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Spectrophotometric indicators of the stability of anthocyanin-containing extracts depending on the color of plant materials

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Abstract. This paper aims at spectrophotometric determination of changes in stability of extractable anthocyanins during drying of plant materials depending on their color. Raw and dried colored parts of 50 plant species from 25 families were used for the study. The extracts were prepared over 95% ethanol acidified with hydrochloric acid (pH ~ 1). The absorption spectra were registered within the range of 210 to 680 nm. The extinction variability factor, coefficient of intensity absorption relative and generalized stability factor were used to determine the anthocyanin degradation. The highest values of the stability factor were obtained for the extracts from fruit shells of burgundy or violet color within the range of 0.934±0.024 to 0.973±0.024, while the extracts from flower petals of the same care featured the stability factor that was 1.19 to 1.44 times less. The values of the stability factor of the extracts from black, red and blue materials are 1.15 to 1.19 times, 1.74 to 2.48 times and 4.65 to 4.84 times less respectively than those of the extracts from violet-burgundy materials. It is appropriate to apply the spectrophotometric factors of anthocyanins stability used in this study to selection of promising plants for industrial cultivation as material of anthocyanin-containing herbal preparations. The most stable anthocyanins are those of burgundy-purple and black fruits.

Keywords: UV-visible spectrophotometry; anthocyanin-containing herbal extract; natural dyes; anthocyanin stability.

1. Introduction

Anthocyanins are one of the groups of plant pigments that give flower petals, fruits and root plants a variety of colors - from pink-red to purple-black.

In higher plants, anthocyanins are represented by six types of flavyliums: cyanidin, delphinidin, pelargonidin, peonidin, malvidin and petunidin. Depending on the radicals R (Fig. 1), the flavyliums form about 600 varieties of anthocyanins [1, 2].

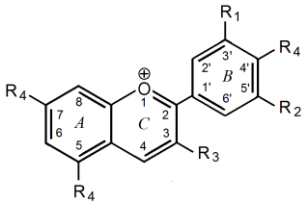

Figure 1. Flavylium cation. Radicals: R_1, R_2 – H, OH or OCH_3, R_3 – glycosyl or H, R_4 – OH and glycosyl

Anthocyanins may be present in both vegetative and generative organs of the plant, where they perform a range of functions. Anthocyanins mainly serve as optical filters protecting photosynthetic apparatus from high energy quanta [3, 4]. They also deactivate radical oxygen and nitrogen forms [5]. Anthocyanins express significant antioxidant activity [6, 7] in the human body and have a therapeutic effect on many diseases, especially on cardiovascular pathology [8-10]. Furthermore, anthocyanins are becoming increasingly attractive as plant pigments for food industry as a natural alternative to synthetic dyes [11].

However, the wide use of anthocyanins in pharmacological, food and other industries is limited by their high lability, since they are highly prone to structural deformation, hydrolysis, destruction or degradation during storage and processing of plant raw materials [12-15]. The stability of different types of anthocyanins is related to their structural that determine the color as well.

The correlation between the stability of extractable anthocyanins and the color of plant materials is not sufficiently studied, although it could serve as a basis for selection of promising plant species and their cultivation on industrial scale to produce herbal preparations and natural dyes. Such correlation can be revealed by optical methods, using drying, which is quite often used in plant material processing technology, as a model "damaging" factor.

This paper aims at spectrophotometric determination of changes in stability of extractable anthocyanins during drying of plant materials depending on their color.

2. Experimental

Colored parts of 50 plant species (Tables 1 and 2) from 25 families were used for this study. Flower petals were collected in the initial phase of flowering, fruits - at full maturity, and root plants and bulbs - in late autumn,

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during the phase of withering of the ground part. The collected materials were divided into two parts, with the first part extracted immediately after collection, the second part was extracted after preliminary drying. The drying was carried out in a drying cabinet at 36 to 38 °C for 10 to 14 hours until residual humidity reached 10 to 12%. The extracts were prepared over 95% ethanol (Merck, Germany) acidified with hydrochloric acid (pH ~ 1). A quantity of plant material was thoroughly grated in the pounder together with extraction agent and quartz sand. Following 5 to 6 minutes infusion, it was filtered through a 0.45-μm PTFE-H filter (Hyundai micro, South Korea) into dark-glass vials.

The absorption spectra of the extracts were recorded using UV-2501PC digital spectrophotometer (Shimadzu, Japan) within the range of 220 to 650 nm.

