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## Спектрофотометрический метод оценки цвета экстрактов из *Sorbus Aucuparia*

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**Аннотация.** В статье представлено описание метода спектрофотометрии. Объектами исследований использовались экстракта из вегетативных частей и плодов рябины красной (*Sorbus aucuparia*) в процессе научного исследования были выбраны диапазоны растворителя с кислой средой, нейтральной и щелочной. Исследование оптических свойств экстрактов проверяли посредством использования спектрометрии, с помощью прибора КФК-2. В процессе работы изучали опытные образцы экстрактов по следующим показателям: интенсивность цвета (I, е.д.), оттенок цвета (T, е.д.), хроматографическая структура (%) и содержание суммы антоцианов (в пересчете на цианидин-3,5-дигликозид, %). В результате экспериментальных исследований была проведена математическая обработка данных с расчетом средних значений и стандартного отклонения. Анализ количественных данных исследования цвета и прозрачности образцов показал зависимость между показателем интенсивность цвета (I, е.д.) и содержанием антоцианов (в пересчете на цианидин-3,5-дигликозид, %), что с увеличением оптической плотности при длине волны  $\lambda=440, 540$  и  $670$  нм возрастает содержание антоциановых комплексов в исследуемых экстрактах *Sorbus aucuparia*. Согласно анализу и обработке данных рекомендовано использование в качестве растворителя воду с щелочной средой для выделения антоциановых соединений при исследовании синего компонента ( $\lambda=670$  нм) определил содержание свободных антоцианов в хинонной форме.

**Ключевые слова:** плоды рябины красной, *Sorbus aucuparia*, интенсивность цвета, антоцианы

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Research article

## The spectrophotometric method for evaluating the color of extracts from *Sorbus Aucuparia*

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**Abstract.** *The article describes the method of spectrophotometry. Materials and methods. The objects of research were extracts from vegetative parts and fruits of red mountain ash (Sorbus aucuparia). In the process of scientific research, solvent ranges with acidic, neutral and alkaline media were selected. The study of the optical properties of the extracts was verified by using spectrometry, using the KFK-2 device. In the course of work, experimental samples of extracts were studied according to the following indicators: color intensity (I, e.d.), color shade (T, e.d.), chromatographic structure (%) and the content of the sum of anthocyanins (in terms of cyanidin-3,5-diglycoside, %). As a result of experimental studies, mathematical data processing was carried out with the calculation of average values and standard deviation. Analysis of quantitative data on the color and transparency of samples showed a relationship between the color intensity index (I, e.d.) and the content of anthocyanins (in terms of cyanidin-3,5-diglycoside, %), that with an increase in optical density at a wavelength of  $\lambda=440, 540$  and  $670$  nm, the content of anthocyanin complexes in the Sorbus extracts under study increases aucuparia. According to the analysis and data processing, it is recommended to use water with an alkaline medium as a solvent for the isolation of anthocyanin compounds in the study of the blue component ( $\lambda=670$  nm) determined the content of free anthocyanins in quinone form.*

**Keywords:** fruits of red mountain ash, Sorbus aucuparia, color intensity, anthocyanins

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**Introduction.** When creating food systems, special attention is paid to the formation of organoleptic abilities, which are most often the promotion of goods on the market. Appearance and color are the first things that the consumer market makes a choice before [1].

In the authors' work, color is described as a qualitative characteristic of light, for visualization of visual sensation [2, 3]. Recently, instrumental methods of assessment have been applied to the study of the indicators "color" and "transparency", where color is decomposed into a luminous flux with wavelengths in the range from 190 to 780 nm [4-8].

Foreign researchers believe that traditional organoleptic assessment of color characteristics is subjective and dependent on the taster's experience, as well as external lighting factors [9]. Therefore, a method of quantitative analysis of intensity in color addition is proposed [10].

In the article by N.S. Anikina and other co-authors, a calculation method is proposed for the analysis of three components at different wavelengths: red ( $\lambda = 540$  nm), yellow ( $\lambda = 440$  nm) and blue ( $\lambda = 670$  nm). The presence of each color is characterized by the decomposition products of phenolic compounds, such as yellow color, the content of tannins (tannins) and anthocyanin, red - free anthocyanins (in the form of flavylum cations and anthocyanin-tannin complex) and the blue component - free anthocyanins in quinone form or combinations of tannins and anthocyanins [11].

The aim of the work is to study the color characteristics of extracts from different parts of Sorbus aucuparia and their dependence on the content of anthocyanins.

