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Studying Lipids of Some Amaranth Varieties of the Amaranthaceae Family

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Annotation
The article deals with the study of lipids of seeds of local varieties of amaranth grown in Uzbekistan. Including the complex processing method of amaranth seed, cake and flour, selection of optimal conditions for obtaining in the supercritical state of the cake to obtain squalene-enriched CO2 extract, chemical analysis of lipids and fatty acids. Modern chemical and physico-chemical methods of analysis of amaranth seeds, lipids, carbohydrates and proteins were used in the work to fulfill the specified tasks.

Keywords: amaranth, CO2-extraction, lipids, squalene.

Featured methods used in the analysis of plant lipids at work, FEC instruments, pH meter, analytical and technical balances, ball mill, sieving device, TIN-1 indicator micrometer, grain cleaner, Agilent Technologies 6890N GC, freeze dryer, magnetic stirrer, rotary evaporators, HPLC device, drying cabinet, laboratory setup for supercritical carbon dioxide extraction (CO_2 extractor). In the course of research, the chemical method of cake processing was improved, the squalene fraction was separated; search for optimal conditions for CO_2 extraction from cake to obtain squalene-enriched extract was carried out, and the chemical composition of CO_2 extract and food was investigated. The high content of flavonoids, proteins, pectins and carotenoids in some types of amaranth indicates that it is used to obtain a number of biologically active compounds that can be used in medical practice and in the organization of functional nutrition. It is an economically important plant that attracts the attention of researchers and agricultural practitioners due to its protein content with a balanced set of essential amino acids, high productivity, and abundance of vitamins and mineral salts. In the work of many researchers, the special importance of amaranth is determined primarily by its nutritional benefits and research on the use of amaranth seeds as raw materials to increase the nutritional value

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of food products, complex production problems are not paid attention to processing of amaranth seeds and development of modern non-traditional industrial technologies.

Amaranth seed oil is widely used in medicine and cosmetology. It is used to correct immunodeficiency conditions in the treatment of diseases of various etiologies: cardiovascular and oncological diseases, metabolic diseases, erosive and ulcerative lesions of the gastrointestinal tract, psoriasis, neurodermatitis, anemia, toxicosis, etc.

Squalene is a hydrocarbon, an isoprene derivative, a precursor of triterpenes and steroid compounds, and it can be used in the production of steroid hormonal drugs, in the prevention and treatment of radiation diseases, oncological and heart diseases, as well as for cosmetic purposes. As the main source of squalene is currently the liver of deep-sea sharks, the development of efficient environmentally friendly technologies for the extraction of squalene from plant materials remains an urgent task.

Currently, foreign specialists have developed and are developing modern methods and technological methods of processing amaranth grain and aerial parts to obtain products with high biological value. A number of works are devoted to complex processing technologies of amaranth raw materials (grains and aerial parts), as well as liquid extraction technologies - liquid carbon dioxide and carbon dioxide mainly in a supercritical state (CO2 extraction).

Researchers have proposed a method of dry separation of crushed seeds, resulting in two fractions [4]. The protein-lipid fraction contains up to 38% protein, up to 18% fat and is prepared to obtain high-quality amaranth oil. The second fraction is a carbohydrate concentrate with a low content of protein, fiber and fat. This fraction is suitable for use in various areas of industrial production (starch, bread, confectionery, perfumery, pharmaceuticals, etc.). Another method of complex processing of amaranth seeds proposed in the work is a biotechnological method, which includes the hydrolytic effect of complex enzyme preparations on amaranth flour. The insoluble residue (protein-lipid concentrate) after hydrolysis with an oil content of up to 27.5% is the raw material for obtaining amaranth oil from it with subsequent production of amaranth protein products.

Much work has been devoted to the development of supercritical **CO_2-** extraction conditions to obtain a squalene-enriched extract (oil) from amaranth grains. Oil industry, especially oil production, accounts for a significant share of all food industry products [5].

The modern technological process of oilseed processing includes preparation of seeds for oil extraction, extraction of oil by pressing and/or extraction, complex purification of oil and its processing. Extraction gasoline oil extraction process ensures maximum oil extraction from seeds, but the negative factor of this technology is the presence of residual solvent in oil and food. One way to solve this problem is to distill the oil at high temperatures [6], which leads to the degradation of the

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thermolabile components of the oil.

