Section 1. Biology

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DETERMINATION OF FLAVONOIDS IN DIFFERENT OILS OF *CANNABIS SATIVA* L (*CANNABACEAE*) AND EVALUATION OF PHYSICO-CHEMICAL INDICATORS

Abstract. The article presents the results of studies of the flavonoid composition of cold-pressed hemp oil, implemented in the trade network of the Republic of Uzbekistan by chromatographic methods, as well as the results of assessing their quality characteristics, verified according to ISO requirements. In the analysis of 5 samples of hemp oils (Cannabis sativa L), 3 samples were found, which, in terms of the quantitative content of quercetin, rutin and gallic acid, are higher than the rest. Among the studied 5 samples of cold-pressed hemp oils, moisture, peroxide value, acid value according to ISO was also determined. In this regard, it follows that in the production and use of cold-pressed hemp oils, it is necessary to constantly monitor moisture, peroxide value, and acid value. Detailed information on flavonoids can be obtained with the complex application of chromatographic methods of analysis.

Keywords: hemp oil, Cannabis sativa L, flavonoids, cold pressed, quercetin, rutin and gallic acid.

Introduction

Flavonoids belong to one of the most significant classes of biologically active substances of natural origin. Natural flavonoids are of great practical significance in the pharmaceutical industry. Most of these compounds have a complex structure, which makes it difficult to obtain them synthetically. As is known, most flavonoids have antiseptic and anti-inflammatory properties, and are also able to remove heavy metals from the body and reduce the concentration of radiation [1–2].

For researchers in this field, of particular interest is the study and identification of flavonoids and their derivatives, present in a significant amount in the

composition of oil obtained by cold pressing from non-narcotic industrial hemp. For example, industrial hemp oil is a useful and nutritious food product, has medicinal properties and has been used by humans since ancient times. But hemp seeds are especially valued, their oil is not only used daily and in various forms for food, but also various medicines are made based on them. Cold-pressed hempseed oil is often perceived by consumers as medicinal and is contrasted with traditional foods. However, they are food products intended for systematic use as part of diets by all age groups of a healthy population [3–6]. Among the food products produced by the oil and fat industry, the most suitable for conversion are emulsion products (spreads, margarines, sauces), in which special ingredients are added to give functional properties, while insufficient attention is paid to the value of hemp oil included in food formulations. Hemp oil are sources of phytocannabinoids, flavonoids, chlorophyll, necessary for the normal functioning of the human body, they are characterized by a high content of phenols and unsaturated fatty acids omega-3, omega-6, omega-9 and macro-microelements and other biologically active components, as well as essential fatty acids. The study of the flavonoid composition of hemp oil will make it possible to predict the possibility of their use in the diet as food and as medicinal [7–14].

Materials and methods

Chromatographic methods are the most effective for the determination of flavonoid compared to other methods of analysis, [3]. The material for the study was Cannabis Sativa L oil, collected after cold pressing. High performance liquid chromatography was used to characterize the flavonoid components of cold-pressed hemp oil [4]. The following method was used for the qualitative and quantitative determination of flavonoids in samples. 96% ethanol was used as a solvent to separate rutin, gallic acid, and quercetin. To do this, the prepared extract and alcohol were mixed in a ratio of 1:10 and extracted with a magnetic stirrer at a temperature of 30 °C for 75 minutes. After that, the HPLC method was used. To determine the amount of rutin, gallic acid and quercetin in hemp oil samples on a Perkin Elmer C18250x4.6 mm 5 mm C18 column (USA). To do this, a calibration curve was built by preparing a solution of 0.5% acetic acid in a ratio of 35:65 and standard solutions of acetonitrile in different concentrations: 1 mg/ml, 1.25 mg/ml and 2.5 mg/ml. The following chromatogram was obtained on an HPLC device (LC2030 C3D plus Shimadzu Japan) based on standard samples with 2.5 minutes of gallic acid, 3.6 minutes of normal and 15 minutes of quercetin. The detection wavelength is 354 nm. The remaining quality parameters for hemp oil are determined strictly according to ISO. For example, moisture and volatile matter according to ISO 662–2016, acid value ISO 660–2020, peroxide value ISO 3960–2017.

