Colour Changes and Instability of Anthocyanins

Hana Křížová
* Jakub Wiener

Technical University of Liberec
Faculty of Textile
Department of Textile Finishing
Studentská 2, 461 17, Liberec 1, Czech Republic
hana.krizova@tul.cz

* Technical University of Liberec
Faculty of Textile
Department of Textile Finishing
Studentská 2, 461 17, Liberec 1, Czech Republic
jakub.wiener@tul.cz

Abstract

Anthocyanins are plant pigments of special interest for their colour changes in different acidic media. In the food industry they are used as colourants and as antioxidants, in food they play the role of natural antioxidants and historically they were used in textile dyeing.

The advantage of anthocyanins is their medical safety, easy availability of renewable resources, and the possibility of recovery of canning and wine pressings.

The disadvantage is a colour instability and susceptibility to physical and chemical conditions that limits their use and further processing. This study looks at testing the rate of decomposition of anthocyanins in the juice of blueberry, elderberry and extract of blue grape vine remnants by alkaline medium exposure, high temperatures and UV radiation and discusses the suitability of their use in textile dyeing.

Introduction

Anthocyanins are an interesting group of natural pigments that occur in different parts of plants, typically in fruits such as grapes, elderberries, blueberries, in red cabbage leaves, in petals of red roses, in skins of red varieties of potato tubers etc. They are responsible for a wide variety of red, purple and blue shades of flowers, fruits and seeds and they perform many functions such as protecting tissue against UV radiation, protecting the seed's embryo against oxidative stress, and attracting insects for pollination.

Anthocyanins are one class of flavonoid compounds and they are an important part of a large group of polyphenols. The structural basis makes a flavan skeleton consisting of two aromatic rings (A, B) and of one pyran cycle (C) (*Fig. 1*) This one is responsible for the typical reaction of flavonoids. It contains a three binding oxygen and its positive charge enables the formation of oxonium salts with anions. [1]

Anthocyanins have a substance glycoside nature – they consist of aglycone anthocyanidins (according to substituents A, B and C series which are besides the hydrogen mostly hydroxyls and methoxy groups – usually cyanidin, malvidin, delphinidin, petunidin, peonidin or pelargonidin, and their representation in plants is different (e.g. elderberry contains mainly derivatives of cyanidin, blue vine derivatives of malvidin, black currants contains derivatives of delphinidin and cyanidin, strawberries derivatives of pelargonidin and cyanidin etc.). [3]
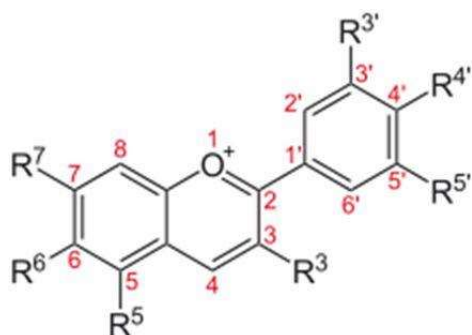
Source: [2]
Fig. 1: *Basic structure of anthocyanins*

In nature, however, anthocyanidins themselves are very rare – they represent only trace amounts of degradation products of anthocyanins. In plants there are found mainly mono-, di- and oligosaccharide glycosides which are often still acylated by organic acids. [3]

A typical property of anthocyanins is a colour change according to the pH (*Fig. 2*), generally from the red in strongly acidic environment (flavylium salt), with increasing pH (pH 4 to 5) comes to the formation of colourless carbinol (in real systems, however, we can see only a drop in colour), then follows purple chinoid base and with further increasing of the alkalinity it comes up to a blue coloured anion. [4]

In certain pH range these transformations are completely reversible and very fast - ten thousandth of a second in the conversion of flavyl salt/chinoid base (proton transfer), and only seconds in the conversion of flavylium salt/carbinol (hydration). [5]

At high pH values it comes up to the opening of pyran ring and to irreversible formation of yellow chalcones (in model systems around pH 8, in real systems comes due to an influence of other substituents such as organic acids and metal ions to the changes only in strongly alkaline medium). By splitting the pyran ring the conjugation is maintained only in twoisolated aromatic rings and the excitation of these electrons is possible only with higher energy photons, i.e. in the UV light. Therefore the visible field is reflected only by the edges of very intense UV absorption bands which produce yellow colouring.