As is known [15], the absorption spectrum of the anthocyanin-containing extract in acidic media usually includes three maxima. The y-axis of the third maximum M3 (Fig. 2) is most variable under external impacts. The y-axis of the third absorption maximum for the extracts from dried plant parts was indicated as *DA*, while the similar y-axis for raw parts was indicated as *RA*. The extinction variability factor (*EVF*) was used to characterize the degradation of anthocyanins inside the extracts as follows:

$$EVF = \frac{DA}{RA}$$

The integral absorption intensity of the third maximum band for raw material was calculated using Simpson formula [16, 17] as the RS area (Fig. 2, R, vertical hatching) within the x-axes λ_b and λ_c of the bend points *b* and *c*. The *DS* area (Fig. 2, D, oblique hatching) was similarly calculated for dried materials within the x-axes λ_f and λ_g of the bend points *f* and *g*. The coefficient of intensity absorption relative (*CIAR*) was determined using the following formula:

$$CIAR = \frac{DS}{RS}$$

The generalized stability factor (*SF*) characterizing the variability of the third maximum for both extinctions and absorption intensities in the absorption spectra of anthocyanin-containing extracts was calculated as the product of the foregoing factors as follows:

$$SF = EVF \times CIAR$$

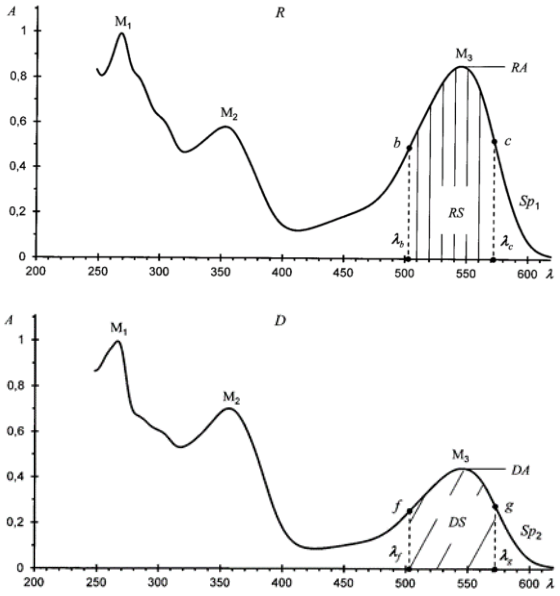

Figure 2. Absorption spectra (Sp1 and Sp2) of the extracts soaked in acid ethanol and produced from raw (R) and dried (D) flower petals of *Impatiens roylei* Walp. M1, M2 and M3 – maxima, RA and DA – y-axes, RS and DS – areas, b, c and d, f –bend points, λ_b, λ_c and λ_d, λ_d– x-axes of the absorption band boundaries

3 to 5 independent samples were taken to study each part of the plant. The results were statistically processed using small sample technique [18].

3. Results and discussion

Wavelengths (λM3) of the third maxima in the absorption spectra of extracts of the examined plant parts are within the green-yellow range of 513 to 548 nm (Tables 1 and 2). The mean λM3 values of the extracts from flowers and fruit shells of burgundy or violet color coincide ($p > 0.05$), while those of the extracts from red plant parts differ by 11.8 nm.

Table 1. Wavelength of the third absorption maximum (λM3), extinction factor (*EVF*), intensity coefficient (*CIAR*) of the absorption spectra of the extracts from different colored parts of various plant species

Color	Plant species	Plant part	λM3	*EVF*	*CIAR*
Violet	*Lonicera dulis* Turcz. ex Freyn.	Fruit shell	536	0.998	0.998
	Vaccinium corymbosum L.		546	1	0.924
	Vaccinium myrtillus L.		548	1	0.999
	mean ± statistical error of mean		543.3 ±3.7	0.999 ±0.005	0.974 ±0.025
Burgundy	*Allium cera* L. Variety «Karmen»	Bulb	536	0.892	0.964
	Beta vulgaris L. Variety «Zilindra»	Root plant shell	543	0.963	0.947
	Raphanus sativus var. radicula Pers. Variety «Gara»		513	1	0.967
	Prunus domestica L.	Fruit shell	538	1	0.999
	mean ± statistical error of mean		532.5 ±6.7	0.964 ±0.025	0.969 ±0.011
Black	*Aronia mitschurinii* A.K. Skvortsov & Maitul.	Fruit shell	534	1	0.998
	Padus avium Kom.		538	0.884	0.916
	Padus maackii Rupr.		537	0.725	0.727
	Ribes nigrum L. Variety «Chempion»		543	0.969	0.928
	mean ± statistical error of mean		538 ±1.9	0.894 ±0.194	0.892 ±0.059

Red	*Berberis amurensis* Rupr.	Fruit shell	518	0.555	0.568
	Berberis thunbergii DC		517	0.771	0.76
	Crataegus canguinea Pall.		536	0.821	0.817
	Crataegus pinnatifida Burge.		536	0.819	0.816
	Lonicera maakii (Rupr.) Maxim.		532	0.571	0.667
	*Ribes rubrum*L.		536	0.857	0.742
	Rubus idaeus L.		535	0.797	0.786
	Viburnum sargentii L.		533	0.696	0.608
mean ± statistical error of mean			530.4 ±2.8	0.736 ±0.041	0.720 ±0.033

