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RESEARCH OF BIOLOGICALLY ACTIVE SUBSTANCES OF HEMP SEEDS, HEMP SEED OIL AND HEMP POMACE

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For the time being, the use of cannabis for medical purposes is more and more relevant. A review of literary sources shows that Ukrainian varieties of hemp are insufficiently studied. Therefore, the variety «Glesia» was chosen for the study, as it is the most promising Ukrainian variety. Fatty oil from hemp seeds is the leading pharmaceutical and food product produced from this raw material in Ukraine. During its production, the pomace remains, which is used for feeding animals. At the same time, it still contains many other BAS and can be a valuable raw material for creating pharmaceutical products. Therefore, developing technologies for the complex processing of this raw material is an urgent task of modern pharmaceutical science.

The aim of this work *was a phytochemical study of biologically active substances of hemp seeds, hemp seed oil and hemp pomace in order to develop the new phytoremedies.*

Materials and methods. *Non-narcotic hemp seeds of the «Glesia» variety, hemp seed oil and hemp pomace were the objects of research. The elemental analysis was made using inductively coupled plasma atomic emission spectrometry - iCAP 7000 Duo; the study of amino acids was made using ion exchange chromatography; the study of fatty acids was made using gas-liquid chromatography. In addition, the content of vitamin E (α-, β- and γ-tocopherols) was studied using high-performance liquid chromatography (HPLC) with UV detection; the content of protein was studied using A.I. Ermakov method in O. O. Sozinov and F. O. Poperelia modification.*

Research results. *The analysis of the qualitative characteristics of the obtained fatty oils shows that all indicators met the requirements of the State Standard of Ukraine. For the first time, the transition of macro- and microelements from hemp seeds of the «Glesia» variety into fatty oil was determined, and their residue in the pomace was established. The content of 16 amino acids was determined. The content of saturated and unsaturated fatty acids in oil samples was established. The content of α- β- γ-tocopherol in hemp seeds, hemp oil and hemp pomace was investigated using GC/MS. It was found that the protein content in the pomace was in the range of 32.8–34.6 %.*

Conclusions. *We conducted a complex study of biologically active substances of non-narcotic hemp seeds of the «Glesia» variety that was harvested in 2019 and 2020, the hemp oil and hemp pomace. It was established that the content of macro- and microelements in the studied raw material of Cannabis sativa L. corresponds to the following order: Ca>Mg>Si>Fe>Al>Mn Zn>Sr>B>Cu>Ba>Cr and Ni>Se>Co>Mo>Cd>Be>I>Pb. The content of 16 amino acids was determined. Of them, 7 amino acids are essential (leucine, valine, threonine, lysine, methionine, isoleucine, phenylalanine), 2 amino acids are essential for children (histidine and arginine), and 7 amino acids are replaceable (alanine, tyrosine, proline, glycine, glutamic and aspartic acids). It was found that the main fatty acids of all samples were linoleic, oleic and linolenic. The content of α- and γ-tocopherol predominated in the studied samples. Hemp seeds of the «Glesia» variety and hemp pomace contain protein. The protein content in the pomace ranged from 32.8 to 34.6 %*

Keywords: *Cannabis sativa L., seeds, fatty oil, pomace, macroelements, microelements, fatty acids, amino acids, tocopherol, protein*

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1. Introduction

Cannabis sativa L. is one of the most ancient crops in the history of Mankind. *Cannabis sativa L.* has been cultivated by humans for more than 10,000 years to obtain edible fatty oil and textile fibre. Hemp fatty oil is the only natural oil with the optimal ratio of linoleic and linolenic acids necessary for the human body [1].

Cannabis sativa L. of the family *Cannabinaceae* is an annual herbaceous dioecious plant up to 4 m high. In Ukraine, hemp is cultivated as an oil and textile plant [1, 2].

In folk medicine, hemp oil is used for treating infectious and inflammatory diseases of the upper respiratory tract, skin, joints, and gallbladder; for treating hormonal disorders, decreased immunity and tuberculosis. In addition, hemp oil helps to reduce fat plaque on the walls of blood vessels, thins the blood and prevents the development of varicose veins. As early as 400 BC Hippocrates said, “Let food be your medicine” [3].

Hemp oil from industrial (non-narcotic) hemp seeds contains polyunsaturated fatty acids (Omega-3 and Omega-6), essential amino acids, vitamin E, and macro-

and microelements [4]. The ratio of unsaturated fatty acids Omega-3 and Omega-6 in hemp oil is the ideal ratio for the human body (3:1). It is balanced for human health and meets the recommendations of the World Health Organization [5–7].

In cosmetology, hemp oil is used as a component of medical cosmetics with an emollient and moisturizing effect. The pomace is a by-product of hemp seed processing. Its production is quite large. In Ukraine and the world, the pomace is mainly used for feeding livestock because of the content of easily digestible protein, polyunsaturated fatty acids (Omega-3, Omega-6), amino acids, and macro- and micronutrients [8].

