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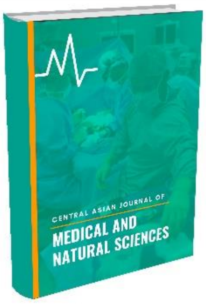

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GC-MS COMPOSITION ANALYSIS OF OILS EXTRACTED FROM SILKMOTH PUPES

Dustov S.I.[1], Normurodov B.R.[1], Mukhamadiev N.K.[2], Knyazev A.A.[3]

EMAIL:
bobomurod_normurodov@bk.ru,
sayfullo_do'stov@bk.ru,
m_nurali@mail.ru,
lavopash283741@yandex.ru

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[1]Bukhara Institute of Engineering and Technology, Uzbekistan
[2]Samarkand state university, Uzbekistan.
[3]Kazan National Research Technological University, Kazan, Russia

ABSTRACT: The paper presents the results of GC-MS analysis of fatty acids of oils isolated from silkworm pupae. The oil extracted from silkworm pupae contains a high ratio of essential fatty acids, [a-linoleic acid (ω-3 fatty acid)] + linoleic acid (40.73%), and also contains other essential fatty acids such as oleic (31, 46%), palmitoleic (0.72%), palmitic (18.6%), stearic (5.83%), and eicosatetraenoic acids (0.96%). The content of a sufficient amount of fatty acids allows the use of oils as a raw material for perfumery and cosmetics and as a fat-liquefying component for processing the skins of cattle, sheep, and fur animals.

KEYWORDS: GC-MS analysis, composition, oils, pupae, silkworm, fatty acids.

INTRODUCTION

According to the International Sericulture Commission, Uzbekistan increased silk production from 2008 to 2014 by 42% (up to 1100 tons of silk), its share in world production is relatively small - about 0.6%, since the lion's share of world silk is produced by China - 82 %, and India - 16%. Despite this, Uzbekistan ranks third in the world in terms of silk production, after China and India. Since the share of the republic among the CIS countries in the total volume of silk production is over 85% [1].

Uzbekistan is expanding its silk production. The waste of silkworm pupae is about 10 thousand tons per year, which can serve as a source of biologically active substances. Silkworm pupae are the main waste after the extraction of silk threads, and constitute 60% of the weight of the dry cocoon [2]. However, the resources of silkworm pupae are used only as fertilizer and feed, or even as industrial waste. Waste disposal is a serious issue because waste decay has a negative impact on the environment [3]. The high content of protein and fat in silkworm pupae determines their nutritional value. Only fat makes up about 30% of the total dry mass of the pupa. The oil extracted from silkworm pupae contains more than 70% unsaturated fatty acids, the majority of which are linoleic acid and oleic acid [4]. According to the authors of the work, the content of unsaturated fatty acids (oleic, linoleic, and linoleic) is 75% [5].

In the works [6], the analysis of the fatty acid composition of silkworm pupae oil was studied by high-pressure liquid chromatography and gas chromatography—mass spectroscopy. It was discovered that the oil contains 49.0% essential fatty acids [a-linoleic acid (-3 fatty acid)] + linoleic acid, as well as oleic (19.9%), palmitoleic (2.5%), palmitic (19.7%), stearic (8.6%), and eicosapentaenoic acids (0.3%). The oil also contains amino acids, antioxidants, quercetin diglucoside, and riboflavin (vitamin B_2), which indicates that silkworm pupae are a valuable nutritional product with essential amino acids, essential fatty acids, antioxidants, and vitamins.

Oils were isolated from five Thai silkworm (Keaw Sakon, Nangnoi Srisaket, Sam Rong, Nang Luang and None Ruesee) varieties in [7]. The yield of oils according to the Soxhlet method and maceration was between 24 and 29, or 5% and 7%, respectively. The oils had high activity in scavenging free radicals and inhibiting tyrosine. Silkworm pupa oil extraction by Soxhlet contain 72–79% unsaturated fatty acids, and the content of -linoleic acid is 32–44%. Because of maceration extraction, the content of unsaturated fatty acids is 75–80%, and the content of -linoleic acid is 40–46%.

Results of the authors [8] Have shown that the oil extracted from silkworm pupae has good quality parameters, namely to contain 25% ω-3 acids (α-linoleic acid), of the total fatty acids, with an approximate level of cholesterol 109 mg/100 g oil. The amounts of β-carotene and α-tocopherol were approximately 785 and 9434 μg/100 g oil, respectively.

