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Carotenoids and carotenes in the fruits of *Cucurbita maxima*, *C. moschata* and *C. pepo* under the conditions of Northwestern Russia

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Cucurbits are among the most valuable vegetable crops widely used for food all over the world, being an important source of carotenoids and carotenes, biologically active substances with antioxidant and other types of activity. Their content largely depends on the *Cucurbita* species and varietal features. The *Cucurbita* collection of the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR) contains more than 3,000 accessions of five cultivated *Cucurbita* spp. and serves as the most important source of source material for developing new cultivars in order to expand the range of functional food products. The present research was aimed at studying the content of carotenoids and carotenes in the fruits of *Cucurbita maxima* Duch., *C. pepo* L. and *C. moschata* Duch. ex Poir. grown in Northwestern Russia, which is characterized by a short growing season and a low sum of active temperatures. The performed study revealed significant differences between the *Cucurbita* species and cultivars in terms of the content of carotenoids, total carotenes and *β*-carotene. In contrast to *C. pepo*, the accessions of *C. maxima* and *C. moschata* were characterized by a wide range of variation of these characters. A statistically significant effect of the flesh and fruit skin color on the content of carotenoids, carotenes and *β*-carotene in them was observed ($p < 0.001$). The minimum amount of carotenoids, carotenes and β-carotene was found in the fruits with light yellow flesh, and the maximum in those with dark orange flesh. Cucurbit fruits with red skin accumulated more target compounds in contrast to the fruits with white skin. The identified *Cucurbita* accessions with high content of carotenes and carotenoids can be used as valuable source material for breeding cultivars with improved biochemical composition, which can broaden the range of functional food products when grown in the regions with unfavorable conditions for the production of this crop.

Keywords: *Cucurbita*, functional nutrition, carotenoids, carotenes, cultivar breeding

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КОЛЛЕКЦИИ МИРОВЫХ ГЕНЕТИЧЕСКИХ РЕСУРСОВ КУЛЬТУРНЫХ РАСТЕНИЙ ДЛЯ РАЗВИТИЯ ПРИОРИТЕТНЫХ НАПРАВЛЕНИЙ СЕЛЕКЦИИ

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Каротиноиды и каротины в плодах *Cucurbita maxima*, *C. moschata* и *C. pepo* в условиях Северо-Запада России

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Тыква – одна из наиболее ценных овощных культур, которая широко используется в пищу во всем мире, являясь важным источником каротиноидов и каротинов, биологически активных веществ с антиоксидантной и другими видами активности. Их содержание в значительной мере зависит от видовых и сортовых особенностей тыквы. Коллекция Всероссийского института генетических ресурсов растений имени Н.И. Вавилова (ВИР) насчитывает более 3000 образцов пяти культурных видов тыквы и является важнейшим источником исходного материала для получения новых сортов с целью расширения ассортимента продуктов функционального питания. Задачей нашего исследования было изучение содержания каротиноидов, каротинов, в том числе β-каротина, в плодах тыквы *Cucurbita maxima* Duch., *C. pepo* L. и *C. moschata* Duch. ex Poir., выращенных в условиях Северо-Западного региона России, который характеризуется коротким вегетационным периодом и невысокой суммой активных температур. Проведенное нами исследование с последующим статистическим анализом позволило выявить достоверные отличия между видами и сортами тыквы из коллекции ВИР по содержанию каротиноидов, суммы каротинов и β-каротина. Для образцов *C. maxima* и *C. moschata*, в отличие от *C. pepo*, был характерен широкий диапазон варьирования этих признаков. Выявлено статистически значимое влияние цвета мякоти и цвета коры плодов на содержание в них каротиноидов, каротинов и β-каротина ($p < 0{,}001$). Минимальное количество каротиноидов, каротинов и β-каротина установлено для плодов со светло-желтой мякотью, максимальное – с темно-оранжевой. Плоды тыквы с красной корой накапливали больше целевых соединений, в отличие от плодов с белой корой. Выделенные нами образцы *Cucurbita* с высоким содержанием каротинов и каротиноидов являются ценным исходным материалом для селекции сортов с улучшенным биохимическим составом для расширения линейки продуктов функционального питания, пригодных к выращиванию в регионах с условиями, нетипичными для производства данной культуры.

Ключевые слова: *Cucurbita*, функциональное питание, каротиноиды, каротины, селекция сортов

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Introduction

Cucurbita L. species have been widely cultivated and used for food since ancient times. Over the centuries of cultivation, a broad genetic diversity of forms has been selected due to their economic importance. They served as a basis for the development of many cultivars significantly differing in biological and morphological characters, and in biochemical composition. The *Cucurbita* collection held by VIR, one of the largest in the world, is the most important source of material for breeding. The formation of the collection started in the 1920s under the guidance and with direct participation of N. I. Vavilov. This work was initiated in 1922 when cultivars released in Western Europe and the United States were acquired, and local varieties were collected in Russia and other countries, especially those used in ancient agriculture. Currently, VIR's collection includes over 3,000 accessions of five cultivated *Cucurbita* species: *Cucurbita maxima* Duch., *Cucurbita pepo* L., *Cucurbita moschata* Duch. ex Poir., *Cucurbita ficifolia* Bouche, and *Cucurbita mixta* Pang. In Russia, the three *Cucurbita* species cultivated by the agricultural industry are *Cucurbita maxima*, *Cucurbita pepo* and *Cucurbita moschata*.

