

# Physico-chemical Indicators of food color pigments derived from *Ocimum basilicum* (Rahon) vegetation

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**Abstract.** At this work the physico-chemical Indicators of food color pigments derived from *Ocimum basilicum* vegetation is presented. This is known that in addition to color pigments, natural food coloring substances contain other biologically active components: vitamins, trace elements, organic acids. The raw material of the plant was extracted for 18 hours and the obtained solution was filtered, solid particles were separated in a centrifuge. The dry matter content of the purified natural dye-preserving solution, pH medium, specific density was determined. The solution was concentrated in a vacuum rotor evaporator for condensation, and its physical and chemical parameters were determined. The amount of fructose in the dye extract obtained in a 20% carbohydrate solution was 5.94 mg/l, the maximum value was 9.12% in the 40% extract, and the fructose content in the dye obtained in a 50% solution was 7.14%. - organized. It was found that the amount of vitamins in the concentrate is higher in 40% and 50% alcohol solution. The safety of the natural dye was analyzed. Heavy metals and their salts, dangerous substances for human health were analyzed.

## 1 Introduction

The main method of obtaining dyes from natural objects is extraction using solvents. The extract is purified from bound compounds, pigments are stabilized. Ethyl alcohol, water, vegetable oil, etc. are used as solvents - extractants. According to their chemical nature, coloring substances synthesized by plants are divided into 3 groups: flavonoids, carotenoids, chlorophylls [1].

According to their origin, substances intended for coloring food products are divided into the following 3 groups such as natural which is derived from plant or animal raw materials; Synthetic and inorganic mineral paints [2-3].

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Special attention is always paid to natural food colors. Due to the restriction of the use of synthetic dyes, the need to develop methods for obtaining natural food dyes is increasing dramatically [4].

This is due to the strict regulation of the use of synthetic dyes, and the need for manufacturers to ensure the naturalness of food products. In most cases, the source of natural dyes is secondary raw materials in the processing of plant products, including vegetables and fruits [5].

In most cases, natural food colors are fruit and berry juices and extracts used to color food products. Natural food dyes are produced from vegetable raw materials and secondary raw materials from fruit and vegetable processing, which in itself is a resource-saving technology [6-7].

The production of food coloring depends on the type of raw material, properties and solubility of the main extractable coloring pigment. The source of food coloring is non-traditional plant raw materials: berries, flowers, leaves, secondary raw materials of canning plants, etc [8-10].

Special attention is always paid to natural food colors. Due to the restriction of the use of synthetic dyes, the need to develop methods for obtaining natural food dyes is increasing dramatically [11-13].

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## 2 Methods

As an experiment, a local basil (*Occimum basilicum L.*) plant was selected and carried out in the following sequence: extraction of the plant into an alcohol solution; centrifugation; filtering; concentration (condensation); precipitation of additives (1:2 ratio); filtering; get a natural dye.

The raw material of the plant was extracted for 18 hours and the obtained solution was filtered, solid particles were separated in a centrifuge. The dry matter content of the purified natural dye-preserving solution, pH medium, specific density was determined. The solution was concentrated in a vacuum rotor evaporator for condensation, and its physical and chemical parameters were determined. KFK-2 UXL FEK was used to determine the amount of dyes in the condensed solution. All the obtained results are included in Table 1.

Determination of dry matter content in paint extracts. The amount of dry matter in paints is determined by the URL-2 refractometer according to the standard method. When using alcohol solutions of paint, it is removed from ethanol by evaporation under vacuum. For this, 10 cm<sup>3</sup> of paint solution is evaporated to 1 cm<sup>3</sup>, and then this amount of paint is dissolved in a 1000 cm<sup>3</sup> flask [19-22].

The amount of solids in alcohol extracts of carotenoid dyes is determined by gravimetric method by drying the dye solution.

Determination of the amount of dry matter is carried out according to the method specified in GOST ISO 2173-2013.

*Refractometric method* - The method is based on determining the mass fraction of water-soluble solids at a temperature of 20°C on a refractometer scale. When testing liquid products, 2-3 drops of the sample are applied to the lower prism with a glass rod. If the product is a mass containing solid particles, then a small amount of the sample is taken on a piece of gauze folded in half, a few drops are squeezed out with slow pressure, discarded,

and the next ones are installed on the prism of the refractometer. The upper part of the prism is lowered, firmly attached to the lower fixed part, and the calculation is taken.

