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Effect of heat treatment and storage on anthocyanins levels in food plants

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ABSTRACT

Introduction: **Anthocyanins, the polyphenolic plant pigments, have high antioxidant activity (AOA), reduce the risks of many pathological conditions in the human body. However, the wide medical and preventive use of anthocyanins is limited by their degradation during processing of plant raw materials. The objective of the work was to study the anthocyanins' stability by spectrophotometric method during heat treatment and storage of vegetable and berry plants.**

Study objects and methods: **Purple potatoes, eggplants, red cabbage, purple carrots, blue onions, red raspberries and blue honeysuckle were used in the study. Anthocyanins' stability was determined by numerical values of extracts' absorption spectra from the studied plants.**

Results and discussion: **High stability indices of 0.623–0.986 were obtained for the anthocyanins of purple carrots, blue onion bulbs or red cabbage leaves whose main component is the antioxidant cyanidin with a relative AOA equal to 3.49. Low stability indices of 0.229-0.23 were obtained for anthocyanins of red raspberry berries and purple potato tubers containing pelargonidin or malvidin with 2.49–3.36 times lower relative AOA than for cyanidin. A regular correlation between stability and AOA of anthocyanins with a rank correlation coefficient of 0.91 ($p<0.05$) was established.**

It was established that during three months of storage in a domestic refrigerator, the content of anthocyanins in purple carrots and purple potatoes decreased by 10–15% of the initial one, and this decrease was 2-3 times greater when other studied plants were stored for 1.5–2 months.

Conclusion: **It is advisable to use the developed spectrophotometric methods in the express-analysis at selection of perspective plants for industrial cultivation as raw material for anthocyanin-containing herbal formulation.**

Keywords: **spectrophotometry, anthocyanin resistance, anthocyanin-containing specimens, purple carrot, purple potato**

Содержание антоцианов в пищевых растениях при тепловой обработке и хранении

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Резюме

Цель: **Полифенольные растительные пигменты антоцианы имеют высокую антиоксидантную активность, снижают риски многих патологических состояний в организме человека. Однако широкое лечебно-профилактическое использование антоцианов ограничивается их деградацией при обработке растительного сырья. Целью работы являлось исследование устойчивости антоцианов спектрофотометрическим методом при тепловой обработке и хранении овощных и ягодных растений.**

Методы: **В исследованиях использовали фиолетовый картофель, баклажаны, краснокочанную капусту, фиолетовую морковь, синий лук, красную малину и жимолость голубую. Устойчивость антоцианов определяли по числовым показателям спектров поглощения экстрактов из исследуемых растений.**

Результаты: **Высокие показатели устойчивости 0.623-0.986 получены для антоцианов корнеплодов фиолетовой моркови, луковиц синего лука или листьев краснокочанной капусты, в составе которых основным является антиоксидант цианидин с относительной антиоксидантной активностью, равной 3.49. Низкие показатели устойчивости 0.229-0.23 получены для антоцианов ягод красной малины и клубней фиолетового картофеля, в составе которых присутствуют пеларгонидин или мальвидин с меньшей в 2.49-3.36 раза антиоксидантной активностью по сравнению с цианидином. Установлена закономерная взаимосвязь между устойчивостью и антиоксидантной активностью антоцианов при коэффициенте ранговой корреляции 0.91 ($p<0.05$). Получено, что за три месяца хранения в бытовом холодильнике содержание антоцианов в фиолетовой моркови, фиолетовом картофеле снижается на 10-15% от исходного, а при хранении других исследованных растений в течение 1.5-2 месяцев это снижение в 2- раза больше.**

Заключение: **Разработанные спектрофотометрические методы целесообразно применять в экспресс-анализе при отборе перспективных растений для промышленного культивирования как сырья для антоциансодержащих фитопрепаратов.**

Ключевые слова: **спектрофотометрия, устойчивость антоцианов, антоциансодержащие препараты, фиолетовая морковь, фиолетовый картофель**

INTRODUCTION

Anthocyanins constitute a vast group of polyphenolic water-soluble plant pigments that give flowers, fruits, leaves, and root crops a reddish-blue to purplish-black coloration. Chemically, anthocyanins are a glycosylated form of hydroxy- and methoxy-substituted derivatives of 2-phenylchromene or anthocyanidins. Anthocyanins are widespread in nature, mainly in flowers, vegetables and fruits, in which more than 600 derivatives of the six main anthocyanidins – cyanidin (Cy), delphinidin (Dp), pelargonidin (Pg), peonidin (Pn), malvidin (Mv) and petunidin (Pt) – were found [1].