According to the colouristic classification anthocyanins pigments are classified into groups of pyran dyes. Anthocyanins are known as pigments in the food industry where they are used as a relatively harmless substance labeled E163 for colorization of jams, juices, yoghurts, sweets etc.Anthocyanins have also been long used for textile dyeing, and although they can not compete with the diversity in the shades of synthetic dyes in the fiber pull-out percentage and in modern times, they are still experiencing a certain renaissance at present due to an increased interest in ecology and a return to natural substances.

The aim ot this work was to study the behavior of selected plant anthocyanins depending on different physico-chemical conditions to which the plant anthocyanins could be exposed during further processing and to assess their degree of instability and the possibility of their practical use.
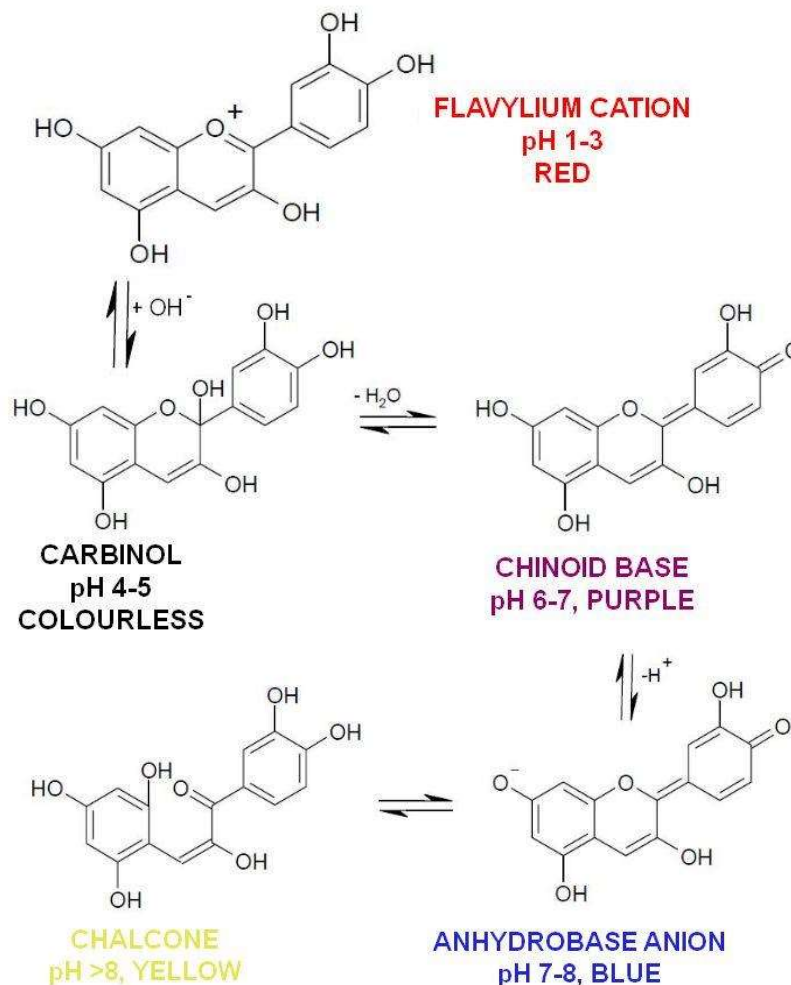
Source: Own based on [6]
Fig. 2: *Structural changes of anthocyanins according to pH*

1   Methods

To determine the approximate content of anthocyanins in the plant material the Giusti and Wrolstad method was used. [7]

This is a spectrophotometric method based on measurement of the absorption of anthocyanins' solution at pH 1 and pH 4.5, i.e. in terms of maximal absorption of flavyl salt, and in the area where the colour disappears.