According to the authors [9], The lipids extracted from silkworm pupae are primarily triacylglycerol, phosphatidylethanolamine, and phosphatidylcholine, with -linoleic acid accounting for 40% of the total. 20:3n-3 was also present in the pupa, but in very small quantities. Total tocopherol was 125.2 μg/g lipid for males and 224.1 μg/g lipid for females, respectively. In addition, the silkworm pupa contained carotenoids such as lutein and neoxanthin, which can act as antioxidants.

The authors of the work [10], extracted the oil of silkworm pupae using various solvents and found that hexane is an effective extractant. The oil yield is 25.9%. The physicochemical characteristics of the oil were established: density (0.943 g/ml), specific gravity (0.991 g/ml), saponification number (235.147 g KOH/g oil), iodine number (131.653 g I/100 g), and free fatty acids (5.057 percent).

In recent years, research has focused on various biomedical applications of silkworm pupae proteins. Studies have shown that silkworm pupae have a positive effect on liver protection, immunity enhancement, anti-apoptosis, and anti-tumor action, regulation of blood glucose and lipid levels, and lowering blood pressure. Therefore, in [11], the authors summarize the nutritional composition of domestic silkworm pupae.

The authors of [12] found that pupal proteins effectively act in wound dressings, have hepatoprotective and antiapoptotic activity, antigenotoxicity, regulate blood glucose and lipid levels, antitumor agent, etc.

In [13], a new application of a protein extracted from silkworm pupae for dyeing silk fabrics was proposed.

The role of unsaturated fatty acids (EFAs) is diverse. Therefore, the obtained oils can be widely used in various areas of the economy, in particular, the perfumery, paint and varnish, and medical industries [5].

Thus, silkworm pupae are considered a good source of oil and can be used for various purposes, including food, medicine, cosmetics [14], and other areas. In this regard, a comprehensive study of the chemical composition of silkworm pupa oil is relevant from the point of view of its application for various purposes.

THE PURPOSE OF THIS WORK is to study the chemical composition of oils isolated from the silkworm pupa using the GC-MS method.

MATERIAL AND RESEARCH METHODS

Silkworm pupae were used as raw materials for oil production (harvest 2021, Bukhara region). Oils from crushed silkworm pupa were isolated by extraction with hexane on a Soxhlet apparatus. The yield was 26.1%, which is consistent with the literature [10].

Study of the fatty acid composition in the form of their methyl esters. To do this, the oil sample was transesterified with a 2 M HCl solution in methanol. The hexane extract of the obtained products was used for gas chromatography–mass spectrometric (GC–MS) analysis. A GC-MS YL6900 was used for analysis with an HP 5 capillary column 30 m long, 0.32 mm ID, and 0.25 µm stationary phase thickness.

Chromatography conditions: initial thermostat temperature of 60°C for 3 min (isothermal mode); heating at a rate of 15°C/min (temperature programming mode) up to 250°C and at 250°C (isothermal mode) for 3 min. Temperature of the injector: 250°C, flow rate of helium gas: 1 ml/min, SplitRatio: 1/100 Detector mass parameters: solvent delay of 3 minutes, emission of 50 mA, scanning range - 30-350 amu, scanning speed of 1600 amu per sec, ion source temperature of 230°C, transfer temperature of 280°C. Analysis time: 21 min.

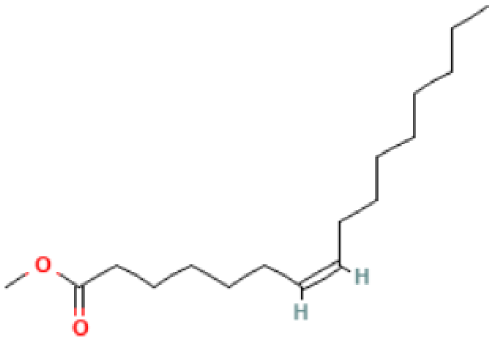
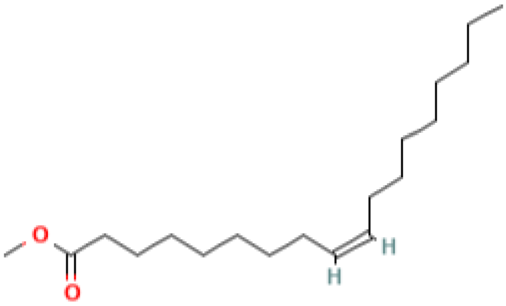
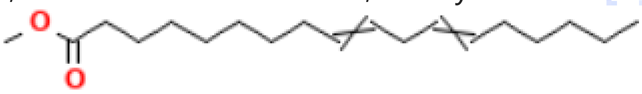
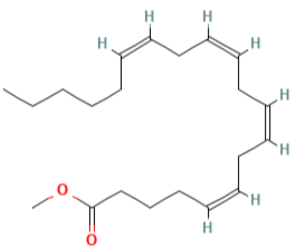
By comparing the obtained mass spectra with the NIST mass spectral library and by retention time, components were identified. For quantitative analysis, the method of internal normalization was used.