Anthocyanins exhibit significant antioxidant activity (AOA) [2, 3], due to which, they counteract oxidative stress in the human body, the main factor in the development of many pathological conditions [4, 5]. Vegetable and fruit supplements rich in anthocyanins reduce the risks of cardiovascular, cancer and degenerative disorders [6]. Recently, anthocyanins that prevent functional disorders of the body have received more attention [7]: international programs of their in-depth study are being introduced [8], target regulations for anthocyanin-containing foods and special berry-based diets are being developed [9].

However, despite their potential, the widespread introduction of natural anthocyanin-containing supplements and components is limited by high lability of anthocyanins, their degradation during the processing and storage of plant raw materials, which significantly reduces their effectiveness [10].

Anthocyanins stability varies within significant limits, depends both on external factors and to a large extent on the chemical nature and the ratio of anthocyanins in different tissues [11].

Selection of the most promising plant varieties with high content of resistant anthocyanins for industrial cultivation is relevant [12], as well as development of express methods to determine their resistance. Among the existing methods for analyzing anthocyanins, the fastest and relatively simple is spectrophotometry [13], which is advisable to choose as an express method to determine resistance. As model external influencing factors of interest is the use of heat treatment and storage of plants, usually used in the technology of processing of raw materials and having a destructive effect on anthocyanins.

The objective of the work was to experimentally study the anthocyanins stability by spectrophotometric method during heat treatment and storage of berry and vegetable plants.

When planning the research, it was quite natural to focus not only on blue onion, red cabbage, red raspberry, and blue honeysuckle, which are familiar to Russians in their diets, but also on “exotic” purple carrots and purple potatoes not yet widespread in Russia.

STUDY OBJECTS AND METHODS

Plant material was used as parts of vegetable and berry plants (Table 1) grown by the authors at the territory of the Ocean Horticultural Noncommercial Partnership in Kiparisovo plot, Nadezhinsky District, Primorsky Krai, RF in the summer of 2021 (43°27 '37", 131°58 '3").

Plant material was selected by the random number method [14], from 3 to 5 PM, in dry sunny weather. The collected material was examined immediately fresh (raw), as well as after heat treatment (boiled) or during storage. During heat treatment, samples of plant material were covered with boiling water 1:10, placed in a boiling water bath for 20 min, removed and dried with filter paper after cooling to room temperature. The collected plant parts were stored in a domestic refrigerator FR-415W (Ocean,

Table 1. Plant materials
Таблица 1. Растительный материал

Item No.	Plant			Supplier of planting material
	Name, genus, family	Cultivar	Part and coloration	
1	Potato (calamus sticks) – *Solanum tuberosum* L., *Solanum* L., *Solanaceae* Juss.	Purple	Dark purple flesh, skin	A.G. Lorch Federal Potato Research Centre
2		Lobella	Red skin	Ovoshchevod Co., Ltd., Artyom city
3	Eggplant – *Solanum melongena* L., Solanum L., *Solanaceae* Juss.	Diamond	Purple-black skin	Scientific Production Association Gardens of Russia, Chelyabinsk city
4	Cabbage – *Brassica oleracea* L. , *Brassica* L., *Brassicaceae* Burnett	Red Jewel	Purple-red leaf	
5		Mikhnevskaya		
6	Garden carrot – *Daucus carota subsp. sativus* Hoffm., *Daucus* L., *Apiaceae* Lindl.	Purple Haze	Purple root	
7	Common raspberry – *Rubus idaeus* L., *Rubus* L, *Rosaceae* Juss.	Monomakh Cap	Red berry	
8	Onions – *Allium cepa* L., *Allium* L., *Amaryllidaceae* J.St.-Hlt	Carmen	Purple bulb	
9	Blue honeysuckle – *Lonicera caerulea* L., *Lonicera* L., *Caprifoliaceae* Juss.	Dlinnoplodnaya	Purple-blue berry	Omskiy Sadovod Co., Ltd., Omsk city

Russia) at (4 ± 2) °C for 1.5–3 months. Every 15 days, the anthocyanin content was recorded, which was expressed as a percentage of the initial value taken as 100%.