The difference is calculated at 520 and 700 nm and using the extinction coefficient of the relevant anthocyanin there is calculated the concentration of anthocyanin in the solution by applying the Lambert-Beer law. According to the dilution there is afterwards calculated its content in the sample. For the calculation there was used the extinction coefficient of "universal" and common monoside cyanidin 3 - glucoside ($\varepsilon = 26\ 900$ litre cm^{-1} mol^{-1}, chrysantemin).

The absorbance was measured on a spectrophotometer HP 8452 diode array (DAD) using 1cm quartz cuvette in the range of the VIS spectrum (380-760 nm), in the environment of buffer KCl / HCl (pH 1) and sodium acetate / HCl (pH 4.5). The total absorbance was calculated from relations (1) and (2):

$$A = \left(A_{VIS\,max} - A_{700}\right)_{pH1,0} - \left(A_{VIS\,max} - A_{700}\right)_{pH4,5} \qquad (1)$$

$$c_{ACY} = \frac{A \cdot M \cdot DF \cdot 1000}{\varepsilon \cdot 1} \qquad (2)$$

A	absorbance [-] at pH 1.0 and pH 4.5 at λ 520 nm (VIS max) and 700 nm

1   optical path length (cuvette widths) (1 cm)

ε absorption coeffecient of cyanidin 3-glucoside (26 900 litre cm^{-1} mol^{-1})

M anthocyanin molar weight (450 g mol^{-1})

DF total dilution (at extraction * at photometrology ...)

c_{ACY} final concentration of monomeric anthocyanins (g litre^{-1} or g kg^{-1} of fresh weight)

The degree of elimination of anthocyanins was determined by the degradation anthocyanins index (DI) which is used in the food industry to monitor and evaluate the quality of fruit juices, stewed fruit, etc. [8] There is also the possibility to access the degradation anthocyanins degree during processing and storage.

Fresh juices which contain anthocyanins have degradation index around 1.0. With values of 6-7 they are already significantly brown.

The method is based on measurement of the absorbance of solutions at pH 1 and pH 4.5 in the absorption maximum from 510 to 530 nm, and degradation index is calculated by means of this formula (3):

$$DI = \frac{A_{1,0}}{A_{1,0} - A_{4,5}} \qquad (3)$$

$A_{1,0}$ absorbance at pH 1.0

$A_{4,5}$ absorbance at pH 4.5

The colour changes were determined by measuring the fabric on a Datacolor remission spectrophotometer in the range of 380-760 nm visible spectrum which registers the light reflected from the fabric and gives information about colour coordinates in the XYZ space, calculated on the value of the CIE-Lab values and K/S according to the relationship (4):

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \qquad (4)$$

ΔE shade tolerance (total colour difference)

ΔL variation in luminance (black / white)

Δa red (+) / green (-) shift of the axis

Δb yellow (+) / blue (-) shift on the axis in the colour space CIE-Lab

The values of K/S are based on the Kubelka-Munk function relation for systems in which more than 50% of incident light is reflected and less than 20% of incident light passes through the material (paper, textile fibers). [9]

The Kubelka-Munk function (5) is analogous to the Lambert-Beer law used to measure solutions in the coloured surfaces. For lighter shades is applicable (as with weaker colour solutions) a linear dependence of the share of coefficients K / S on the concentration of dye in the fiber (6).

$$\frac{K}{S} = \frac{(1-R)^2}{2R}$$ (5)

K absorption coefficient

S scattering coefficient

R degree of remission (1 = absolute reflection, 0 = total absorption of light of a wavelength)

$$\frac{K}{S} = kc$$ (6)

K experimentally determined constant [g mol^{-1}]

C dye concentration in the fiber [mol g^{-1}]

2   Materials

For the experiments juices were pressed from fruits of elder (*Sambucus nigra),* cranberry blueberry (*Vaccinium myrtillus*) and aqueous extract of grape berry skins (*Vitis vinifera*) of blue table grapes Alden.