**Materials and research methods.** The objects of the research were extracts obtained from dry vegetative parts (leaves and branches) and fruits of red rowan (Sorbus aucuparia). Purified water was used as a medium for dissolving (solvent) the studied plant raw materials and reducing the error of the research, the acidity of the aqueous medium was regulated using a 1% solution of citric acid (pH = 1.98 units) and 1% sodium bicarbonate (pH = 9.1 units). Purified water was obtained in accordance with the requirements for type II water according to GOST 52501-2005 "Water for laboratory analysis" (by filtration through the UPVA-159 unit, Belgorod, Russia). The plant materials were collected in the Novosibirsk region in dry weather, the collection period was September 2022. The raw materials were dried: leaves and branches by natural drying ( $T = 20 \pm 2$  ° C, humidity 75%), fruits by convective drying (drying oven with forced ventilation "Binder", Germany) ( $T = 55 \pm 3$  ° C, convection 100%), the raw materials were dried until the moisture content in the final product was  $6.0 \pm 1.0\%$  (using the Chizhov method). The samples were stored at a temperature of  $t = 20 \pm 3$  ° C, air humidity  $W = 67 \pm 2\%$ .

For the study, samples of plant materials were taken in dry crushed form (fraction diameter  $\approx 0.5$  cm) crushing was carried out in a laboratory mill LM 201 of the company "Plaun" (Russia, Moscow) and in the form of extracts. For the studies, extracts were prepared from crushed plant materials by mixing the sample with a solvent in the ratio of 1:20 (g: ml). Extraction was carried out for 180 and 240 min at a temperature of  $60 \pm 2$  °C in a water bath of the company "Loip" (Russia, St. Petersburg).

The coding of the samples was carried out in the following sequence and is presented in Table 1.

**Table 1 – The code of the extract samples**

Sample code	Type of raw material	Solvent environment	Duration, min
<b>p.0/180</b>	fruit	neutral	180
<b>p.0/240</b>		neutral	240
<b>p.k/180</b>		sour	180
<b>p.k/240</b>		sour	240
<b>p.s./180</b>		alkaline	240
<b>p.sh/240</b>		alkaline	180
<b>l.0/180</b>	leaves	neutral	180
<b>l.0/240</b>		neutral	240
<b>l.k/180</b>		sour	180
<b>l.k/240</b>		sour	240
<b>l.sh/180</b>		alkaline	180
<b>l.sh/240</b>		alkaline	240
<b>v.0/180</b>	branches	sour	180
<b>v.0/240</b>		sour	240
<b>v.k/180</b>		alkaline	180
<b>v.k/240</b>		alkaline	240
<b>v.sh/180</b>		sour	180
<b>v.sh/240</b>		sour	240

The extraction study was carried out using optical methods: measuring the optical density (D, units), transmittance (T, %) and absorption coefficient ( $\epsilon$ , units) using a KFK-2 photoelectric concentration colorimeter (ZOMZ, Russia). Blue ( $\lambda=440$  nm), green ( $\lambda=540$  nm) and red ( $\lambda=670$  nm) light filters were used in the work. Distilled water was used as a comparison solution.

The color intensity (I, units) taking into account all pigments was expressed according to formula (1) [1,11,12]:

$$I = D_{440} + D_{540} + D_{670} \quad (1)$$

Where D – optical density of the absorption spectrum, rel. units,

It was established that color intensity I less than 0.10 characterized the extracts as weakly colored, from 0.11 to 0.30 as moderately colored, from 0.31 to 0.50 as well colored, and more than 0.51 as intensely colored [11].

The proportion of red color in the color composition was determined as the dA (%) indicator using formula (2) [14]:

$$dA(\%) = \left(1 + \frac{D_{440} + D_{670}}{2 \cdot D_{540}}\right) * 100 \quad (2)$$

The color shade (T, units) was expressed according to formula (3) [1,11,12]:

$$T = \frac{D_{440}}{D_{540}} \quad (3)$$

Where D – optical density of the absorption spectrum, rel. units,

The chromatographic structure (%) was calculated using formula (4) [1]:

$$D_{440,540,670}(\%) = \frac{D_{440} + D_{540} + D_{670}}{I} * 100 \quad (4)$$

Mathematical data processing using regression analysis was performed using the MS Excel program, the experiments were carried out in triplicate. The adequacy of the regression equations was checked using Fisher's F-criterion. The significance of the regression coefficients was assessed using Student's t-criterion.

The following indicators were taken as controlled parameters of the extracts: active acidity (pH, units), upper limit of thermodynamic stability of the solution ( $\phi_{st}$ ), temperature (t, °C), absorption coefficient ( $\epsilon$ , units), transmittance coefficient (T%), color intensity (I, units), color shade (T, units), chromatographic structure.