To prepare all extracts, we ground a sample of plant material to 1.5 to 2 mm, placed a sample of about 0.5 g (precise sample weight) in a dark glass vial, added 5 ml of 95% ethanol (Constanta Farm Co., Ltd., Russia) acidified with hydrochloric acid (NevaReactiv, Russia) in the 100:1 ratio (pH = 1–1.2), kept it for 5–6 hours and filtered through a 0.45 μm PTFE-H membrane filter (Hyundai micro, South Korea).

The extracts' absorption spectra (AS) were recorded on a UV-2501PC digital spectrophotometer (Shimadzu, Japan) in the wavelength range of 200–650 nm in 1 nm increments and were adjusted to 1% extract. The given AS were processed according to the previously described method [14], and the coordinates of the maximums and inflection points of the spectral line contours were determined. The integral absorption intensity, numerically equal to the area under the spectral line, was calculated by the Simpson's formula [15], the wavelengths of the spectral line contour inflection points in the vicinity of the "anthocyanin" maximum were taken as the integration limits. For example, for the potato skin extract, the integral absorption intensity corresponds to the area S (Figure 1, oblique shading) within the λ_b, λ_c inflection points *b* and *c* wavelengths.

The stability factor (*SF*) was calculated using the original formula:

$$SF = [AM_2 x AM_1]/[S_2 x S_1],$$

where AM_1, AM_2 are absorptions of "anthocyanin" maxima, S_1, S_2 are areas under the spectral line, index 1 refers to raw samples, and index 2 to cooked samples.

The total amount of anthocyanins (*TA*) in 100 g of the sample was determined spectrophotometrically, calculated by the formula:

$$TA= 100x[AMxKxV_1x(V_2+V_3)]/[V_2xM],$$

where *AM* is the absorption of the "anthocyanin" maximum in the spectrum reduced to 1% extract, rel. un.; *K* is the conversion factor of 0.004, mg/ml·unit; V_1 is the volume of extractant used to prepare the extract, ml; V_2 is the extract volume taken into the cuvette, ml; V_3 is the extractant volume added to the cuvette, ml; *M* is the sample weight, g.

Five independent randomized samples were taken in the study of each part of the plant. The experimental material was statistically processed using the small sample and correlation analysis methods [16], the differences were considered statistically significant or reliable at the null hypothesis significance level $p < 0.05$.

RESULTS AND DISCUSSION

Anthocyanin content in plant material.

The results are presented in Table 2 and Figure 2.

The highest values of anthocyanin content were obtained in fresh blue honeysuckle berries compared with other plants studied. According to B. Kusznierewicz et al [17], blue honeysuckle belongs to the food products that are the richest in anthocyanins, the content of which can reach 284–780 mg100g-1 of raw weight. For the other studied plants, it was found that the content of anthocyanins in raw red raspberry berries, eggplant shells and red cabbage leaves was 5.4–8.9 times lower, in raw purple potato skins, blue onion bulbs and purple carrots 10.5–15.1 times lower, and in potato purple pulp and red skin 37.6–48.1 times lower than in honeysuckle berries. According to the published data, the ranges of anthocyanin content are 35.1–175.8 in raspberries [18], 41.3–155.3 in eggplant skin [19], 28.3–60 in red cabbage leaves [20, 21], 7.89–49.53 in purple potato tubers [22], 8.32–30 in blue onion bulbs [23, 24], 20–50 in purple carrots [25, 26], and 4.81–30.71 mg100^{-1} g in red potatoes