When calculating the indicators of the device, the temperature at which the tests are carried out is taken into account, since the indicators of the device scale are correct only at 20°C. If the tests are conducted at a different temperature, the appropriate correction is made using the International Temperature Correction Table. The following method is used for the study of dark-colored products, the liquid phase of which is difficult to separate for prism application: 5-10 g of the sample is weighed with an accuracy of 0.01 g, about 4 g of quartz sand and distilled water are added, the mass is equal to the mass of the obtained sample. The mixture is rubbed quickly and thoroughly, a part of it is applied to a double-folded gauze, a few drops are squeezed out, thrown away, and the next ones are applied to the prism of the refractometer and the calculation is carried out.

$$x = 2a \quad (1)$$

Where 2 is the degree of dilution;  $a$  is the refractometer reading, taking into account the temperature correction.

Arithmetic average of two parallel determinations is taken as the final result, their difference should not exceed 0.2%.

Modern refractometer RE40 (Mettler Toledo) (OKP 42 1522) a small amount of the test solution is placed to determine the dry matter by the method. Conducting the analysis. In a refractometer with a lens, the lid of the device is closed. After that, wait for 20-30 s to automatically adjust the temperature of the measurement environment (measuring chamber of the device) to 20°C (thermal condensation), then press the "measurement" button and take refractometer readings.

*Processing the results* - On the refractive index scale, dry matter is set as a percentage.

*Photometric analysis of dye composition in anthocyanin dye solution.* Determination of the amount of red dyes in anthocyanin dyes is carried out using a standard solution of cobalt sulfate  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$  (in cobalt numbers) (method 1) according to the method described in the literature and in accordance with standard samples of anthocyanin dyes.

*Method 1.* This approach involves the addition of an anthocyanin dye solution, which is equivalent to a solution containing 22 mg of dye (enine), to 1 dm<sup>3</sup> of an aqueous solution containing 20 g of crystalline cobalt sulfate. The purpose is to color the cobalt sulfate solution. A measured amount of 1 g of the dye being examined is dissolved in distilled water and completely transferred to a 1000 cm<sup>3</sup> flask. The flask is then filled with distilled water until it reaches the desired level. The solution under analysis is transferred into an optical cuvette with a width of 10 mm. The optical absorption is then measured using a KFK-2 photocolormeter, specifically at the wavelength of light where maximum absorption occurs ( $\lambda_{\text{max}} = 490 \text{ nm}$ ).

The content of dyes in the dye solution is calculated according to the following formula:

$$S = (0.022 \cdot A_2 \cdot 1000) / (m \cdot A_1) \quad (2)$$

Where: S- concentration of dye in g/dm<sup>3</sup> max; Optical absorption max of A1-cobalt sulfate standard solution; A<sub>2</sub> - optical absorption max of the analyzed dye solution; t is the weight of the paint sample in g; 0.022 is the concentration of epin dye solution, equal to 0.022 g in 1 dm<sup>3</sup> of the standard solution.

*Method 2.* The concentration of anthocyanins is evaluated by measuring the optical absorbance of the investigated solutions at a wavelength of 490 nm using a 10 mm thick optical cuvette. The sample is prepared by diluting 1 cm<sup>3</sup> of the examined solution with a buffer solution of pH = 1.0 to a final volume of 10 cm. A buffer solution with a pH of 1.0 is

created by combining a 0.2 N potassium chloride solution and a hydrochloric acid solution in a ratio of 25:67.

The amount of anthocyanins in the dye solution is calculated according to the following

$$C_A = [A_{490}^{(pH=1)}]/49 \quad (3)$$

Where SA is the concentration of anthocyanins in the dye solution, mg/100 cm<sup>3</sup>:  $[A_{490}^{(pH=1)}]/49 = a$  sample of the analyzed dye solution at pH=1.0 for a cuvette with a solution layer thickness of 10 mm Absorption of light at  $\lambda_{max} = 490$  nm: calibration drawn in CA - A (OEP) coordinates The coefficient calculated from the slope of the graph is 49.

The calibration graph for determining the content of anthocyanin dyes in a solution at pH=1.0 for  $\lambda_{max} = 490$  nm is calculated for the optical absorption of pure aronia anthocyanin solutions at pH=1.0 and  $\lambda_{max} = 490$  nm as a function of their concentration and has the form:

$$A_{490}^{(pH=1)} = 49.05 \cdot C_A - 8.26 \cdot 10^4 \quad (4)$$

(The coefficients of 49.05 and  $8.26 \cdot 10^4$  were calculated by the method of least squares with a correlation coefficient of K - 0.99).

