

## RESEARCH ON THE FATTY ACID COMPOSITION OF SILKWORM PUPAE OIL OBTAINED BY MICROWAVE-ASSISTED EXTRACTION

<sup>1</sup> *M.X. Jumaniyazova,*

<sup>2</sup> *D.T.Ro'zmetova,*

<sup>3</sup> *Sh.R.Kurambayev,*

<sup>4</sup> *B.B.Hakimova*

<sup>1</sup> *Phd student, Department of Food Technology, Urgench State University*  
[jumaniyazovamaxliyo21@gmail.com](mailto:jumaniyazovamaxliyo21@gmail.com)

<sup>2</sup> *Assoc. Prof., Department of Food Technology, Urgench State University, PhD.,*

<sup>3</sup> *Professor, Department of Food Technology, Urgench State University, DcS.*

<sup>4</sup> *Senior lecturer, Department of Food Technology, Urgench State University. Ph.D.,*

**Abstract:** *This study used from silkworm pupae, which are waste products from local silk factories. Scientific literature claim that silkworm pupae oil is safe to consume, and that the oil is rich in 60–70% unsaturated fatty acids, including oleic acid and alpha-linolenic acid. During the study, oil was extracted from silkworm pupae using microwave-assisted extraction with two different organic solvents, and the resulting samples were analysed by gas chromatography. First, the samples were dried, then ground, and finally extracted using microwave-assisted extraction. According to the results obtained, the oil yield during oil extraction in 96% ethyl alcohol for 30 minutes was 19.8% at a microwave power of 30%. The highest oil yield (26.4%) was achieved using grade A extraction gasoline in 20 minutes at 30% microwave power. The oil samples were then analysed using GC analysis. According to the results, mulberry silkworm pupae oil contains the following unsaturated fatty acids: C18:1 (31.465%) and C18:2 (32.374%).*

**Key words:** *microwave-assisted extraction, ethyl alcohol, grade A extraction gasoline, gas chromatography, omega-3*

**Introduction.** Scientists around the world are currently focusing their attention on studying the effects of the saturated and unsaturated fatty acids found in fats and oils used in the food industry on the human body. For human health, it is very important that there is a balance of omega-3 and omega-6 fatty acids, especially in vegetable oils. The development of inflammatory diseases has been linked to the high content of omega-6 and relatively low content of omega-3 in vegetable oils. One of

the most pressing issues in solving this problem is to identify alternative sources of omega-3 oils and to study the possibility of using them in food. Mulberry silkworm pupae oil is one such oil that is rich in omega-3 fatty acids. The life cycle of the silkworm *Bombyx mori* L. involves a series of stages, one of which is the cocoons phase. This marks the transition from larva to adult. The silkworm is one of the oldest insects to be domesticated by humans. It has long been used for silk production and as a valuable resource for medicinal and culinary purposes. However, as with other insect resources, the use of silkworm pupae is still relatively low [1]. Consequently, the transformation of silkworm pupae oil into functional foods could be a viable approach to boost insect consumption. An  $\alpha$ -linolenic acid content of approximately 33% is possessed by silkworm pupae oil, which is rich in unsaturated fatty acids [2]. Previous research has shown that silkworm pupae oil is more digestible than most cooking oils when tested in vitro, and that modified mulberry silkworm pupae oil significantly improves digestibility performance [3]. In addition, human metabolism is more capable of processing fatty acids found in glycerophospholipids than those found in triglycerides [4].

Extensive research has shown that increasing levels of long-chain omega-3 polyunsaturated fatty acids (n-3 LCPUFAs), such as  $\alpha$ -linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are found in mulberry silkworm pupae oil, can lower the risk of developing chronic diseases, including heart disease, cancer and arthritis [5-7]. ALA (C18:3) is an essential fatty acid in the human diet and the main omega-3 long-chain polyunsaturated fatty acid (LCPUFA) found in food and medicine. It effectively prevents various diseases, including cardiovascular disease, hypertension, inflammation, autoimmune disease, depression and impaired neurological function [8-10]. Recently, the polyunsaturated fatty acid  $\alpha$ -linolenic acid, which has attracted considerable attention for pharmaceutical and dietary purposes, has been identified as a good source in silkworm (*Bombyx mori* L.) pupae oil [11-13]. However, only a small proportion of silkworm pupae are used as fertiliser for agriculture and as animal feed. A large amount of silkworm pupae is considered industrial waste [14] and is not used to its full potential. If the enrichment process could be carried out at a reasonable cost, extracting  $\alpha$ -linolenic acid concentrate from silkworm pupae oil would be very profitable. The methods used to enrich natural oils with polyunsaturated fatty acids mainly depend on the number of double bonds in the carbon chain and the degree of unsaturation [5]. The sericulture industry in our republic is highly developed. However, despite its rich nutritional content, the silkworm pupae, a waste product of this industry, still does not have its own application system in the food industry. This scientific study involved

conducting experiments to investigate the possibility of producing a food product from this waste material that retains its nutritional value.

