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DETERMINATION OF THE TOTAL ANTHOCYANINS AND CAROTENOIDS CONTENT IN LEAVES OF FRUIT AND BERRY CROPS COMMON FOR WESTERN KAZAKHSTAN

Annotation. The total anthocyanins content, total carotenoid content and total content of chlorophylls *a* and *b* in leaves of fruit and berry crops common to the Western Kazakhstan region were analyzed using UV-vis spectrophotometry. Twelve plant species were studied in this work. Anthocyanins level in leaves varied from 0.041 to 0.532 mg/g, carotenoid levels were from 0.56 to 3.87 µg/g dry weight. The range of chlorophylls *a* and *b* content is significantly wider (2.15 to 14.07 and 1.8 to 9.1 µg/g respectively). Among the investigated plants *R.canina* has the highest levels of natural pigments. It has been observed that *P.armeniaca* is the plant richest in anthocyanins, in turn *R.canina* and *A.ovalis* was found to be richest in carotenoids. It was also found that the pigments content depends on plant species and the content of chlorophyll *a* exceeds the content of chlorophyll *b* from 1.19 to 2.75 times.

Keywords: fruit and berry crops; plant leaves; anthocyanins; carotenoids; chlorophyll.

Introduction

Anthocyanins and carotenoids are two groups of pigments with different chemical structures. They are present in fruit and berry crops, give them a special color, and can also have a healing effect on human health [1]. In the past few decades the interest in the investigation of anthocyanins and carotenoids in fruit and berry crops due to their possible health benefits was significantly increase [2].

Anthocyanins are phenolic water-soluble glycosides or acyl-glycosides of anthocyanidins. These compounds are secondary plant metabolites, protecting them against biotic and abiotic stresses, being the most abundant cyanidin delphinidin and pelargonidin derivatives [3]. Anthocyanins are responsible for the pink, red, blue and purple colors in flowers, fruits and vegetables, with the color being related to the substitution pattern (position and chemical groups) on the aromatic rings. They have been used as natural food coloring agents and are emerging as promising ingredients in food and nutraceutical industries. Anthocyanins are also recognized as possessing anti-microbial, anti-cancer and anti-diabetic activities and as potent antioxidant and anti-inflammatory agents [4].

Carotenoids are another set of naturally occurring plant pigments, mostly responsible for the red, yellow and orange colors of vegetables and autumn leaves (when all the chlorophyll has already been degraded), but can also be found in dark green vegetables [5]. All carotenoids are a polyisoprenoid structure, a long-conjugated chain of double bond and a near bilateral symmetry around the central double bond. The most common types of carotenoids in plant leaves are lutein, β-carotene, violaxanthin and neoxantin [6].

The consumption of these compounds by humans, who are not able to synthetize them, seems to have a prominent role in reductions in several diseases such as eye-related cancer, immune disorders and cerebrovascular and cardiovascular diseases. Carotenoids are also quite relevant in industry, being used as food colorants, cosmetic products and nutraceuticals [7].

Chlorophylls are another relevant pigment class, that present in photosynthetic organisms such as plants, algae and cyanobacteria. Chlorophylls are the pigments that make plants green and are arguably the most important compounds on earth as they are required for the harvesting and transduction of light energy in photosynthesis [8]. These pigments are large molecules with a cyclic part (chlorine ring) bound to a metal ion (magnesium), which reflects green light. Five forms of chlorophyll are known (chlorophylls a, b, c, d and f), presenting slightly distinct functions during the photosynthetic processes undertaken by the different organisms. In plants, during senescence and fruit ripening, the programed chlorophyll breakdown occurs to allow the remobilization of nutrients to parts of the plant that are still growing. This phenomenon unmasks the presence of carotenoids and anthocyanins in green plant leaves, which are mainly observed in autumn [9]. In addition, chlorophylls and carotenoids are known with their antioxidant properties [10]. Furthermore, chlorophylls as other porphyrins are used in photodynamic therapy of tumors and exhibit anti-mutagenic activity in short-term genotoxicity assays [11].

In this work, 12 species of fruit and berry crops common in the West Kazakhstan region were investigated. The content of anthocyanins and carotenoids in leaves were determined using UV-vis-spectrophotometry.

Materials and methods

Reagents and solvents.