With the help of an organic solvent, triacylglycerides, phospho- and glycolipids, pigments, free fatty acids, pesticides, etc. are partially extracted from seed cells together with oil, will come. Oxidation of unsaturated fatty acids, oil color change when heated, etc., are negative indicators that affect the quality indicators of oil [7]. Therefore, there is a need for further refining and processing of oil.

The use of sub- and supercritical fluids as solvents and extractors in extraction processes is one of the ways to solve the problems of energy saving and to meet the increasing demands for environmental cleanliness of food products, materials and technological processes in general [8]. Supercritical technology has a number of advantages over traditional methods, such as ease of recovery of the solvent and the possibility of recycling it, greater yield and higher quality of the product to be obtained, absence of solvent residue in the extract, one-step operation, etc. selectivity of extraction [9]. The most commonly used solvent is carbon dioxide (**CO_2-**), which has a relatively low critical temperature (35 ° C), high volatility, regeneration and diffusion coefficient, is non-toxic, does not harm the environment and does not burn. In addition, this extractor is cheap, convenient and environmentally friendly [8]. **CO_2** extraction (liquid) of oil is an innovative replacement of traditional methods of its extraction with organic solvents.

Researchers were the first to extract oil from amaranth seeds with supercritical carbon dioxide at temperatures of 30-35 °C in the pressure range of 8-33 MPa. A new approach to the isolation of squalene from amaranth seeds has been proposed, which consists in establishing the optimal, in terms of selectivity, conditions for supercritical oil extraction and subsequent saponification of the extract with the release of unsaponifiable substances (US) with a squalene content close to 100%. The author experimentally confirmed the possibility of concentrating the target component - squalene by fractionating the oil from amaranth seeds with supercritical **CO_2**. An extract with 35.5% squalene content was obtained [10].

A sample of **CO_2** extract from amaranth seeds was obtained at a pilot plant at the experimental plant of the Kazan Research Institute of Chemical Industry. The authors carried out the oil extraction process at a temperature of 20-22°C and a pressure of 5.8-6.0 MPa. The yield of the extract under these conditions turned out to be low and amounted to only 3.5%. The authors did not determine the squalene content in the extract [11].

Some researchers isolated amaranth oil with liquid carbon dioxide (subcritical **CO_2**) in a batch chamber extraction unit with the following technological parameters: pressure - 5.8 MPa, extraction temperature - 18°C. The mass fraction of extractive substances in this case was 5.41% [12]. The disadvantages of this method include the low content of squalene in the oil (7.08%). However, according to recommendations for rational nutrition [13], the more adequate level of consumption of amaranth oil is 0.4 g/day, which corresponds to 4% squalene content in the oil. Other scientists have

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developed a method for CO_2 extraction of amaranth seeds, in which the crushed seeds are extracted with carbon dioxide under supercritical conditions at a temperature of 35-45°C, a pressure of 8.3-12.3 MPa and a mass ratio of the extractant and raw materials in the range (50-75):1 [14]. The supercritical extraction process was carried out in a flow-through experimental setup [15]. The authors obtained amaranth oil as follows. At atmospheric pressure, 18.5 g of crushed amaranth seeds were loaded into the extractor in a special mesh cartridge, after which all containers and communications were washed with carbon dioxide, then the pressure and temperature were brought to supercritical values: the operating pressure P = 8.3 MPa was set, and the liquid thermostat system was started, designed to maintain the process temperature T=35°C.

The duration of the extraction process was determined by achieving a mass ratio of extractant and raw material of up to 75:1. The amount of extractant was determined by gravimetric method. The resulting extract was separated, followed by regeneration of the extractant and isolation of amaranth oil. The yield of amaranth oil using this method was 5-5.3% based on the feedstock. The squalene content in oil is 20-23%. Next, unsaponifiable substances were isolated from the oil, in which the squalene content was determined by GC (gas-liquid chromatography) and mass spectroscopy. The authors point out that the isolation of squalene from the unsaponifiable fraction of oils and fats is a classic way to obtain this valuable product. Receive 15-23% NV with a squalene content of 75-80%.

Popova I.Yu. and Vodyanik A.R. supercritical fluid extraction of amaranth seeds with carbon dioxide was carried out at a temperature of 50 °C and a pressure of 30.0 atm.[16]. Using this technology, a product with a squalene content of about 8% was obtained.