For the time being, the use of cannabis for medical purposes is more and more relevant. A review of literary sources shows that Ukrainian varieties of hemp are insufficiently studied. Therefore, the variety «Glesia» was chosen for the study, as it is the most promising Ukrainian variety. Fatty oil from hemp seeds is the leading pharmaceutical and food product produced from this raw material in Ukraine. During its production, the pomace remains, which is used for feeding animals. At the same time, it still contains many other BAS and can be a valuable raw material for creating pharmaceutical products. Therefore, developing technologies for the complex processing of this raw material is an urgent task of modern pharmaceutical science. Taking this into account, the objects of our research were the seeds, fatty oil and pomace of the «Glesia» variety.

The aim of the study. The phytochemical study of biologically active substances of hemp seeds, hemp oil and hemp pomace to develop the phytoremedies.

2. Planning (methodology) of research

Fig. 1 shows a graphical representation of the research planning process.

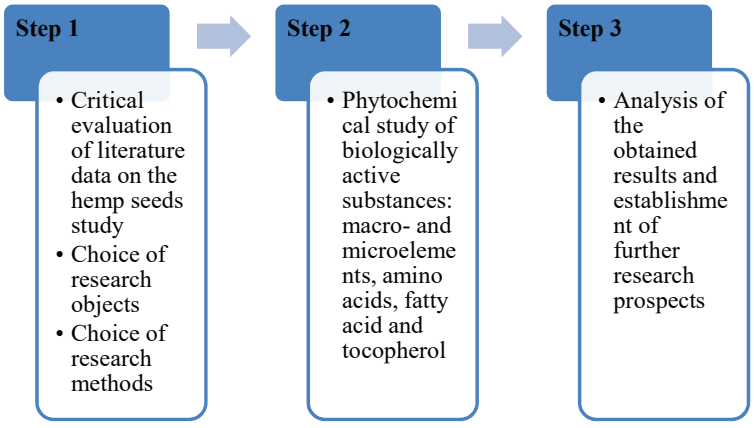


Fig. 1. Planning of the research

3. Materials and methods

3. 1. Research objects

The seeds of the non-narcotic hemp «Glesia» variety from the Institute of Bast Crops of NAAS, Hlukhiv, Sumy region, the hemp oil and the hemp pomace were the objects of the research.

The samples of seeds harvested in 2019 (No. 1) and 2020 (No. 2), hemp oil No. 1 and No. 2, respectively, and cake No. 1 and No. 2, respectively, were used for the research. The seeds were stored in cardboard boxes in a dark cabinet at a stable temperature.

We obtained fatty oil from the seeds of «Glesia» sowing hemp by cold pressing method at a temperature of up to 50 °C on a laboratory screw press. According to organoleptic characteristics, hemp oil is dark green in colour with a pleasant nutty aroma.

For the study of non-narcotic hemp seeds of the «Glesia» variety, Industrial (technical) hemp was obtained. It is an annual bast plant that is grown for fibre and seeds.

Nowadays, industrial hemp is one of the agricultural crops that most fully meets the strategic goals and objectives of the state environmental policy of Ukraine until 2030, approved by the Law of Ukraine of February 28, 2019, No. 2697-VIII.

Employees of Bast Crops Research Station of the Institute of Agriculture of the North – East NAAS of Ukraine were the first in the world who create varieties of hemp without narcotic substances. These varieties are included in the Register of Plant Varieties of Ukraine, Russia, EU and Canada. Such hemp cannot be used as a raw material for the manufacture of narcotic drugs; their cultivation is not socially dangerous [9].

The absence of alkaloids was confirmed by an adverse reaction with Dragendorff's reagent [10]. We used samples of technical hemp of "Glesia" variety for the study. Using gas-liquid chromatography, the content of tetrahydrocannabinol up to 0.0023 % was determined in the samples. This result confirms that hemp seeds are non-alkaloid. The content of the psychoactive substance tetrahydrocannabinol does not exceed 0.3 % (according to European legislation 0.2 %).

3. 2. Methods of analysis

We obtained fatty oil from hemp seeds of the "Glesia" variety using a laboratory screw press (Patent of Ukraine No. 114066) at temperatures up to 50 °C [11]. According to organoleptic characteristics, hemp oil is a dark green liquid with a pleasant nutty aroma. Therefore, we have obtained the fatty oil of seed hemp. 189 g of fatty oil (18.9 %) and 790 g of cake (79 %) were obtained from 1 kg of hemp seeds.

To determine the qualitative characteristics of the oil, the following indicators were used: acid and iodine numbers and saponification numbers. The requirements for hemp seeds used in industrial processing are set out in GOST 9158-76 [11]. In addition, hemp oil quality indicators are specified in the State Standards of Ukraine [12–19].