In recent decades, the concept of healthy nutrition has become generally accepted. This has led to the need to expand research on the quality and functionality of vegetable products. Cucurbits are among the vegetable crops of the highest demand in the world. According to the latest FAOSTAT data, the world production of cucurbit fruits is almost 230 million tons. The leading producers are China and India, while Russia produces 1.2 million tons annually and holds the third place in terms of gross harvest.

Cucurbit fruits contain substances that are important for the healthy human nutrition, such as amino acids, vitamins C, B1, B2, B6, E, carnitine, as well as folic and pantothenic acids. The pectins contained in cucurbit fruits purify the human body from heavy metal ions by forming insoluble complexes with them and promoting their excretion (Oloyede et al., 2012). Due to the presence of many compounds, cucurbit flesh has well-proven antioxidant properties, for example, it helps to neutralize free radicals (Fu et al., 2006; Yadav et al., 2010; Gutierrez, 2016).

Carotenes and carotenoids are a group of bioactive compounds most well-represented in pumpkin (Solovyova et al., 2014). Along with anthocyanins, carotenoids belong to a class of the most widespread plant pigments. The major carotenoid in pumpkin (> 80%) is *β*-carotene, with lesser amounts of lutein, lycopene, α-carotene and cis-*β*-carotene (Seo et al., 2005). The daily requirement for vitamin A is on average 900 mcg for an adult male, 700 mcg per day for a woman, with an allowable consumption threshold of 3000 mcg per day (Dietary reference intakes…, 2001). Based on the Institute of Medicine bioconversion rates of provitamin A carotenoids (PVACs) to retinol (Dietary reference intakes…, 2001): 1 mg of retinol is equivalent to 12 mg, 24 mg and 24 mg of *β*-carotene, *α*-carotene and *β*-cryptoxanthin, respectively (Buzigi et al., 2022). The content of *β*-carotene in pumpkin ranges from 402 to 154,760 μg/100g, depending on the cultivar and place of reproduction (Buzigi et al., 2022), which covers the daily requirement for vitamin A, which is 5 mg per day for a healthy adult (Shashkina et al., 2009).

The physiological role of carotenoids is quite diverse. They have an extensive list of pharmacological properties, that is, radioprotective, provitaminic, anticarcinogenic and other types of activity (Fu et al., 2006; Yadav et al., 2010; Gutierrez, 2016). It has been established that the consumption of cucurbit fruits with high content of *β*-carotene has an immunostimulating effect due to the production of Th1 cytokines (Kim et al., 2016).

The accumulation of carotenoids and carotenes in cucurbit fruits largely depends on the species and varietal features. The dependence of these indicators on the climate characteristics of the cultivation zone, soils, and the use of fertilizers was confirmed (Oloyede et al., 2012). The conditions and duration of storage after the harvest can also markedly affect carotenoid levels in cucurbit fruits (Maťová et al., 2019).

In connection with the above, the study of specific features of carotenoid and carotene accumulation in cucurbit fruits of different species keeps being relevant. Golubkina et al. (2021) revealed differences in the content of carotenoids and carotenes between different parts of the cucurbit fruit (flesh, skin). Exclusively lutein was found in the flesh of the pumpkin cultivar 'Konfetká; lutein, zeaxanthin and *β*-carotene were detected in the skin, while *β*-carotene predominated in the placenta (Golubkina et al., 2021). Whang et al. (1999) observed that the content of *β*-carotene in the flesh and skin of C. *moschata* fruits was the same. According to Kim et al. (2012), the content of *β*-carotene in the fruit skin of three *Cucurbita* species was 5–15 times higher than that in the flesh.

The task of the present research was to study the total content of carotenoids, carotenes, and *β*-carotene in fruits of *C. maxima*, *C. pepo* and *C. moschata*, grown in Northwestern Russia, which is characterized by a short growing season and a low sum of active temperatures, in order to preselect material for breeding programs aimed at expanding the range of biofunctional products.

The research was aimed at revealing the features of carotenoid, carotene and *β*-carotene accumulation in the most economically important *Cucurbita* species from the VIR collection, and at identifying accessions rich in these pigments, which can be used as the source material when developing new *Cucurbit*a cultivars suitable for expanding the range of functional food products when cultivated in the areas with unfavorable climatic conditions.

Materials and methods

Plant materials.

The research was carried out in 2018–2019 in the Biochemistry Lab of VIR. The study involved 69 samples of fruits of three cultivated *Cucurbita* species *C. pepo*, *C. maxima*, and *C. moschata* of different geographic origin from the global collection of VIR (Table 1).

