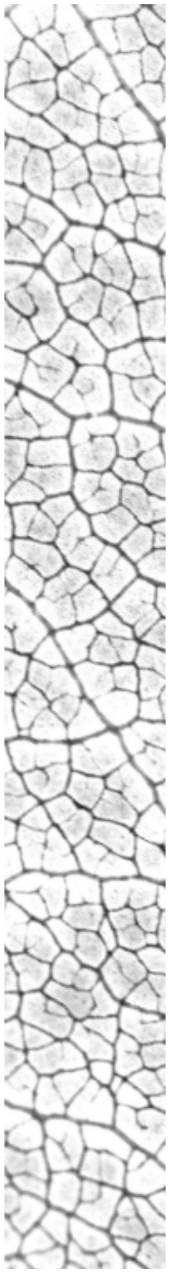

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Environmental study of fatty acid composition of safflower oil received by cold pressing method

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Abstract
Safflower is an olive culture of the sunflower family, the most widespread in the environment of arid regions of Central Asia. Its seeds contain 25-37% (in the core 46-60%) of semi-drying oils and up to 12% protein. As an object of study used safflower seeds of varieties Iyrkas. Aim of the present study was to determine the fatty acid composition of safflower oil received by cold pressing method. In order to do this, an SP-2560 column and a "Chromoteck 5000.1" gas chromatograph were used. When analyzing the chromatograms obtained in the study, the most crucial and complex stage is the identification of peaks. To determine the content of each fatty acid, the area normalization method was used. In the process of producing safflower oil in a cold way, waste such as cake is formed. In the study of the amino acid composition of safflower cake using ion-exchange chromatography with postcolumn derivatization of ninhydrid (without tryptophan), a Shimadzu LC-20 Prominence liquid chromatograph was used, and the determination of tryptophan by ion-exchange derivatization of ninhydrin. It is revealed that the protein of the safflower oil cake contains 18 amino acids. All amino acids that make up the safflower cake are α-amino acids. Mass fraction of essential amino acids in safflower oil was 5.66%, in particular, lysine contains 82.15 nmol/ml, methionine 45.10 nmol/ml. The obtained values for the quality characteristics indicate the prospects of using this type of natural oil: directly for food, as well as for production of oilseeds, such as mayonnaise, sauces, spreads; as biofuel; when designing and optimizing compound feed formulations.

Keywords: cold pressing, safflower, quality, safflower oil, fat-and-acid composition, safflower cake, complex use

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INTRODUCTION

Safflower is an oil-bearing culture of the sunflower family (**Fig. 1**). The most widespread safflower was found in the environment of arid regions of Central Asia (Antipov et al. 2015, Codex Alimentarius 2007). The climate of northwestern Kazakhstan, where safflower is mainly grown, is characterized by sharp temperature contrasts: cold winters and hot summers with a deficit of atmospheric precipitation. The average annual precipitation is 297 mm, incl. for the growing season of plant development - 37% of the annual rate. Deficiency of atmospheric precipitation is accompanied by intensive process of evaporation, great dryness of air and soil. The yield of safflower is 10-12 c/ha. Under favorable conditions of the year, as well as observing agrotechnical methods of cultivation (optimal sowing times, seeding rates and best predecessors), it is possible to obtain up to 15-17 c/ha or more.

Safflower is more drought-resistant, highly yielded, easier on agrotechnical and soil-climatic requirements. Safflower is grown mainly as an oilseed crop. Its seeds contain 25-37% (in the core of 46-60%) of semi-drying oils and up to 12% of protein. Due to the fact that it is very rich in unsaturated fatty acids, oil impregnates the skin faster and is absorbed almost instantly, this has been widely used in cosmetic products (Boyraz et al. 2016, Moses 2014, Nkongho et al. 2014, Rodrigues et al. 2016).

