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RESEARCH ARTICLE

Extraction of Oil from Mulberry and Eri Silkworm Pupae and Analyzing the Physio-Chemical Properties for Commercial Utilization

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ABSTRACT

Silkworm pupal oil was extracted using different solvents from spent dried pupal powder by soxhlet and column extraction methods. The oil content from spent silkworm pupae was maximum recovered from mulberry silkworm pupae (25.90 g/100 g) with hexane solvent in the column extraction method. The better physio-chemical properties *viz*., density (0.943 g/mL), specific gravity (0.991 g/mL), saponification value (235.147 g KOH/ g oil), iodine value (131.653 g I/ 100 g) and free fatty acids (5.057 %) were observed in the eri silkworm pupal oil, whereas, maximum acid number (1.966 mg KOH/g oil) and peroxide value of (2.44 ppm) was noticed in mulberry silkworm pupal oil. Hence, mulberry silkworm pupae were effectively used for oil extraction by column extraction method using hexane, and these physio-chemical properties of pupal oil were achieved the desired level for utilizing the silkworm pupae oil as commercial utilization.

Keywords: *Silkworm pupae;Oil extraction;Physio-chemical properties*

INTRODUCTION

Insects play a crucial role as a source of edible by-products in the history of human nutrition in developing countries all over the world. The consumption of cooked insects has been documented in countries like India, Japan, Thailand, Africa, Latin America, Australia, Mexico, etc., where they represent a cheaper source of good quality protein and fat (Deori *et al.,* 2014). Pupae of sericigenous insects *viz.,* mulberry silkworm, *Bombyx mori,* and eri silkworm, *Samia ricini* are valued as food material in northeastern parts of India, apart from exploitation for silk. The pupae remaining after reeling the silk from the cocoon, goes as waste and are often discarded in the open environment or used for fertilizers (Wei *et al.,* 2009). Silkworm pupae constitute 60 per cent of dry cocoon weight and it is estimated that 40,000 metric tonnes of dry spent silkworm pupae are produced annually (Mahesh *et al.,* 2015). The main ingredients of silkworm pupae are protein (51 %), essential fatty acids (29 %), cholesterol (3 %), chitin, and vitamin A, B_2, and D, with these vitamins being safe and very important to human nutrition (Heo *et al.,* 2007).

Silkworm pupae contain 25-30 per cent of oil; harvesting oil from spent silkworm pupae could help augment the acute shortage for edible oil in the food and feed industry in the country (Longvah *et al.,* 2012). Several studies have focused on lipid extraction methods applied to insects. Most were performed on wild-caught edible insects using organic solvents for lipid extraction (Laroche *et al.,* 2019). Additionally, silkworm pupae oil can be extracted using different methods, such as mechanical pressing, volatile solvents, and supercritical fluid extraction. The main disadvantage of mechanical pressing is the relatively low yield of oil and solvent exaction not validated with different organic solvents. Owing to the operational complexity, long-term exposure to alkali is harmful to human health and the high cost of the apparatus, supercritical fluid extraction is limited in practice (Chen *et al.,* 2016). Searching for an efficient technique with low cost is vitally essential for the extensive utilization of spent silkworm pupae. Hence, the present study focused on the extraction of oil from mulberry and eri silkworm pupae and studying the physico-chemical properties.

MATERIAL AND METHODS

Collection of silkworm pupae

Spent mulberry silkworm pupae were collected from multi-end silk reeling unit, adavalli, Coimbatore district, and silver mine silk processors Udumalapet, Tirupur district, Tamil Nadu. Eri silkworm pupae were procured from Central Sericultural Germplasm Resource Center, Hosur, Tamil Nadu.

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Effect of drying time of silkworm pupae

The collected fresh spent silkworm pupae were subjected to the drying in hot air oven at 85 - 90° C to remove moisture content. Silkworm pupae were weighed at an interval of two hours and reduction of moisture content was estimated, to determine the effect of drying time (Ravinder *et al.,* 2015).

Extraction of oil from silkworm pupae

The dried pupae were ground to powder and oil extraction was done using organic solvents *viz.,* acetone, chloroform, xylene, hexane, and petroleum ether.

Soxhlet extraction

The cellulose thimbles were loaded with dried silkworm pupal powder and kept into the main chamber of the soxhlet extractor and refluxed for 8 hours at 65-70°C. The above-mentioned organic solvents were used for oil extraction and the oil was separated from the solvent using a rotary vacuum evaporator (Thirupathaiah *et al.,* 2016).

Column extraction method

Hundred grams of dried silkworm pupal powder was filled in a 500 mL column and 300 mL of solvent was added. The column was covered with a lid and kept undisturbed at room temperature for seven days to prevent the solvent evaporation. After seven days, the contents were filtered with filter paper and oil separated from solvent by a rotary vacuum evaporator (Thirupathaiah *et al.,* 2016).

Estimation of physio-chemical properties

The specific gravity was examined at 25° C using a specific gravity bottle. Moisture content, acid value, peroxide value, iodine value, free fatty acids, and saponification value of the extracted oil were examined by using standard methods (AOCS, 1997 and 2003). Density was tested by ASTM (1998) method. All these tests were done in 15 replicas to minimize the errors.