*The method of determining the amount of sugar.* The method is based on determining the mass fraction of water-soluble sugars at a temperature of 20°C on the scale of a sugar meter. The test solution is poured into a 500 ml cylinder with a diameter of 5 cm, a clean and dry saccharometer is carefully immersed in the liquid deeper than the required division, then it is left for 5-7 min and the saccharometer readings are taken. The indicator fluid stops looking at the level of the meniscus along the lower edge of the meniscus. Accuracy is carried out at a temperature of 20°C.

*Method for determining the amount of fructose.* Determination of fructose is based on the Selivanov reaction: when fructose or other ketose is heated with hydrochloric acid, oxymethylfurfural is formed. Oxime-tilfurfural forms a cherry-red compound with resorcinol. In the reaction of heating fructose with hydrochloric acid, the rate of formation of oxymethylfurfural is much higher than that of aldehydes, which determines the specificity of the Selivanov reaction for fructose.

The extinction value of a solution containing the oxymethylfurfural condensate product formed from fructose with resorcinol is determined photometrically. Standard fructose solution (control) is prepared for quantitative determination of fructose content.

Reagents: test fructose solution (10-100 mg/ml), standard fructose solution (25 mg/ml), 0.1% resorcinol solution in 96% ethyl alcohol, 30% hydrochloric acid solution (1.5 ml), Selivanov reagent (0.5 ml).

Equipment: glass rods, test tubes with polished air flow condenser, pipettes, test tube rack, water bath, clock, laboratory thermometer, spectrophotometer.

Work process. 1 ml of fructose test tube solution (sample) per test tube to the second, 1 ml of distilled water is added (control). Then 1 ml of resorcinol solution and 3 ml of hydrochloric acid solution are added to both test tubes. The contents of the tubes are mixed and heated in a water bath at a temperature of 80°C for 20 min. After heating, the solutions are cooled and colorimetric at 490 nm. After that, the result is viewed on the calibration graph. The mass concentration of fructose in the test sample (mg/ml) is calculated according to the following formula.

The concentration of fructose in the studied mass ( $\mu\text{g/ml}$ ) is found by the following formula:

$$S = QE1/E2 \quad (5)$$

Where E1 and E2 are the extinction of the test and standard solutions, respectively; The Q-factor is the ratio of the mass concentration of a standard sample to the volume of the sample.

The technique of inductively coupled plasma mass spectrometry (ICP-MS) is used to quantitatively determine the presence of micro- and macroelements in dye extracts. An accurately measured sample ranging from 0.0500 to 0.5000 of the substance being studied is weighed using an analytical balance. The weighed sample is then transferred to Teflon autoclaves. The autoclaves are subsequently filled with the necessary quantity of purified concentrated mineral acids, specifically nitric acid (chemically pure) and hydrogen peroxide (chemically pure). The autoclaves are sealed and positioned within a Berghof microwave melter MWS-3 + software or a comparable microwave melter. The decomposition program for a given test substance determines the degree of decomposition and the number of autoclaves required, with a maximum of 12 units.

After decomposition, the contents of the autoclaves are quantitatively transferred to 50 or 100 ml flasks and nitric acid is poured in until the volume reaches 0.5%.

To study the substance under investigation, ICP is performed on an MS or similar device with the optics of an inductively coupled argon plasma emission spectrometer. In this method, the optimal wavelength for the maximum emission of micro- and macroelements determined by this method is indicated.

When compiling a series of analyses, the quantity is expressed in milligrams (mg) and the level of separation is measured in milliliters (ml). Upon receiving the data, the device automatically calculates and records the precise quantitative amount of the substance in the test sample. The results are expressed in units of mg/kg or  $\mu\text{g/g}$ , with the error limits indicated by the relative standard deviation (RSD) in percentage.

The instruments and vessels utilized include the ISP MS NEXION-2000 or a comparable mass spectrometer, a microwave disintegration device from Germany or equivalent autoclaves, and Teflon volumetric flasks.

The reagents utilized in the experiment include multi-element standard #3, which contains 29 elements for mass spectrometry analysis. Additionally, the reagents consist of mercury (Hg), nitric acid, hydrogen peroxide, bidistilled water, and argon gas with a purity level of 99.995%.