Safflower oil approximates the taste of sunflower and olive oil, it is used for food purposes for the production of margarine, spreads and mayonnaise. Safflower oil contains very little saturated fat and a lot of unsaturated fat. This makes it an excellent dietary product for people

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a - inflorescence of safflower b - seeds of safflower c - safflower cake

Fig. 1. Safflower

Table 1. Fatty acid composition of safflower oil

Fatty acid	*Group*	*Safflower oil (Codex Alimentarius 2007)*	*High-oleic safflower oil (Codex Alimentarius 2007)*	*False saffron oil (Ustenova et al. 2016)*	*Safflower oil (Wood et al. 2018)*
Lauric	C 12:0	HO	HO-0,2	-	-
Myristic	C 14:0	HO-0,2	HO-0,2	0,2	0,1
Palmitic	C 16:0	5,3-8,0	3,6-6,0	6,3	6,9
Palmitoleic	C 16:1	HO-0,2	HO-0,2	0,1	0,1
Stearic	C 18:0	1,9-2,9	1,5-2,4	2,8	2,6
Oleic	C 18:1	8,4-21,3	70-83,7	10,5	15,4
Linoleic	C 18:2	67,8-83,2	9,0-19,9	78,5	75,3
Linolenic	C 18:3	HO-0,1	HO-1,2	0,7	0,1
Arachic	C 20:0	0,2-0,4	0,3-0,6	0,1	0,4
Gondoinic	C 20:1	0,1-0,3	0,1-0,5	0,4	0,2
Behenic	C 22:0	HO- 1,0	HO-0,4	0,4	0,2
Erucidic	C 22:1	HO – 1,8	HO–0,3	-	-
Lignoceric	C 24:0	HO - 0,2	HO–0,3	-	0,1
Erucylacetic	C 24:1	HO - 0,2	HO–0,3	-	0,1

HO – not determined, taken as ≤ 0.05%

suffering from cardiovascular disease (Davooabadi and Aghajani 2013, Paramesha et al. 2011, Wood et al. 2018, Zubkov et al. 2014b).

By Zubkov et al. (2014a, 2014b) the prospects of using safflower dye oil in the food and pharmaceutical industries were studied. According to his research, the fatty acid composition is a complex of ten fatty acids, the predominant is linoleic (78.5%), which suggested the presence of biological activity in safflower oil, namely, hypocholesterolemic, which makes it promising raw material for use in scientific medical practice.

According to the research of the Kazakh National Medical University named after S.D. Asfendiyarov (Ustenova et al. 2016), the oil content of safflower seeds is similar to that of olive oil and includes 72% linoleic acid and seven antioxidant derivatives of serotonin. The use of safflower oil as an antioxidant is justified. Due to the high content of vitamin K, safflower oil is shown to people with skin disease, as a means of activating vascular repair, affecting the structural pattern and density of capillaries and the gastrointestinal tract (Antipov et al. 2015, Codex Alimentarius 2007, Ukhanov et al. 2016).

Analysis of oil from safflower seeds isolated from approximately three thousand seeds lots from two different varieties of low-oleic and high-oleic grades, given in the works of Australian scientists, testifies to the content of fourteen fatty acids. Linoleic acid contains 75.3%, omega-3 fatty acids - 0.2%, omega-6 fatty acids - 75.3%, omega-9 fatty acids - 13.2%. Studies of fatty acid composition indicate the presence of monounsaturated acids in safflower oil 93%, 1.5% polyunsaturated and 4% saturated (Ahmed et al. 2014).

MATERIALS AND METHODS

Seeds of safflower of grade “Iyrkas” were used as an object of research. The fatty acid composition of safflower oil is the basic quality characteristic. According to the literature, **Table 1** shows the fatty acid composition of safflower oil and safflower oil with a high content of oleic acid.

The pressing regime affects the chemical composition of the oil. The test sample was obtained on the experimental unit “Screw oil-press” on the basis of the department of technology of fats of processes and apparatuses of chemical and food productions FSBEI of HE “VSUET” (**Fig. 2**) with the following parameters: annular clearance of the pressing chamber is: 0.7 mm; the optimal speed of the screw is 6-7s-1, while the temperature is 328-333 K.

Physicochemical parameters were determined in accordance with GOST 18848-73 “Vegetable oils. Quality indicators”.