Samples for the study were grown in the fields of Pavlovsk and Pushkin Laboratories of VIR (St. Petersburg, Pushkin). The soils of the experimental field were soddy, weakly podzolic, sandy loam in texture, with neutral acidity (pH 7.1–7.6). The humus horizon thickness was 23 to 47 cm, and the humus content 2.1–3.0%. The supply of mobile forms of potassium was medium, and of phosphorus high. The climate in this agroclimatic region is characterized by moderately warm and, in some years, cool summers. The warmest month of the year is July with an average long-term air temperature of 16.5–17.7°C. The sum of positive temperatures is 2100–2300°C. The period with temperatures above 10°C lasts for 105–115 days. The amount of precipitation during the growing season is 550–600 mm per year. The air temperature during the growing season of 2018 was 2–4°C above the average long-term values. The amount of precipitation in May and June was below normal, while in July, August and September it was at the average level. In 2019, the air temperature and the amount of precipitation during the growing season were

Table 1. ***Cucurbita*** **L. species from the VIR collection used in the research**

Таблица 1. **Виды** ***Cucurbita*** **L. из коллекции ВИР, взятые в исследование**

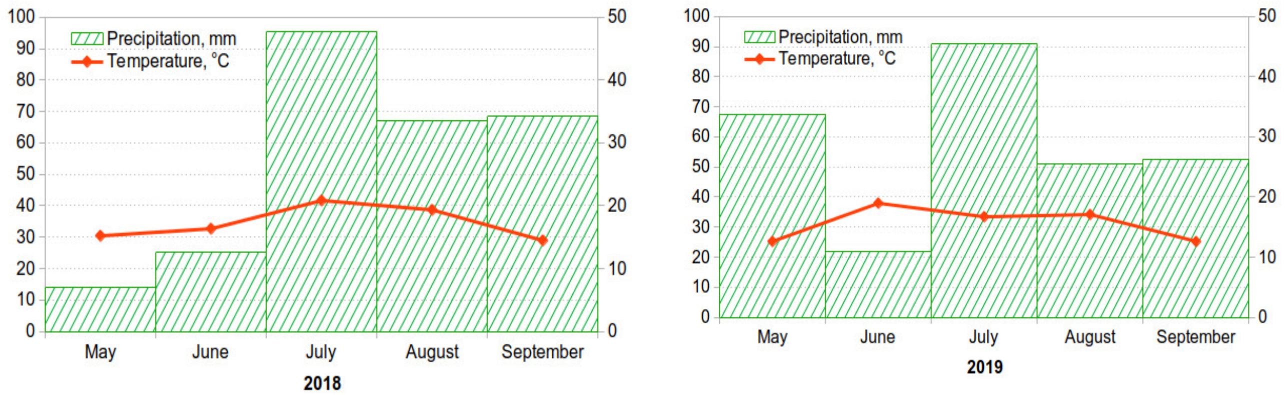
Species	Origin	Quantity
C. maxima	Azerbaijan, Argentina, Bulgaria, Italy, Kazakhstan, China, Poland, Russia, USA, Tajikistan, Ukraine, France, Chile, Sweden	48
C. moschata	Azerbaijan, Argentina, Armenia, Bangladesh, Bulgaria, China, The Netherlands, USA, Turkey, Uruguay, Japan	14
C. pepo	Armenia, Bulgaria, Hungary, Russia, USA, France	7

close to the long-term average values, with the exception of small precipitation amount in June (Fig. 1A, B).

Sowing of the accessions, agricultural techniques for plant care as well as plant material study, harvesting and preparation for biochemical analysis were carried out according to the guidelines developed at VIR (Fursa et al., 1988). Plants were grown in two-row plots, 5 plants in a row, 10 plants in total per plot. The planting distance was 2 × 1.4 m. Sowing was carried out on May 23–25. The emergence of sprouts was recorded on days 6–8. Fruits were harvested when completely ripe.

amount found on the calibration curve, mg), V (volume of the initial extract, cm^3); V_1 (volume of the extract applied to chromatographic paper, cm^3); V_2 (volume of the extract after chromatographic separation, cm^3); m (weight of the sample, g).

For the separation of carotenes, chromatographic paper (Whatman No. 3A) was used as the stationary phase and petroleum ether as the mobile one. Carotenes were identified as a separate band. After separation, the paper was completely dried, and the carotenes eluted with small portions of acetone. The measurements were carried out on a spectrophotometer at a wavelength of 454 nm, the total carotene content was calculated in accordance with equation (B).



A B

Fig 1. **Climatograms for 2018 (A) and 2019 (B), Pushkin and Pavlovsk Laboratories of VIR (St. Petersburg, Pushkin)**

Рис. 1. **Климатограммы за 2018 г. (A) и 2019 г. (B), научно-производственная база «Пушкинские и Павловские лаборатории ВИР» (Санкт-Петербург, Пушкин)**

Sample preparation and spectrophotometric analysis.

Sample preparation and biochemical analysis were performed according to methodological guidelines developed at VIR (Ermakov, 1987). Each cucurbit sample was composed from three fruits. One-sixth or one-eighth part was taken from each fruit, depending on its size, chopped into 2 × 5 mm pieces and mixed. A 5 g sample was taken from the mixture. The pigments were extracted with small portions of 90% acetone until the complete discoloration of the flesh. Absorbance of chlorophylls, carotenoids, carotenes and *β*-carotene was measured at the 440, 645, 663 and 454 nm wavelengths by the UV spectrophotometer Ultrospec II (Biochrom, England) using a 90% acetone solution as a control. Total carotenoids (A), total carotenes (B), and *β*-carotene (C) content were calculated by the following equations (Ermakov, 1987):

$$\text{(A) Ccar(mg/100g)}=\frac{(4.695 \cdot A440 - 0.268 \cdot (Cla + Clb)) \cdot V \cdot 100}{m};$$

$$\text{(B) } X\left(\frac{mg}{100g}\right) = \frac{a \cdot V \cdot V2 \cdot 100}{m \cdot V1};$$

$$\text{(C) } \textit{beta-Carotene (mg/100g)}=\frac{37.453 \cdot (A454) + 0.1846}{10},$$

where: Ccar (carotenoids, mg/100 g), A (pigment's absorption rates), X (carotene content, mg/100g), a (the pigment

Finally, the amount of carotenoids was calculated taking into account the dilutions made during the pigment extraction process. The content of carotenoids, carotenes and *β*-carotene was expressed in mg/100 g of wet weight of cucurbit fruit flesh. Each analysis was performed in duplicate, and the obtained means were statistically processed.