A CO2 extract of amaranth seeds stabilized with rosemary extract (Rosmarinus officinalis) with high antioxidant activity is commercially produced. The extract contains 12.1% NV, including 2.7% phytosterols and 0.076% total tocopherols (in terms of α-tocopherol). The squalene content in NV is 8.7%. The oil contains 44% omega 6-linoleic and 28% oleic acids. It has been experimentally proven that the CO2 extract of this composition helps maintain skin hydration, elasticity, and firmness; has antioxidant potential; reduces skin moisture loss, restoring its barrier properties; improves skin condition in patients with psoriasis. From 19-20 kg of raw materials, 1 kg of extract is produced[17].

English scientists have studied the influence of temperature, pressure, extractant flow rate and preliminary sample preparation on the speed of the supercritical CO_2 extraction process and the yield of the extract. The extraction rate is found to be a function of the solvent flow rate, while the oil yield depends on the flow rate, sample preconditioning, and pressure[18]. In this work, extraction of amaranth seeds was carried out at a pressure of 10-30 MPa and a temperature of 35-50 °C. Pre-treatment of amaranth seeds is undertaken due to their physical properties. The raw seeds are lens-shaped in shape and have a diameter of 1.0-1.5 mm. Before extraction, the seed coat must be broken to improve extraction efficiency. It has been found that, due to the hardness of the shell, it is

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better to grind the seeds in a special mill, which completely destroys this shell, than to crush or crush them. From seeds ground to flour, extraction proceeds faster and the extract yield increases.

Solvent pressure, temperature, and density have interdependent effects on extract yield. At low pressure (about 10 MPa), the extract yield decreases with decreasing temperature due to the low density of the solvent and therefore low solubility. At higher pressures (>20 MPa), the yield increases with temperature due to the enhanced solubility effect, where the increase in solute vapor pressure compensates for the decrease in solvent density. Extraction rate and yield decrease with increasing solvent flow rate, which is due to decreased solvent contact time with the seeds.

The authors [19] compared the yield of amaranth oil NV components (squalene, tocopherols, phytosterols, etc.) using different oil extraction methods. Supercritical **CO_2** extraction, extraction with a mixture of chloroform and methanol, and cold pressing were used. The content of squalene and tocopherols was determined by HPLC, and phytosterols were determined by GC/MS. during cold pressing (5.74%). The amount of tocopherols in the oil was the highest even in liquid extraction (131.7 mg/100 g of oil), β-tocopherol being the dominant homologue (40%). The oil obtained by liquid extraction was also characterized by a high content of phytosterols (2.49%). The major sterols in all samples were the sum of α-spinasterol and β-sitosterol.

The current situation regarding the processing and use of amaranth is that the efforts of producers are mainly focused on the production of expensive products from amaranth seeds - amaranth oil, squalene and **CO_2** extract and by-products of such production. For example, the **CO_2** puree obtained from amaranth seeds has practical value and is not yet used. The technology of extracting **CO_2** from amaranth seeds mainly involves the separation of the lipid part. Data from literary sources show that the conditions for carbon dioxide extraction of amaranth grains differ significantly among different authors, and this is reflected in the yield of the extract, its chemical composition, and especially in the content of squalene. To increase the concentration of squalene in the extract and its fractions, it is necessary to further improve the method of CO_2 extraction of grain[20].

Podbor optimalnyx usloviy SO2-ekstraktsii jmykha s polucheniem ekstrakta, obogashchennogo squalenom

SO2-extractsii jmykha

Apparatus, material and reactivity

In the work, a laboratory facility was used for the extraction of vegetable raw materials with carbon dioxide in a supercritical state (China), chromatograph VEJX, column chromatography (KX), silica gel brand KSK, extraction gasoline (tkip 72-76 °C), methanol, diethyl ether, hexane, heptane, benzene, diethyl ether, acetic acid, 50% sulfuric acid.

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Implementation experience

And the extractor is equipped with izmelchenny and kaffemolke jmyx so sredney maslichnostyu 4.0-4.8 % i soderjaniem squalene v jmyxe 5.0-5.5 % i v raznykh uzloviyax provodiyu. V syrom extract s pomoshchyu VEJX opredelyali soderjanie squalene, a zatem vydelyali iz than squalene for use and kachestve standard. S cellu polucheniya fractsii, enriched with squalene, iz extracta vydelili neomylyaemye veshchestva (NV) i specified ix soderjani.