Statistical analysis

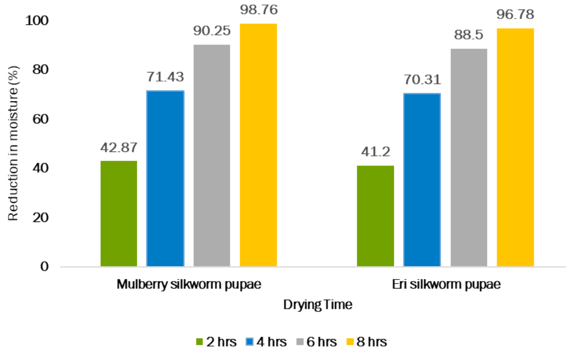
The obtained results are the means of independent values expressed in bar graphs and statistical significance was analyzed by paired t-test with $p<0.005$ level of significance (Gupta, 2002).

RESULTS AND DISCUSSION

Effect of drying time of silkworm pupae

The effect of drying time at a constant temperature of 85 - 90° C on the moisture content of pupae for oil extraction was studied and presented in Figure 1. The initial moisture content of mulberry and eri silkworm pupae was found to be 73 and 75 per cent, respectively. Gradual reduction of moisture content in silkworm pupae was observed every 2 hours. The results of moisture reduction in mulberry and eri silkworm pupae at two hours (42.87 and 41.20 %) four hours (71.43 and 70.31 %) six hours (90.25 and 88.50 %) and eight hours (98.76 and 96.78 %). After eight hours of drying time, moisture reduction was observed in both the silkworm pupae. The obtained final dry weight of mulberry and eri silkworm pupae is 9.42 and 9.35 per cent, respectively. The results showed that 8 hours of drying time is necessary to reduce the moisture content of silkworm pupae suitable for oil extraction. Ravinder *et al.* (2015) reported that oil extraction from silkworm pupae was efficient when the moisture level was in the range of 1.2 to 11 per cent. A similar observation was observed by Niveditha *et al.* (2020) who reported that reduction of moisture content is recommended for oil extraction from silkworm pupae.

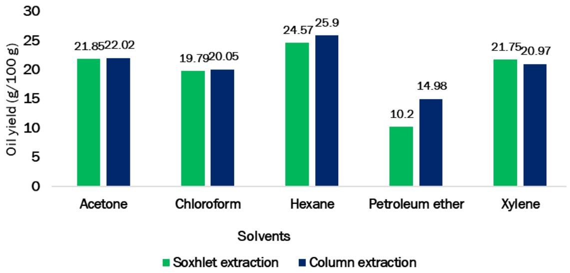
Figure 1. Effect of drying time of silkworm pupae for moisture reduction



Optimization of solvent and method of extraction of oil from mulberry silkworm pupae

The amount of oil recovered from mulberry silkworm pupae by soxhlet and column extraction methods using different solvents was presented in Figure 2. There was a significant variation observed in the amount of oil recovered from the soxhlet and column extraction method. A higher amount of pupal oil (g/100g) was recovered from the column extraction method using hexane solvent (25.90 g/100g) followed by acetone (22.02 g/100g), xylene (20.90 g/100g), and chloroform (20.05 g/100g).The least amount (14.98 g/100g) of oil was recovered in solvent petroleum ether, whereas, soxhlet extraction method yielded a comparatively lower amount of oil with different solvents such as hexane (24.57 g/100g), acetone (21.85 g/100g), xylene (21.75 g/100g), chloroform (19.79 g/100g) and petroleum ether (10.20 g/100g). Irrespective of different solvents used for oil extraction, hexane yielded a higher amount of oil in both column and soxhlet extraction method than other solvents.

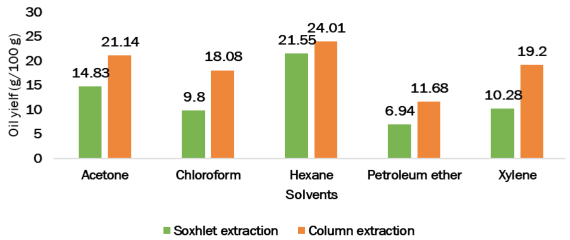
Figure 2. Optimization of solvent and method of extraction for oil recovery from mulberry silkworm pupae



Optimization of solvent and method of extraction of oil from eri silkworm pupae

Optimization of solvent and method of extraction of oil from eri silkworm pupae was presented in Figure 3. There was significant variation found between soxhlet and column extraction methods in oil yield from eri silkworm pupae. The maximum amount of oil was recorded from solvent, hexane (24.01 g/100g) followed by acetone (21.14 g/100g), xylene (19.20 g/100g), chloroform (18.08 g/100g), and petroleum ether (11.68 g/100g) in column extraction method. In the case of soxhlet extraction, hexane yielded 21.55 g/100g of oil followed by acetone (14.83 g/100g), xylene (10.78 g/100g), chloroform (9.80 g/100g), and the least amount of oil (6.94 g/100g) was recorded from petroleum ether solvent. Among the two methods, the column extraction method yielded a higher amount of oil compared to the soxhlet extraction method.

