UDC 615.332:582.951.4
DOI 10.37000/abbsl.2023.108.09

CONTENT OF BIOLOGICALLY ACTIVE COMPOUNDS IN FRUITS OF CAPSICUM ANNUUM L

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The study of the chemical composition of natural raw materials allows not only to obtain data on the peculiarities of its structure and organization, but also to find new chemical compounds with useful qualities. The search for new biologically active compounds that can be used in pharmacology and pharmacy, food chemistry, and experimental research in various areas is particularly effective.
The aim of the work was to determine the quantitative content of capsaicinoids and carotenoids in the fruits of Capsicum annuum L.
Ripe fruits of Capsicum annuum L. varieties Ukrainian bitter, Kharkiv bitter, Astrakhansky, Kharuz were selected for the study. The fruits were dried to a constant weight. The study of the content of capsaicinoids was carried out with their preliminary separation using thin-layer chromatography, after which the optical density was measured by the photocalorimetric method. To study the content of carotenoids, they were pre-cleaned from other extractive substances, after which the optical density was measured by the photocalorimetric method.
It was established that the fruits of all studied varieties meet the state standard for the content of capsaicinoids. The predominant capsaicinoid was capsaicin (60-80%). The content of dihydrocapsaicin ranged from 15 to 30%. The total content of carotenoids for most varieties exceeded 2 mg / 1 g of fruit pulp. The predominant fraction was red (60-70%).
The fruits of all studied varieties of Capsicum annuum L. can be used as plant raw materials for the further production of pharmaceutical preparations.

Key words: *capsaicinoids, carotenoids, Capsicum annuum L., anti-inflammatory.*

PROBLEM

The study of the phytochemical composition of plant raw materials is necessary for its further use in various spheres of human activity - food and chemical industry, technical industries, pharmaceutical production and others. A significant variety of biologically active compounds found in plant raw materials make it an ideal candidate for the search for new useful substances, including those with medicinal properties.

ANALYSIS OF THE LATEST RESEARCH

Among the possible directions of searching for new substances with anti-inflammatory activity, the study of compounds of plant origin, which have numerous biological and pharmacological effects, including anti-inflammatory ones, is promising [1, 2]. As a source of anti-inflammatory substances, the attention of researchers is drawn to the fruits of Capsicum annuum L. (allspice) [3]. The most interesting biologically active components of allspice fruits are capsaicinoids [4] and carotenoids [5]. According to their chemical structure, capsaicinoids belong to alkaloids and have analgesic [6], anticarcinogenic effects [7], have a positive effect on lipid metabolism [8], protect the cardiovascular and gastrointestinal systems [9]. Carotenoids, in turn, are powerful antioxidants that contribute to the normalization of metabolic processes during inflammation [5]. Fluctuations in the content of capsaicinoids and carotenoids depending on the variety, level of ripeness, conditions of cultivation and storage, make the process of standardization of the fruits of different varieties of one-year-old pepper necessary before possible use as medicinal raw materials.

THE AIM OF THE STUDY

To investigate the content of capsaicinoids and carotenoids in the fruits of Capsicum annuum L., the most common varieties in Ukraine, and to study the anti-inflammatory properties of the liquid extract based on the most promising variety.

MATERIALS AND METHODS OF RESEARCH

Ripe fruits of Capsicum annuum L. of the 2016 harvest of four varieties were used as plant material: Ukrainian bitter, Kharkiv bitter, Astrakhan, Kharuz. All plant material was obtained from a private farm, where it was grown from varietal seed material.

Before starting the analysis, the fruits were dried at a temperature of 55°C to a constant weight, after which they were divided into three components - fruit pulp, seeds and seed placenta. The peduncles and calyxes were moving away. The obtained plant tissues were crushed.

Extraction of capsaicinoids from tissue homogenates was carried out in a Soxhlet apparatus. To determine the amount of capsaicinoids, a previously known amount of ethanolic extracts was evaporated to dryness on a rotary evaporator, after which the dry residue was dissolved in 5 ml of a chloroform-methanol mixture (1:1). An aliquot of the obtained solution was applied to Sorbfil "analytical" chromatographic plates of the PTSH-AF-A type. The separation was carried out in the absolute methanol:acetic acid (49:1) system. The adsorbing layer was removed from the area of the plate containing the "spot" of capsaicin and dihydrocapsaicin (Rf = 0.8 and Rf = 0.6, respectively, determined in advance) and transferred to test tubes containing 3 ml of 0.05 M borate buffer, pH 9.4 and 0.5 ml of a saturated aqueous solution of 2,6-dichloro-4-chloroimide. The resulting mixture was intensively shaken and left for 30 minutes in the dark to develop the color. The following quantitative determination of capsaicin and dihydrocapsaicin was carried out on a KFC-3 photocolorimeter at a wavelength of 600 nm. The concentration of capsaicinoids was found using calibration curves [10].

