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THERMAL STABILITY OF ANTHOCYANINS FROM COMMON DOGWOOD (*CORNUS SANGUINEA* L.) FRUITS

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ABSTRACT. The Common dogwood (*Cornus sanguinea* L.) fruits are known for their high antioxidant activity, partially due to their anthocyanin content. The aim of this study is to examine the storage stability of anthocyanins from these fruits, at different temperatures (2°C, 22°C and 75°C). The results show that thermal degradation reaction of anthocyanins from Common dogwood fruits complies with first-order reaction kinetics. Degradation parameters, such as half-life $t_{1/2}$, reaction rate constant k and activation energy Ea values were determined. Anthocyanin extract stored at 2°C presented the highest stability with a degradation constant rate of $1.0 \cdot 10^{-3}$ h^{-1}. The calculated thermal degradation activation energy of the investigated fruits extract was Ea = 40.35 kJ/mol.

Keywords: *Cornus sanguinea* L.*,* anthocyanins, degradation kinetics

INTRODUCTION

The consumer acceptability and the appearance of food products are directly affected by their colour. Recent studies alert on the severe toxicity of synthetic dyes used in the food industry [1, 2]. Consequently, the food industry is now searching for natural food colorants as a safety alternative to the artificial ones. Plant sources are widely used to develop food additives that have both colouring and health beneficial properties. Anthocyanins, betacyanins, chlorophylls and carotenoids are the main groups of natural pigments. As red synthetic dyes are the most toxic and some of them, such as Amaranth Red and Ponceau GR, banned in the USA and Europe, finding new natural sources for red pigments significantly increased lately. Anthocyanins are the largest class of red water soluble natural colours that offer alternatives to synthetic toxic dyes.

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Anthocyanins are naturally occurring flavonoids which play an important role in the attractive colour (red, pink, orange, purple and blue) of fruits, flowers, leaves and vegetables. Studies have shown that they also possess a number of potential health benefits for humans, such as antioxidant, anticancer, anti-diabetic, anti-inflammatory and anti-obesity properties [3-5]. Many plants such as red grapes, red cabbage, Roselle calyx, blood orange are the main sources of anthocyanins for the food industry. All these plants present a high economical value, so looking for alternative anthocyanin sources is of great interest. Less consumed fruits, such as Black elderberry, European cranberrybush, Cornelian cherries gained lately attention for this purpose [6, 7].

Common dogwood (*Cornus sanguinea* L.) belongs to *Cornaceae* family, being native to most of Europe and Western Asia. The fruit is a black berry, containing a single seed, eaten especially by some mammals and birds [8]. Reported studies on *Cornus sanguinea* L. fruits and their properties are limited. Dogberries extracts are known to possess a high antioxidant activity, even higher than *Ginkgo biloba* extract [8]. Anthocyanins rich dogberries extract can be regarded as promising candidate for value-added ingredients for a variety of functional foods and nutraceuticals.

The colour of anthocyanins depends essentially on their different structural forms, strongly related to the pH value.