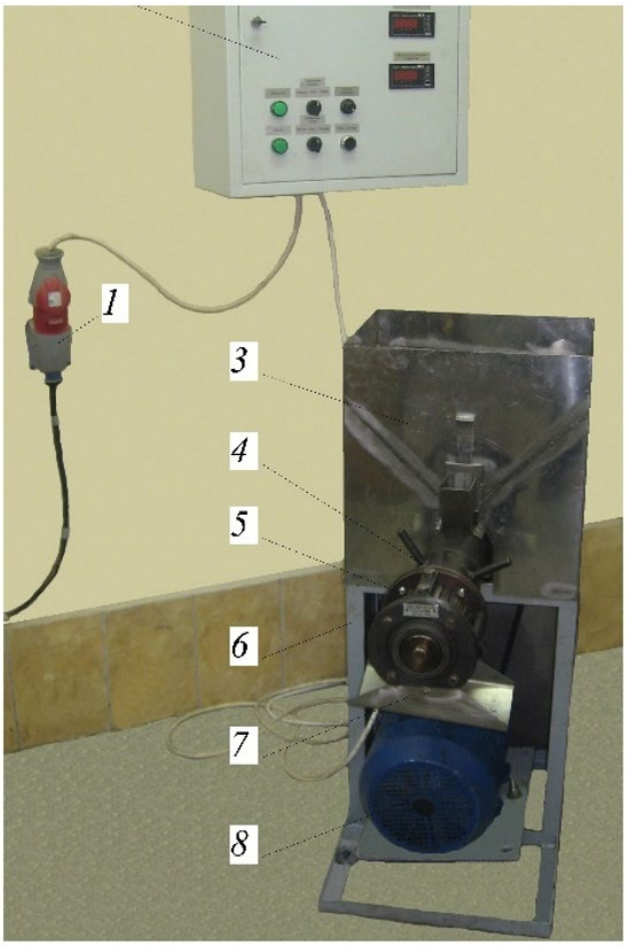

Fig. 2. Experimental installation of MPE-1: 1 - power supply; 2 - control panel, 3 - charging hopper; 4 - regulating device of the clearance; 5 - pressing chamber; 6 - mount; 7 - oil collector; 8 - electric coupling

To determine the fatty acid composition, an SP-2560 column and a "Chromoteck 5000.1" gas chromatograph were used (**Fig. 2**). When analyzing the chromatograms obtained in the study, the most crucial and complex stage is the identification of peaks. To determine the content of each fatty acid, the area normalization method was used. **Table 2** shows the results of calculations for the components of safflower oil.

Fig. 3. Chromatograph "Chromoteck 5000.1"

The weight fraction of each of the acids was calculated by the formula

$$\chi_i = \frac{S_i \cdot 100}{\sum_i S_i} \quad (1)$$

where S_i - the area of the peak of ethyl ether, mm2; $\sum_i S_i$ - the sum of the areas of all peaks in the chromatogram, mm2.

RESULTS AND DISCUSSION

From the diagram (**Fig. 5**) and **Table 2** it is evident that fatty acids of 18 and 16 groups prevail in safflower oil, the content of other fatty acids in the total is 1.2%.

In the test sample, the prevalence of omega-6 fatty acids (concentration of 80% of linoleic and γ-linolenic fatty acids) is observed.

Omega-6 fatty acids help the body burn excess fat, instead of postponing it for future use. Natural fatty acid kits are bricks of human prostaglandins, hormone-like substances that help normalize blood pressure, control muscle contractions and participate in the immune response of the organism.

The obtained data of fatty acid composition of the analyzed sample of safflower oil is well correlated with the literature data (Antipovet al. 2015, Codex

Table 2. Calculation of the components of safflower oil

Time, min	Group	Content, mm²	Height, mm	Concentration, %
38,294	14:0	32,992	7,095	0.122
42,415	16:0	1735,589	341,807	6.460
43,925	16:1	5,507	1,146	0.020
44,162	16:1	16,572	3,405	0.062
44,813	16:1	9,405	1,780	0.035
47,746	18:0	653,760	98,144	2.433
49,711	18:1	2613,986	295,746	9.729
49,963	18:1	172,445	24,437	0.642
52,071	18:2	19,851	2,301	0.074
52,939	18:2	21342,949	1467,863	79.434
53,809	20:0	95,015	17,102	0.354
55,901	20:1	42,382	7,301	0.158
56,293	18:3	31,454	4,764	0.117
60,641	22:0	69,204	14,173	0.258
67,335	24:0	27,737	5,091	0.103