Extraction and quantitative determination of carotenoids was carried out only in the fruit pulp. For this purpose, 25 g of fruit pulp homogenate was poured into 500 ml of cooled acetone (5 °C) for several minutes. The obtained extract was filtered under a vacuum pump through paper filters No. 4. The filtrate was transferred to a separatory funnel containing petroleum ether (a mixture of heptane and hexane 1:1) and water. Mixing was carried out without shaking, after which the aqueous phase was removed. The remaining mixture was washed several times with distilled water, after which the remaining water was removed using anhydrous sodium sulfate. Later, the solution was stored in a hermetically sealed vessel, in a dark place, at a temperature ≤ 5 oC.

Determination of the concentration of carotenoids in different fractions was carried out as follows. A precisely known amount of a solution of carotenoids in petroleum ether was concentrated on a rotary evaporator until an oily residue was obtained, which was dissolved in acetone. After that, the optical density was measured at the global wavelength of 472 and 508 nm, on a KFK-3 photocolorimeter. The concentration of carotenoids in the yellow and red fractions was calculated according to the following formulas:

$$C_{red\ fraction,\ mcg/ml} = \frac{21444 \times A_{508} - 403{,}3 \times A_{472}}{270{,}9}$$

$$C_{yellow\ fraction,\ mcg/ml} = \frac{1724{,}3 \times A_{472} - 2450 \times A_{508}}{270{,}9}$$

Where: A_{508} and A_{472} are the optical density of the solutions for the corresponding wavelengths [5].

Determination of the anti-inflammatory properties of the alcoholic extract of the fruits of Capsicum annuum L. was performed on young white male Wistar rats, which were kept in standard vivarium conditions with free access to food and water. All studies complied with the rules of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and the principles of the National Congress of Bioethics of Ukraine (Kyiv, 2001).

The inflammatory reaction was induced by injecting 0.2 ml of 0.2% carrageenan solution under the plantar aponeurosis of the right limb. For treatment, the inflamed area of the limb was immersed in an alcoholic extract of the fruits of Capsicum annuum L., variety Ukrainian bitter for 20 minutes, once a day. Treatment was started the next day after inducing inflammation and was carried out until the external manifestations of the inflammatory process disappeared. The therapeutic protocol for the animals of the control group was similar to the experimental one, but ethyl alcohol was used as a drug. The therapeutic effectiveness of the applied treatment was assessed by the dynamics of changes in the morphological signs of inflammation - the volume

and thickness of the inflammatory edema. Edema volume was measured using a digital plethysmometer 37140, Ugo Basile (China), edema thickness was measured with an electronic caliper YT-7201, YATO (Poland) [11]. Statistical processing was performed with determination of average probable intervals.

RESULTS OF RESEARCH AND DISCUSSION

Data on the quantitative content of capsaicinoids and carotenoids in different parts of the fruits of Capsicum annuum L. Ukrainian varieties are given in table 1 and 2.

Table 1. **Content of capsaicin and dihydrocapsaicin in fruits of one-year-old pepper of different varieties, mg / 100 g of fresh fruits (calculated from values for dried tissue)**

Name of the variety	Capsaicin content			Dihydrocapsaicin content		
	Fruit pulp	Seeds	Seed placenta	Fruit pulp	Seeds	Seed placenta
Ukrainian bitter	51	50	220	10	10	48
Kharkiv bitter	41	28	150	12	9	42
Astrakhan	110	24	160	37	10	62
Haruz	56	47	79	26	23	12

Table 2. **The content of the red and yellow fractions of carotenoids in the fruits of one-year-old pepper of different varieties (calculated from the values for dried tissue)**

Name of the variety	Total content of carotenoids, μg / 1 g of fruit pulp	Red fraction, %	Yellow fraction, %
Ukrainian bitter	2150	70,5	29,5
Kharkiv bitter	2012	67,8	32,2
Astrakhan	1769	72,6	27,4
Haruz	2234	62,5	27,5

The total content of capsaicin and dihydrocapsaicin was 389 mg/100 g for the Ukrainian bitter variety, 282 mg/100 g for the Kharkiv bitter variety, 403 mg/100 g for the Astrakhan variety, and 243 mg/100 g for the Kharuz variety. The percentage content of capsaicin ranged from 60 to 80%, dihydrocapsaicin - from 15 to 30%. The largest amount of capsaicinoids was found in the seed placenta, the smallest amount was in the seeds. The highest content of carotenoids was determined in the fruits of the Kharuz variety, the lowest - in the fruits of the Astrakhansky variety. The predominant carotenoid fraction is red, on average, the content of carotenoids in the red fraction is twice as high as the content of carotenoids in the yellow fraction.
The dynamics of changes in the thickness and volume of carrageenan-induced edema during treatment with alcoholic extracts of the fruits of Capsicum annuum L. of the Ukrainian bitter variety are shown in Fig. 1.