Data processing.

To calculate the Kruskal–Wallis and Mann–Whitney criteria, the Past 2.17c program was used. The descriptive statistics indicators (medians, quartiles, mean, and standard deviation) were determined using the LibreOffice 7.0.2.2 program.

Results

The study of 69 cultivars of *C. pepo*, *C. maxima* and *C. moschata* from the VIR collection with subsequent statistical analysis made it possible to establish differences between these species in terms of carotenoids, carotenes, and *β*-carotene content.

According to the results of the analysis, the highest values of carotenoids (8.28 ± 7.59), carotenes (4.25 ± 5.18) and *β*-carotene (1.64 ± 2.32) content were found for the accessions of *C. maxima*, the medium level of carotenoids (5.11 ± 4.72) and carotenes (2.85 ± 3.53) for *C. moschata*, and the minimum levels (3.38 ± 2.95 and 1.80 ± 1.85, respectively) for *C. pepo*. The minimum *β*-carotene value of 0.59 ± 0.55 was found for *C. moschata* and the medium one (0.65 ± 1.00) for *C. pepo* (Fig. 2, Table 2).

The accessions of *C. pepo* differed from the other studied ones by the minimal variability in the content of carotenoids and carotenes (0.63–8.24 and 0.45–5.22; respectively), and medium for *β*-carotene (0.08–2.61 mg/100 g) (see Table 2, Fig. 2).

The highest average two-year values of carotenoid, carotene and *β*-carotene content were found for the local acces-

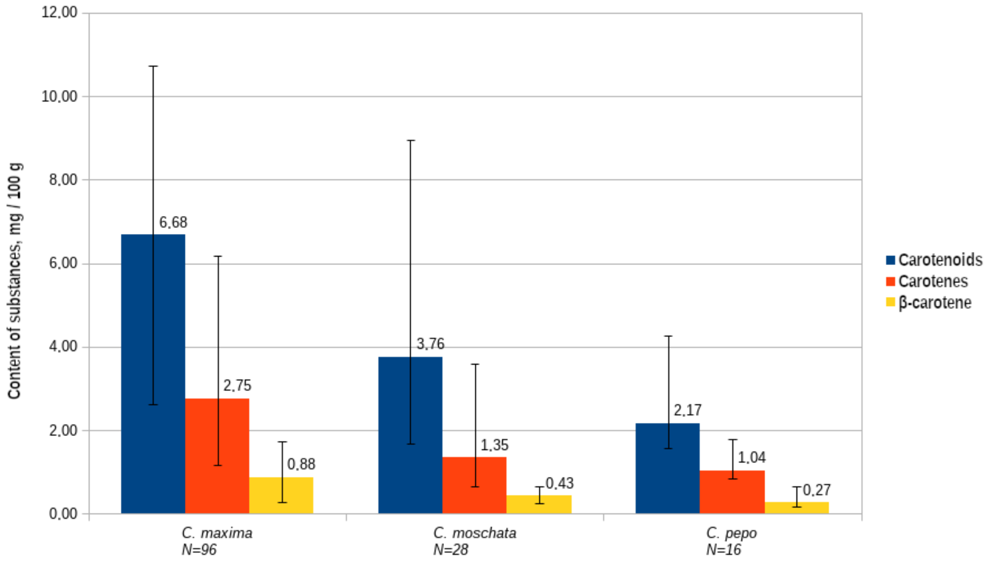


Fig. 2. The content of carotenoids, carotenes, and *β*-carotene in fruits of *Cucurbita maxima* Duch., *C. moschata* Duch. ex Poir. и *C. pepo* L. (medians)

Рис. 2. Содержание каротиноидов, каротинов и β-каротина в плодах *Cucurbita maxima* Duch., *C. moschata* Duch. ex Poir. и *C. pepo* L. (медианы)

Table 2. The content of carotenoids, carotenes, and *β*-carotene in the flesh of fruits of *Cucurbita* L. accessions from the VIR collection, mg/100 g (mean values ± SD)

Таблица 2. Содержание каротиноидов, каротинов и β-каротина в мякоти плодов образцов *Cucurbita* L. из коллекции ВИР, мг/100 г (средние значения ± стандартное отклонение)

Cucurbita species	Years of study	Carotenoids	Carotenes	*β*-carotene
C. maxima	2018	10.25 ± 9.42	5.60 ± 6.77	2.25 ± 2.92
	2019	6.31 ± 4.43	2.90 ± 2.14	1.02 ± 1.25
Two year average		8.28 ± 7.59	4.25 ± 5.18	1.64 ± 2.32
C. moschata	2018	6.49 ± 6.06	3.67 ± 4.58	0.77 ± 0.68
	2019	3.73 ± 2.33	2.04 ± 1.85	0.42 ± 0.29
Two year average		5.11 ± 4.72	2.85 ± 3.53	0.59 ± 0.55
C. pepo	2018	2.79 ± 2.17	2.00 ± 2.26	0.79 ± 1.47
	2019	3.84 ± 3.50	1.64 ± 1.58	0.53 ± 0.45
Two year average		3.38 ± 2.95	1.80 ± 1.85	0.65 ± 1.00

The widest range of variability of carotenoids (0.61–34.1), carotenes (0.33–23.1) and *β*-carotene (0.07–6.75 mg/100 g) during the study period was observed in the accessions of *C. maxima* (see Table 2, Fig. 2).

