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ANTHOCYANS OF SOME PLANTS OF UZBEKISTAN AND THE CREATION OF FOOD DYES FROM THEM

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The chemical composition of anthocyan pigmentary substances of some plants of Uzbekistan have been studied. From the plant species investigated various flavonoids and glycosides of cyanidin and delphinidin have been isolated. A method has been developed for obtaining food dyes and this has been used to organize their production.

Until recently, in all sectors of the food industry synthetic dyes — indigo carmine, amaranth, tartrazine, fuchsine, and chrysoidine — have been used for coloring food products. However, special investigations have shown that synthetic dyes have properties harmful for human health.

In order to discover promising species of dye plants and to create from them natural food dyes, we have made a chemical study of the qualitative and quantitative compositions of the dye plants and a study of the physicochemical properties of the pigmentary substances isolated.

Quantitative analysis of the plants studied for their total content of anthocyans showed that it ranged over wide limits (0.5-12%) [1-3].

As a result of the investigations performed, it was found that the greatest value as sources of natural dyes are the red double form of the hollyhock, hybrid hibiscuses, the cotton plant, and the pressing residues of dark varieties of grape and mulberry, which are distinguished by a high content of anthocyan pigments [1, 3].

It has been established with the aid of two-dimensional paper chromatography, using the solvent systems 1) butan-1-ol–acetic acid–water (40:12:28); and 2) 6% acetic acid, that the pigmentary substances obtained from the flowers of cotton plants of varieties Tashkent-1, Tashkent-2, and Tashkent-3 include two anthocyans and several other phenolic compounds.

By chromatographing ethyl acetate fractions of these pigments on a silica gel column using a) a mixture of diethyl ether with ethyl acetate and b) water-saturated ethyl acetate as eluents, we have isolated three compounds of flavonol nature: compound (**1**) with mp 249-250°C, (**2**) with mp 220-222°C, and (**3**) with mp 230-231°C.

By comparing their physicochemical constants and spectral characteristics with those given in the literature and by a direct comparison with authentic specimens, compounds (**1-3**) were identified as quercetin 7-O-glucopyranoside (quercimeritrin), quercetin 3-O-*β*-*D*-glucopyranoside (hirsutrin), and quercetin 3′-O-glucoside, respectively.

By chromatographing the total pigmentary substances of butanol fractions on a column of cellulose powder (with water–acetic acid–hydrochloric acid (82:15:3) as eluent) we isolated two anthocyans: anthocyan A-1 with mp 239-241°C, $C_{26}H_{29}O_{15}Cl\cdot 4H_2O$, UV spectrum (MeOH + 0.01% HCl, λ_{max}, nm): 523 (log ε 2.26), with the addition of aluminum chloride, 558 nm; and anthocyan A-2, with mp 215-217°C, $C_{21}H_{21}O_{11}Cl\cdot 2H_2O$, UV spectrum (MeOH + 0.01 HCl, λ_{max}, nm): 525 (log ε 2.26), with the addition of aluminum chloride, 578 nm.

On the basis of the results of a study of acid hydrolysis, alkaline cleavage, and spectral characteristics, and from their physicochemical constants, anthocyans A-1 and A-2 were identified as cyanidin 3-O-*β*-*D*-glucopyranosyl-*β*-*D*-xylopyranoside (gossypicyanin) and cyanidin 3-O-*β*-D-glucopyranoside (chrysanthemin), respectively. These anthocyans have also been isolated from the total pigments of the flowers of *Tulipa gregii* and *Verbena hybrida* and from the pigments of the fruit of *Cerasus avium* L. (Pushti, Napoleon, and Fransis varieties) cultivated in Uzbekistan.