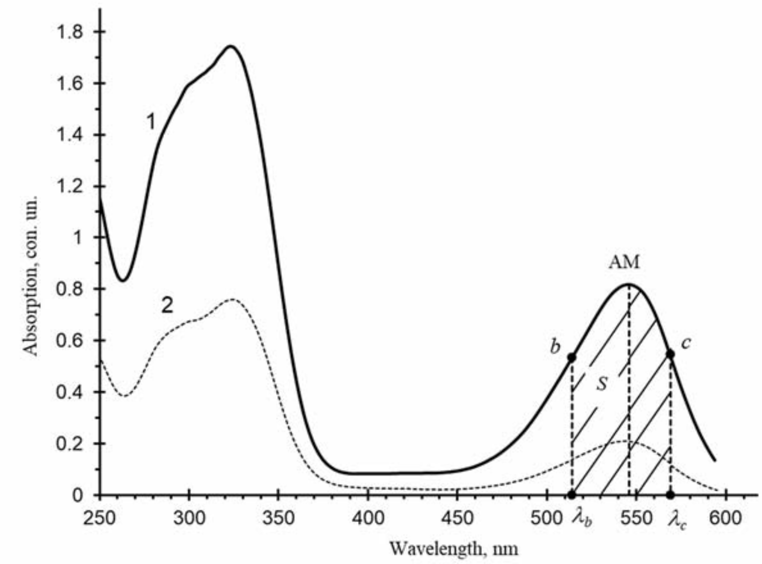

Fig.1. Absorption spectra reduced to 1% extracts from raw (1) and cooked (2) purple potato skins. AM – "anthocyanin" maximum, b, c – inflection points, λ_b, λ_c – inflection point wavelengths or integration limits, S – area corresponding to the integral absorption intensity

Рис. 1. Спектры поглощения, приведенные к 1% экстрактам из сырой (1) и вареной (2) кожуры фиолетового картофеля. AM – «антоциановый» максимум, b, c – точки перегиба, λ_b, λ_c – длины волн точек перегиба или пределы интегрирования, S – площадь, соответствующая интегральной интенсивности поглощения

Table 2. Total content of anthocyanins and its relative change in raw and cooked plant parts
Таблица 2. Общее содержание антоцианов и относительное его изменение в сырых и вареных частях растений

Item No.	Plant, variety	Plant part	Anthocyanins content*, mg 100 g^{-1}		Change in content, %
			Raw	Cooked	
1	Potatoes, “Purple”	Tuber pulp	8.65±0.63	4.22±0.08	51.21
		Tuber peel	31.02±2.53	7.87±0.39	74.63
2	Potatoes, “Lobella”	Tuber peel	6.77±0.44	5.05±0.28	25.41
3	Eggplant, “Almaz”	Vegetable shell	55.38±4.12	9.06±0.74	83.64
4	Cabbage, “Red Jewel”	Leave	36.35±1.48	13.09±0.65	63.98
5	Cabbage, “Mikhnevskaya”		38.94±2.34	11.97±0.87	69.26
6	Carrots, “Purple Haze”	Root	21.54±1.25	20.01±1.24	7.09
7	Onions, “Carmen”	Bulb	27.96±1.26	5.05±0.23	81.94
8	Raspberry, “Shapka Monomakha”	Berry	59.58±4.21	13.71±0.96	76.98
9	Blue honeysuckle, “Dlinnoplodnaya”		325.1±2.68	209.7±1.45	35.49

** — mean value of 5 samples ± error of mean*

[22]. The analysis of the obtained results and comparison with literature data show that the values of total anthocyanin content obtained in the studied plant parts fall within the ranges presented in the relevant literature, which indicates the adequacy and validity of the method used in this study to determine the anthocyanin content.