A number of buffer solutions with increasing pH of 1 to 10 (KCl / HCl, citrate, phosphate, acetate and borate buffer) was prepared from the chemicals. Solutions 0.1 M of sodium hydroxide and 0.1 M of sodium carbonate were also prepared.

3   Experimental part and discussion

3.1   Content of anthocyanins in the tested material

Tab. 1 shows the contents of anthocyanin in the tested juice from elderberries, blueberries and aqueous extract of blue grape skins. High levels of anthocyanins content in the grape skins are of good promise for further research into the use of this material given the considerable volumes of waste from wine production which could be further exploited. Tests were done on fresh blue grape and it should be noted that the content of anthocyanins in the waste material is still quite high. The amount of anthocyanins differs according to the degree of maceration and fermentation. [10]

Tab. 1: *Contents of anthocyanins in the measured materials*

Plant	pH 1.0 A_{520}	pH 1.0 A_{700}	pH 4.5 A_{520}	pH 4.5 A_{700}	Total absorbance	Dilution (DF)	c ACY [g litre^{-1}] *[g kg^{-1}]
Elderberries	0,1999	0,0189	0,0783	0,0233	0,1260	100	0,211
Blueberries	0,1555	0,0000	0,0828	0,0552	0,1279	1000	2,140
Blue grapes	0,2476	0,0514	0,0820	0,0313	0,1455	3000	*7,301

Source: Own

3.2   Measuring of the absorption spectra of anthocyanins at different pH

The plant anthocyanins are not a very stable substance. They show a higher stability in acidic pH - in this state they are often used in the food industry for colorisation of jams, juices and yogurts. However, numerous wine studies show that even in an acidic environment red wine (pH around 3.6) anthocyanins are subject to degradation over several months.They become stable only during ripening of wine when it comes to copigmentation with tannins. The *Fig. 3* shows the change in colour of anthocyanins in various acidic environments. Although the spectra obtained from various juices are a little different, for all of them an absorption of maximum around 520 nm in strongly acidic conditions is typical.
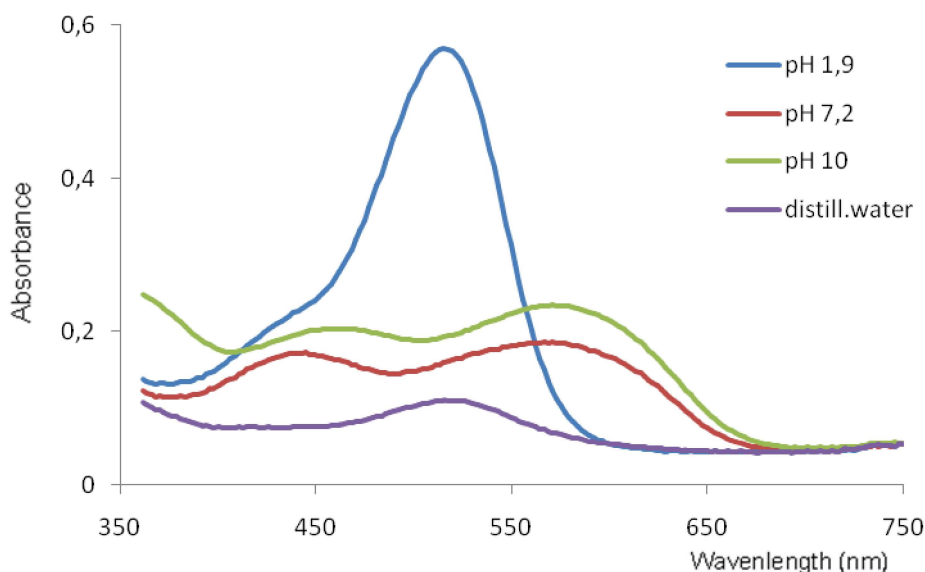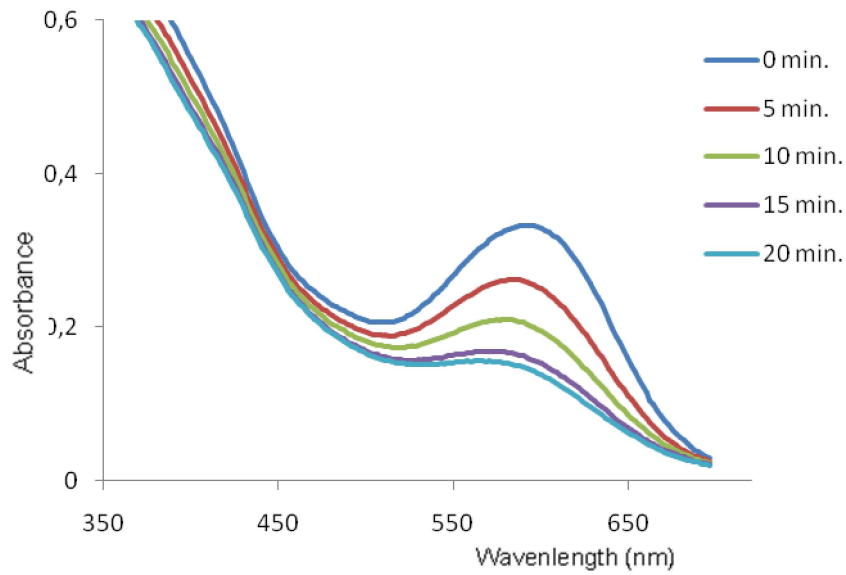