**Research Objectives and Methods**

1. The mulberry silkworm pupae was obtained from the Khorezm Ipagi silk processing enterprise in June 2025. The experiments were carried out in the laboratory of the Department of Food Technology at Urgench State University. The samples were dried in a KERN DBS 1.1 version 2/2013 drying oven at 60 °C for six hours until the moisture content was 5%. They were then ground in an FW 135 laboratory mill (Taisite, Tianjin, China) and the resulting powder was placed in plastic containers and stored at -10 °C for further experiments.

2. The microwave extraction equipment (WBFY-205, Yuhua China) has a maximum power of 1000W to 20W variable, with a microwave frequency of 2.45 GHz. The dimensions of the inner cavity are 330 mm × 365 mm × 235 mm. During the experiment, the time, irradiation power, and stirring speed are controlled by an electronic control panel[15]. To carry out the process, 2 different organic solvents were used in different proportions. 1. Extraction gasoline grade A; 2. Ethyl alcohol -



96%.

For each extraction in the experiments, a 30 g sample was extracted using grade A extraction gasoline and 96% ethanol. To prepare for extraction, the required volume of solvent (150 ml for each experiment) was poured into the flask, along with the silkworm pupae powder. The flask was placed inside the microwave oven and connected to the cooling system via a hole in its top. The microwave oven was switched on and the power level and extraction time were set using the digital panel. After extraction, the micelles were filtered, and the organic solvents contained were removed at 35-40 °C in a rotary evaporator ((DiChTe Gmbh Siemensring 9147877 Willich Germany) under vacuum (KNF Neuberger Gmbh Alte Weg3 79112 Freiburg Germany). The obtained oil was dried in a Memmert GmbH + Co. KG drying oven at 105 °C for 15 minutes. The oil yield was calculated using the following formula [14]:

$$X = ((P1 - P2) \times 100) / P$$

Here: P1-weight of the oiled tube, g; P2-weight of the empty tube, g; P- weight of the test sample, g;

The microwave extraction method is highly effective in obtaining lipid-rich fractions from silkworm pupae. This step, as well as microwave extraction, was extracted with ethyl alcohol (96%). The process data is presented in Table 1.

Table 1.

**Oil separation using microwave extraction with ethyl alcohol.**

Solvent	(Power), %	Time, min	Oil yeild, %	Temperature , °C
Ethyl alcohol 96 % li	20	10	12,01	65,05
		20	12,35	68,55
		30	13,5	70,01
	30	10	13,9	71,02
		20	14,06	72,25
		30	19,8	76,01
	40	10	t.o.y.i*	-

t.o.y.i\*The experiment did not end there.

The highest oil yield (19.8%) was achieved in 30 minutes at a Power-30% when the oil extraction process was carried out in 96% ethyl alcohol. The lowest oil yield was 9.13% after 10 minutes at power 40%. In the next experiment, gasoline grade A was used as an oil separation solvent. The results of this process are shown in table 2.