Figure 3. Optimization of solvent and method of extraction for oil recovery from eri silkworm pupae



In the present study, maximum oil recovery was obtained from mulberry and eri silkworm pupae by column extraction method (25.90 and 24.01 g/100g) than soxhlet extraction method (24.57 and 21.55 g/100g) using hexane as solvent. These results corroborate with Thirupathaiah *et al.* (2016) who reported that column extraction method yielded higher oil content from mulberry silkworm pupae than the soxhlet extraction method. Supanida *et al.* (2008) reported that oil content varied from 24-29 per cent in five native varieties of *B. mori.*

Different solvents used for the extraction of pupal oil, hexane yielded a high amount of oil with a clear and pleasant odor. Ricochon and Muniglia (2010) reported that better oil yield 96 per cent was obtained from edible insects using hexane as a solvent. The findings also showed that the hexane was quite efficient in obtaining the lipid part of the insects (Choi *et al*., 2017). The other important point is the selection of hexane as solvent is being easy to remove due to its high volatility and hexane has the ability to mix with the oil aggressively without affecting the other nutrients like protein. Jeevan kumar *et al.* (2019) reported that the non-polar nature of hexane resulted in high percentage of oil recovery than other non-polar and polar solvents used.

Physio-chemical properties

The estimated physio-chemical properties of mulberry and eri silkworm pupal oil was presented in Table 1. There was no significant variation in the physio-chemical properties such as density (0.880 and 0.943 g/mL), specific gravity (0.892 and 0.991 g/mL) and moisture content (0.100 and 0.090 %) were observed in mulberry and eri silkworm pupal oil, respectively. The other physio-chemical properties such as saponification value (187.784 and 235.147 g KOH/ g oil), iodine value (104.651 and 131.653 g I / 100 g), acid number (1.966 and 1.990 mg KOH/ g oil), peroxide value (2.424 and 2.229 ppm) and free fatty acids (3.913 and 5.057 %) were showed significant difference between mulberry and eri silkworm pupal oils.

Table 1. Physiochemical properties of mulberry and eri silkworm pupal oil

Properties	Mulberry silkworm pupal oil	Eri silkworm pupal oil	t value	P value (<0.05)
Density (g/mL)	0.880	0.943	6.640	0.021*
Specific gravity (g/mL)	0.892	0.991	8.368	0.013*
Moisture content (%)	0.100	0.090	6.808	0.020*
Saponification value (g KOH/g oil)	187.784	235.147	14.900	NS
Iodine value (g I/100 g)	104.651	131.653	58.206	NS
Acid number (mg KOH/g oil)	1.966		19.665	NS
Peroxide value (ppm)	2.424	2.229	40.138	NS
Free fatty acid (%)	3.913	5.057	14.504	NS

*Significant at the p≤0.05 level (2-tailed).

Some of the physio-chemical properties such as density, specific gravity, saponification value, iodine value, and free fatty acids were found to be higher in eri silkworm pupal oil (0.943, 0.991, 235.147, 131.653 and 5.057) than mulberry silkworm pupal oil. Significantly higher acid number (1.966 mg KOH/ g oil) and peroxide value (2.424 ppm) in mulberry silkworm pupal oil compared to eri silkworm pupal oil (1.190 mg KOH/ g oil and 2.229 ppm). Free fatty acids were found to be higher in eri silkworm pupal oil compared to mulberry silkworm pupal oil.

Sarma and Ganguly (2016) reported the physio-chemical properties *viz.,* specific gravity, moisture content, acid value, saponification value, and iodine value for muga silkworm pupal oil. Oils having higher saponification and iodine value is suitable for making soap and shampoos (Aremu *et al.,* 2015). Hu *et al.* (2017) and Tamborrino *et al.* (2014) analyzed physico-chemical properties of mulberry silkworm pupal oil and recorded lower acid value and peroxide value less than three and it can be regarded as good quality oil. Similar results of reduced acid number (1.190 and 1.966 mg KOH/ g oil) and peroxide values (2.229 and 2.424 ppm) were also reported in the present study for mulberry and eri silkworm pupal oil. Hence, it can be considered a good quality oil. Significantly, both the oils had a good amount of saturated fatty acids (5.057 % and 3.913 %) in the form of oleic acid. Results from the present study fall in line with Azlan *et al.* (2010).

CONCLUSION

The present study let out that the eight hours drying period at the temperature of 85-90° C is required to reduce the moisture content of silkworm pupae from the fresh weight. Extraction of oil with two different extraction methods showed that higher oil recovery from the column extraction method. Among the solvents used hexane was found to be the best solvent. Analysis of physiochemical properties of oil exhibited a significant difference between mulberry and eri silkworm pupae with optimum ranges. Based on these results, both mulberry and eri silkworm pupal oil could be used as potential edible oil and for the development of by-products.

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