The fruits of *C. moschata* demonstrated the medium degree of variability in the content of carotenoids (1.08–13.2), carotenes (0.38-10.5) and the minimal for *β*-carotene (0.06-1.55 mg/100 g) (Table 2, Fig. 2).

sions and cultivars of *C. maxima:* 'Slastyona' (k-4935, Russia) – 17.1; 8.8 and 6.2 mg/100 g, respectively, k-2787 (Russia) – 12.9, 9.4 and 6.8 mg/100 g; *C. moschata:* Local k-4585 (Bangladesh) – 11.95, 7.7 and 1.4 mg/100 g, 'Muskatnaya 51-27' (k-3362, Bulgaria) – 13.2, 10.5 and 1.6 mg/100 g, *C. pepo:* k-4936 (Bulgaria) – 8.2; 5.2 и 2.6 mg/100 g; of carotenoid and carotene content for the cultivars of *C. maxima:*'Lesnoy Orekh' (tk-2283, Russia) – 34.2 and 23.0 mg/100 g, respectively, 'Krasnaya Novinka' (k-5384, China) – 12.8 and 8.9, 'Krasnaya Malenkaya' (tk-2079, China) – 12.5 and 9.0, and 'Zelenaya Zvezda' (k-5339, China) – 15.4 and 8.1, respectively; *C. moschata:* Tiana F1 (k-5388, the Netherlands) – 7.7 and 5.4 mg/100 g, respectively; of carotenoid content for the cultivars of *C. maxima:*'Izobiliye' (k-5032, Russia) – 2.1 mg/100 g, 'Chernaya Tsennaya' (k-5340, China) – 15.3, 'Krasnaya Dragotsennost' (k-5383, China) – 14.9, 'Zelenaya Stolovaya' (k-5342, China) – 13.2, 'Krasnaya' (tk-2372,China) – 12.5 mg/100 g; *C. moschata:* 'Berkanush' (k-4510, Armenia) – 7.7 mg/100 g; of carotene content for the accession of *C. maxima:* Local k-4919 (Kazakhstan) – 7.3 mg/100 g, and *β*-carotene content for the cultivar of *C. maxima* 'Zhdana' (k-5017, Ukraine) – 6.3 mg/100 g.

Although no reliable dependence of carotene, carotenoid and *β*-carotene content in the *Cucurbita* accessions on climate conditions during the two years of research could be established, still it was possible to trace the trend of this relationship. Virtually all accessions of *C. maxima*, *C. moschata* and *C. pepo* demonstrated higher values of carotene, carotenoid and *β*-carotene content in 2018, except for the content of carotenoids in *C. pepo* accessions, which was higher in 2019. Apparently, the higher temperatures in 2018, compared to the long-term average data, contributed to the accumulation of target substances in the flesh of cucurbit fruits (see Table 2). Our data are consistent with the results of other researchers (Cavalcante, Rodriguez-Amaya, 1992; Kimura et al., 1991), which showed that high temperature and intense insolation enhance fruit's carotenogenesis.

The influence of the species of the studied cucurbit accessions on the content of pigments in it was assessed using the Kruskal–Wallis test. As a result, a statistically significant relationship between the species and indicators of carotenoid, carotene and *β*-carotene content in the cucurbit fruit flesh was established ($p < 0.01$, $p < 0.05$ and $p < 0.05$; respectively). The post hoc analysis using the Mann–Whitney test (with Bonferroni correction) revealed statistically significant differences between *C. maxima* and *C. pepo* in the content of carotenoids ($p < 0.01$), carotenes ($p < 0.05$), and *β*-carotene ($p < 0.05$).

C. maxima was found to be the most polymorphic species in comparison with others, and was distinguished by a variety of colors of the skin and flesh of the fruit. To assess the influence of the color of the skin and flesh of *C. maxima* fruits on the content of pigments (carotenoids, carotenes, and *β*-carotene) in them, the Kruskal–Wallis test was used (pairwise comparison using the Mann–Whitney test with the Bonferroni correction for the post hoc analysis). The considered flesh color options included light yellow, yellow-orange, yellow, creamy, orange, light orange, dark yellow, dark orange, bright yellow; bright orange, and those for the skin included white, yellow (including creamy-yellow and light yellow), green (including brown-green, light green, gray-green, dark green and black-green), red (including orange-red), orange (including pink-orange, light orange and bright orange), pink (including light pink), gray (including also whitish-gray, light gray, gray with pink spots and dark gray).

The performed statistical analysis revealed the effect of the color of the fruit flesh on the content of carotenoids, carotenes and *β*-carotene in them ($p < 0.001$ in all cases) (Fig. 3, A, B, C).