Opredelenie soderjaniya neomylyaemyx veshchestv v SO2-extracte

Apparatus, material and reactivity

Vydelenie NV is carried out by the recommended method [26] in double replication. Analytical scales used for work, 2 flasks with a capacity of 250 ml, C2H5OH, KOH, air cooler, electric plate, 2 separating funnels with a capacity of 500 ml, extraction gasoline (temperature 72-76 °C), phenolphthalein, filter paper, rotornyy isparitel, drying cabinet.

Conducting an analysis

In a flask, add about 5 g of extract, add 30 ml of alcohol and 5 ml of a 50% aqueous solution of KON, add an air cooler, and add 1 teaspoon of full-fledged alcohol. After cooling, the contents of the flask are quantitatively transferred to a dividing funnel, in which 20 ml of warm distilled water is pre-filled, and then 5 ml of alcohol and 20 ml of cold water are added. The flask is rinsed with 50 ml of extraction gasoline (temp. 72-76 °C), which has plums and a separatory funnel after cooling and can be brought to room temperature.

The separating funnel was closed with a stopper, shaken for 1 min (periodically removing gasoline vapors through the tap) and left to stand until the liquid in the furon relaxed. Drain the soap solution and flask, extractor and second separatory funnel. NV extraction is repeated 6 times, using 50 ml of gasoline each time.

Combined flushing part for a gasoline engine (25 ml each) 50% alcohol and neutral washing water (after phenolphthalein). The gasoline solution was filtered into a pre-dried and weighed conical flask. The autogonal solvent and rotary evaporator and restock and flask were dried and dried in an oven to constant weight at a temperature of 80 °C. The first weighting is done from 1 hour, then 15 minutes.

NI content v%(X) was calculated using the formula:

$$X = \frac{P_1 \times 100}{P}$$

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where P1 is the weight of the residue after drying, in g;

P – weight of oil, in g. The average of two parallel determinations was taken as the final result.

Table 1. Effect of conditions of CO2 extraction of amaranth cake on the yield of the extract and the content of squalene in it.

№	Conditions	experience			
		1	2	3	4
1	**Pressure, MPa**	20-25	20-21	20-22	22-25
2	**Temperature, °C**	30-40	30-40	40-50	40-50
3	**Extraction time, min**	90	60	60	60
4	**Yield of crude extract,**	1,0	0,5	2,5	2,7
5	**% by weight of raw materials**	3,8	3,5	5,1	6,8
6	**% squalene in extract, HPLC % fraction**	1,5	0,8	1,3	1,2
7	**squalene from extract % squalene in fraction**	17,0	20,0	43,0	45,0

As can be seen from the table, in the conditions of experiments 3 and 4, compared to experiments 1 and 2, a higher yield of CO2 extract with a higher (2 times) squalene content (6.8%) was obtained. These results are comparable. Data in[12] and[19]. Comparing our results with those published in[17], we can see that under the conditions of experiment 4 we isolated a 10-fold less squalene fraction, but the amount of squalene in this fraction was 5-fold higher. It should be noted that amaranth oil with a squalene content of at least 6% has a pharmacological effect. This oil effectively normalizes cholesterol metabolism and is comparable to the effects of drugs in the class of statins, but without any side effects [21]. To obtain a standard sample of squalene, the crude **CO_2** extract was treated once with gasoline, the extract was filtered, the filtrate was evaporated, and the composition of the gasoline extract was determined. The extract was then chromatographed on a silica gel column, eluting squalene with a 99.5:0.5 benzine-ether solvent system. Squalene was obtained with 95% purity for use as standard for HPLC. Figure 1 shows the HPLC chromatograms of the **CO_2** extract and the squalene standard.