All the analytical grade chemicals were purchased from commercial suppliers and used directly without any purification.

Collection and preparation of plant material.

List of plants chosen for our investigation with Russian and local names is given in Fig. 1.

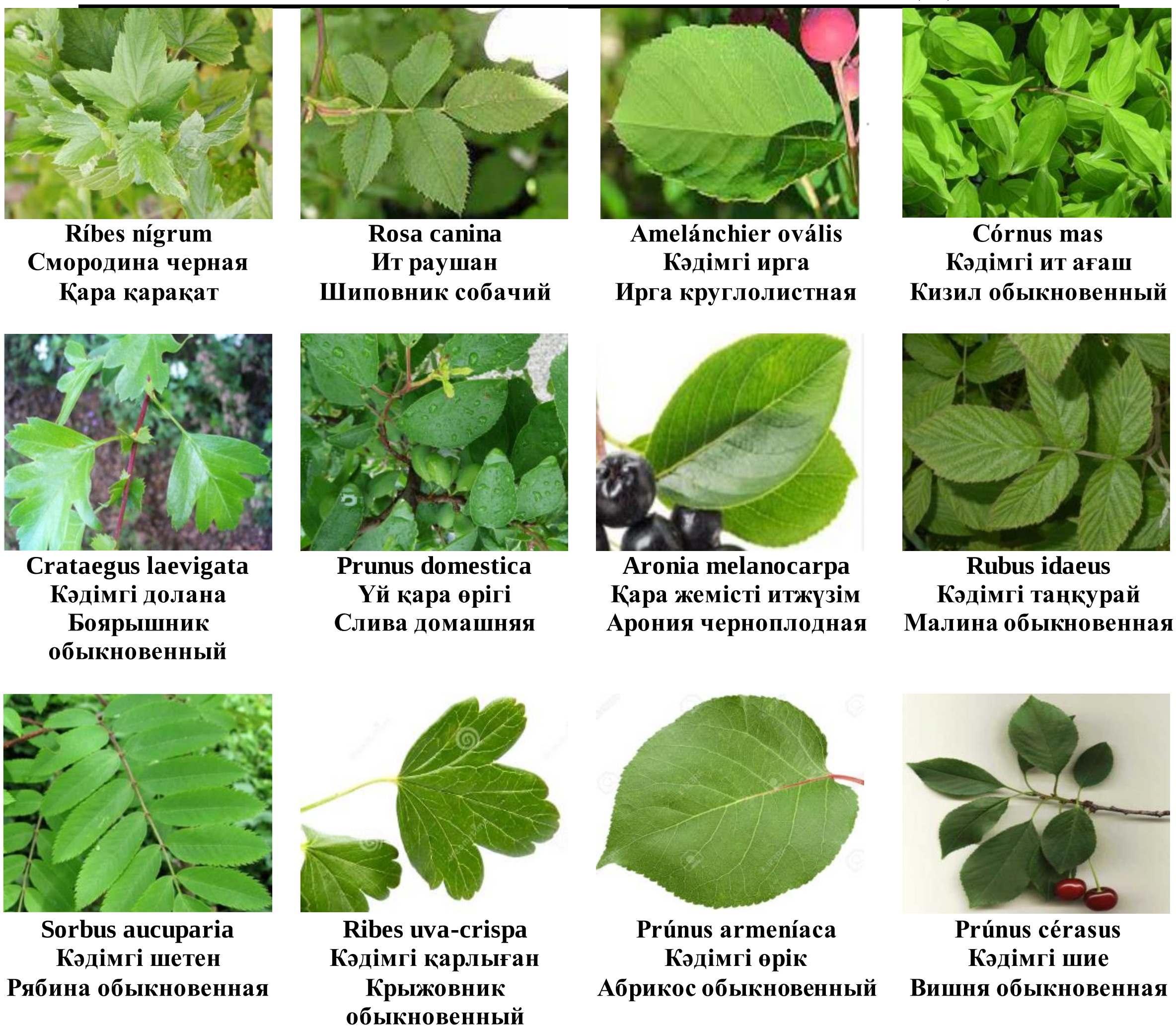

Figure 1 - Fruit and berry crops species used in this investigation.