Source: Own
Fig. 3: *Anthocyanins absorption spectra at different pH (blueberry)*

3.3   Degradation of anthocyanins in alkaline medium

The alkaline environment is absolutely devastating for anthocyanins, the yellow chalcones are formed with increasing pH in the order of minutes to seconds. *Fig. 4* shows the rate of degradation of anthocyanins from elderberry at pH 12. In terms of chemical kinetics it is the first order chemical reaction. Based on the linear dependence of concentration and absorbance of the solution and the rate of change of absorbance there were calculated anthocyanins degradation rate constants and their degradation half-lives (7) - (13)

Source: Own
Fig. 4: *Degradation of anthocyanins in pH 12 (elderberry)*

According to Lambert-Beer law the absorbance is linearly dependent on the concentration so it can be replaced in the kinetic equation of chemical decomposition by this concentration: (7)

$$\frac{-dA_0}{dt} = kA \tag{7}$$

After the separation of variables is integrated the equation (8) (9)

$$\int \frac{-dA_0}{A} = -k \int dt \tag{8}$$

$$\ln A = -kt + C \tag{9}$$

and the integration constant is calculated from the absorbance values at time $t = 0$ (10)

$$C = \ln A \quad \text{and} \quad \ln A = -kt + \ln A_0 \tag{10}$$

So rate constant is (11)

$$k = \frac{\ln A_0 - \ln A}{t} \tag{11}$$

The half-life of decomposition is characterized by the relationship (12)

$$A_{1/2} = \frac{1}{2} A_0 \tag{12}$$

and then (13)

$$t_{1/2} = \frac{\ln 2}{k} \tag{13}$$

k rate constant $[s^{-1}]$

A absorbance of the solution

A_0 initial absorbance at time t_0

t time [s]

$t_{1/2}$ half-life decomposition of anthocyanins, i.e. time in which a half of the original amount of pigment decomposes [s]

Tab. 2 compares the kinetics of decomposition in a black elderberry pigment at pH 11, 12 and 13 (absorbance is measured at 520 nm which is a typical wavenlength for anthocyanins).

