby decreasing the p-Aniside value of the Soxhlet extracted oil. The results revealed that p-Anisidine value (p-AV) of silkworm pupal oil extracted by both the Soxhlet and macerated methods were in the standard range i.e. $p-AV < 6$ (Tavakoli *et al.*, 2019) [58].

4.2.5 TOTOX value

The silkworm pupal oil extracted by Soxhlet displayed a significantly ($p < 0.01$) higher TOTOX value (9.8) and macerated silkworm pupal oil demonstrated lower (9.2). Idrissi *et al.*, (2022) [25] also reported higher TOTOX value in Soxhlet as compared to maceration in Virginia and Valencia-Type peanut oil. The TOTOX calculation gives double importance to primary oxidation products and the primary oxidation products were found more in Soxhlet leading to a greater overall TOTOX value compared to the cold maceration technique. The TOTOX value of high-quality oil is generally less than 10 (Tavakoli *et al.*, 2019) [58]. Silkworm pupal oil has TOTOX value less than 10 and therefore can be regarded as good quality oil.

4.2.6 Iodine value (gI₂/100 g)

In the silkworm pupal oil, the iodine value was significantly ($p < 0.01$) higher in Soxhlet method (120 gI₂/100 g) and in the maceration method it was lower (119 gI₂/100 g). These results correlate well with the reports of Yeasmin *et al.*, (2024) [66] and Susirirut *et al.*, (2023) [57]. Uahomo *et al.*, (2023) [62] also reported higher iodine value in Soxhlet extraction method as compared to maceration extraction method. The combination of high temperature and solvent flux in Soxhlet resulted in co-extraction of additional unsaturated compounds that contributed to a higher iodine number in Soxhlet. Silkworm pupal oil extracted by both the methods showed iodine value well within the range reported for commonly available edible oils i.e. canola oil has iodine value in the range 110-126 (Gunstone 2009) [22], fish oil has

iodine value in the range of 190-205 (Thomas A 2002) ^[61], walnut oil has iodine value in the range of 132-162 (Prasad 2003) ^[46], cod liver oil has iodine value in the range of 148-183 (Holmes 1929) ^[23].

5. Conclusion

Silkworm pupal oil demonstrated desirable quality characteristics when extracted using both Soxhlet and maceration methods. The extraction methods significantly influenced the physicochemical, compositional, and oxidative stability indices of silkworm pupal oil. Therefore, the selection of an appropriate extraction method needs to be adopted depending upon the intended application of the oil. Nevertheless, silkworm pupal oil exhibited good overall characteristics and can be regarded as a good quality oil having significant potential for a broad spectrum of applications, including cosmetic formulations, nutraceutical products, animal feed supplementation, and biomedical applications.

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