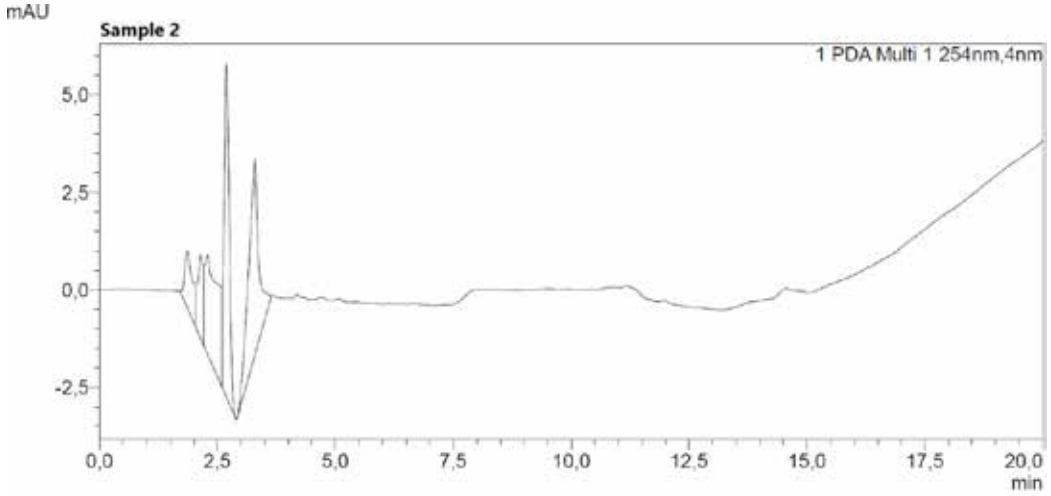
Results and discussion

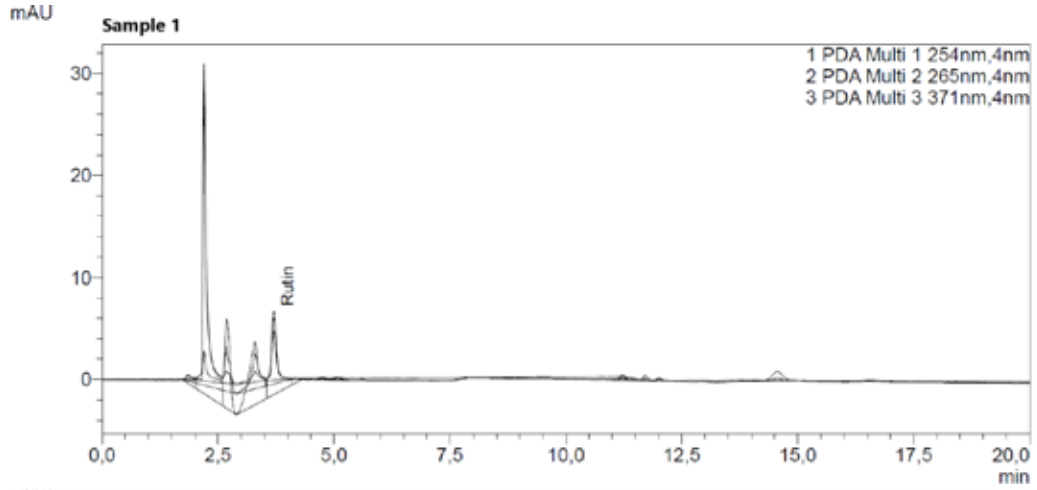
The study of the component composition of flavonoid compounds of hemp oil was carried out. Chromatogram of the separation of a mixture of flavonoid compounds of the standard extract (a) and industrial hemp oil (b): quercetin, rutin and gallic acid. Mobile phase: 0.5% acetic acid-acetonitrile mixture (35:65 vol.). Mobile phase rate 1 ml/min. Detection wavelength 354 nm