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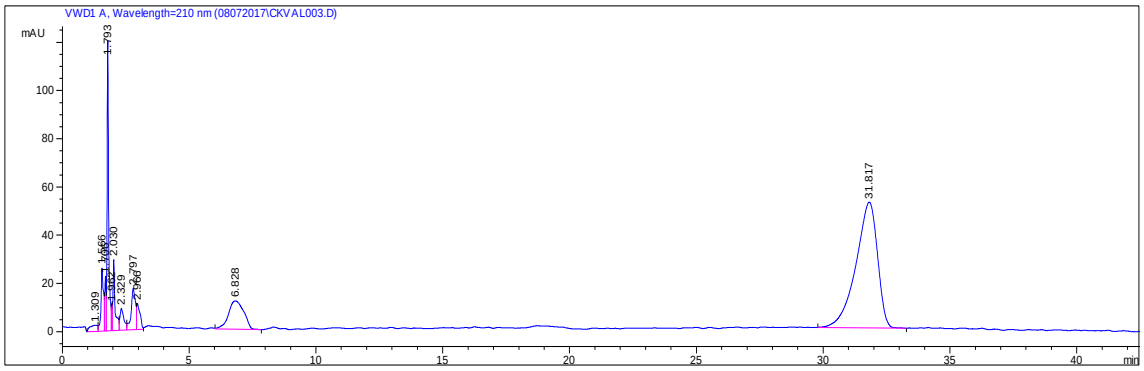
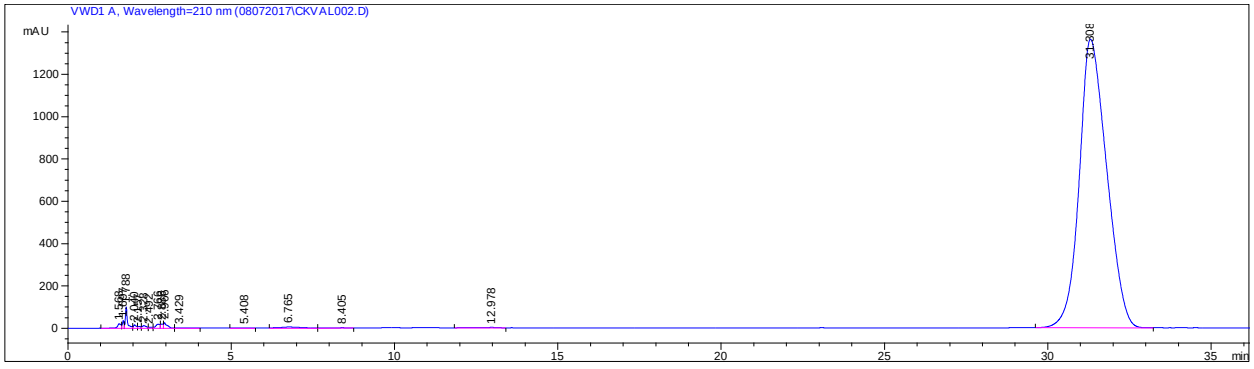
CO_2 extraction



Signal 1: VWD1 A, Wavelength=210 nm

Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
1	1.309	VV	0.3061	54.24141	2.50871	1.0826
2	1.566	VV	0.0917	176.15149	26.15968	3.5157
3	1.706	VV	0.0564	87.02128	22.68448	1.7368
4	1.793	VV	0.0604	504.67148	120.84757	10.0724
5	1.962	VV	0.0352	27.32892	10.87013	0.5454
6	2.030	VV	0.0834	173.31592	29.35682	3.4591
7	2.329	VV	0.1488	97.52563	8.96178	1.9464
8	2.797	VV	0.1636	199.74120	17.14228	3.9865
9	2.966	VV	0.1223	100.28603	10.71101	2.0015
10	6.828	VP	0.6271	499.21954	11.81031	9.9635
11	31.817	BP	0.9020	3090.95581	52.19321	61.6901
Totals :				5010.45870	313.24599	

Стандарт сквалена



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Peak #	RetTime [min]	Type	Width [min]	Area mAU *s	Height [mAU]	Area %
14	8.405	VV	0.6613	93.23624	1.73656	0.1141
15	12.978	VV	0.8136	174.51616	2.69551	0.2135
16	31.308	VB	0.8818	7.94408e4	1364.14221	97.2058
Totals :				8.17244e4	1649.35041	

Pic. 1. Chromatogram of VEJX SO_2 extract and standard squalene.

Chemical Analysis of Lipids, Fatty Acids, and Squalene Content in Carbon Dioxide Extracts and Carbohydrates and Proteins in Foods after **CO_2** Extraction

Chemical analysis of lipids

Instruments, Materials and Reagents

To analyze the lipids of the carbon dioxide extract, we used thin-layer Silufol silica gel, hexane, diethyl ether, acetic acid, 50% sulfuric acid, ready-made thin-layer chromatography (TLC) plates with sample classes of plant lipids. (Saturated and unsaturated carbohydrates, carotenoids, FA esters with alcohols, TAG, FFA, TF, phytosterols).