Table 2. Wavelength of the third absorption maximum (*λ*M3), extinction factor (*EVF*), intensity coefficient (*CIAR*) of the absorption spectra of the extracts from different colored flower petals of various plant species

Color of flower fetal	Plant species	*λ*M3	*EVF*	*CIAR*
Burgundy	*Cosmos bipinnatus* Cav.	536	0.778	0.934
	Cosmos atrosanguineus Cav.	536	0.952	0.916
	Petunia × *hybrid* Hort. ex. Vilm.	545	0.714	0.716
	Rhododendron dauricum L.	543	0.899	0.903
	Weigela praecox (Lemoine) Bailey	537	0.989	0.966
mean ± statistical error of mean		539.4 ±1.9	0.867±0.052	0.887±0.044
Violet	*Allium nutans* L.	542	0.968	0.929
	*Allium schoenoprasum*L.	541	0.786	0.790
	Aquilegia olympica Boiss.	547	0.645	0.669
	Aquilegia vulgaris L.	546	0.902	0.882
	Iris tenuifolia Pall.	547	0.712	0.713
	Lathyrus sylvestris L.	543	0.717	0.699
	Lupinus polyphyllus Lindl.	548	0.943	0.944
	Petunia × *hybrida* Hort. ex E. Vilm.	547	0.643	0.629
	Primula siebolgii E. Morren	541	0.991	0.874
	Syringa vulgaris L.	543	0.868	0.999
	Vicia crassa L.	545	0.784	0.801
mean ± statistical error of mean		544.5±0.8	0.814±0.038	0.812±0.037
Red	*Impatiens roylei* Walp.	545	0.515	0.539
	Paeononia anomala L.	528	0.671	0.477
	Paeononia tenuifolia L.	527	0.412	0.645
	Prunus tribola Lindl.	536	0.592	0.566
	Polygonum persicaria L.	533	0.751	0.685
	Rhododendron schlippenbachii Maxim.	528	0.784	0.806
	Rosa majalis Herrm.	531	0.359	0.805
	Rosa rugosa Thunb.	534	0.544	0.674
	Tagetes patula L.	532	0.797	0.799
	Trifolium pratense L.	545	0.594	0.457
	Valeriana officinalis L.	537	0.621	0.654
mean ± statistical error of mean		542.2±1.9	0.603±0.043	0.646±0.038
Blue	*Iris sibirica* L.	543	0.416	0.435
	Commelina communis L.	546	0.405	0.406
	Lathyrus komarovii Ohwi	543	0.596	0.594
	Symphytum officinale L.	542	0.319	0.335
mean ± statistical error of mean		543.5±0.9	0.434±0.058	0.442±0.055

The highest *EVF* and *CIAR* values within the range of 0.924 to 1 (Table 1) were obtained for the extracts from fruit shells of *Vaccinium corymbosum*, *Vaccinium myrtillus*, and *Prunus domestica*. In case of other studied fruit shells of purple and burgundy color these indicators were 1.4 to 4.5% less ($p > 0.05$).

The extracts from black fruit shells of *Aronia mitschurinii*, *Padus avium*, and *Ribes nigrum* featured values of the similar indicators that were 7.3 to 7.9% less comparing with the extracts from violet fruit shells ($p < 0.05$).

The highest *EVF* and *CIAR* values obtained for the extracts from fruits of *Berberis amurensis*, *Crataegus pinnatifida*), *Lonicera maakii* and other studied red fruits were 23.6 to 25.7% lower comparing with those of the extracts from violet fruit shells ($0.001 < p < 0.05$).

Similar correlation between the *EVF* and *CIAR* indicators and the color of plant material was also obtained for flower petals of different coloration. For instance, the extracts from burgundy petals of *Weigela praecox* and purple petals of *Allium nutans* had also high *EVF* and *CIAR* values (Table 2), yet they were 0.8 to 4.6% less comparing with fruit shells of similar color. These indicators of the extracts from other studied petals of the same color were 9 to 12% less.

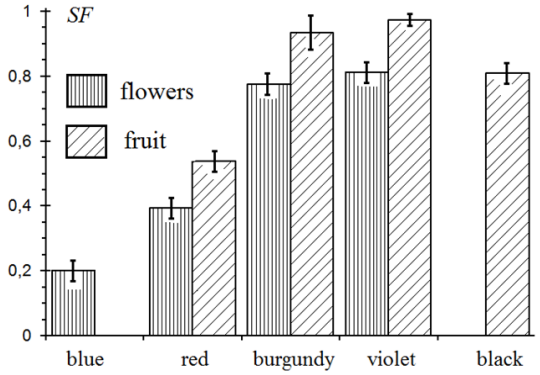

Figure 3. Stability factor (SF) of anthocyanins in extracts of flower petals and fruits depending on their color

The values for the extracts of red petals were 27.2 to 30.4% lower than those for burgundy petals and 10.2 to 17.9% lower than red fruit shells. The lowest *EVF* and *CIAR* values were registered in the extracts from blue flower petals, which, on average, were 49.9 to 50.2% less than those for burgundy petals and 53.6 to 54.7% less than those for purple fruit shells.