The post hoc analysis showed that in terms of the total carotenoids in fruits of *C. maxima* there existed significant differences between fruits with yellow flesh and those with dark yellow and bright orange flesh ($p < 0.01$ and $p < 0.001$, respectively) as well as between fruits with light yellow flesh and those with dark yellow and bright orange flesh ($p < 0.05$ and $p < 0.01$, respectively). As can be seen from Figure 3A, the lowest content of carotenoids is characteristic of the fruits with light yellow flesh, while the highest to those with dark orange flesh. In terms of the total carotene content, the fruits with yellow flesh were explicitly opposed to those with bright orange flesh ($p < 0.01$), and it also refers to fruits with light yellow flesh vs. fruits with dark yellow and bright orange flesh ($p < 0.05$ and $p < 0.01$, respectively) (see Fig. 3, B). In terms of the total content of *β*-carotene, significant differences between fruits with light yellow flesh and those with bright orange flesh ($p < 0.01$) were clearly revealed (see Fig. 3, B). As can be seen from Figures 3A, B, C, the lowest content of carotene in general, *β*-carotene included, was detected in fruits with light yellow and yellow-orange flesh, while the highest was found in fruits with dark orange flesh.

A statistically significant effect of *C. maxima* fruit skin color on the content of carotenoids, carotenes and *β*-carotene in them ($p < 0.001$ in all cases) was revealed (Fig. 4, A, B, C). The post hoc analysis showed that in terms of the total content of carotenoids, fruits with yellow skin considerably differed from those with red ($p < 0.01$), and fruits with pink skin from those with green and red skin ($p < 0.05$) (Fig. 4, A). The values of total carotene content clearly separated the fruits with red skin from those with pink skin ($p < 0.05$) (Fig. 4, B). In terms of the total *β*-carotene content, fruits with green and red skin significantly differed from those with pink skin ($p < 0.05$ in both cases) (Fig. 4, C).

It is demonstrated in Figs. 4: A, B, C that the lowest content of carotenoids, carotenes and *β*-carotene is characteristic of fruits with white skin, while the highest for the fruits with red skin.

Discussion

Carotenoids belong to the group of provitamin A, being its metabolic precursors. This is the most abundant class of plant pigments, the most important of which is *β*-carotene. The bulk of provitamin A is obtained from leafy green, orange and yellow vegetables, tomato products, fruits and some vegetable oils (Seo et al., 2005). Cucurbits are an important source of carotenoids – antioxidants with recognized pharmaceutical potential. Studies by a number of authors indicated considerable differences in the content of carotenoids in *Cucurbita* species and cultivars (Murkovic et al., 2002; Azevedo-Meleiro, Rodriguez-Amaya, 2007; Kim et al, 2012; Kulczyński, Gramza-Michałowska, 2019a, b).

The post hoc analysis of the obtained results (the content of carotenoids, carotenes and *β*-carotene) showed significant differences between *C. maxima* and *C. pepo*, it being consistent with the data previously obtained by Kim et al. (2012), who revealed differences between the species in the content of *β*-carotene in the flesh of fruits grown in Gunsan, Naju and Kochang regions of South Korea. Kulczyński and Gramza-Michałowska (2019a, b) found statistically significant differences ($p = 0.05$) in biochemical composition between *C. pepo* and *C. moschata* reproduced in western Poland (Wolkowo).

The highest *β*-carotene content was found by Kim et al. (2012) in *C. maxima* (17.4 mg/kg wet weight), medium in *C. moschata* (5.7 mg/kg), and low in *C. pepo* (1.48 mg/kg), which is somewhat different from the findings of the present research, in the course of which it was found that the maximum content of *β*-carotene (1.64 mg/100 g) indeed corresponded to the accessions of *C. maxima*, while the medium (0.65) and low (0.59) contents were found in *C. pepo* and *C. moschata*, respectively. The values obtained in the present