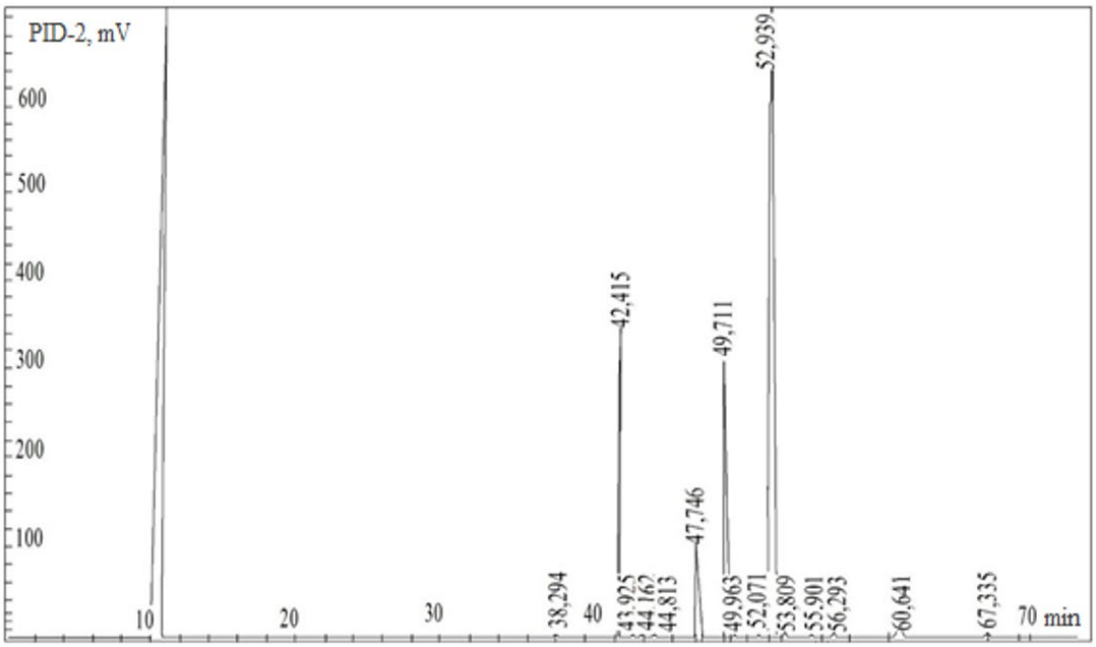

Fig. 4. Chromatogram of determination of fatty acid composition

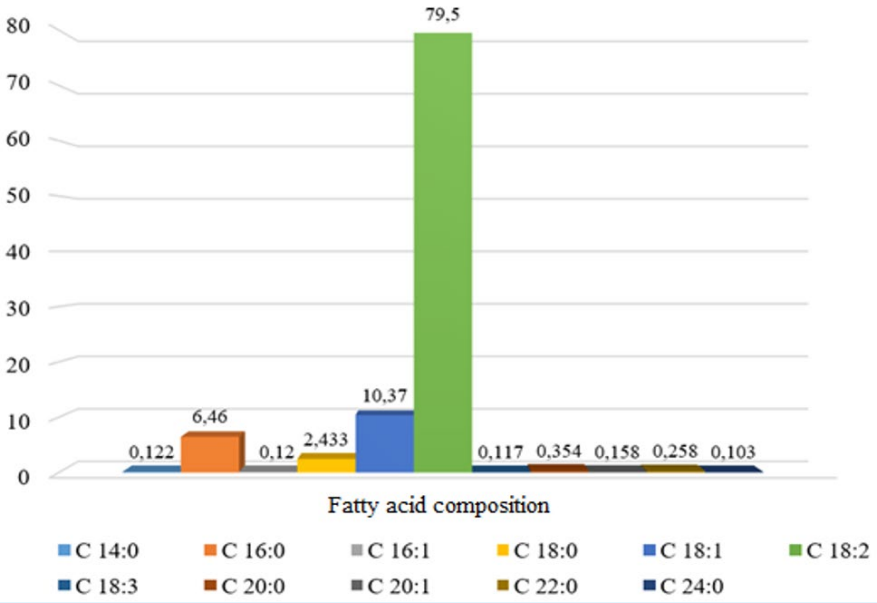

Fig. 5. Fatty acid content

Alimentarius 2007, Zubkov et al. 2014b), which indicates the high accuracy of the conducted studies.

The qualitative characteristics of vegetable oil are also physicochemical indicators. The acid number of safflower oil was AN = 1.07 mgKOH/g, peroxide number of PN = 8.09 mmol/kgO_2, anisidine number of safflower oil AdN = 3.25. The humidity of the safflower oil is 0.03%. The obtained data indicate the possibility of using this oil directly in food, as well as for the production of oilseeds, such as mayonnaise, sauces, spreads. Storage temperature for safflower oil is not regulated, but loading and unloading of oil should be carried out in the temperature range from 10-20 ° C.

In the process of production of safflower oil, a waste such as cake is produced in a cold way. At present, there are more than 400 oil producing enterprises in Russia with a productivity of 50 t/day to 3000 t/day. The most effective innovations are developments in the field of utilization and processing of waste products of fat and oil production.

Processing of oil cake and oil meal significantly reduces the costs of exporting production waste, additional income from the sale of new products obtained during the processing of waste, and improvement of the environmental situation. Taking this into account, it is necessary to analyze the composition

Table 3. Amino acid content in the sample

№	Name	Name of amino acid	C, nmol / ml	Mass fraction, %
1	ASP+ASN	aspartic acid + asparagine	387,97	1.64
2	THR	threonine	140,17	0.53
3	ALA	alanine	258,46	0.73
4	VAL	valine	203,71	0.76
5	SER	serine	228,99	0.76
6	GLU+GLN	glutamic acid + glutamine	659,56	3.07
7	GLY	glycine	386,72	0.92
8	LEU	leucine	253,81	1.05
9	CYS	cysteine	15,11	0.12
10	MET	methionine	45,10	0.21
11	ILEU	isoleucine	134,43	0.56
12	TYR	tyrosine	72,58	0.42
13	PHE	phenylalanine	138,74	0.74
14	HYS	histidine	88,99	0.43
15	LYS	lysine	82,15	0.38
16	ARG	arginine	239,20	1.32
17	PRO	proline	184,86	0.67
18	TRP	tryptophan	20,66	0.11