The **elements** of hemp seeds, hemp oil and hemp pomace samples were studied using inductively coupled plasma atomic emission spectrometry – iCAP 7000 Duo [20].

Sample preparation involved homogenization, weighing, the addition of nitric acid, and transferring the appropriate sample to a microwave oven. Under the influence of pressure and temperature, the decomposition of the samples occurs. The resulting sample is diluted with deionized water and injected into an inductively coupled plasma

atomic emission spectrometer, which includes a computer-controlled background-corrected atomic emission spectrometer, a radio frequency generator, and an argon supply system. Atomic emission was measured by optical spectroscopy. The sample was sprayed, and the formed aerosol was transported to the plasma torch, where the excitation occurred. Characteristic atomic emission lines were generated by radio frequency inductively coupled plasma. The measurement spectrum was spread out on the diffraction grating of the spectrometer, and the detector recorded the intensity of the lines. The signals from the detectors were monitored and processed by a computer system.

The **amino acids** of hemp seeds, hemp oil and hemp pomace samples were established using ion exchange chromatography. The method is based on the separation of amino acid mixture using an ion exchange column. The elution of amino acids was carried out with various buffer solutions with a constantly increasing pH value (from 3.25 to 5.28).

The determinations were carried out using ninhydrin reaction and photometric detection at a wavelength of 570 nm. The analysis was made using the amino acid analyzer T-AAA 339 M (Czech Republic) in comparison with the standards of amino acid hydrolysates by SSTU (State Standard of Ukraine) ISO 13903: 2005 [21–23].

The **fatty acids** of hemp seeds, hemp oil and hemp pomace samples were determined according to SSTU ISO 5509: 2001 "Animal and vegetable fats and oils. Preparation of methyl esters of fatty acids" [24]. The fatty acids of hemp seed oil samples were studied using gas-liquid chromatography. The sum of lipophilic compounds was removed from the air-dry raw material using the Soxhlet apparatus and exhaustive extraction with hexane. Then it was released from hydrophilic substances and methylated with acetyl chloride in the medium of methyl alcohol. The obtained fatty acid esters were extracted with hexane and analyzed using Hewlett Packard chromatograph, USA (sorbent – polyethene glycol 20 M; injector and detector temperature – 220 °C; oven temperature – 65 °C; holding time – 10 minutes; carrier gas – nitrogen, carrier gas velocity – 2 ml/min, flow distribution – 1:10, injection volume – 1 μl). The methyl esters were identified by the retention time of the peaks compared to the time of standard mixture methyl esters release and the time of standardized olive oil methyl esters release [24].

The determination of **vitamin E** content (α-, β- and γ-tocopherols) of hemp seeds, hemp oil and hemp pomace samples was carried out in comparison with the standards of amino acid hydrolysates following GOST EN 12822-2014 from 01.01.2016 (Food products) using high-performance liquid chromatography (HPLC) with photometric (ultraviolet) detection. To prepare the sample solution, it is necessary to saponify the sample material with the extraction of analytes. The comparison sample of tocopherol was weighed to the nearest 1 mg and dissolved in a specific volume of the appropriate solvent – *n*-hexane (when the method of normal-phase HPLC is used) and methanol (when the method of reversed-phase HPLC is used) [25, 26].

The study of **protein** of hemp seeds, hemp oil and hemp pomace samples was carried out according to A. I. Ermakov method in O.O. Sozinov and F.O. Poperelia modification. The determination of protein fractions is based on the extraction of proteins by solutions used in two sequences:

1) salt, alkaline and alcohol;

2) salt and alcohol [27].

A portion of the test sample weighing not more than 0.5 g was taken from freshly ground seeds, and water-salt, alcohol-soluble and alkali-soluble protein fractions were successively removed from it. Aqueous solutions were used for this purpose: 2 % sodium chloride solution, 70 % ethyl alcohol and 0.2 % sodium hydroxide solution. Samples were added to the flasks, and 10–15 times the amount of solution was added, shaken for 1 h at room temperature. After shaking, the extracts were separated from the precipitate by centrifugation at 6000 rpm. Extraction of each fraction was made in triplicate [27]. The protein content of the extracts was determined by the Kjeldahl method. First, when adding the catalyst and concentrated sulfuric acid, the extracts were evaporated in a Kjeldahl flask to obtain 1 ml. Then the combustion, distillation and titration according to standard methods were carried out. In parallel, the protein content in cannabis seeds was determined. The difference between the amount of protein in all fractions and the protein content of whole seeds did not exceed 5 % [27]. When converting the nitrogen content into the protein content, the coefficient of 6.25 was used (as for high-protein crops). The separated fractions were determined as a percentage of the total protein content of the sample. The difference between the parallel definitions did not exceed 0.3 % [27].

The research was conducted based on the State Enterprise «Ivano-Frankivsk Scientific and Production Center for Standardization, Metrology and Certification» (accreditation certificate No. 2H098 dated 20.06.2014).