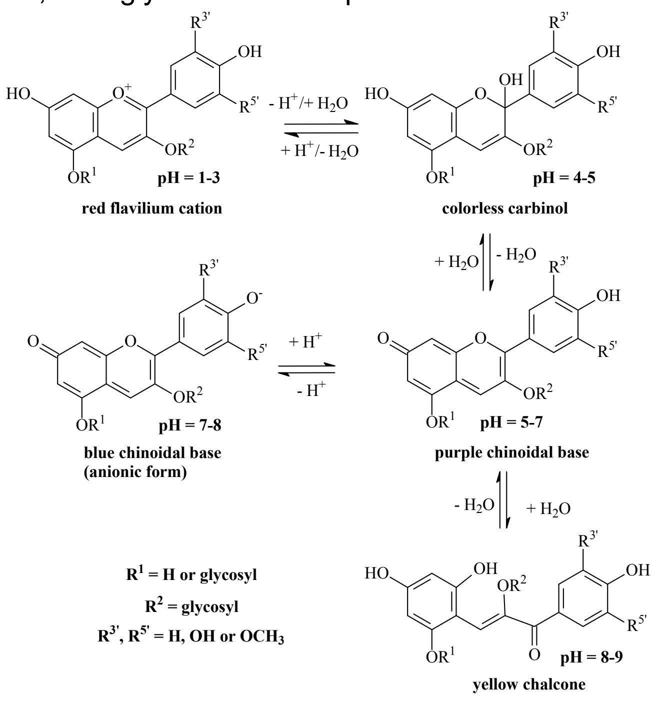

Figure 1. Chemical structures of anthocyanins as a function of pH

A mixture of equilibrium forms of anthocyanins: red flavylium cation, colourless carbinol, purple quinoidale base and yellow chalcone coexists (Figure 1) at pH values between 3 and 6 [9].

The use of anthocyanins as colorants in food systems is limited by their low stability. The degradation reaction of these red pigments results in appearance of undesirable colourless or brown compounds which affect the physicochemical properties of foods. The most important factor that affects the stability of anthocyanins is the temperature. Colour degradation can be observed when the temperature increases [10, 11].

Thus, investigation of anthocyanin degradation and measurement of their content at various intervals of storage is useful for the food industry.

However, to date, no information is available in the literature on the degradation kinetics of dogwood berries anthocyanins. Consequently the aim of this study was to evaluate the effect of temperature on the stability of these natural pigments and the accurate determination of the kinetic parameters for their degradation process in order to use *Cornus sanguinea* L. berries as source for natural red colorants.

RESULTS AND DISCUSSION

The total anthocyanin content of Common dogwood fruits extract was determined using the pH differential method developed by Giusti and Wrolstad [12]. This method measures differences in absorbance of the anthocyanin extracts at two different pH values – pH 1 and 4.5 - resulted as structural change of the red flavylium cation, the chemical form which predominates at pH = 1 in the colorless carbinol, the major structural form at pH = 4.5. This method allows an accurate determination of total monomeric antocyanin content and is widely used for measuring this parameter in fruit extracts. The pigment content was calculated from the experimental data according equation (1) and expressed as cyanidine-3-glucoside (Cy-3-Glu) equivalents:

$$TA = \frac{A \cdot MW \cdot DF \cdot 1000}{\varepsilon \cdot l} \quad (1)$$

where: TA = total anthocyanin content (mg/L)

A = absorbance, calculated as: (equation 2)

$$A = (A_{pH\ 1.0} - A_{pH\ 4.5})_{506\ nm} - (A_{pH\ 1.0} - A_{pH\ 4.5})_{700\ nm} \quad (2)$$

MW = molecular weight (g/mol)

DF = dilution factor

l = path length (cm)

ε = molar extinction coefficient (L/mol/cm)

1000 = conversion factor from gram to milligram.

The initial content of the total monomeric anthocyanins in the dogberries extract was 114.8 mg Cy-3-Glu equivalents / L.

The changes in the total anthocyanin content during storage of Common dogwood fruits extracts at different temperatures was investigated in order to establish the degradation reaction kinetic order as well as the kinetic parameters of this reaction. The graphical method was used to determine the order of reaction for the degradation of anthocyanins by action of temperature. The degradation process of anthocyanins was strongly influenced by temperature. The decreases of the anthocyanin content of the investigated extracts during storage at 2°C and 22°C were plotted as a function of time (Figure 2).