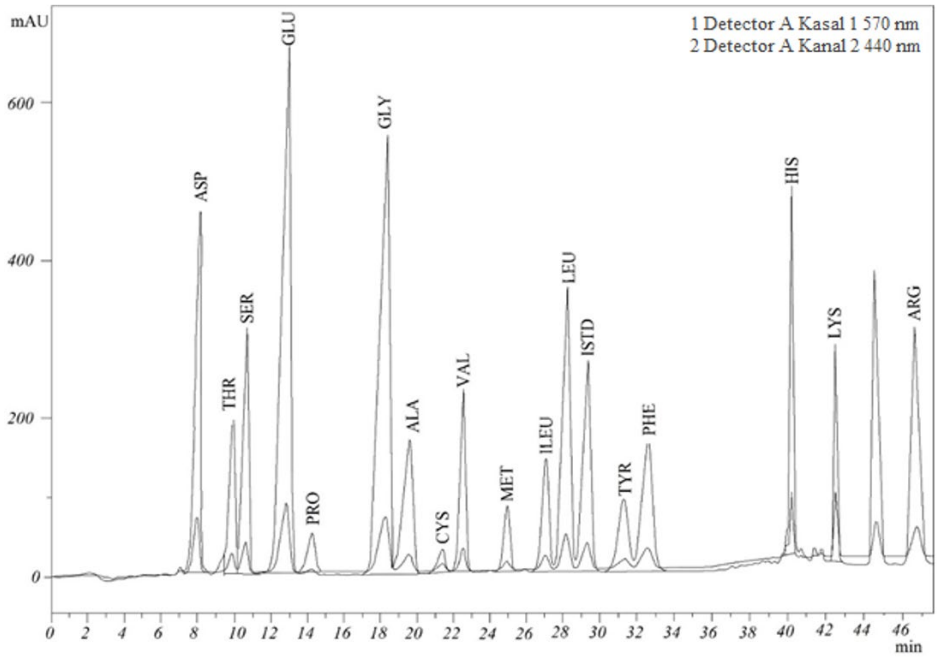

Fig. 6. Chromatogram of determination of amino acid composition

of waste products for the development of actual promising compound feed formulas, using safflower cake.

The liquid chromatograph Shimadzu LC-20 Prominence was used to study the amino acid composition. Determination of the amino acid composition was carried out by the method of ion exchange chromatography with postcolumn derivatization of ninhydrid (without tryptophan), and determination of tryptophan by ion-exchange derivatization with ninhydrin.

Ion exchange chromatography refers to liquid-solid phase chromatography, in which the mobile phase is the eluent (liquid), and the stationary phase is the ion exchanger (solid).

The ion exchange chromatography method is based on the process of substitution of ions bound to the stationary phase by the ions of the eluent entering the column. Separation occurs due to the different affinity for the ion exchanger of ions in the mixture, which leads to different rates of their movement across the column. Ion chromatography includes all high-performance liquid chromatographic ion separation in columns combined with direct detection in a flow detector and quantitative processing of the obtained analytical signals.

As a result of the research, it has been established that the composition of the safflower oil cake protein includes 18 amino acids (**Table 3**, **Fig. 6**). The mass fraction of these fatty acids is given in **Table 3**. The nutritional value of plant proteins is determined by their digestibility and the content of essential amino acids.

The mass fraction of crude protein in rapeseed cake was determined by the Kjeldahl method. The moisture content of the cake, which was received for research, was determined at 7.4%.

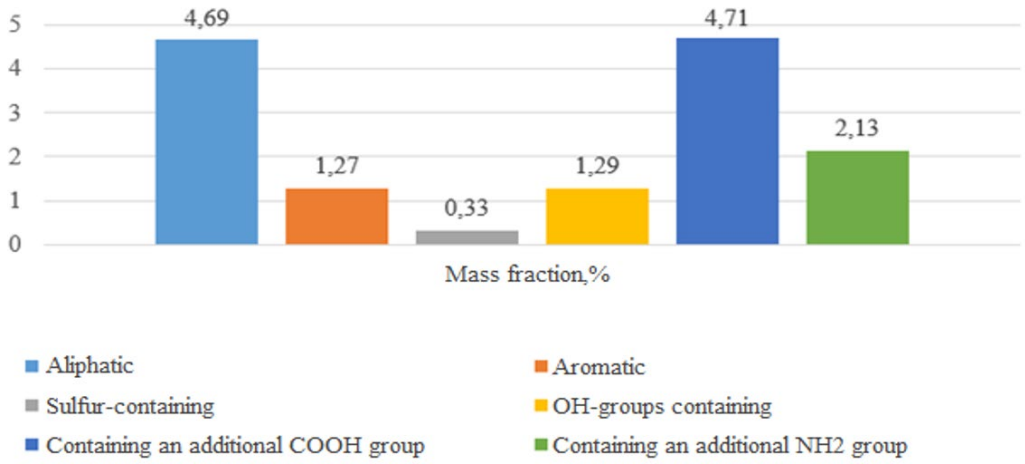

Fig. 7. Distribution of amino acids contained in safflower oil in the structure of the lateral radical