Statistical analysis. All results are processed according to the State Pharmacopoeia of Ukraine, using the method of variation statistics with the calculation of the arithmetic mean and its standard error. Student's test evaluated the reliability of the compared values, and the probability level was taken as $p \leq 0.05$.

4. Research results

We obtained the fatty oil of hemp seeds. 189 g of fatty oil (18.9 %) and 790 g of the pomace (79 %) were obtained from 1 kg of hemp seeds of the «Glesia» variety. According to organoleptic characteristics, hemp oil is a dark green liquid with a pleasant nutty aroma.

The established qualitative characteristics of hemp oil are presented in Table 1.

The analysis of the qualitative characteristics of the obtained fatty oils No. 1 and No. 2 shows that all indicators were within the norm according to SSTU. Therefore, the results indicate that these oils meet the requirements of the State Standard of Ukraine.

The results of the study of macro- and microelements in the samples of *Cannabis sativa L.* are given in Table 2.

The results of the study of amino acids in hemp seeds, hemp oil and hemp pomace are presented in Table 3.

The results of fatty acid determination in hemp oil No. 1 and No. 2 are presented in Table 4 and Fig. 2.

Table 1

Physical and chemical parameters of hemp oil

Indicator	The norm, according to the normative document	Hemp oil from the seeds of the 2019 harvest	Hemp oil from the seeds of the 2020 harvest	Regulatory document for the test method
Acid value, mg KOH	3.0	2.1	1.8	SSTU 4350
Moisture and volatile matter, %	0.2	0.16	0.15	SSTU 4603
Insoluble Impurities, %	0.05	0.02	0.01	SSTU 5063
Phosphorus-containing substances, in terms of P_2O_5, %	0.04	0.03	0.028	SSTU 7082
Peroxide value, mmol/kg, no more	6.0	1.8	1.8	SSTU 4570
Colour number, mg of iodine, no more	100	145	145	SSTU 4568
The degree of transparency, fem, no more	25	8.5	8.3	SSTU 8842

Table 2

Macro- and microelements of *Cannabis sativa L.* raw materials

The element	*Cannabis sativa L.* raw material					
	Hemp oil No. 1	Hemp oil No. 2	Hemp pomace No. 1	Hemp pomace No. 2	Hemp seeds No. 1	Hemp seeds No. 2
mg/kg of raw material						
Ca	1939.20±84.33	1939.62±72.82	4171.05±124.36	4171.75±152.42	3884.03±111.93	3884.67±109.76
Mg	391.90±12.92	391.93±14.46	842.89±24.12	842.97±31.36	784.89±24.82	784.96±28.54
Si	81.45±3,36	81.77±4,01	175.18±4,85	175.88±4,62	163.13±3,96	163.78±3,59
Fe	53.90±1.96	54.69±2.11	117.33±3.02	117.64±2.53	109.26±2.31	109.54±2.58
Al	28.79±1.08	28.89±1.13	61.42±2.47	62.15±1.99	57.19±2.11	57.87±1.36
Mn	23.52±1.13	23.65±0.95	50.59±2.02	50.86±1.96	47.11±0.83	47.36±1.11
Zn	21.03±0.96	21.25±1.02	45.22±1.16	45.71±1.36	42.11±1.14	42.56±2.08
Sr	9.88±0,28	9.93±0,31	21.24±0,93	21.36±1,05	19.78±0,94	19.89±0,87
B	4.98±0.21	5.02±0.19	10.69±0.22	10.76±0.38	9.95±0.65	10.02±0.47
Cu	3.75±0.12	3.91±0.06	8.05±0.07	8.40±0.25	7.50±0.21	7.82±0.18
Ba	1.99±0.07	2.01±0.09	4.27±0.15	4.33±0.21	3.98±0.17	4.03±0.22
Cr	0.14±0.01	0.14±0.01	0.30±0.04	0.30±0.03	0.28± 0.02	0.28±0.02
μg/kg of raw material						
Ni	299.58±12.01	299.66±14.32	644.34±11.87	644.39±23.45	600.00±21.22	600.08±31.24
Se	74.90±3.21	74.93±2.87	161.09±4.53	161.11±4.11	149.96±3.57	150.03±3.87
Co	19.93±0.84	19.99±0.56	42.94±0.76	42.97±1.37	39.99±1.49	40.04±1.65
Mo	9.97±0.34	10.01±0.51	21.46±1.12	21.49±1.37	20.01±1.24	20.07±0.95
Cd	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
Be	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
I	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
Pb	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002