### 3 Results and discussion

The extraction process of anthocyanins was studied in the following experimental procedure. The leaves of basil plant are dried, crushed, taken in a certain amount, in solutions of different concentrations of alcohol (1:10) at room temperature, the first extraction is for 5 hours, the second extraction is for 18 hours, and anthocyanins are isolated. The resulting solutions are combined, filtered, solid particles are separated in a centrifuge. The amount of dry matter, medium, and specific density of solutions containing purified natural dyes are determined.

The resulting solutions were concentrated in a 1/7 ratio in a rotary evaporator. The amount of dry matter in each concentrate obtained. In the determination method specified in GOST ISO 2173-2013, acidity, density were determined, the obtained results are presented in Table 1.

**Table 1.** Indicators of dye concentrate from basil leaves.

Alcohol solution, (%)	Comparison weight, (kg/m <sup>3</sup> )	Dry matter quantity, (%)	Solution pH of the environment
	Concentration	concentration	concentration
10%	1,056	11.4	3.5
20%	1,063	18.5	3.3
30%	1,071	21.0	3.2
40%	1,075	21.4	4.5
50%	1,078	21.6	4.5
60%	1,085	15.8	4.5
70%	1,091	16.0	4.4

As can be seen from the table, the specific gravity of the concentrate increased from 1.056 to 1.091. The increase in the density of the concentrate is due to the decrease in its percentage due to the evaporation of alcohol, and the increase in the percentage of water. The concentration of the product obtained in the rotor vacuum-evaporation apparatus increased from 11.4 to 21.6% of dry matter content, the increase in concentration has an exponential characteristic, but its maximum value should be higher, because alcohol as a solvent has the property of rapid evaporation. In the graph, the concentration increased by 7.1% from 10 to 20% solution and by 2.5% from 20 to 30% solution. A sharp decrease in the increase in concentration was caused by the evaporation of essential oils, which are considered dry substances, and the accumulation of comfora oil on the surface of the solution. Concentrate contains mainly anthocyanins.

The acidity of the solution is the concentration of the alcohol-water mixture in the extract. It changes to the alkaline side only when it exceeds 60%, which is due to the neutralization of some organic acids, while in the concentrate it first decreased and then increased.

In addition, the amount of sugar and vitamins in the paint concentrates obtained from basil leaves was determined. The obtained results are presented in Table 2.

Fructose, glucose and sucrose are found in the basil leaf extract, and the fructose content is 1.3-2.1 times higher in the product obtained using different extractants. Therefore, this product has the properties of diet for people with diseases such as diabetes, atherosclerosis, stroke, heart attack.

**Table 2.** Amount of carbohydrates and vitamins in basil leaf concentrate.

Con-concentrate	Carbohydrates, mg/l			Vitamins mg/l				
	Fructose	Glucose	Sucrose	B1	B2	B6	B12	Bs
20%	5.94	-	-	27.52	341.67	-	-	-
30%	8.46	6.94	2.73	22.20	376.36	-	-	-
40%	9.12	4.76	4.59	274.40	375.40	-	524.17	651.40
50%	7.14	5.6	6.82	265.70	265.70	-	526.32	617.20

The acidity, amount of dry matter, specific density, amount of coloring matter in the extract, amount of carbohydrates and vitamins in the concentrate were determined using general methods. The amount of fructose in the dye extract obtained in a 20% solution was 5.94 mg/l, the maximum value was 9.12% in the 40% extract, and the amount of fructose in the dye obtained in a 50% solution was 7.14%. The change in the amount of glucose has the same dynamics. Sucrose content increased with increasing percentage of alcohol in the solution. This means that the amount of monosaccharides in the lower concentration of the solution is relatively high.

A polymer chain consisting of sugars precipitates in alcohols with a high concentration, that is, precipitation depends on the molecular mass of polysaccharides. The amount of vitamins in the concentrate is higher in 40% and 50% alcohol solutions, which proves that

vitamins dissolve better in water compared to organic solvents. The irrigation of plant also plays a major role in the final output of the plant [23].

## 4 Conclusion

In the study, the physico-chemical parameters, carbohydrate and vitamin content of the dye-rich concentrate obtained from the basil (*Occimum basilicum L.*) plant were studied. The amount of fructose in the dye extract obtained in a 20% carbohydrate solution was 5.94 mg/l, the maximum value was 9.12% in the 40% extract, and the fructose content in the dye obtained in a 50% solution was 7.14%. - organized. It was found that the amount of vitamins in the concentrate is higher in 40% and 50% alcohol solution. The safety of the natural dye was analyzed. Heavy metals and their salts, dangerous substances for human health were analyzed.

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