The highest stability factor (*SF*) values were obtained for the extracts from fruits of burgundy or violet color (Fig. 3) within the range of 0.934±0.024 to 0.973±0.024, while those for the extracts from flower petals of the same color were 1.19 to 1.44 times less. The stability factor for the extracts from black materials were 1.15 - 1.19 times less, those from red materials – 1.74 to 2.48 times less, and those from blue materials were 4.65 to 4.84 times less in than the factor values for the extracts from violet-burgundy materials.

The distribution of material color by stability of anthocyanins it contains may be expressed in the following order:

[burgundy - violet] > [black] > [red] > [blue].

Thus, it is plausible to argue that it is anthocyanins of blue plant materials that are prone to the largest degradation in the course of drying. Anthocyanins of burgundy-violet materials feature the least degree of change. The stability of anthocyanins of black and red materials is in the middle between that of "burgundy-violet" and "blue" materials.

Discussion

As follows from their presented research results, the resistance of anthocyanins from fruits is slightly higher compared to that of anthocyanins from flower petals with the same color. One of the reasons for this is probably due to an increase in the sugar content in the fruits during ripening. The effect of sugars on the stability of anthocyanins has not been sufficiently studied, but it is indicated [14] that glycosylation prevents the degradation of anthocyanins due to the blockade of fermentation. The increase in sugar residues (especially glucose) seems to give a higher stability of anthocyanins in the fruit compared to anthocyanins in the petals of the flowers. There are probably other reasons, which requires special studies.

The *EVF*, *CIAR* and *SF* indicators used in this study have not previously been applied to spectrophotometric analysis of the stability of anthocyanins of plant material extracts, so direct comparisons and comparisons with the results other studies are excluded. However, there may be parallels with known data on the presence of anthocyanins in plant materials of different coloration. Normally, several anthocyanins of different types are present in the colored part of a plant, yet among them, as a rule, only one or two of them have a predominant concentration, and it is them that give plant organs the relevant color [2].

Blue color is given to plant parts mainly by delphinidin derivatives [2, 19]. Among anthocyanins, delphinidin features the highest antioxidant capacity [1, 6, 8], shows the most active participation in redox processes, degrades rapidly and has low stability. The lowest *SF* values for the extracts from flower petals of *Commelina communis* and other blue plant parts obtained by the authors fully conform to these data.

Peonidin and malvidin [2] give burgundy and violet color to flowers and fruits, e.g. blueberry fruit shells (*Vaccinium myrtillus*) [20, 21]. The highest *SF* values were obtained for the extracts from burgundy and violet plant parts of plants, so it is plausible to argue that peonidin and malvidin are the most stable substances.

The black color of fruits is due to the presence of many types of anthocyanins, including delphinidin [2], e.g. *Aronia mitschurinii*, *Padus avium* and *Ribes nigrum* [22, 23]. The presence of poorly stable delphinidin causes a decrease in the overall stability of anthocyanin group of black fruits comparing with burgundy ones.

Pelargonidin, peonidin or cyanidin color plant parts in red [2, 22], e.g. *Vaccinium erythrocarpum* and *Ribes rubrum* [2]. Since the *SF* values obtained for the extracts from red materials are lower than those of for burgundy-purple ones, the stability of pelargonidin and cyanidin is lower comparing with them.

Thus, anthocyanins can be arranged in descending order of stability as follows:

[peonidin, malvidin] > [petunidin, pelargonidin, cyanidin] > [delphinidin].

As comparison of the obtained data with the material color depending on the presence of anthocyanins shows, that the most stable anthocyanins are those that give plant parts burgundy-purple or black color.

4. Conclusions

The stability of anthocyanins is determined by the spectrophotometric indicator *SF*, which is calculated as the product of the extinctions and absorption intensities of the third maximum absorption spectra of anthocyanin-containing extracts from plant raw materials.

Anthocyanins of extracts from fruits with burgundy purple and black color have the highest *SF* value.

Anthocyanins of extracts from flower petals are less stable compared to anthocyanins of extracts from fruit shells with the same color.

SF can be used in practice in the selection of promising plants for industrial cultivation and production of anthocyanin-containing phyto preparations.

Conflict of interest

Authors have no conflict of interest to declare.

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