Fresh plant leaves were collected from their native habitat, away from roads and industrial enterprises. Plants were thoroughly washed with tap water and double distilled water and then kept in a shaded ambient atmosphere to total remove the moisture. The dried samples were then ground in a stainless-steel mill, sieved through a 1.0 mm and stored at 4°C until further use.

Determination of total carotenoids content.

Total carotenoid content was determined *via* measurement of the absorbance of acetone extract as follows [12].

To 0.05 g of dry sample 5 ml of acetone were added and pulverized in a porcelain mortar in ice bath. Then, 1.0 g of anhydrous sodium sulfate was added and the solution mixed slowly followed by increasing the volume of acetone to 10 ml. The mixture was centrifuged at 26,000 rpm for 10 min. The supernatant was then removed and the absorbance measured at 662, 645 and 470 nm in 10 mm quartz cuvette. The total carotenoid content was calculated by the following formulas:

$$C_a\ (\mu g/g) = 11.24A_{662} - 2.04A_{645}$$

$$C_b\ (\mu g/g) = 20.13A_{645} - 4.19A_{662}$$

$$C_t\ (\mu g/g) = (1000A_{470} - 1.9C_a - 63.14C_b) / 214$$

where C_a stands for chlorophyll *a*, C_b for chlorophyll *b* and C_t is total carotenoid content. A_{470} is absorption at 470 nm (related to carotenoids), A_{645} - at 645 nm (related to chlorophyll *a*) and A_{662} - at 662 nm (related to chlorophyll *b*) [12].

Determination of anthocyanins content.

Anthocyanins content was spectrophotometrically measured from 1% hydrochloric acid extract of dried sample as described below [12].

To 0.02 g of dry sample 4 ml of 1% hydrochloric acid containing methanol were added and pulverized in a porcelain mortar. Solution was kept for 24 h in the refrigerator (4ºC). Then, after centrifugation for 10 min at 13,000 rpm the absorbance of supernatant was measured at 530 and 657 nm in 10 mm quartz cuvette against blank. The blank solution was 4 ml of 1% hydrochloric acid solution containing methanol. The anthocyanins content (mg/g DW) was calculated by the following equation:

$$\text{Anthocyanins (mg/g)} = A_{530} - (0.25 \cdot A_{657})$$

where ‘‘A’’ stands for absorbance at 530 nm and 657 nm respectively

Statistical analysis

Each experiment was carried out in triplicate (n = 3) and the data presented as an average of three independent determinations.

Research results

The results of chlorophyll *a* and chlorophyll *b* content determination are shown in Fig. 2.

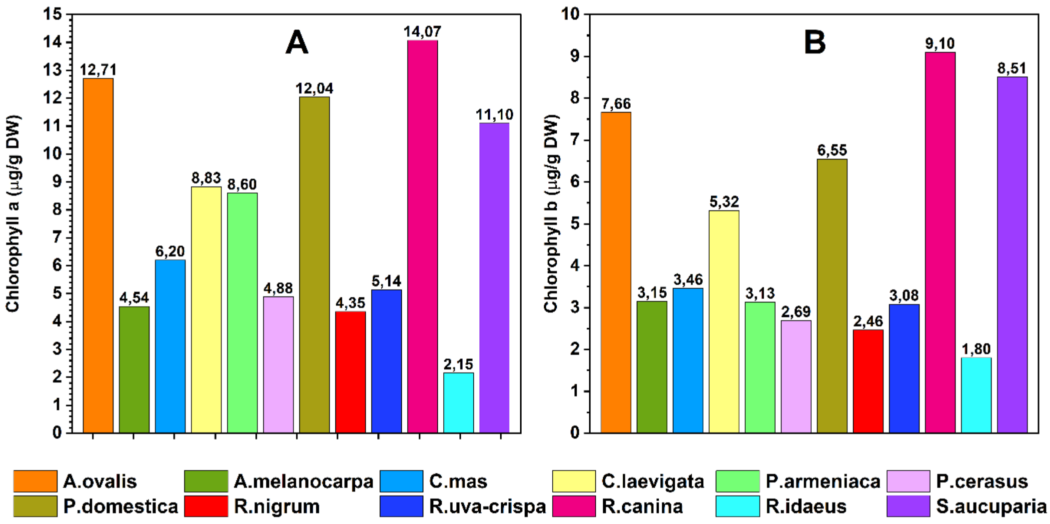

Figure 2 – Content of Chlorophyll *a* (A), Chlorophyll *b* (B) in leaves of the investigated plants.