Determination of anthocyanins.

The volume of extracts in the amount of 0.5-1.0 cm<sup>3</sup> was placed in a measuring test tube, 10 cm<sup>3</sup> of 1% hydrochloric acid solution was added and kept in a water bath at 40-45 °C for 20 minutes. After cooling, the contents were filtered through a paper filter and the optical density of the solution was measured on a spectrophotometer at wavelengths of  $\lambda = 510$  nm and 670 nm (in the spectra, detection of a green and red light filter). As a comparison solution, use a 1% hydrochloric acid solution.

The content of the sum of anthocyanins (in terms of cyanidin-3,5-diglycoside, %) is calculated using formula (5) in % of dry matter [13]:

$$X, \% = \frac{(D_{510} - 0,33 * D_{670}) * V}{453 * m * (100 - W)} \quad (5)$$

V - volume of extract, cm<sup>3</sup>; 453 - specific absorption index of cyanidin-3,5 - diglycoside;  $D_{490,670}$  optical density of absorption spectrum, rel. units; m - weight of plant material in extract, g.

**Research results and their discussion.** The studied samples differed in optical density indices, and therefore in the calculated characteristics – color intensity, color shade. Unlike the work of Nilova L.P. and other co-authors [14], and Rozhnov E.D. [12], the research was conducted not only in a neutral and acidic environment, but also in an alkaline one. The study of extracts from various initial plant materials was analyzed according to several indices, presented in Table 2.

Table 2 – Study of color characteristics

Samples	I, e.d. <sup>1</sup>	calculation dA% <sup>2</sup>	T, e.d. <sup>3</sup>	$D_{440}(\%)^4$	$D_{540}(\%)^5$	$D_{670}(\%)^6$	Anthocyanins <sup>7</sup> , %
fruit							
p.0/180	0.20±0.01	21.37±1.07	1.53±0.08	46.80 ±2.34	30.54±1.53	<b>22.66± 1.13</b>	0.008±0.001
p.0/240	0.20±0.01	21.05±1.05	1.50±0.08	46.73 ±2.34	31.16±1.56	<b>22.11± 1.11</b>	0.007±0.001
p.k/180	0.08±0.00	21.30±1.07	<b>1.74±0.09</b>	<b>53.33 ±2.67</b>	30.67±1.53	16.00± 0.80	traces
p.k/240	0.08±0.00	21.78±1.09	<b>1.78±0.09</b>	<b>52.98 ±2.65</b>	29.80±1.49	17.22± 0.86	traces
p.s./180	<b>0.35±0.02</b>	20.40±1.02	1.38±0.07	44.93 ±2.25	32.46±1.62	<b>22.61± 1.13</b>	<b>0.020±0.001</b>
p.sh/240	<b>0.36±0.02</b>	19.79±0.99	1.29±0.06	43.66 ±2.18	33.80±1.69	<b>22.54± 1.13</b>	<b>0.021±0.001</b>

leaves							
<b>l.o/180</b>	0.26±0.01	23.46±1.17	2.29±0.11	61.90±3.09	27.08±1.35	11.03± 0.55	0.016±0.00 1
<b>l.o/240</b>	0.28±0.01	21.03±1.05	1.84±0.09	57.35±2.87	31.18±1.56	11.47± 0.57	0.010±0.00 1
<b>l.k/180</b>	0.20±0.01	25.31±1.27	<b>2.65±0.13</b>	<b>65.33±3.27</b>	24.62±1.23	10.05± 0.50	0.006±0.00 1
<b>l.k/240</b>	0.20±0.01	26.11±1.31	<b>2.87±0.14</b>	<b>67.95±3.40</b>	23.68±1.18	8.37± 0.42	0.007±0.00 1
<b>l.sh/180</b>	<b>0.39±0.02</b>	17.73±0.89	1.04±0.05	40.82±2.04	39.29±1.96	<b>19.90± 0.99</b>	<b>0.039±0.00</b> 2
<b>l.sh/240</b>	<b>0.42±0.02</b>	18.23±0.91	1.01±0.05	38.28±1.91	37.80±1.89	<b>23.92± 1.20</b>	<b>0.047±0.00</b> 2
branches							
<b>v.o/180</b>	0.34±0.02	22.24±1.11	1.58±0.08	45.86 ±2.29	28.99±1.45	<b>25.15± 1.26</b>	0.009±0.00 1
<b>v.o/240</b>	0.37±0.02	20.42±1.02	1.29±0.06	41.89 ±2.09	32.43±1.62	<b>25.68± 1.28</b>	0.010±0.00 1
<b>v.k/180</b>	0.04±0.00	28.71±1.44	<b>3.47±0.17</b>	<b>73.20±3.66</b>	21.09±1.05	5.71± 0.29	traces
<b>v.k/240</b>	0.04±0.00	27.17±1.36	<b>3.13±0.16</b>	<b>70.65±3.53</b>	22.55±1.13	6.79± 0.34	traces
<b>v.sh/180</b>	<b>0.39±0.02</b>	17.99±0.90	1.07±0.05	41.34 ±2.07	38.50±1.93	<b>20.16± 1.01</b>	<b>0.027±0.00</b> 1
<b>v.sh/240</b>	<b>0.43±0.02</b>	18.54±0.93	1.02±0.05	37.65 ±1.88	36.94±1.85	<b>25.41± 1.27</b>	<b>0.036±0.00</b> 2
Note: <sup>1</sup> Color intensity, units according to formula (1); <sup>2</sup> share of red color in color addition % according to formula (2); <sup>3</sup> color shade, units according to formula (3); <sup>4,5,6</sup> Chromatographic structure (%); <sup>7</sup> anthocyanins in terms of cyanidin-3,5-diglycoside, % according to formula (5)							