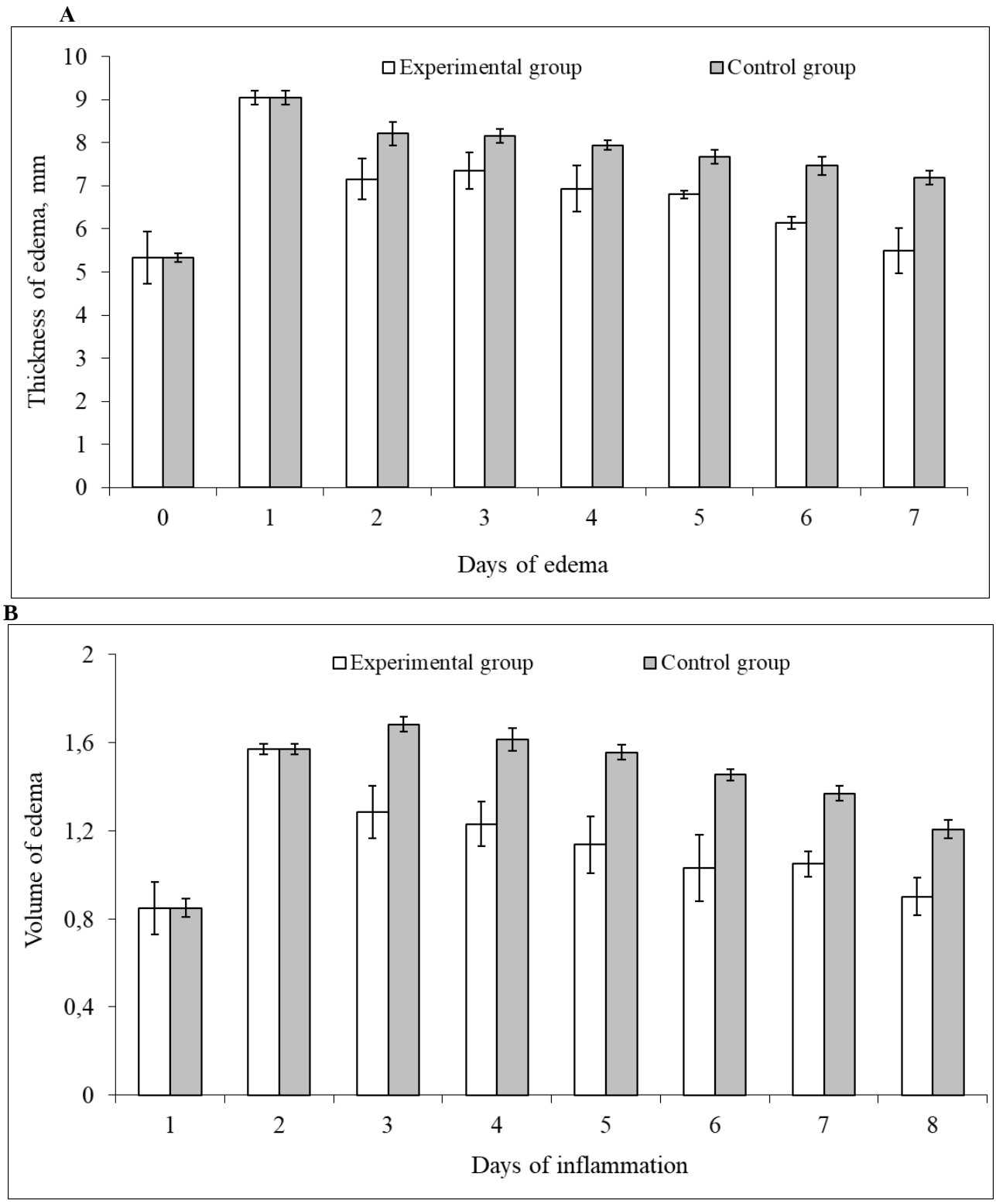

Fig. 1. Dynamics of changes in thickness (A) and volume (B) of the area of carrageenan-induced inflammation in rats treated with alcoholic extract of Capsicum annuum L.

The volume and thickness of the limb in the area of introduction of phlogogen under the experimental conditions increases by ~ 2 times. When using the alcoholic extract of Capsicum annuum L. as a therapeutic agent, a significant reduction in inflammatory edema (23-25%) was observed already after the first day of treatment. By the seventh day after inducing inflammation, treatment with pepper extract led to the disappearance of edematous phenomena; upon palpation, no pain reaction was detected, and recovery of functional activity was also observed (animals freely leaned on the injured limb). In contrast, in the control group of animals, the volume and width of the inflammatory area exceeded the intact values by 28-32%, and the final disappearance of the manifestations of inflammation was observed only after 12 days.