***Tab. 2:** Decomposition kinetics of anthocyanins (elderberry)*

Time [min]	pH 11 A_{520}	pH 12 A_{520}	pH 13 A_{520}
0	0,1989	0,2104	0,2031
5	0,1973	0,1913	0,1327
10	0,1955	0,1740	0,0871
Rate constant [s^{-1}]	$2,57*10^{-5}$	$3,20*10^{-4}$	$1,42*10^{-3}$
Half-life of decomposition	7,5 h	36 min.	8 min.

Source: Own

3.4   Thermal degradation of anthocyanins

Anthocyanins undergo heat treatment e.g. in the preservation of food and if they are to be used for dyeing textiles which takes place for the best possible extraction of the dye from the bath and for dye penetration into the fibers in a time-temperature mode where the colouring bath is brought to boiling point, the extraction of dyes for fiber as well as the thermal degradation of anthocyanins is parallel. The thermal stability of individual anthocyanin species varies as shown in the *Tab. 3*. The content of anthocyanins and degradation indices (DI) were measured in the material at the beginning and during boiling after 1, 2 and 4 hours in small closed containers.

According to *Tab. 3* the content of anthocyanins in the bath decreases at various plant juices at different rates, the most significant decrease in anthocyanins was observed in blueberries – after the first hour of boiling the contents dropped to almost half of the original amount. The elderberry seems in the first hour of boiling still relatively stable, the berry then shows a drastic decrease of anthocyanins. The blue grape extract which contains mainly glucosides of malvidin and cyanidin seems to be most resistant to thermal degradation although according to the literature malvidin itself is subject to thermal degradation much easier than cyanidin. Although the mechanisms of thermal degradation of anthocyanins is not fully explored it is associated with the emergence of chalcones, dihydroflavonol and acids with one aromatic ring (benzoic and cinnamic acid) and also dihydroflavonols and catechins. So most produce colourless products. [5]

Tab. 3: *Thermal degradation of anthocyanins*

Boiling time [hours]	Elderberries c ACY [g litre⁻¹]	Elderberries DI	Blueberries c ACY [g litre⁻¹]	Blueberries DI	Bluegrapes c ACY [g kg⁻¹]	Bluegrapes DI
0	0,296	1,28	1,037	1,12	6,336	1,12
1	0,259	2,63	0,603	1,23	5,392	1,21
2	0,142	6,59	0,359	1,43	4,016	1,36
4	0,062	6,74	0,181	2,04	2,294	2,44

Source: Own

3.5 Decomposition of anthocyanins by exposure to light

Tab. 4: *Light degradation of anthocyanins on textiles*

Plant / fabric	L*	a*	b*	K/S	Fastness to light [degree of blue scale]
Elderberries / cotton	67,82	7,28	5,22	0,61	1
Elderberries / wool	41,19	12,33	8,14	4,00	1 - 2
Blueberries / cotton	53,24	6,33	-6,32	2,14	1 - 2
Blueberries / wool	32,57	12,42	2,95	9,88	3
Blue grapes / cotton	43,40	11,42	-5,29	3,23	2
Blue grapes / wool	28,18	12,63	2,79	10,19	3

Source: Own

The decomposition of anthocyanins by the light has not been tested directly on solutions but on the fabrics dyed with anthocyanins. The tests were performed on the device UVCON (Atlas type UC-327-2) which combines the energy of fluorescent radiation in the wavelength range below 250 nm, and the water condensation and temperature (50 °C). Samples were irradiated together with standard which represents so called blue scale. It is a series of eight-speed colour blue woolen cloth when the next dye always decays slower than the previous one. The worst light fastness value is 1, level 6-8 represents an excellent light stability.

The test results of anthocyanins resistance to UV radiation are summarized in *Tab. 4* which is a comparison of cotton and wool fabrics dyed with tested material that was exposed to UV radiation. It is evident that the lowest resistance (grade 1-2 of the blue scale at which the dye has decayed only over several hours) showed a lighter colour shade, the deepest colour ones (dyed wool fabric and blueberry extract from the skins of blue grapes) survived stage 3, which is in the case of clothing fabrics an insufficient lightfastness value.