Table 3

Amino acids of *Cannabis sativa L.* raw materials

Amino acid	The content in the test sample, % *n*=3					
	Hemp seeds No. 1	Hemp seeds No. 2	Hemp oil No. 1	Hemp oil No. 2	Hemp pomace No. 1	Hemp pomace No. 2
Alanine	1.20±0.0206	1.23±0,0344	1.25±0.0515	1.28±0.0550	1.39±0.0790	1.42±0.0481
Arginine	3.05±0.0378	3.19±0.0412	3.50±0.0653	3.76±0.1375	4.19±0.0859	4.33±0.0859
Aspartic acid	3.12±0.0274	3.59±0.0412	2.99±0.0722	3.47±0.0619	4.56±0.0894	4.82±0.0859
Cysteine	0.41±0.0206	0.49±0.0309	0.41±0.0412	0.48±0.0515	0.63±0.0550	0.76±0.0343
Glutamic acid	5.47±0.1718	5.40±0,0447	5.46±0.0790	5.38±0.0756	7.99±0.0722	7.96±0.0652
Glycine	1.20±0.0274	1.23±0.0412	1.23±0.0378	1.25±0.0790	1.51±0.0928	1.55±0,0481
Histidine	0.96±0.0550	0.95±0.0790	0.95±0.0722	0.94±0.0825	1.22±0.0515	1.29±0,0893
Isoleucine	1.15±0.0515	1.19±0.0481	1.16±0.0447	1.18±0.0653	1.51±0.0825	1.73±0,0481
Leucine	1.95±0.0309	2.05±0.0550	1.92±0.0653	2.01±0.0722	2.28±0.0756	2.26±0.0412
Lysine	0.94±0.0412	0.95±0.0756	0.94±0.0515	0.95±0.0756	1.21±0.0447	1.23±0.0412
Methionine	0.59±0.0309	0.61±0.0447	0.59±0.0790	0,61±0.0619	0.89±0.0412	0.92±0.0446
Phenylalanine	1.05±0.0447	1.04±0.0447	1.18±0.0481	1.16±0.0481	1,32±0.0412	1.31±0.0481
Threonine	1.01±0.0481	1.04±0.0309	1.05±0.0481	1.06±0.0722	1,36±0.0378	1.38±0.0378
Serine	1.54±0.0378	1,57±0.0481	1.68±0.0584	1.67±0.0687	1,91±0.0584	1.90±0.0756
Tryptophan	1.06±0.0412	1.08±0.0412	1.06±0.0550	1.08±0.0928	1,38±0.0481	1.39±0.0825
Valine	1.44±0.0412	1,42±0.0447	1.48±0.0549	1.46±0.0515	1,78±0.0412	1.76±0.0756

Table 4

Fatty acids of hemp oil

No.	Fatty acid	The content of methyl esters of fatty acids, %	
		Hemp oil No. 1	Hemp oil No. 2
1	Palmitic acid	6.66	6.67
2	Palmitoleic acid	0.02	0.16
3	Stearic acid	2.79	2.94
4	Oleic acid	16.23	19.20
5	Linolic acid	57.74	55.85
6	γ – linolenic acid	1.16	1.14
7	Linolenic acid	14.81	13.44
8	Eicosenoic acid	0.60	0.59

We studied the content of α- tocopherol, β- tocopherol and γ-tocopherol in hemp seeds, hemp oil and hemp pomace using GC/MS method.

The results of the study are presented in Fig. 3.

Many indicators determine the quality of plant products. The protein content is one of the main.

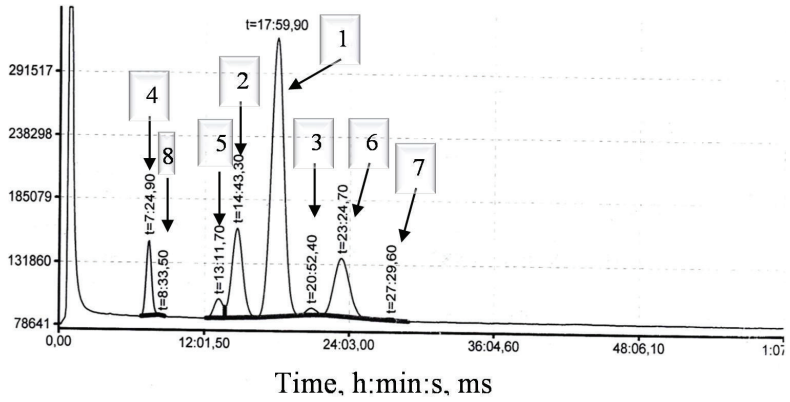
The results of the study of the protein content in hemp seeds and hemp pomace are shown in Table 5.

The results of the study show (Table 6) that hemp seeds and hemp pomace contain protein. The protein content in the hemp pomace was in the range of 32.8 to 34.6 %. The highest protein content was found in the hemp pomace of the 2020 harvest.