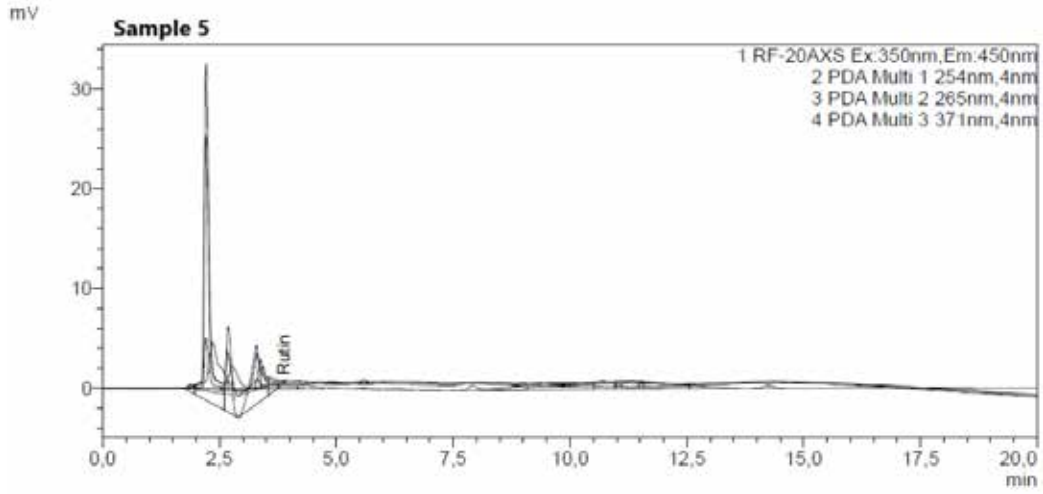
Table 1.– Flavonoid composition of hemp oil

№	Name		Ret. Time	Area	Height	Conc. mg/ml
	Samples	Flavonoids				
1	*2*	*3*	*4*	*5*	*6*	*7*
1	Standard	Quercetin	2.493	898349	92205	0.050
		Rutin	3.635	1616920	232739	0.050
		Gallic acid	15.891	5600155	226403	0.050
2	Sample 1	Rutin	3.700	85001	8179	0.003

1	*2*	*3*	*4*	*5*	*6*	*7*
3	Sample 2	–	–	–	–	–
4	Sample 3	Gallic acid	2.685	93983	8692	0.005
5	Sample 4	Rutin	3.584	3480	459	0.0001
		Gallic acid	2.676	33500	4766	0.002
6	Sample 5	Rutin	3.573	18341	1439	0.001