After heat treatment, the total anthocyanin content decreased least in purple carrot roots – by 1.08 times, in red Lobella potato peel – by 1.34 times, and in honeysuckle berries – by 1.55 times, and most in purple potato peel – by 3.94 times, in raspberry berries – by 4.34 times, and in eggplant skin by – 6.11 times compared to

Table 3. Own data of anthocyanins’ stability indicator (SF) and literature data of anthocyanins with the highest content, their antioxidant activity (AOA) in vegetable and berry plants
Таблица 3. Собственные данные показателя устойчивости антоцианов (SF) и литературные данные антоцианов с наибольшим содержанием, их антиоксидантная активность (AOA) в овощных и ягодных растениях

Item No.	Plant, part	Own data	Literature data	Literature data		
		Variety	SF*	Variety	Highest content of anthocyanins	AOA** [25]
1	Purple carrot, root	Purple Haze	0.986±0.064	*Daucus carota ssp. sativus var. atrorubens* Alef. [27]	Cyanidine	3.49
2	Blue onions, bulb	Carmen	0.725±0.056	W1201 [24]	Cyanidine	3.49
3	Red cabbage, leaf	Red Jewel	0.623±0.051	Cairo [28]	Cyanidine	3.49
		Mikhnevskaya	0.689±0.048			
4	Blue honeysuckle, berry	Dlinnoplodnaya	0.417±0.028	Wojtek, Brazowa, Zielona, Jolanta, 44, 46 [17]	Cyanidine	3.49
					Peonidin	1.69
				Zarnitsa, stenantha, pallassi, Selec. 2-32, Selec. F1-9-58, Bluebird, Berry Blue, Magadan, Selec. 8-18 [29]	Cyanidine	3.49
					Peonidin	1.69
5	Red raspberry, berry	Shapka Monomakha	0.230±0.016	*R. jamai censis, R. rosifolius, R. racemosus, R. acuminatus, R. idaeus cv. Haritage* [30]	Cyanidine	3.49
					Pelargonidin	1.07
6	Purple potatoes, whole tuber	Purple	0.229±0.023	Salad Blue, Vitelotte, Valfi, Blue Congo [22]	Malvidin	1.4

**SF — anthocyanins’ stability index, the average of 5 samples ± error of the mean,*
*** — relative antioxidant activity compared with trolox*

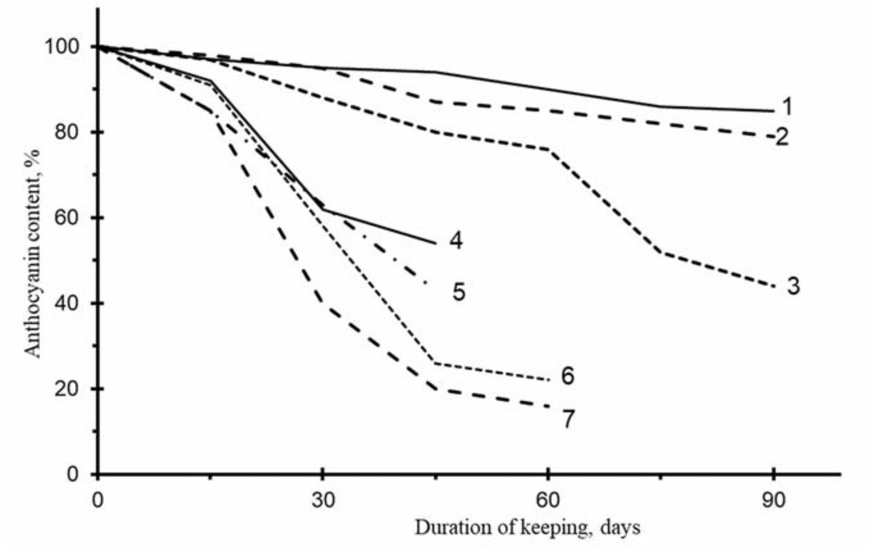

Figure 2. Changes in the anthocyanin content in parts of vegetable and berry plants during storage. 1 – purple carrot roots, 2 – purple potato tubers, 3 – blue onion bulbs 4 – blue honeysuckle berries, 5 – red raspberries, 6 – red cabbage leaves, 7 – eggplant shells
Рис. 2. Изменение содержания антоцианов в частях овощных и ягодных растений при хранении (1 – корнеплоды фиолетовой моркови, 2 – клубни фиолетового картофеля, 3 – луковицы синего лука 4 – ягоды голубой жимолости, 5 – ягоды красной малины, 6 – листья краснокочанной капусты, 7 – оболочки плодов баклажана

fresh samples, respectively. The anthocyanin content reduction values in potato pulp and whole tubers by 2.05 to 2.07 times and in red cabbage leaves by 2.77 to 3.18 times have intermediate values relative to fresh ones.