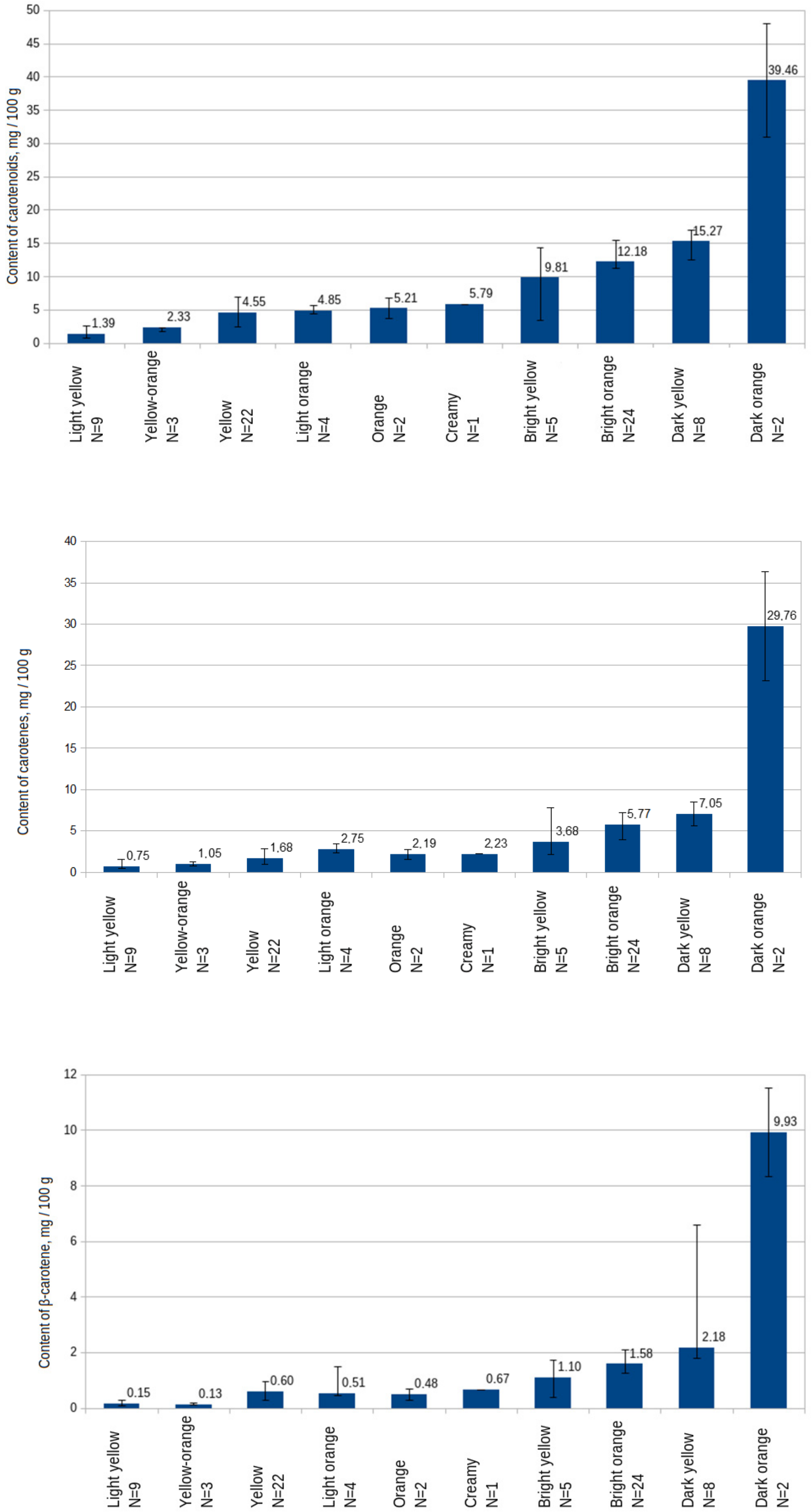


Fig. 3. Content of carotenoids (A), carotenes (B) and *β*-carotene (C) in cucurbit fruits with differently colored flesh from the VIR collection taken in the study

Рис. 3. Содержание каротиноидов (А), каротинов (Б) и β-каротина (В) в плодах *Cucurbita* spp. с разноокрашенной мякотью из коллекции ВИР, взятых в исследование