As can be seen, the highest content of chlorophylls *a* and *b* was determined in *R.canina*, (14.07 µg/g and 9.10 µg/g respectively). Significant amount of the pigments was also observed for *S.aucuparia*, *A.ovalis* and *P.domestica*. In general, the content of chlorophyll *a* varies from 2,15 µg/g (*R.idaeus*) to 14,07 µg/g (*R.canina*). The content of chlorophyll *b*, in turn, is within 1.80 µg/g (*R.idaeus*) to 9.10 µg/g (*R.canina*). In addition, content of the chlorophyll *a* exceeds the content of chlorophyll *b* in all plants tested. The Chl a/b ratios for investigated plants are given in Fig.3.

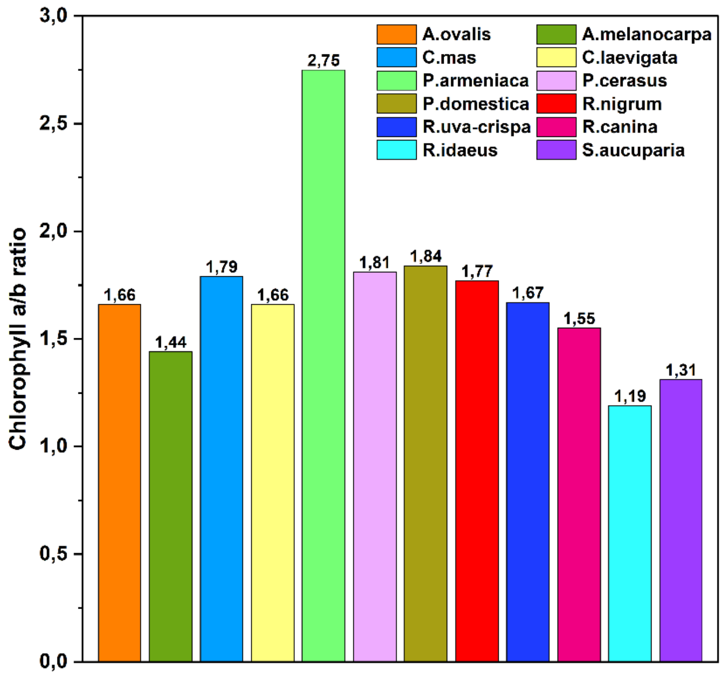

Figure 3 – The Chlorophyll a/b ratio in in leaves of the investigated plants.

Fig. 3 illustrates that Chl a/b ratio in investigated plants varies from 1,19 (*R.idaeus*) to 2.75 (*P.armeníaca*). This ratio of chlorophylls content is typical for the plant world and can vary from 1 to 3 [13]. Examples of high and low Chl a/b ratios in leaves have been previously determined for different developmental stages of leaves grown under low or high light conditions [14].

The total content of anthocyanins in studied plants is given in Fig.4

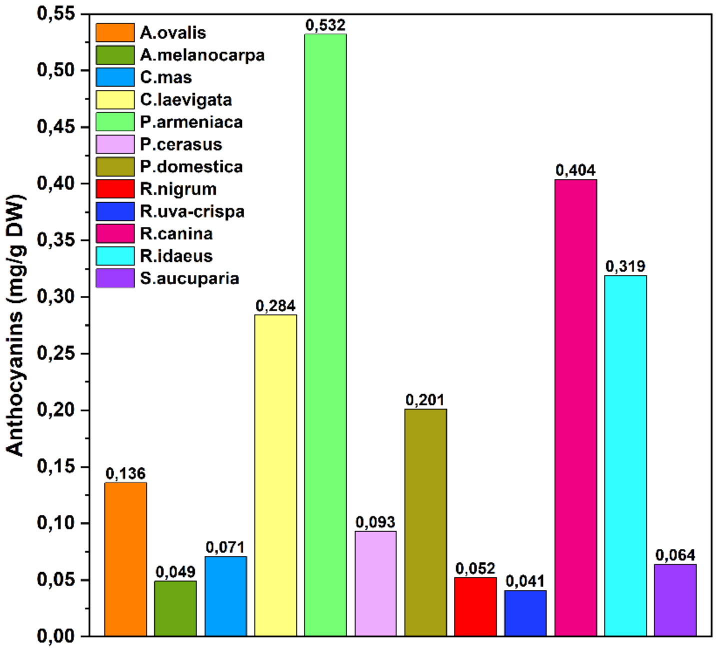

Figure 4 – Total anthocyanins content in leaves of the investigated plants.