It should be noted that the decrease in the anthocyanin content after heat treatment in purple potato pulp is 1.92 times less than in the peel. The obtained result is probably due to the presence of starch in the potato pulp, which may contribute to stabilization of anthocyanins [11] due to the fermentation inhibition.

The correlation coefficient between the initial content of anthocyanins in the studied raw plant material and the decrease in their content after cooking has a low, unreliable value: $r=-0.2\pm0.35$ ($p=0.58 > 0.05$), which shows a rather weak relationship between the compared values.

Anthocyanins' stability.

The highest value of the stability index, almost one (Table 3), was obtained for anthocyanins from purple carrot roots, while for anthocyanins from blue onion bulbs and red cabbage leaves *SF* was 1.6 to 1.67 times lower. The lowest *SF* values are seen in the eggplant skin anthocyanins – 37.92 times less than the SF values of purple carrot anthocyanins. The stability values of anthocyanins from blue honeysuckle berries, red raspberries, and whole purple potatoes tubers relative to the SF of purple carrots' root crops anthocyanins are 2.36, 4.29, and 4.3 times lower, respectively.

Usually there are several anthocyanidins of different types in any colored part of the plant, but among them, as a rule, only one or two have a predominant concentration, and they mainly give the corresponding color to this part of the plant [12]. Individual anthocyanins, depending on their individual structure, have different AOAs [26] (Table 3). The literature indicates that cyanidin-3-glycoside is the predominant anthocyanin in purple carrot roots [27], blue onion bulbs [24], and red cabbage leaves [28], which has the highest AOA. In addition to cyanidin, blue honeysuckle berries [29] and red raspberry berries [30] contain less active peonidin and pelargonidin with an AOA 2.06–3.26 times lower than the cyanidin AOA. Malvidin prevails in purple potato tubers [22] and has a 2.49-fold lower AOA than cyanidine.

Thus, a comparison of our own data and literature shows that the stability of anthocyanins during heat treatment is higher the greater their AOA.

The rank correlation coefficient between *SF* and AOA has a statistically significant value of 0.91 ($p < 0.05$), indicating that there is a significant relationship between the stability of anthocyanins and AOA.

Content of anthocyanins during storage.

During the vegetable raw materials' storage for 3 months, the content of anthocyanins decreased in purple carrot roots by 1.18 times, in purple potato tubers by 1.26 times and in blue onion bulbs by 2.27 times relative to the initial content (Figure 2). The decrease in anthocyanin content is more significant in the remaining studied plants. Over 2 months of storage, the anthocyanin content decreased by 4.54 and 6.25 times relative to the initial one in red cabbage leaves and eggplant skins, and over 1.5 months' storage by 1.85 and 3.32 times relative to the initial content in honeysuckle and raspberry berries.

The data presented in Figure 2 confirm the above conclusion about the relationship between the stability of anthocyanins and AOA. It should be kept in mind that raw purple potato tubers contain 16.29–23.41 $g100^{-1}$ of starch [22] that may play a protective role [11]. It should be noted that during heat treatment, as a result of starch gelatinization [32], the anthocyanin's protective effect probably decreases, therefore, low *SF* values were obtained in cooked potatoes (Table 3). During storage of potatoes in conditions of reduced positive temperature, the starch contained in the tubers' tissues in the form of granules has a protective effect and maintains the anthocyanins' stability.

CONCLUSION

The spectrophotometric methods used to determine the anthocyanin content and stability are reasonable to use as express methods for the selection of promising plants for industrial cultivation as raw materials for anthocyanin-containing herbal formulation.

Anthocyanins' stability during the plant raw materials heat treatment is closely related to the AOA, with the increase of which the stability also increases.

Purple carrots and purple potatoes are anthocyanin-containing plants suitable for long-term storage. Blue honeysuckle and red raspberry berries, as well as red cabbage and eggplants are not suitable for storage longer than 30 days.

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