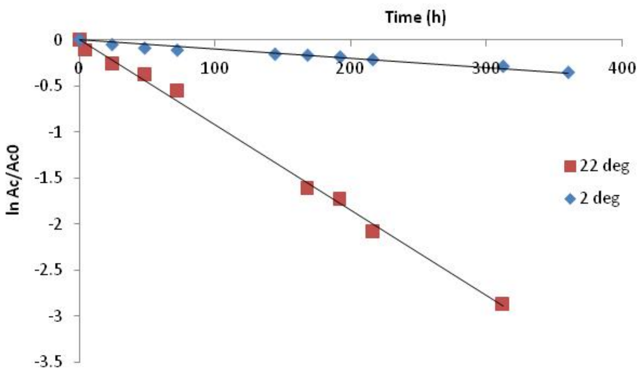

Figure 2. Influence of temperature on the degradation reaction of anthocyanins from Common dogwood fruits extract at 2°C and 22°C

The high linear correlation coefficients R^2 (Table 1) clearly indicate that the degradation of dogberries anthocyanins from aqueous extract stored at low temperatures followed first order reaction kinetics.

Table 1. Kinetic parameters of the degradation process of anthocyanins from dogberries extract at different temperatures

Temp. (°C)	k (h^{-1})	$t_{1/2}$ (h)	R^2
2	0.001	693	0.9467
22	0.0093	74.52	0.9956
75	0.0504	13.75	0.9903

This kinetic model was reported by other researchers [13-16]. The monomeric anthocyanin content decreased 80% when the extract was stored at room temperature (22°C), while storage at refrigerator (2°C) resulted in cca. 15% loss of the anthocyanins in one week. Increasing the storage temperature from 2°C to 22°C caused a 9.3 fold increase of the reaction rate constant, from $k_{2°C} = 1 \cdot 10^{-3}$ h^{-1} to $k_{22°C} = 9.3 \cdot 10^{-3}$ h^{-1} (Table 1).

As expected, the anthocyanins degradation process was greater when the extract was exposed to elevated temperatures (75°C). Figure 3 presents the linear regression of the anthocyanin content from the extract stored at the above mentioned temperature. The half-life value of the degradation reaction determined from the experimental data was 13.75 h, the process being 50 fold faster than at 2°C. The decrease of anthocyanins content over time fitted a first order kinetics with a good regression coefficient.

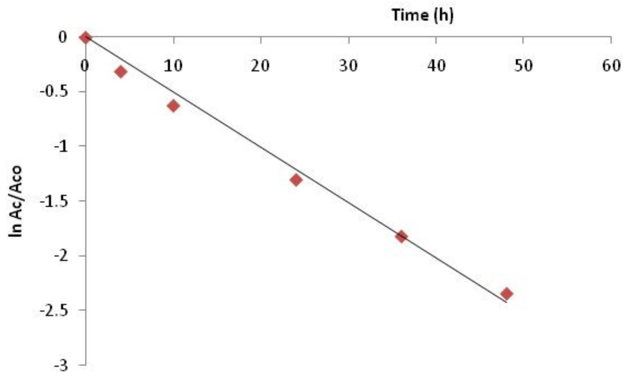

Figure 3. Influence of temperature on the degradation reaction of anthocyanins from Common dogwood fruits extract at 75°C

The temperature dependence of the degradation process was described by the Arrhenius equation (3):

$$k = K_0 e^{-Ea/RT} \tag{3}$$

where: k = rate constant (h^{-1})

K_0 = frequency factor (h^{-1})

E_a = activation energy (kJ/mol)

R = universal gas constant (8.314 J/mol/K)

T = absolute temperature (K)

Plotting lnk as a function of 1/T (Figure 4) the Arrhenius activation energy Ea was calculated. The activation energy for the dogberries anthocyanins degradation Ea = 40.35 kJ/mol was comparable to the values found in the literature. Other researchers reported similar values for the degradation process of plums anthocyanins (Ea = 37.48 kJ/mol) [17] but also higher values were found for anthocyanins from other sources, e.g. 65.32 kJ/mol for grape pomace [18] and 58.55 kJ/mol for Cornelian cherries pigments [7], proving that the natural colorants from *Cornus sanguinea* L. fruits are less susceptible to degradation by exposure to elevated temperatures.