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TABLE 1. Characteristics of Extracts, Fruit Juices, and Concentrates of Pigments

	Extracts, fruit juices, and concentrates of pigments	Indices		
		Relative density at 20°C	Anthocyan content, g/liter	Dry matter content, %
1.	Extract from the flowers of hybrid hibiscuses using as extractant the diffusion juice of mulberry pressings		5.30	4.38
2.	Mulberry juice		6.80	5.25
3.	Combined diffusion juice of the flowers of hibiscuses and mulberry juice		5.60	4.57
4.	Concentrate of pigment from flowers of hybrid hibiscuses and mulberries	1.200	57.10	44.2
5.	Extract from hollyhock flowers using as extractant the diffusion juice of grape pressings		5.84	3.73
6.	Combined extracts of hollyhock flowers with mulberry juice		6.21	3.98
7.	Concentration of the pigment from hollyhock flowers, mulberries, and grape pressings	1.19	59.4	46.0
8.	Concentrate of the pigment from the form of hollyhock flowers of the double	1.21	58.3	46.1
	Concentrate of the pigment from cottonplant flowers	1.18	34.6	43.2

From the flowers of *Verbena hybrida*, in addition to anthocyans A-1 and A-2, we isolated the dark violet compound A-3 with mp 182-183°C, $C_{21}H_{21}O_{12}Cl \cdot H_2O$, UV spectrum (MeOH + 0.01% HCl, λ_{max}, nm); 536 (log ε 2.24), with the addition of 5% of aluminum chloride, 570 nm. The hydrolysis of anthocyan A-3 with 5% hydrochloric acid led to the formation of glucose and an aglycon — delphinidin —, while its alkaline cleavage gave phloroglucinol and gallic acid. By its physicochemical indices, the products of acid hydrolysis and alkaline decomposition, and direct comparison with an authentic sample, anthocyan A-3 was identified as delphinidin 3-O-β-*D*-glucopyranoside.

A technology for obtaining concentrates of red food dyes and their blends from the flowers of hollyhock, hybrid hibiscuses, dark red mulberries and grape pressings has been developed (Table 1) and has been introduced into the Chartak experimental factory for food concentrates.

The dependence of the stability of the food dyes and of the structure and composition of the dye substances on the pH of the medium, the temperature, and the components accompanying them has been studied. It has been found that the dyes, especially those containing peonidin glycosides, are relatively stable. The presence in the dyes of various flavonols, catechins, leucoanthocyanidins and proanthocyanidins and also gallo- and ellagotannins promotes the stability of the food dyes.

EXPERIMENTAL

The amounts of anthocyan pigments in the plant materials, extracts, and finished product in the form of concentrate and powder were determined by a spectrophotometric method from a calibration graph drawn up with standard preparations of the total anthocyans isolated from hollyhock flowers, rose flowers, and high mallow and also according to OST [Sector Standard] 18-405-83 by comparing the intensities of the coloration of solutions of cobalt sulfate and the solutions under investigation. The concentrates of pigments from various plant species were obtained by the procedure described in [1, 4].

Isolation and Separation of the Anthocyans. The anthocyans were extracted fron the plant material under investigation with water and with aqueous ethyl alcohol containing 0.7-1% of hydrochloric acid. The combined extracts were

concentrated in vacuum to a dry-matter content of 15-18% and were treated with chloroform to eliminate accompanying substances, and the aqueous residue obtained was extracted successively with ethyl acetate and *n*-butyl alcohol. The total anthocyans were isolated from the concentrated butanolic extract by precipitation with diethyl ether. Chromatography of the total flavonols was carried out on KSK silica gel with the use as eluents of ether–ethyl acetate (1:9), (1:5), and (1:1), and water-saturated ethyl acetate.

The separation of the total anthocyans and the isolation of individual anthocyans were conducted on a column of cellulose powder with the eluents ethyl acetate–formic acid–water (70:15:15) (system 1) and (3:1:3) (system 2), butan-1-ol–acetic acid–water (4:1:5; upper phase) (system 3), and water–acetic acid–hydrochloric acid (82:15:15) (system 4).

The structures of the anthocyans and anthocyanidins isolated were established with the aid of chemical (acid and enzymatic hydrolysis and alkaline cleavage) and physicochemical methods [4].

The main fragments and the structures of the anthocyans and anthocyanidins isolated were determined from the results of a study of the product of stepwise and complete acid hydrolysis, oxidative degradation with hydrogen peroxide, and degradation in an alkaline medium.

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