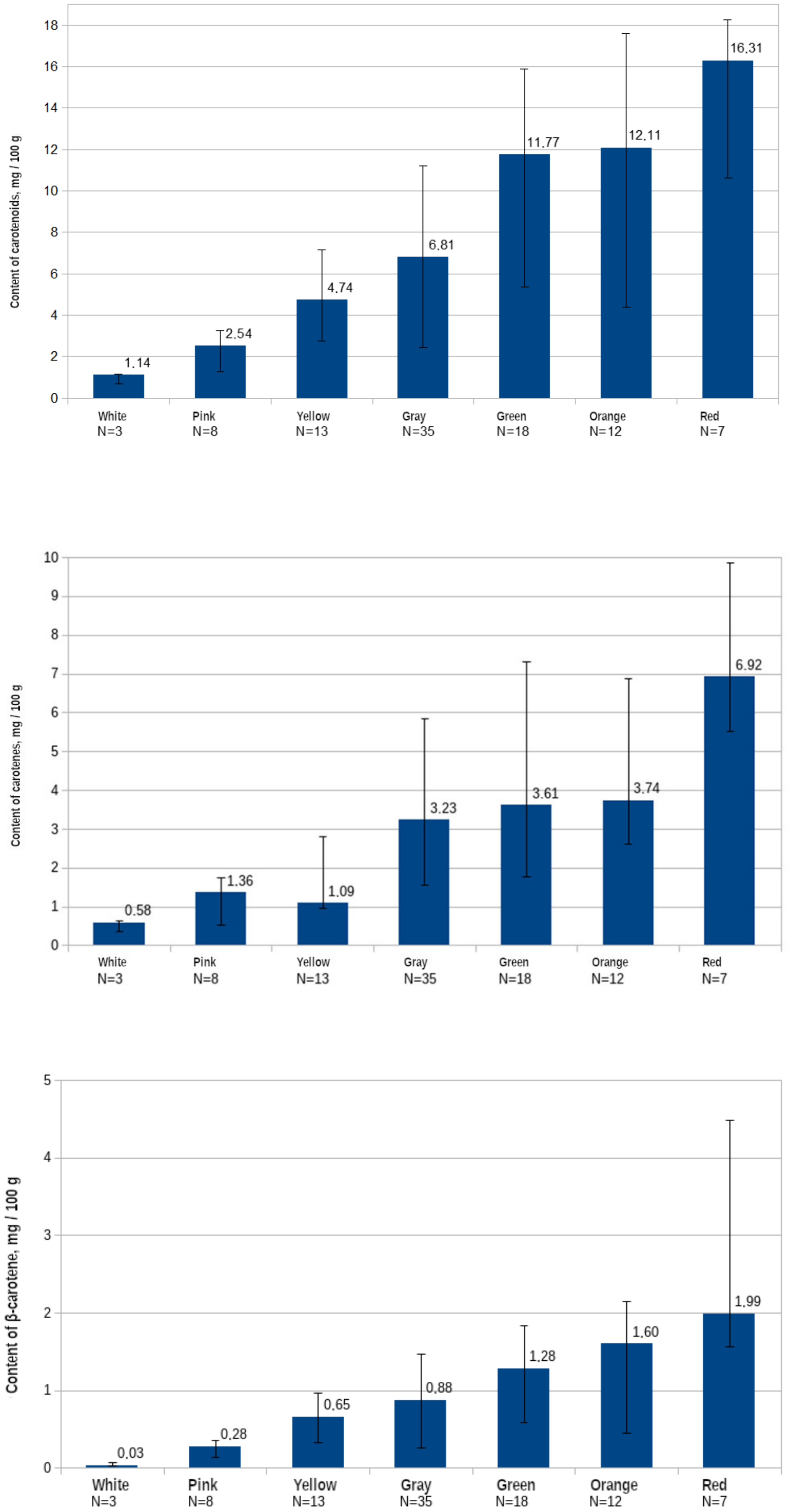


Fig. 4. **Content of carotenoids (A), carotenes (B) and *β*-carotene (C) in cucurbit fruits with differently colored skin from the VIR collection taken in the study**

Рис. 4. **Содержание каротиноидов (А), каротинов (Б) и β-каротина (С) в плодах *Cucurbita* spp. с разной окраской кожуры из коллекции ВИР, взятых в исследование**

research and by Kim et al. (2012) were comparable. Kulczyński and Gramza-Michałowska (2019a, b) confirmed that the maximum values of carotenoids and *β*-carotene were typical for the cultivars of *C. maxima*, which agrees with the data obtained in the present research. However, Kulczyński and Gramza-Michałowska noted that medium values of carotenoid and *β*-carotene content were characteristic of *C. pepo* and *C. moschata*, respectively. According to our data, the medium level of carotenoids and carotenes was established for *C. moschata*, and the minimum one for *C. pepo*, while the values of *β*-carotene content were minimal for *C. moschata* and medium for *C. pepo*. It was shown in the works by Kulczyński and Gramza-Michałowska (2019a) that the highest *β*-carotene content of 11.53 mg/100 g was found in cv/ 'Melonova Zolta' (*C. maxima*), which was comparable with our data of 2018 on the highest results for *β*-carotene content in the accession k-2787 (13.1) and cv. 'Slastyona' (10.4 mg/100 g).

Murkovic et al. (2002) analyzed 12 cultivars of *C. maxima*, 5 cultivars of *C. pepo*, and 4 cultivars of *C. moschata* as well as the *C. maxima* × *C. moschata* hybrid grown in Austria. The content of carotenoids in the *Cucurbita* accessions studied by them ranged from 0.06 to 7.4, and of *β*-carotene from 0 to 7.5 mg/100 g. The range of carotenoid variability established in the present research was found to be wider (0.61–34.15), and the maximum values higher. The *β*-carotene data from the present study are comparable with those by Murkovic et al. (0.07–6.28 mg/100 g).

The difference in the figures obtained by us and Kim et al. (2012), Kulczyński and Gramza-Michałowska (2019a, b), Murkovic et al. (2002) can be explained by the differences in climate conditions between the locations of *Cucurbita* fruit growing, that is, Northwestern Russia (Pushkin), South Korea, western Poland (Wolkowo), and southern Austria.

Our conclusions about the high statistical significance ($p < 0.001$) of the effect of the cucurbit fruit flesh color on the level of accumulation of carotenoids and carotenes, including *β*-carotene, were confirmed by the studies of Murkovic et al. (2002). They also found that the flesh color characteristics correlate with the content of carotenoids in fruit flesh. The high-carotene cultivars in the mentioned study had orange flesh, while the low-carotene ones had bright yellow flesh.

The statistically significant ($p < 0.001$) effect of the cucurbit fruit skin color on the content of carotenoids, carotenes and *β*-carotene in fruit flesh, which was established using the post hoc analysis in the present research, could not be compared with the data of other researchers due to the absence of such data.

Conclusions

A study of the main pigments (carotenoids and carotenes, including *β*-carotene) in the fruit flesh of different *Cucurbita* spp. from the VIR collection made it possible to reveal statistically significant differences between the accessions of *C. maxima*, *C. moschata*, and *C. pepo* with different flesh and fruit skin colors.

The two-year study period made it possible to identify the trend in the relationship between the accumulation of main pigments in cucurbit fruits and peculiarities of the climate parameters in Northwestern Russia as well as to select the *Cucurbita* accessions which, when grown under these conditions, can accumulate the largest amount of carotenoids and carotenes, including *β*-carotene, which makes it possible to use such accessions for expanding the range of biofunctional nutrition products in the regions with a short growing season and a low sum of positive temperatures.

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