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THE INFLUENCE OF pH TO THE VISIBLE ABSORPTION OF CYANIDIN FROM THE BLACK CHERRY AND BETANIN FROM BEETROOT

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Abstract

The goal of this study is the extraction and the characterization of the anthocyanic pigments from the black cherry and beetroot (cyanidin is the main aglycon from the black cherry and betanin form beet root) at different pH values.

Keywords: *black cherry, beetroot, pH, stability, anthocyans, spectrophotometry*

Introduction

Anthocyans are vegetable pygments that are responsible for the red, violet, and blue colours of flowers, fruits, and seeds (Gabelman, 1994).

The skeleton (figure 1) is represented by the tetrahydroxyflavilium cation, which is substituted with hydroxy, methyl, or methoxy groups (Bodea, 1964; Ciulei, 1993; Dewick, 1997). The anthocyanidine aglycons are obtained by anthocyans hydrolysis (the saccharides from the anthocyan structures are: glucose, galactose, rhamnose). Pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin are the main known anthocyanidins (Fennema, 1996; Hendry, 1996; Kaufman, 1999). In table 1, the significance of radicals R and R' are presented.

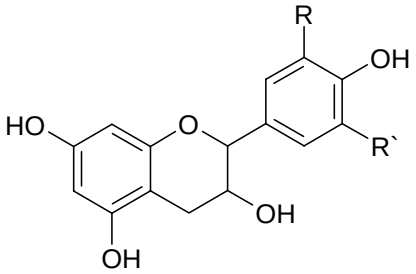

Fig. 1. The skeleton of anthocyans

Table 1. The significance of radicals R and R' from anthocyans skeleton

Radical	Pelargonidina	Cianidina	Peonidina	Delfinidina	Petunidina	Malvidina
R	H	OH	OCH_3	OH	OCH_3	OCH_3
R`	H	H	H	OH	OH	OCH_3

The goal of this study is the extraction and the characterization of the anthocyanic pygments from the black cherry and beetroot (cyanidine is the main aglycon from the black cherry and betanin from beet root) at different pH values.

Experimental

Materials: The beetroot (*Beta vulgaris*) and black cherry was purchased from the market from west zone of Romania. The buffer solutions used for extraction at different pH values were obtained from H_3BO_3, NaOH, and HCl solutions according to the "Tables for the Laboratory-Merck Chemical Co". H_3BO_3 was purchased from Fluka Chemie AG, BioChemika Analytika, Sweden, NaOH was purchased from Lachema, Czech Republic, the 36% HCl and 96% H_2SO_4 solutions were obtained from Merck&Co., Inc., New Jersey, lead acetate and methanol (*p.a.*) were obtained from Chimopar, Bucharest.

Extraction: 50 g Fine grounded raw black cherry, beetroot and 100 ml warm distilled water (50°C approximately) were added in a 500 ml Berzelius flask. The mixture was vigorously stirred for 10 minutes and then filtered, a coloured solution being obtained as a clear filtrate. For the anthocyanic pigment crystallization 5 g lead acetate was added to filtrates, and colours were changed. Precipitates were filtered, washed with methanol for three times (3 x 10 ml methanol), and then dried at 20°C for 24 h. The dried pigment was dissolved in 50 ml distilled warm water (50°C approximately) and then 2.5 ml concentrated sulfuric acid was added in order to precipitate the lead excess as sulfate. The precipitate was separated by centrifugation and filtration.

Anthocyans identification: Anthocyans identification was performed by studying the colour changes *vs.* pH values, by the spectrophotometric analysis of the extracts that provide the wavelength corresponding to the maximal absorption (λ_{max}). The UV-VIS analysis was performed on a Specord Carl Zeiss spectrophotometer and the pH values were verified with a digital pH-meter.

Results and discussion

The solutions acquired after removing the excess of lead have a pH value of 2 (for both extracts – black cherry and beet root – at pH 2 the color was red). The modification of the color as a result of alkalinization is explained by the modification of anthocyan structures.

Anthocyan chloride and anthocyanidine (red colour) is transformed into a colourless pseudobase – leucocyanidine – that is dehydrated and transformed into a violet quinolone derivative. By treatment of alkalis (NaOH buffer solution) a blue coloured sodium salt was obtained.

Anthocyans isolated from the black cherry have a cyanidine aglicon, that influence the hue of colours acquired with the change of the pH value from 1-3 to 12-14.

In the case of beet root *(Beta vulgaris*) the variation of the colour *vs.* pH was unclear, the presence of cyanidine being unclear.