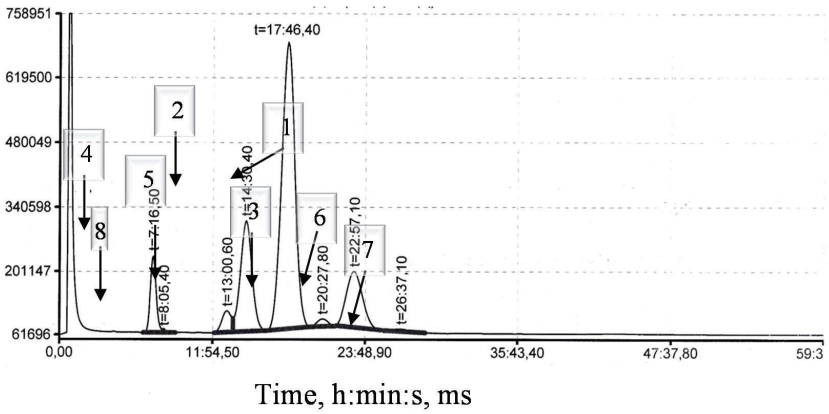
Table 5

The protein content in *Cannabis sativa L.* raw materials

Sample	Hemp seeds No. 1	Hemp seeds No. 2	Hemp pomace No. 1	Hemp pomace No. 2
	$\overline{x} \pm \Delta\overline{x}$, n=3			
Protein content, %	24.8±0.31	26.6±0.24	32.8±0.51	34.6±0.36



a



b

Fig. 2. The chromatogram of hemp oil methyl esters identified using the GC method: *a* – No. 1, 2019; *b* – No. 2, 2020; 1 – linoleic acid, 2 – oleic acid, 3 – linolenic acid, 4 – palmitic acid, 5 – stearic acid, 6 – linolenic acid, 7 – eicosenic acid, 8 – palmitoleic acid

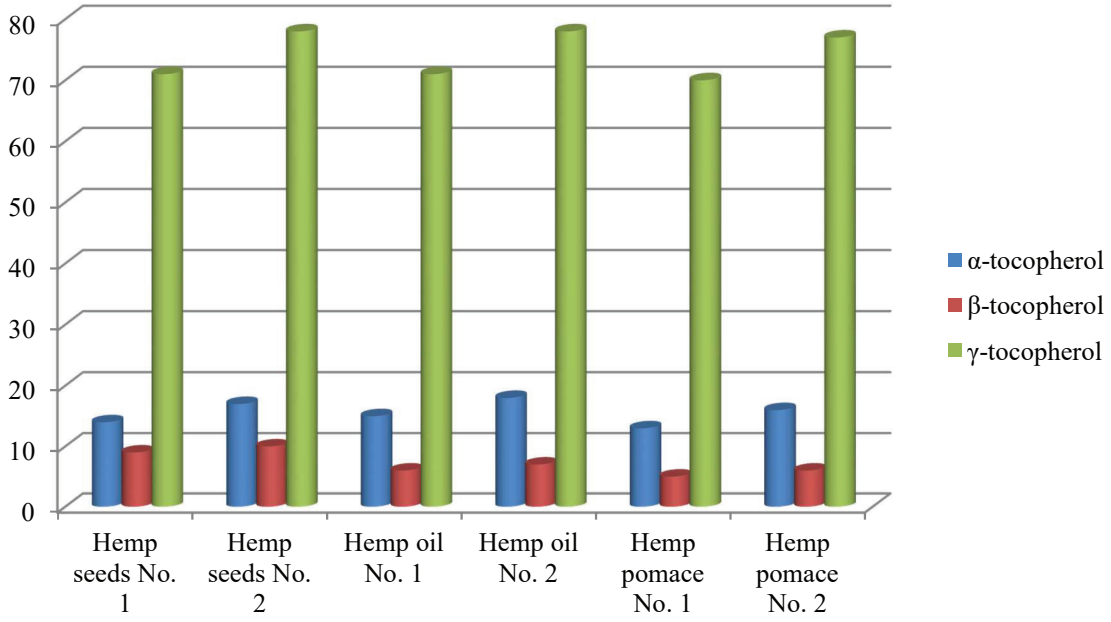


Fig. 3. The content of tocopherols in *Cannabis sativa L.* raw materials

5. Discussion of research results

For the first time, we determined the transition of macro- and microelements from seeds to fatty oil of *Cannabis sativa L.* and established their content in the pomace.

The results (Table 2) indicate that there are 20 inorganic elements in *Cannabis sativa L.* raw materials.

Thus, the highest content in *Cannabis sativa L.* raw materials was established for the macronutrient Ca. Its content ranged from 1939.20 to 4171.75 mg/kg of raw material, depending on the studied object. The content of macronutrient Mg was from 391.90 to 842.97 mg/kg of raw material, depending on the object under study. The highest content of Si (175.88 mg/kg of raw material) accumulated in the pomace sample 2. The content of Fe and Al in the tested samples was detected in the range from 53.90 to 117.64 mg/kg and from 28.79 to 62.15 mg/kg of the studied object, respectively. The Mn content was from 23.52 to 50.86 mg/kg of raw material. The highest content of Zn (45.71 mg/kg of raw material) was found in the pomace sample 2. Sr was found in the range from 9.88 to 21.36 mg/kg of raw material, depending on the object of study. The highest content of B, Cu and Ba was found in the pomace sample 2 and was 10.76, 8.40 and 4.33 mg/kg of raw material, respectively. The content of Cr was from 0.14 to 0.30 mg/kg, depending on the object of study. In the studied objects of *Cannabis sativa* L, the traces of Cd, Be, I and Pb were also detected.