According to Fig. 4, the anthocyanins content varied in a fairly wide range from 0.041 mg/g in *R.uva-crispa* to the highest value of 0.532 mg/g in *P.armeniaca*. Significant amounts of anthocyanin was observed for *P. domestica* (0.201 mg/g), *C.laevigata* (0.284 mg/g), *R. idaeus* (0.319 mg/g) and *R. canina* (0.404 mg/g). Other plants contain less than 0.14 mg/g of anthocyanins.

The total carotenoid content determined in plant leaves is given in Fig.5.

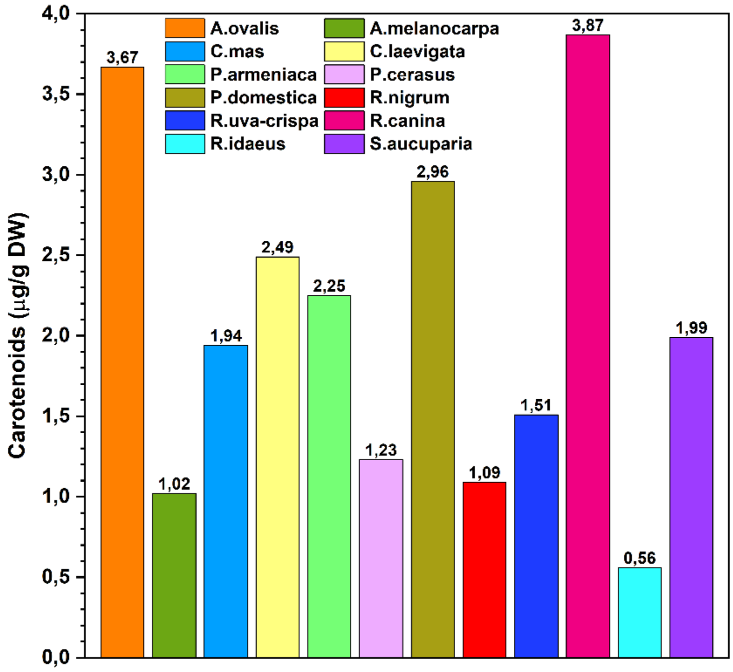

Figure 5 – Total carotenoids content in leaves of the investigated plants.

Fig. 5 shows that total carotenoid content is in the same trend as the content of chlorophylls *a* and *b*. The highest values of total carotenoids corresponds to *R.canina* (3.87 µg/g) and *A.ovalis* (3.67 µg/g). The lowest – to *R.idaeus* (0.56 µg/g). In the leaves of most of the plants studied, the total carotenoid content varies from 1,0 to 3,0 µg/g.

A comparison of the total content of carotenoids in the leaves of similar plant species growing in another countries is shown in Table 1.

Table 1- Comparison of total carotenoids content (µg/g) in leaves of the investigated plants.

Country	Total carotenoids content (µg/g)			References
	R. canina	*S. aucuparia*	*R. idaeus*	
Tunisia	4.68	-	-	[15]
Bagheria, Palermo, Italy	19,76	-	-	[16]
Komi Republic, Yoshkar-Ola	-	1.50	-	[17]
Warsaw, Poland	-	-	2.61	[18]
Uralsk, Kazakhstan	3.87	1,99	0.56	This work

As can be seen from the Table 1, the total carotenoid content for identical species significantly varies depending on the region of growth. Similar data are available in previously published works on the phytochemical composition of leaves of

R. canina, R. idaeus and *S. aucuparia* growing in Tunisia, Italy, the Komi Republic, and Poland. These results are differed from those obtained in our investigation. Comparison of available data allow to suggest that the reason of these differences is due to the fact that the pigments content is directly depends on climatic conditions and soil type.