Figure 2 contents the visible spectra of the watery solution before the treatment with solid lead acetate. A maximum absorption appear at 560 nm for the watery filtrates from the black cherry, but no relevant absorption could be observed for the watery filtrates from beet root in the range of 400-600 nm.

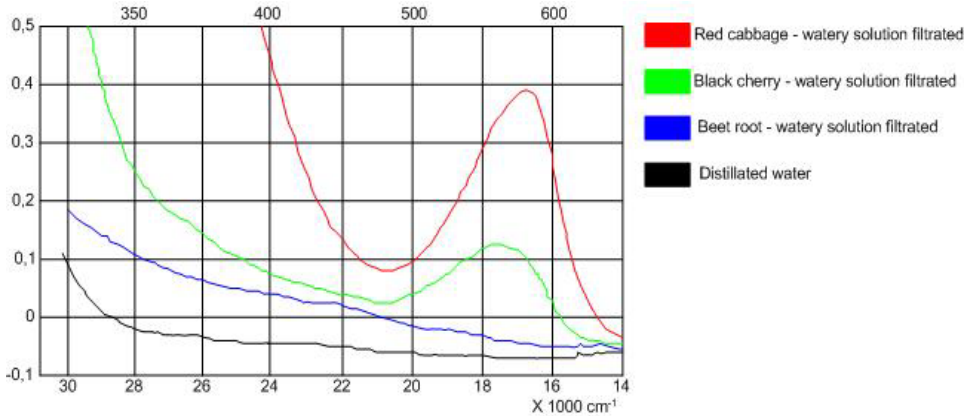

Fig. 2. The visible spectra of the watery solutions before the treatment with lead acetate.

Figure 3 shows the visible spectra for the residual solutions after haltering the pigments with lead acetate. It can be observed a maximum of absorption at 580 nm for red cabbage, at 560 nm for the black cherry, but for the beetroot the absorption was unclear.

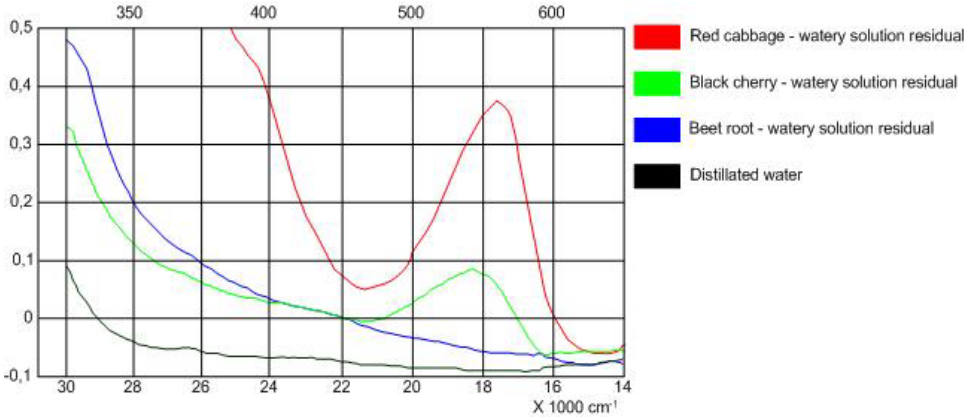

Fig. 3. The visible spectra for the residual solutions after filtering of the pigment

Figure 4 and 5 show the visible spectra for the black cherry at the value of pH of 2, 5, 7, 8, and 11. At pH 2 a maximum absorption at 560 nm was observed; the maximum absorption was observed at 510 nm for the pH 5, 570 nm for the pH 7, and no maximum absorption was observed in the range of 400-600 nm for pH 8 and 11.

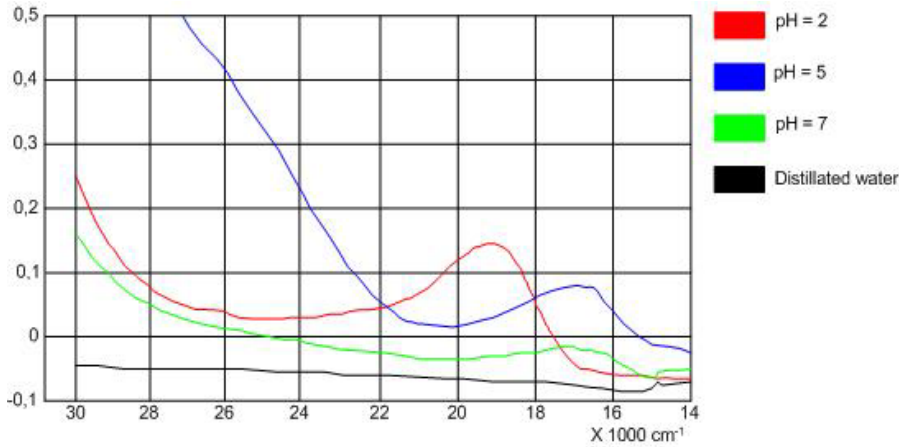

Fig. 4. The visible spectra for anthocyans from the black cherry for the pH values of 2, 5, and 7

Figure 6 and 7 show the visible spectra for beetroot at the pH values of 1, 3, 5, 10; a maximum absorption was observed at 500 nm only at pH value of 1.