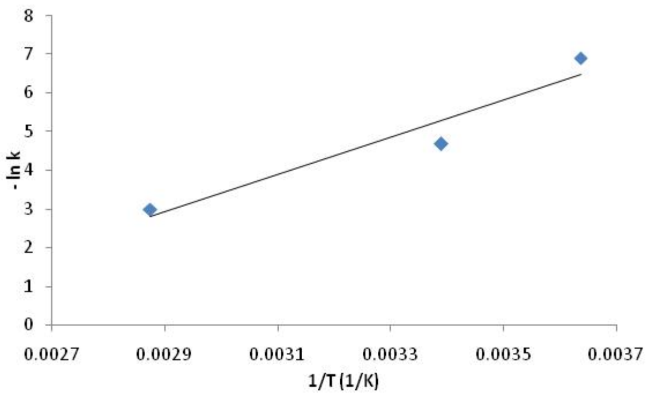

Figure 4. The Arrhenius plots for degradation of anthocyanins in dogberries extracts

All these results clearly indicate that low storage temperatures are required to minimize the loss of anthocyanins from Common dogwood fruits.

CONCLUSIONS

The results from the present research provide accurate information regarding the kinetic parameters of the degradation process of anthocyanins from *Cornus sanguinea* L. fruits. The reaction kinetics of this process fitted to a first-order model in all the investigated conditions. Increasing temperature resulted in higher degradation rate constants values which ranged from $1 \cdot 10^{-3}$ h^{-1} to $50.4 \cdot 10^{-3}$ h^{-1}. The determined Ea value for the investigated process suggests that the anthocyanins from dogberries are rather stable, fact that indicates that these berries can be used as an important alternative source of natural red colorants for the food industry.

EXPERIMENTAL SECTION

Chemicals and reagents

All chemicals and reagents were purchased from Merck (Darmstadt, Germany), were of analytical grade and were used without further purification. A TYPDP1500 Water distiller (Techosklo LTD, Držkov, Czech Republic) was used to obtain the distilled water.

Plant material

Samples of Common dogwood fruits (dogberries) were harvested in September 2014 from Cluj-Napoca, Romania. Fruits were packed in polyethylene bags and kept frozen at −18°C.

Extract preparation

The frozen dogberries were crushed in a mortar. Sixty g of fruit puree were transferred to an Erlenmeyer flask and 250 mL of distilled water were added. After stirring for 1 h at room temperature, the mixture was filtered under vacuum. The filtrate was quantitatively transferred to a 250 mL volumetric flask and made up to the mark with distilled water.

Determination of anthocyanin content

The crude fruit extract was mixed with the corresponding buffer solution (potassium chloride buffer, pH = 1.0 or sodium acetate buffer, pH = 4.5).

Aliquots of the extract (5 mL) were transferred to a 10 mL volumetric flask and made up to 10 mL with corresponding buffer (pH = 1 and pH = 4.5) and allowed to equilibrate for 15 minutes. Three identical samples were prepared for each pH value and anthocyanin content was measured one time for each replicate.

Visible spectra of the obtained solutions were recorded by scanning the absorbance between 400 and 700 nm, in order to determine $\lambda_{VIS\ max}$ of the samples, using an UV-VIS Perkin Elmer Lambda 25 double beam spectrophotometer. Quartz cuvettes of 1 cm pathlength were used. Absorbance readings were made against distilled water as a blank.

The absorbances of the samples were measured at 512 nm ($\lambda_{VIS\ max}$) and 700 nm.

Pigment content was calculated as equivalents of cyanidin-3-glucoside using MW = 449.2 g/mol and ε = 26,900 L/mol/cm.

Degradation studies

The extract was divided into 50 mL portions and stored in the dark, well capped to avoid evaporation, at 2°C (in refrigerator), at room temperature (22°C) and in a thermostatic water bath, preheated to 75°C, respectively (±1 °C). At regular time intervals, samples were removed and analysis were conducted immediately.

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