Conclusion

The main goal of this study was to determine the anthocyanins and carotenoids content in leaves of some berry and fruit crops locally grown in West Kazakhstan region. The research has shown that investigated plants are significantly differ on total anthocyanins and carotenoids contents and the content of all pigments varies in wide range. Considering the fact that all plants were collected in the same area and at the same time, this difference is most likely due to the species belonging and physiology specifics of each plant species. The difference in pigment content from similar species studied in other countries indicates that their content is also influenced by the region of growth, climate characteristics and soil type. *R.canina* stood out with the highest natural pigment content among others. In general, obtained results of our research indicate that leaves of the most common berry and fruit crops are the reach source of natural pigments.

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Максотова А., Исағат Р., Ұзақбай Г., Мендіғалиев Е., Акатьев Н.

ОПРЕДЕЛЕНИЕ ОБЩЕГО СОДЕРЖАНИЯ АНТОЦИАНОВ И КАРОТИНОИДОВ В ЛИСТЬЯХ ПЛОДОВО-ЯГОДНЫХ КУЛЬТУР, ТИПИЧНЫХ ДЛЯ ЗАПАДНОГО КАЗАХСТАНА

Аннотация. Методом УФ-вид-спектрофотометрии проанализировано общее содержание антоцианов, общее содержание каротиноидов и общее содержание хлорофиллов *a* и *b* в листьях плодово-ягодных культур, распространенных в Западно-Казахстанской области. В работе изучено двенадцать видов растений. Содержание антоцианов в листьях варьировалось от 0,041 до 0,532 мг/г, каротиноидов – от 0,56 до 3,87 мкг/г сухой массы. Диапазон содержания хлорофиллов *a* и *b* был значительно шире (от 2,15 до 14,07 и от 1,8 до 9,1 мкг/г соответственно). Среди исследованных растений *R.canina* имеет самый высокий уровень содержания натуральных пигментов. Отмечается, что *P.armeniaca* является растением, наиболее богатым антоцианами, а *R.canina* и *A.ovalis*, в свою очередь, оказались наиболее богаты каротиноидами. Также установлено, что содержание пигментов зависит от вида растений, а содержание хлорофилла *a* превышает содержание хлорофилла *b* от 1,19 до 2,75 раз.

Ключевые слова: плодово-ягодные культуры; листья растений; антоцианы; каротиноиды; хлорофилл.

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БАТЫС ҚАЗАҚСТАНҒА ТӘН ЖЕМІС-ЖИДЕК ДАҚЫЛДАРЫНЫҢ ЖАПЫРАҚТАРЫНДАҒЫ АНТОЦИАНИНДЕР МЕН КАРОТИНОИДТАРДЫҢ ЖАЛПЫ ҚҰРАМЫН АНЫҚТАУ

Аңдатпа. УК-спектрофотометрияны қолдану арқылы Батыс Қазақстан облысында кең тараған жеміс-жидек дақылдарындағы антоциандардың жалпы мөлшері, каротиноидтардың жалпы мөлшері және *a* және *b* хлорофиллдердің жалпы мөлшері талданды. Бұл жұмыста өсімдіктің он екі түрі зерттелді. Жапырақтардағы антоциандардың құрғақ салмақтарының жалпы мөлшері 0,041-ден 0,532 мг/г, каротиноидтар – 0,56-дан 3,87 мкг/г дейін өзгерді. Хлорофилл *a* және *b* мәндерінің диапазоны кеңірек болды (тиісінше 2,15-тен 14,07-ге дейін және 1,8-ден 9,1 мкг/г-ге дейін). Зерттелген өсімдіктердің ішінде табиғи

пигменттердің ең жоғары деңгейі *R. canina* өсімдігіне тән. *P.armeniaca* антоцианиндерге ең бай өсімдік екені атап өтілді, өз кезегінде *R.canina* және *A.ovalis* жалпы каротиноидтарға ең бай болды. Сондай-ақ, өсімдік құрамындағы пигменттердің мөлшері өсімдік түріне байланысты, ал хлорофилл а мөлшері хлорофилл б мөлшерінен 1,19-2,75 есе артық екені анықталды.

Кілт сөздер: жеміс-жидек дақылдары; өсімдік жапырақтары; антоцианиндер; каротиноидтар; хлорофилл.