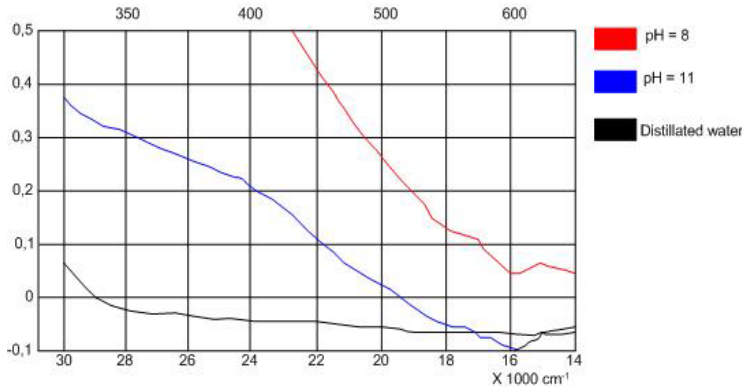

Fig. 5. The visible spectra for anthocyans from the black cherry for the pH values of 8, 11

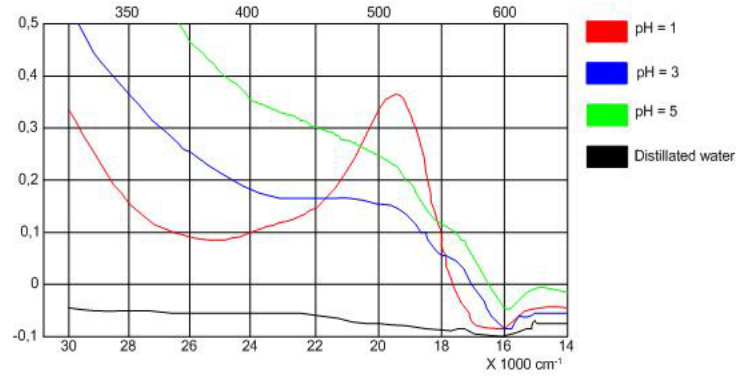

Fig. 6. The visible spectra for the anthocyans from beetroot at the pH values of 1, 3, 5

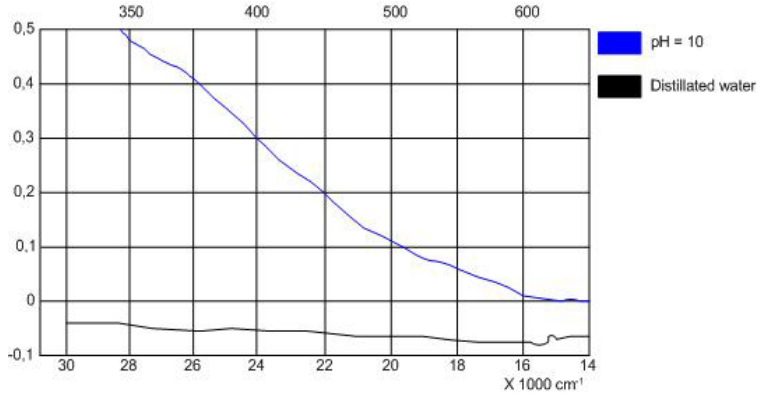

Fig. 7. The VIS spectrum for the anthocyans from beetroot at pH 10

Conclusions

The purpose of the experiment was to determinate the influence of the pH values to the visible spectra of cyanidine from the black cherry. A relevant difference for the absorption intensity is revealed at various concentrations after the correction of the pH values with HCl and NaOH solutions. A pH value in the range of 9-11 conducts to a smaller concentration of cyanidine with a lower intensity of absorption. The beetroot analysis reveals that the maximum absorption is concluding only at pH 1, due to a higher concentration of the betanin in the anthocyan mixture from the beetroot. At pH 3 and 5 an unclear maximum absorption was observed; at pH 10 no absorption was observed in the range of the wavelength of 400-600 nm, probably due to the higher concentration of the betanin in the anthocyan mixture from the beetroot, which has no absorbance in the range of 400-600 nm.

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