Since capsaicin and dihydrocapsaicin are capsaicinoids, the mass fraction of which usually exceeds 90% in the capsaicinoid composition of pepper fruits [12], determining their total amount allows making a sufficiently accurate assumption about the total content of capsaicinoids. All studied varieties have a mass fraction of capsaicinoids that exceeds 0.15%, which meets the requirements of DESTU 14260-89. The European pharmacopoeia has stricter requirements for the content of capsaicinoids. The mass share of capsaicinoids in bitter pepper fruits should not be lower than 0.4%. According to the criteria of the European Pharmacopoeia, the Astrakhan variety and, possibly taking into account the content of capsaicinoids other than capsaicin and dihydrocapsaicin, the Ukrainian bitter variety can be used as raw materials for the production of medicinal products. For the investigated pepper varieties, the highest accumulation of capsaicin and dihydrocapsaicin is observed in the seed placenta.

Considering the ratio of carotenoid fractions, it is possible to conclude about the predominant accumulation of capsanthin (red fraction) and lower accumulation of lutein (yellow fraction) in the fruit pulp of pepper. Also, pepper fruits contain a significant amount of β-carotene, which is present in both fractions [5].

Significant anti-inflammatory properties of Capsicum annuum L. extract, which were found in the carrageenan inflammation model, are explained, in our opinion, by the high content of both capsaicinoids and carotenoids in pepper fruits. The most studied anti-inflammatory mechanism of action of capsaicinoids consists in the desensitization of TRPV-1 receptors, which leads to a decrease in the pain sensitivity of thin C-type nerve fibers, as well as a decrease in the release of pro-inflammatory neuropeptides (substance P, calcitonin gene-related peptide) [13]. The powerful antioxidant effect of carotenoids reduces the level of damage to cellular and subcellular structures by reactive oxygen species and free radicals, which are formed in large quantities in the first, altering, phase of inflammation [14]. For the study of anti-inflammatory activity, the Ukrainian bitter variety was chosen; this choice is due to the very wide distribution of this variety on the territory of Ukraine. In this regard, it is the Ukrainian bitter variety that is the most likely candidate for possible further use as a plant raw material for the industrial preparation of medicinal preparations of one-year-old pepper.

CONCLUSIONS

1. The conducted study showed that varieties of Capsicum annuum L. Astrakhan and Ukrainian bitter, which are widely cultivated in the territory of Ukraine, meet domestic and foreign standards for the total content of capsaicinoids and can be used in the manufacture of medicinal products.
2. Alcoholic extract of the fruits of Ukrainian bitter pepper of the same year variety has high anti-inflammatory properties on the model of carrageenan inflammation and shortens the recovery period of experimental animals from 12 to 7 days.

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ВМІСТ БІОЛОГІЧНО АКТИВНИХ СПОЛУК У ПЛОДАХ *CAPSICUM ANNUUM* L

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Дослідження хімічного складу природньої сировини дозволяє не тільки отримати дані щодо особливостей її структури та організації, але й дозволяє знайти нові хімічні сполуки з корисними якостями. Особливо ефективним є пошук нових біологічно активних сполук, що можуть бути застосовані у фармакології та фармації, харчової хімії, експериментальних дослідженнях різного напряму.
Метою роботи було визначення кількісного вмісту капсаіциноїдів та каратиноїдів у плодах *Capsicum annuum* L.
Для дослідження були відібрані стиглі плоди *Capsicum annuum* L. сортів Український гіркий, Харківський гіркий, Астраханський, Харуз. Плоди висушивались до постійної ваги. Дослідження вмісту капсаіциноїдів проводили з попереднім їх розділенням за допомогою тонкошарової хроматографії, після чого вимірювали оптичну щільність фотокалориметричним методом. Для дослідження вмісту каротиноїдів проводили їх попереднє очищення від інших екстрактивних речовин після чого проводили вимірювання оптичної щільності фотокалориметричним методом.
Було встановлено, що плоди усіх досліджуваних сортів відповідають державному стандарту щодо вмісту капсаіциноїдів. Переважним капсаіциноїдом був капсаіцин (60-80 %). Вміст дигідрокапсаіцину коливався від 15 до 30 %. Загальний вміст каротиноїдів для більшості сортів перевищував 2 мг / 1 г плодової м'якоті. Переважаючою фракцією була червона (60-70 %).
Плоди усіх досліджених сортів *Capsicum annuum* L. можуть бути використані у якості рослинної сировини для подальшого виготовлення фармацевтичних препаратів.

Ключові слова: *капсаіциноїди, каротиноїди, Capsicum annuum L., протизапальний.*