THE RESULTS OBTAINED AND THEIR DISCUSSION

Fatty acids are valuable components from the point of view of the use of oil isolated from silkworm pupae in perfumery, the food industry, cosmetics, and as a fattening agent for skins. Therefore, the main attention was paid to the study of the relative content of fatty acids in the sample. The results of gas chromatographic analysis of fatty acids in the form of methyl esters are shown in table 1.

Table 1.The relative fatty acid content of oils, isolated from silkworm pupae

N	Compound Name, chemical and structural formula	Content, %
1	Methyl tetradecanoate ($C_{15}H_{30}O_2$) 	0,55
2	Pentadecanoic acid, methyl ester ($C_{16}H_{32}O_2$) 	0,18
3	Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$) 	18,6
4	Hexadecenoic acid, methyl ester, (Z)- ($C_{17}H_{32}O_2$)	0,72

		
5	Methyl stearate ($C_{19}H_{38}O_2$) 	5,83
6	9-Octadecenoic acid (Z)-, methyl ester ($C_{19}H_{36}O_2$) 	31,46
7	9,12-Octadecadienoic acid, methyl ester 	7,54
8	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- ($C_{19}H_{32}O_2$) 	33,19
9	Eicosanoic acid, methyl ester ($C_{21}H_{42}O_2$) 	0,16
10	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)- ($C_{21}H_{34}O_2$) 	0,96

From the data in Table 2, it can be seen that the oil contains a high ratio of essential fatty acids: [a-linoleic acid (ω-3 fatty acid)] + linoleic acid (40.73%), and contains other essential fatty acids such as oleic

(31.46%), palmitoleic (0.72%), palmitic (18.6%), stearic (5.83%), and eicosatetraenoic acids (0.96%). The fatty acid composition was compared to the authors' data [15] and the fatty acid composition of fish oil [15]. Comparative characteristics are given in the table. 2.

Table 2.Comparative characteristics of fatty acids isolated from silkworm pupae and fish oil

Fatty acid	Silkworm [15]	Fish oil [15]	Silkworm (uzb)
C(14:0)	0,5	3,8	0,55
C(14:1)	-	0,3	
C(15:0)	0,2	-	0,18
C(16:0)	19,6	11,6	18,6
C(16:1)	0,6	7,0	0,72
C(17:0)	-	0,7	
C(18:0)	6,9	2,3	5,83
C(18:1, n-9)	28,8	16,9	31,46
C(18:2, n-6)	6,0	4,9	6,54
C(18:3, n-3)	32,1	1,2	33,19
C(19:0)	-	0,1	-
C(20:0)	0,1	0,2	0,16
C(20:1)	-	1,3	
C(20:4)	-	-	0,96
C(20:5, n-3)	-	9,6	-
C(22:0)	-	6,8	-
C(22:1)	-	0,4	-
C(22:6, n-3)	-	11,4	-
∑SFA	27,3	25,5	25,32
∑MUFA	29,4	25,9	31,64
∑n=6	6,0	4,9	6,54
∑n=3	32,1	22,2	33,19
∑n=6/∑n=3	0,2	0,2	0,2

From the data in Table 2 shows that in terms of the total content of saturated fatty acids, Silkworm (Uzb) almost does not differ from the data given in [rom the data given in [15], but in terms of the content of monounsaturated fatty acids, it is 7.6% more than Silkworm [15], relative to fish oil by 22.2%. By the ratio ∑n=6/∑n=3 everything is the same, i.e., is equal to 0.2.

The content of a sufficient amount of fatty acids allows the use of oils as a raw material for perfumery, cosmetics, as a fat-liquefying component for processing the skins of cattle, sheep, and fur animals.

CONCLUSIONS

1.The oil extracted from silkworm pupae contains a high ratio of essential fatty acids, [a-linoleic acid (ω-3 fatty acid)] + linoleic acid (40.73%), and also contains other essential fatty acids such as oleic (31, 46%), palmitoleic (0.72%), palmitic (18.6%), stearic (5.83%), and eicosatetraenoic acids (0.96%).

2. Because oils contain a sufficient amount of fatty acids, they can be used as a raw material in perfumery, cosmetics, and other industries. As a fat-liquoring component for the treatment of hides, skins of cattle, sheep, and fur skins.

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