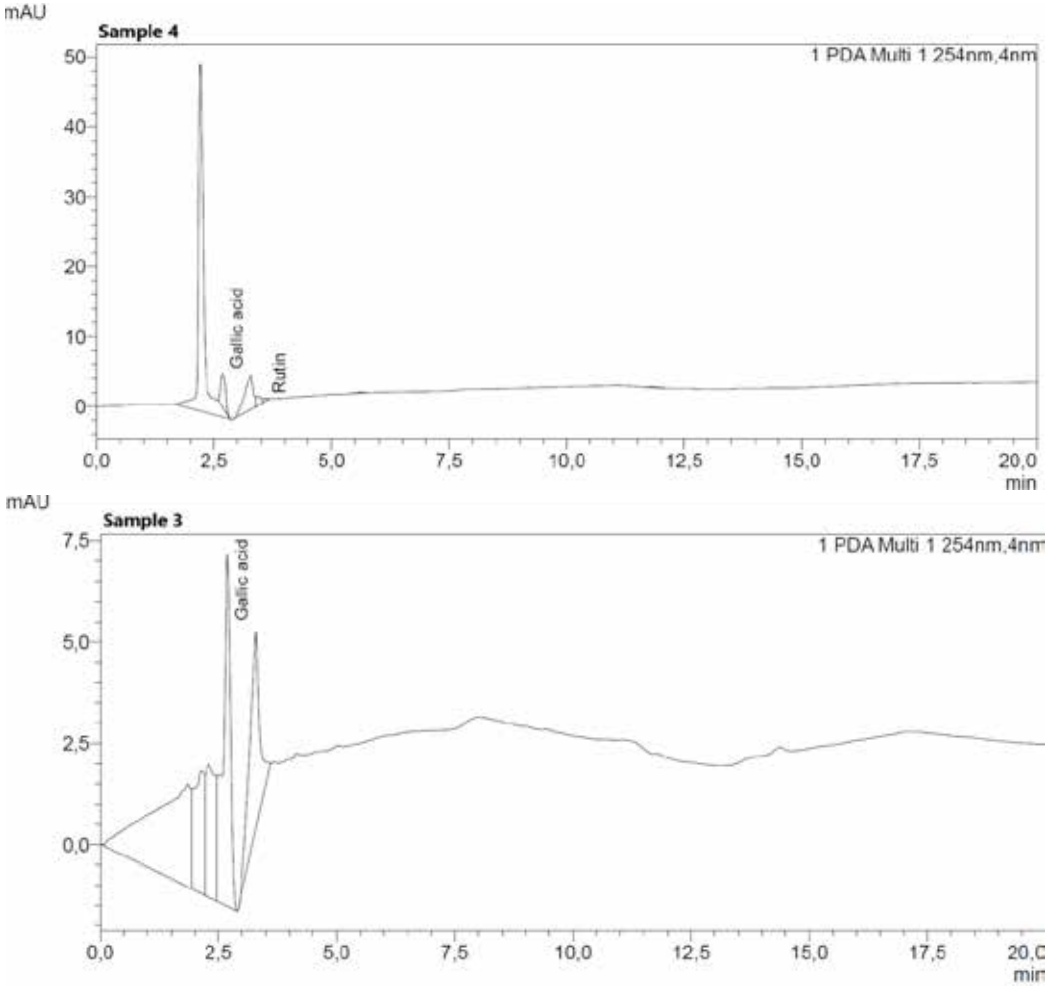


Figure 1.

As can be seen from (table 1), the results obtained indicate the homogeneity and identity of the detected flavonoids. Quercetin, rutin and gallic acid and its derivatives are biologically active substances with antioxidant, antitumor, antimutagenic and antiviral activity.

Table 2.– Physical and chemical indicators of hemp oil according to GOST 8989–73

№	Name indicators	Actual Results					Norm for oil GOST 8989–73			ISO for testing
		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	refined	unrefined		
								1 grade	2 grade	
1.	Moisture%	0.11	0.12	0.10	0.12	0.10	0.12	0.15	0.20	662–2016
2.	Acid number mg/100	0.6	0.7	1.1	0.8	1.0	0.4	2.3	6	660–2020
3.	Peroxide value m/mol	7.0	6.7	7.4	4.5	7.5	No more than 10			3960–2017

After determining the flavonoid composition, we determined the parameters of cold pressing, obtained at a temperature of 55–600 °C. An oil yield from hemp seeds over 30% is considered high. However, hemp oil oxidizes too quickly due to its high content of polyunsaturated fatty acids. As a result of storage, low-molecular compounds, peroxides, aldehydes, free fatty acids, ketones accumulate in hemp oil, which leads to a sharp deterioration in the properties of the product: the oil thickens, a rancid taste, and an unpleasant odor appear. Many products of oxidation of polyunsaturated fatty acids have mutagenic and carcinogenic properties, so quality control of hemp oil is an urgent task (Table 2).

An important characteristic of the quality of hemp oil is its moisture (%), acid (AN) and peroxide (PC) numbers. It should be noted that, although it is usually considered acceptable for edible vegetable oils to have an IC value not exceeding 10 mg-eq O_2/kg, a change in the taste (rancidity) and smell of hemp oil usually begins at IC values less than 6–9 meq O_2/kg oils. The value of the acid number characterizing the content of free fatty acids should not exceed 2 mg KOH/g oil. The results for our oil are shown below (Table 2). The option we have chosen meets the quality standard. Moisture, AN and PC indicators correspond to the requirements of GOST 8989–73.

Conclusion

Flavonoids play a very significant role in the prevention of cardiovascular disease. Flavonoid compounds seal and strengthen blood vessels, increase their elasticity and reduce their permeability, brittleness and fragility. In addition, flavonoids reduce the level of cholesterol in the blood and its deposition on the walls of blood vessels, and also affect the maintenance of normal blood pressure and prevent platelet aggregation and remove toxins from the heart muscle. To check the presence of flavonoids, we carried out qualitative and quantitative analyzes of hemp oil using HPLC methods. The quality of hemp oil meets the requirements of GOST 8989–73. Out of this, we must include hemp oil in our diet.

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