We found that the highest content of macro- and microelements accumulates in the hemp pomace, and the lowest content – is in the hemp oil.

The heavy metals in the studied objects were within the permitted limits (SFU 2.0 – 2.4.27).

The content of macro- and microelements in the samples of *Cannabis sativa L.* raw materials corresponds to the following order: Ca>Mg>Si>Fe>Al>Mn>Zn>Sr>B>Cu>Ba>Cr and Ni>Se>Co>Mo>Cd>Be>I>Pb.

Thus, hemp oil contains macro- and microelements and can be used as their source in human nutrition.

Hemp seeds, hemp oil and hemp pomace are valuable for medicine and pharmacy because of their biologically active substances, which are collected in naturally balanced complexes and are an essential source of macro- and micronutrients for the human body [28]. Identified natural concentrators of macro- and microelements among hemp seeds, hemp oil and hemp pomace can be successfully used in medicine for the prevention of diseases and rehabilitation therapy [28].

In the human body, macro-and micronutrients are not synthesized, and their balance is maintained through the intake of food. Ideally, the daily diet should cover the needs of the body, but, according to scientists, the amount of minerals in food is different and not always sufficient [28].

We determined the content of 16 amino acids in *Cannabis sativa L.* raw materials. 7 amino acids are essential (leucine, valine, threonine, lysine, methionine, isoleucine, and phenylalanine), and 2 amino acids are essential for children (histidine and arginine). Essential amino acids are not synthesized in the human body, so their intake of food is essential. The results indicate the prospects of using *Cannabis sativa L.* raw materials to produce complex phytopreparations.

Amino acids are structural, chemical units that form proteins. Up to 20 different amino acids are most commonly found in proteins, and each is present in the protein in a different amount. The biological value of food proteins depends on the ratio of essential amino acids that should be taken only with food. There are ten essential amino acids – lysine, methionine, tryptophan, phenylalanine, leucine, isoleucine, threonine, valine, arginine and histidine. Lysine, methionine and tryptophan are exceptionally scarce. These amino acids are not synthesized in the human body and must necessarily be taken with food in a particular ratio, that is, to be in balance [29, 30].

It was found that the content of fatty acids in hemp seed oil (Table 4, Fig. 2) corresponds to the following

order: linoleic acid ($C_{18}H_{32}O_2$)>oleic acid ($C_{18}H_{34}O_2$)>linolenic acid ($C_{18}H_{30}O_2$)>palmitic acid ($C_{16}H_{30}O_2$)>stearic acid ($C_{18}H_{36}O_2$)>γ-linolenic acid ($C_{18}H_{30}O_2$)>eicosenoic acid ($C_{20}H_{38}O_2$)>palmitoleic acid ($C_{16}H_{30}O_2$).

The content of saturated and unsaturated fatty acids in oil samples is as follows: palmitic acid ($C_{16}H_{30}O_2$) – from 6.6588 % to 6.6702 %; palmitoleic acid ($C_{16}H_{30}O_2$) – from 0.0157 % to 0.1647 %; stearic acid ($C_{18}H_{36}O_2$) – from 2.7945 % to 2.9361 %; oleic acid ($C_{18}H_{34}O_2$) – from 16.2259 % to 19.2048 %; linoleic acid ($C_{18}H_{32}O_2$) – from 55.8510 % to 57.7376 %; linolenic acid ($C_{18}H_{30}O_2$) – from 13.4426 % to 14.8091 %; γ-linolenic acid ($C_{18}H_{30}O_2$) – from 1.1448 % to 1.1554 %; eicosenic acid ($C_{20}H_{38}O_2$) – from 0.5859 % to 0.6030 %. The main fatty acids of all samples were linoleic, oleic and linolenic. These important components in large quantities are quite rare in nature, and therefore such plants are among the most valuable objects for pharmacy and cosmetology.

Hemp seeds have a high nutritional value and contain almost 20 % of the oil with a high content of polyunsaturated fatty acids. It is the only natural oil that contains unsaturated fatty acids – linoleic (*omega-6*) and linolenic (*omega-3*) in an optimal ratio that are essential for maintaining the protective functions of the human body. These acids clean the blood vessels (arteries), transform cholesterol and inhibit its accumulation. The content in hemp oil of more than 2 % of gamma-linolenic acid, which is contained in breast milk and is quite rare in nature, is precious.