Conclusion

Though the colour changes of anthocyanins appear as an interesting phenomenon, in the case of dyeing textiles they are causing problematic instability shade. Sensitivity to pH, the degradation effects caused by higher temperature and poor light fastness are limiting factors for their use in textile dyeing. Nevertheless, they represent a readily available material in the form of thousands of tons of wine and canning waste a year. This material is already

experimentally used − for example as a source of tannins which are added to synthetic resins and adhesives [11] [12] and it is also sometimes used for dyeing textiles. [10]

In our country the remnants from blue grape are plowed back in the vineyards although they could be gently used i.e. anthocyanins and some tannins could be gently extracted. But as shown by the experiments, for using anthocyanins in textile dyeing one would first need to resolve the problem of insufficient lightfastness.

Acknowledgements

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Ing. Hana Křížová, doc. Ing. Jakub Wiener, Ph.D.

BAREVNÉ ZMĚNY A NESTABILITA ANTOKYANŮ

Antokyany jsou rostlinná barviva, která jsou zajímavá především svými barevnými změnami v různě kyselém prostředí. Pro svou snadnou dostupnost a relativní zdravotní nezávadnost jsou využívána jako koloranty v potravinářském průmyslu, v potravě se uplatňují jako přirozené antioxidanty a historicky též nacházely použití v barvení textilu. V souvislosti s obecně vzrůstajícím zájmem o ekologii a přírodní látky by mohl být využíván jako vydatný a laciný zdroj antokyanů např. odpad z produkce červeného vína.

V této práci byla testována rychlost rozkladu antokyanů ve šťávě z borůvek, černého bezu a v extraktu z výlisků modré révy působením alkalického prostředí, vysoké teploty a UV záření.

FARBVERÄNDERUNGEN UND UNBESTÄNDIGKEIT DER ANTOCYANE

Anthocyane sind pflanzliche Färbemittel, die vor allem mit ihren Farbveränderungen in verschieden sauerer Umgebung interessant sind. Für ihre leichte Zugänglichkeit und einer relativ gesundheitlichen Einwandfreie werden sie als Kolorante in der Lebensmittelindustrie genutzt. In der Nahrung kommen sie zur Geltung, als natürliche Antioxidante und historisch fanden sie Anwendung bei der Färbung von Textilien. Im Zusammenhang mit der allgemein wachsenden Interesse an Ekologie und Natursubstanzen, könnte als ergiebige und billige Quelle der Anthocyane, zum Beispiel Abfall von der Produktion von Rotwein sein.

In der Arbeit wurde die Zersetzungsgeschwindigkeit der Anthocyane an dem Saft der Heidelbeeren, schwarzen Holunder und dem Extrakt der Presslinge der blauen Rebe getestet, durch Wirkung der alkalischen Umgebung, hoher Temperatur und UV Strahlungen.

ZMIANY KOLORU I NIESTABILNOŚĆ ANTOCYJANÓW

Antocyjany są barwniki roślinne, które są interesujące przede wszystkim zmiananie koloru w różnych kwaśnych środowiskach. Dlatego są często stosowane w przemysle spożywczym, gdzie zwiększają zawartość barwnika w produktach końcowych, w diecie są wykorzystywane jako naturalne przeciwutleniacze i w czasach historycznych były wykorzystywane do barwienia wyrobów włókienniczych. Dlatego w związku z tym, ze jest szerokie zainteresowanie o ekologii i substancje naturálne, mogą być stosowane jako tanie i obfite źródło antocyjanów takich jak odpady produkcji czerwonego wina.

W tym badaniu przetestowany szybkości degradacji antocyjanów w soku z borówki, czarnego bzu i wyciąg z winogron purpurowych w zasadowym środowisku, wysokiej temperaturze i promieniowaniu UV.