STUDIA UBB CHEMIA, LVI, 4, 2011 (p. 243 - 248)

DEGRADATION KINETICS OF ANTHOCYANINS FROM CRUDE ETHANOLIC EXTRACT FROM SOUR CHERRIES

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ABSTRACT. Storage stability of anthocyanins in ethanolic extract from dried sour cherry (*Prunus cerasus L.*) fruits was studied. Total anthocyanins content was determined using the pH differential method. Results indicate that the storage degradation of anthocyanins followed first-order reaction kinetics.

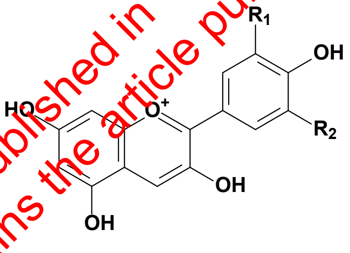
Keywords: *sour cherry extract, anthocyanins, degradation kinetics*

Article published in Volume 56 (LVI), no. 4, 2011, pp. 243-248 is withdrawn.
Remains the article published in Volume 56 (LVI), no. 2, 2011, pp. 189-194.

INTRODUCTION

Anthocyanins are a group of naturally occuring phenolic compound, which have an important role in the color quality of many flowers, fruits, vegetables and related products derived from them. Thus, measurement of anthocyanin content and investigation of their degradation is very useful for the food industry.

The anthocyanins are glycosides of anthocyanidines. Up to now, more than 540 anthocyanins and 23 anthocyanidins were reported [1] of which only six are the most common in vascular plants (Figure 1).



R_1	R_2	Anthocyanidine
H	H	Pelargonidin
OH	H	Cyanidin
OH	OH	Delphinidin
OCH_3	H	Peonidin
OCH_3	OH	Petunidin
OCH_3	OCH_3	Malvidin

Figure 1. The six more commun anthocyanidins structure

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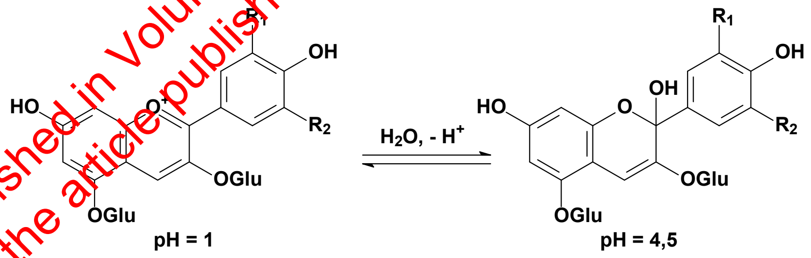
The most commonly found in nature are the glycoside derivatives of the three non-methylated anthocyanidins: cyanidin, delphinidin and pelargonidin.

The isolation and determination of anthocyanin content have been investigated by many authors [2-5]. The anthocyanin content of sour cherries varies largely with genotipe. The most abundant anthocyanins found in the fruits of *Prunus cerasus* are the glucoside forms of cyanidin (Cy) and peonidin (Pn) [6]: Cy-3-glucoside, Cy-3-glucosylrutinoside, Cy-3-sophoroside, Cy-3-rutinoside, Cy-3-xylosylrutinoside, Pn-3-glucoside, Pn-3-rutinoside, Cy-3-gentobioside.

Anthocyanins, like other polyphenolic compounds found in fruits, are known for their antioxidant activity. This plays a vital role in the prevention of cancer, diabetes, cardiovascular and neuronal illnesses [7]. There are several studies focused on the effect of anthocyanins in cancer treatments [8] and also on their antiinflammatory and plateled inhibitory effects [9]. Anthocyanins content in fruits and vegetables is also reported as responsible for their antimicrobial properties [10].

Anthocyanins are very unstable and susceptible to degradation [11]. Their stability is affected by storage and processing conditions, including pH, temperature, light, solvent, oxygen.

Substantial quantitative and qualitative information can be obtained from the spectral characteristics of anthocyanins. Anthocyanin pigments undergo reversible structure transformation as a function of pH, which can be measured using optical spectroscopy. The colored flavilium cation form predominates at pH = 1, while, at pH = 4.5 the colorless hemiketal form is the most stable (Scheme 1).



Scheme 1

The pH differential method is a rapid and easy procedure to determine the total anthocyanins content from various sources [12]. The method is based on the reaction depicted in Scheme 1 and permits an accurate measurement even in the presence of other interfering compounds.

The aim of this study was to determine the degradation kinetic parameters of sour cherry fruits anthocyanins in ethanolic extract during storage at room temperature (25°C).

RESULTS AND DISCUSSION

The experiments were carried out on refrigerated sour cherry fruits (commercially available) which were allow to stay at room temperature until complete drying occurred.