The study results show that the content of α- tocopherol and γ-tocopherol prevailed in *Cannabis sativa L.* raw materials of «Glesia» variety. Therefore, it can be used to develop the drug based on hemp seeds, hemp oil and hemp pomace. The highest content of tocopherols was in the seeds, oil and pomace of the 2019 harvest.

Tocopherols are not synthesized in the organism, and humans get them with food. According to the biological action, all tocopherols are divided into substances with vitamin and antioxidant effects. The maximum vitamin activity is shown by α-tocopherol. Compared with α-tocopherol, the biological activity of β-tocopherol is 40 %, and γ-tocopherol – 8 %. Other forms are inactive. γ-tocopherol has the most significant antioxidant effect, α-tocopherol has the least [31]. Tocopherols are widely used in the pharmacotherapy of various diseases. Therefore, it is advisable to determine the content of γ- and α-tocopherol, which provide regeneration of damaged cells inside the body, protect the body from cardiovascular disease, have protective factors in bone fractures, restore liver cells that were affected by various toxic substances, improve sebaceous gland function, condition of the skin and mucous membranes, reduce hyperpigmentation, increase muscle strength and endurance, increase the mental and physical activity [31].

Using hemp pomace will enrich the body with protein, plant fibre, essential amino acids, vitamin E and trace elements. This food supplement normalizes gastrointestinal tract activity, improves digestion, promotes proper weight loss, and is suitable for health.

This dietary product will normalize intestinal motility, restore its microflora, and remove toxins. The significant fibre content relieves the feeling of hunger and allows to lose extra pounds quickly and maintain average body weight. The fibre is indicated for use in type II diabetics because it normalizes blood glucose levels and prevents insulin spikes. It also normalizes sleep and eliminates headaches.

Study limitations. When studying the amino acids in the samples of hemp seeds, hemp oil and hemp pomace using the amino acid analyzer T-AAA 339 M (Czech Republic) in comparison with the standards of amino acid hydrolysates (ion exchange chromatography method), the number of standards was limited, so not all amino acids could be identified.

Prospects for further research. The development of phytomedicines based on hemp oil and hemp pomace that contain a variety of BAS and the study of their pharmacological effects is promising.

6. Conclusions

The phytochemical study of biologically active substances of hemp seeds, hemp oil and hemp pomace of non-narcotic hemp seeds of "Glesia" variety of 2019 and 2020 harvested have been carried out.

The yield of hemp oil (cold pressing method) was 18.9 %. According to organoleptic characteristics, hemp oil is a dark green liquid with a pleasant nutty aroma. Our analysis of the qualitative characteristics of the obtained fatty oils shows that all indicators were within the norm according to SSTU. Therefore, the results indicate that these oils meet the requirements of the State Standard of Ukraine.

For the first time, we determined the transition of macro- and microelements from hemp seeds of "Glesia" variety into the fatty oil and established their content in the pomace. The results indicate 20 inorganic elements in the seeds, oil and pomace of *Cannabis sativa L.* We found that the highest content of macro- and microelements accumulates in hemp pomace, and the lowest content – is in hemp oil. The content of heavy metals in the studied objects is within the permitted limits (SPU 2.0 – 2.4.27). Thus, hemp oil contains macro- and microelements and can be used as their source in human nutrition.

We have studied the content of 16 amino acids of hemp oils: 7 amino acids are essential (leucine, valine, threonine, lysine, methionine, isoleucine, phenylalanine), 2 amino acids are essential for children (histidine and arginine), and 7 amino acids are replaceable (tyrosine, proline, glycine, serine, glutamic and aspartic acids). Monoaminocarbons include 5 amino acids: valine, isoleucine, leucine, alanine, glycine; oxymonoaminocarbons include: threonine, serine; diaminomonocarbons include: arginine, lysine; sulfur-containing: methionine; aromatic compounds include: phenylalanine, tyrosine; heterocyclic compounds include: histidine, proline; monoaminodicarboxylic compounds include: glutamic and aspartic acids.

We found the content of saturated and unsaturated fatty acids in oil samples. The primary fatty acids of all samples were linoleic, oleic and linolenic. These essential components in large quantities are quite rare in nature;

therefore, such plants are among the most valuable objects for pharmacy and cosmetology. In addition, polyunsaturated fatty acids are necessary to restore the body after severe illness, including SARS-CoV-2 or rehabilitation after chemotherapy. The oil from the industrial (non-narcotic) seeds of *Cannabis sativa L.* contains the unsaturated fatty acids *Omega-3* and *Omega-6* in the ideal for the human body ratio following the recommendations of the World Health Organization (WHO).

It also studied the content of α- tocopherol, β- tocopherol and γ-tocopherol and protein in hemp seeds, hemp oil and hemp pomace in *Cannabis sativa L.* raw materials of the «Glesia» variety.

The research results are relevant, meaningful and necessary for developing new phytoremedies with pre-desired pharmacological effects and creates conditions for the development of complex processing technologies for this raw material.

Conflict of interests

The authors declare that they have no conflict of interest with this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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