*Sorbus aucuparia* fruit extracts in different solvents had different color characteristics. Thus, the "color intensity (I, units)" index when processed in an alkaline medium showed the highest quantitative result and the samples can be described as "well colored", while in an acidic medium the transition of coloring pigments is lower by  $\approx 4.5$  times compared to an alkaline medium and  $\approx 2.5$  times, the samples were described as "weakly colored". According to formula 5, the constituent element when assessing the content of anthocyanins plays a numerical value at  $\lambda = 670$  nm. It was noted that with a high content of red pigments ( $D_{670}$  (%)) in alkaline fruit extracts (samples p.sh.180 and p.sh/240), there was a high content of anthocyanins (in terms of cyanidin-3,5-diglycoside, (%)) - in comparison with the control sample, it is higher by  $\approx 2.5$  times.

Samples of extracts from leaves and branches of *Sorbus aucuparia* in different solvents also had higher color characteristics in a medium with sodium bicarbonate. When comparing all *Sorbus aucuparia* extracts with each other, the maximum release of anthocyanins was noted in the leaves (sample l.sh/240) was  $\approx 0.05\%$ , in the branches (sample v.sh/240)  $\approx 0.04\%$ , in the fruits (sample p.sh/240)  $0.02\%$ .

The calculated characteristic "color shade" (T, units), with a ratio of wavelengths  $\lambda=440$  and 540 nm (more often used to study flavonoids of various natures) indicated the maximum transition in the branches and amounted to  $\approx 3.3$  units for treatment for 180 and 240 minutes within the error limits, thereby indirectly indicating the preservation of phenolic compounds.

Analyzing the data in Table 1, high values were noted for the studied indicators, this fact indicates the preservation of phenolic compounds during drying. When assessing the chromatographic structure  $D_{440}$  (%) nm determined high values in an acidic environment, in comparison with neutral and alkaline in fruits 1.2 times, in branches  $\approx$  1.8 times.

**Conclusion.** Comparative analysis showed that there is a relationship between the color intensity index (I2, units) and the content of anthocyanins (in terms of cyanidin-3,5-diglycoside, %), so with an increase in optical density at a wavelength of  $\lambda = 440, 540$  and  $670$  nm, the content of anthocyanins in the studied *Sorbus aucuparia* extracts also increases.

The aim of the work was to study the color characteristics of extracts from different parts of *Sorbus aucuparia* and their dependence on the content of anthocyanins. During the analysis and generalization of the obtained data, the parameters for evaluating the extracts using different solvents (neutral, acidic and alkaline) revealed significant differences when using the solvent medium and the type of plant material (fruits, leaves, branches). According to the analysis and processing of the data, it is recommended to use water with an alkaline medium as a solvent for the extraction of anthocyanin compounds. A transition in an acidic medium of phenolic compounds in red ( $\lambda = 540$  nm) and yellow ( $\lambda = 440$  nm) color was noted, according to the literature [11], it can be assumed about the transition of tannins and other polyphenolic compounds of the anthocyanin-tannin complex. The study of the blue component ( $\lambda = 670$  nm) and the calculation of anthocyanins (in terms of cyanidin-3,5-diglycoside, %) determined the content of free anthocyanins in the quinone form.

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