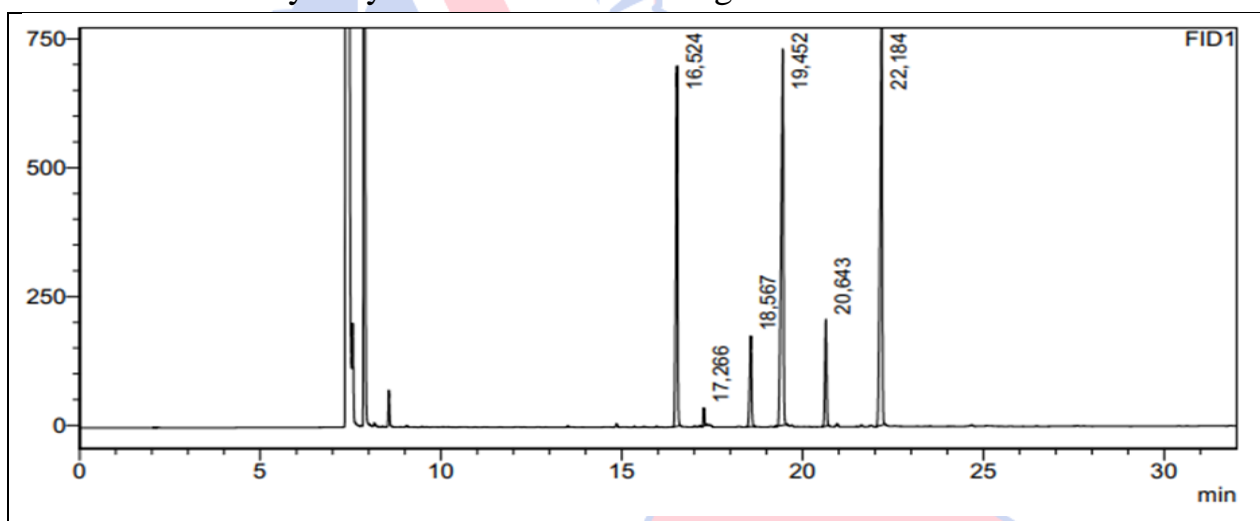
Table 2

**Oil separation using microwave extraction method based on grade A extraction gasoline**

Solvent	(Power), %	Time, min	Oil yeild, %	Temperature, °C
A extraction gasoline	20	10	17,3	38,01
		20	18,2	39,45
		30	25,01	42,01
	30	10	25,3	43,25
		20	26,4	45,55
		30	24,7	44,75
	40	10	24,2	44,01
		20	19,3	43,75
		30	18,4	43,01

As shown in Table 2, the highest oil recovery (26.4%) was achieved in 20 minutes at Power -30% when grade A extraction gasoline was used as a solvent. The lowest oil recovery was 18.4% after 30 minutes at a power -40%.

The next experiments involved performing gas chromatographic analysis on samples of mulberry silkworm pupae oil that had been extracted using a microwave method. Chromatography conditions: Detector temperature - 250°C; Evaporator temperature - 220°C; Thermostat temperature regime: initial temperature 100°C is held for 4 min, then the temperature is heated to 200°C at a rate of 25°C/min and held at this temperature for 8 min, then the temperature is heated to 250°C at a rate of 5°C/min and held at this temperature for 7 min. Hydrogen flow rate - 40ml/min; Air flow rate - 40ml/min; Injection volume - 1 µl. Supelco FAME 37 Mix standard sample was used to identify fatty acids in the chromatogram.



**Figure 1: GC analysis of mulberry silkworm pupae oil extracted using microwave extraction.**

The fatty acid composition of silkworm cocoon oil was analysed using gas chromatography (GC-2030) following extraction. The primary objective was to ascertain the presence of  $\alpha$ -linolenic acid, a vital omega-3 fatty acid that is known to be beneficial for health. GC-FID analysis showed that the oil extracted from silkworm pupae is rich in polyunsaturated fatty acids (PUFA), with  $\omega$ -3 fatty acids accounting for approximately  $\alpha$ -linolenic acid (C18:3) 32.707%, palmitic acid (C16:0) 22.7%, and stearic acid (C18:0) 28.96%. (C18:1)28,96%.

**Table 3**

**Fatty acid profile of Silkworm pupae oil**

No	Fatty acids	Sample-1
1	C16:0	22,463
2	C16:1	0,896
3	C18:0	6,359

4	C18:1	31,465
5	C18:2	6,443
6	C18:3	32,374

### Conclusion

This research project involved the extraction of mulberry silkworm pupae, a waste product of industrial production, using microwave extraction with two organic solvents. 1. Grade A extra gasoline and 2. ethyl alcohol (96%). The fatty acid composition of the extracted oil samples was analysed, yielding the following results: The oil yield obtained by extracting the oil in 96% ethanol for 30 minutes at a microwave power-30%. The highest oil yield of 26.4% was achieved in 20 minutes at a microwave power-30% using grade A extraction gasoline. Gas chromatographic analysis of oil extracted from mulberry silkworm pupae revealed a high concentration of the omega-3 fatty acid  $\alpha$ -linolenic acid (C18:3) (32.374%). This indicates the need for an effective refining process to make this oil suitable for use in the food industry. Our work will be continued with a focus on researching methods for purifying this type of oil.

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