Analysis

The qualitative composition of the lipid fraction of the CO2 extract was determined by TLC on Silufol in a solvent system of 1) hexane: diethyl ether: acetic acid 7: 3: 0.1 (by volume) and 2) heptane: benzene (9: 1).). Lipid stains were developed by spraying the plates with 50% sulfuric acid and then burning them. To identify stains, including squalene, we used the literature [23] and our own data [24] on the nature of chromatographic mobility and expression of classes of plant lipids, as well as model classes of lipids.

In addition to squalene, the lipids of the extract contain fatty acid esters, tocopherols, free fatty acids, triterpenols and phytosterols.

Determination of fatty acid composition

Isolation of fatty acids (FA) for gas-liquid chromatographic analysis

FAs were isolated after saponification of the **CO_2** extract and removal of unsaponifiable matter (NS) from the reaction products.

Instruments, Materials and Reagents

A 250 ml round bottom flask, separating funnel, rotary evaporator, 50% sulfuric acid, methyl orange solution, diethyl ether, anhydrous sodium sulfate, and diazomethane were used in the work.

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Analysis:

The analysis was performed as described above with the following continuation.

After the NV was removed, a drop of methyl orange and 50% sulfuric acid were added to the soap solution in a separatory funnel to decompose the soap until the solution turned pink. The released FAs were extracted with diethyl ether (3 times 10-15 ml). The ether extracts were combined and washed with distilled water (3-5 times 10-15 ml each) until the washings were neutral in methyl orange. The absence of pink color in the washed water indicated complete removal of sulfuric acid from the solution. The ethereal solution was dried over anhydrous sodium sulfate, transferred to a dry round-bottom flask, and evaporated on a rotary evaporator under weak vacuum. The isolated FA was converted to methyl esters (ME) by treatment with diazomethane.

Gas-liquid chromatography (GC) ME LC

Apparatus, materials and reagents:

The analysis was carried out on an Agilent 6890 N gas chromatograph with a flame ionization detector, using a 30 m x 0.32 mm capillary column with a medium polar stationary phase HP-5, helium carrier gas, and column programming temperature from 150 °C to 270 °C. Identification of saturated FAs was carried out using a model mixture of ME fatty acids with a composition of 10:0-24:0. Unsaturated FAs were identified based on comparison of their retention time (RT) with the retention times of FAs isolated from natural sources. Table 2 shows the results of the analysis.

Table 2. Composition of fatty acids in carbon dioxide extract

№	Fatty acid	K acids by weight, %		
		Fabulous	Marhamat	Uzbekistan-M
1	Palmitinovaya, 16:0	26,34	22,76	23,93
2	Stearic, 18:0	3,70	3,75	3,39
3	Oleic18:1 + Linolenic 18:3 (ω_3)	33,94	35,58	35,73
4	Linolevaya, 18:2 (ω_6)	36,02	36,17	33,81
5	Arakhinovaya, 20:0	0,55	0,90	0,71
6	Begenovaya, 22:0	0,28	0,49	0,35
7	Lignoceric, 24:0	0,28	0,35	0,37
Total saturated fatty acids		31,15	28, 25	28,75
Total unsaturated fatty acids		69,96	71,75	69,54

From the data in Table 2 it follows that the CO_2 extract contains more than 22% saturated palmitic acid, the same 35-36% unsaturated oleic and omega-6 linoleic acids and about 2% (1.76%) high

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molecular weight saturated acids of the series 20:0 – 24 :0.

Using a chemical method, a fraction with a squalene content of 53% was consistently obtained from the cake.

The cake was extracted with CO_2 in a supercritical state. After supercritical CO_2 extraction, an extract was obtained containing, according to HPLC data, 6.8% squalene. In addition to squalene, the CO_2 extract contains fatty acid esters, triacylglycerides, free fatty acids (FA), triterpenols, and phytosterols. In total, omega-6 linoleic acid makes up 36% of fatty acids. From the CO_2 extract, a fraction with 45% squalene was isolated, as well as squalene itself of 92% purity.

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