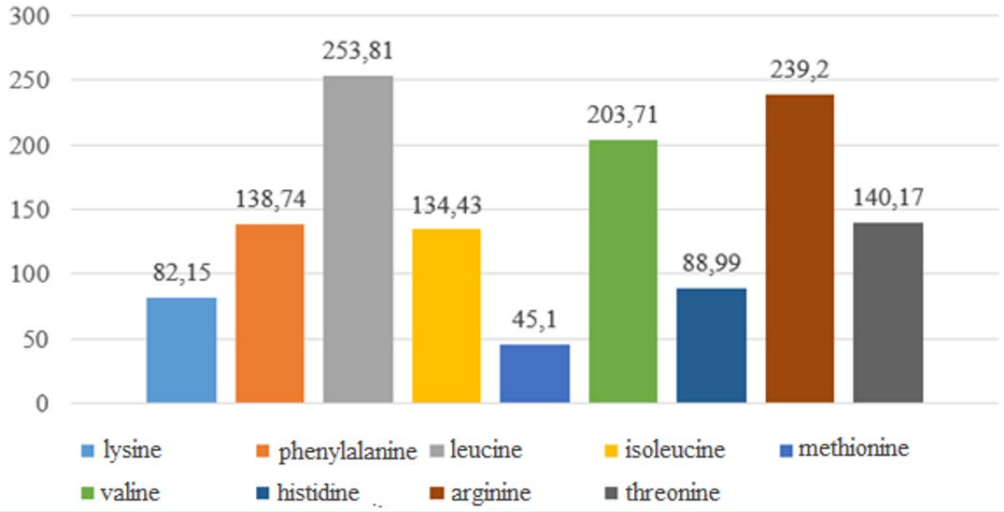

Fig. 8. Essential amino acids of safflower oil, nmol/ml

To determine the protein content, the following equipment was used: the protein decomposition plant Turbotherm (Gerhardt), the ammonia distillation unit Vapodest (Gerhardt). As a result of the studies it was established that the mass fraction of crude protein in safflower cake was 19.9%.

Amino acids in structure are organic carboxylic acids, in which at least one hydrogen atom is replaced by an amino group. In amino acids, a carboxyl group (COOH), an amino group (NH2), an asymmetric carbon atom and a side chain (radical R) are necessarily present. It is the structure of the side chain of amino acids that differ from each other.

Aliphatic, aromatic, sulfur-containing OH-groups containing an additional COOH group and an additional NH2 group are distinguished by the structure of the lateral radical. The distribution of amino acids contained in safflower oil according to the structure of the lateral radical is shown in **Fig. 7**.

By the participation of amino acids in protein synthesis, proteinogenic and non-proteinogenic amino acids are isolated. All proteinogenic amino acids are α-amino acids. Non-proteinogenic amino acids - amino acids that do not participate in protein biosynthesis (not part of protein), many of them are toxins and inhibitors of enzymes of various metabolic reactions. All the amino acids that are part of the composition of the safflower cake are α-amino acids, which are the compounds from which the protein molecule is built.

Some amino acids are not synthesized in humans and animals. They got the name essential. Such amino acids only ten. Four of them are limiting - they most often limit the growth and development of animals. For example, in rations for poultry, the main limiting amino acids are methionine and cysteine, in rations for pigs - lysine. The body should receive a sufficient amount of the main limiting acid with food so that other amino acids can be effectively used for protein synthesis. Of the eighteen amino acids presented in the safflower cake, nine are essential (**Fig. 8**).

The mass fraction of essential amino acids in safflower oil was 5.66%, in particular, lysine contained

82.15 nmol / ml, methionine 45.10 nmol / ml. Safflower cake will help replenish protein deficiency in animal diets.

The results indicate the possibility of using waste products of safflower oil obtained by the cold pressing method in the production of mixed fodders and premixes.

CONCLUSION

The obtained values for qualitative characteristics testify to the prospects of using this type of oil directly in food, as well as for the production of oilseeds, such as mayonnaise, sauces, spreads.

Safflower oil can be used as biofuel, the lowest heat of its combustion is 36,978 MJ/kg; density - 913 kg/m^3; kinematic viscosity 85.6 mm^2/s. In comparison with rapeseed oil, the specific effective fuel consumption is reduced by 2.08%.

The obtained results of studies of safflower cake show that it can be used in the design and optimization of compound feed formulas.

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