Total anthocyanin content was determined using the pH-differential method [12], using two buffer systems: potassium chloride buffer (0.025 M, pH = 1.0) and sodium acetate buffer (0.04 M; pH = 4.5). The extract was mixed with the corresponding buffer and the absorbance of the samples was measured at 525 nm ($\lambda_{VIS\ max}$; see Figure 1) and 700 nm (for haze correction).

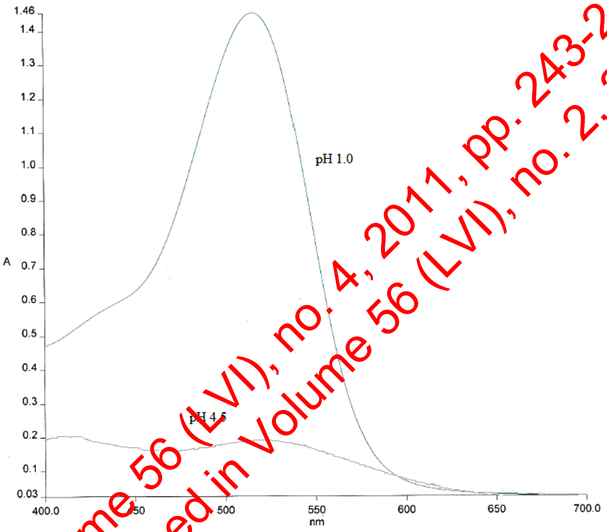


Figure 1. UV-VIS spectra of anthocyanins in buffer solutions at pH =1 and pH =4.5

The following equation was used to calculate the total anthocyanins as cyanidin-3-glucoside equivalents [12]:

$$TA = \frac{A \cdot M \cdot F \cdot 1000}{\varepsilon \cdot l} \tag{1}$$

where:

TA = total anthocyanins content (mg/l)

A = absorbance, calculated as;

$$A = (A_{pH\ 1.0} - A_{pH\ 4.5})_{525\ nm} - (A_{pH\ 1.0} - A_{pH\ 4.5})_{700\ nm} \tag{2}$$

M = molecular weight

F = dilution factor

l = pathlenght (cm)

ε = molar extinction coefficient

1000 = conversion factor from gram to miligram

All measurements were done in triplicate (n = 3).

The anthocyanin content during storage at 25^0C was plotted as a function of time (Figure 2). The linear dependence of ln [A] = f(t) demonstrates that the degradation process of monomeric anthocyanins follows first order reaction kinetics, results which are in agreement with those from the previous studies, reporting a first order reaction model for the degradation of these flavonoidic compounds from various sources [13,14].

The kinetic parameters were calculated using the following equations:

$$\ln[TA] = \ln[TA_0] - kt \tag{3}$$

$$t_{1/2} = \frac{0.693}{k} \tag{4}$$

where:

[TA] = total anthocyanin content (mg/l) at time t
$[TA_0]$ = initial total anthocyanin content (mg/l)
k = reaction rate constant (h^{-1})
t = reaction time (h)
$t_{1/2}$ = half-life (h)

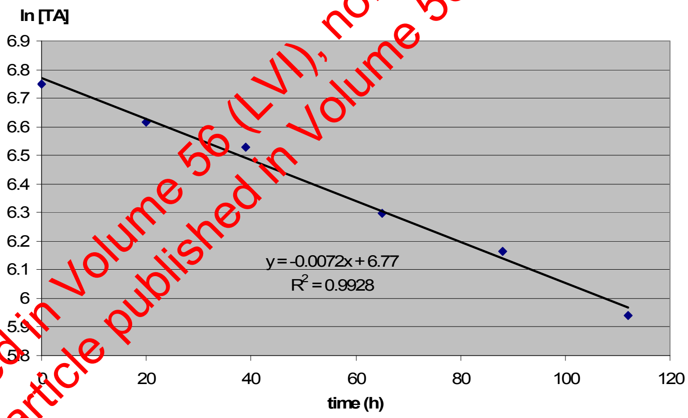


Figure 2. Degradation of anthocyanins in sour cherries ethanolic extract during storage at 25^0C

The determined value for the kinetic rate constant was $7.2 \cdot 10^{-3}$ h^{-1} and the half-life value was $t_{1/2}$ = 96.25 h. First order reaction kinetics for the degradation process of anthocyanins in sour cherry concentrates with a 38 days value for $t_{1/2}$ at 20^0C was reported [15]. In comparison, the alcoholic extract of anthocyanins from sour cherries is significantly less stable ($t_{1/2}$ = 4 days).

CONCLUSIONS

The total anthocyanin content in the ethanolic extract of sour cherries was determined (850.97 mg/kg dried fruits, in agreement with literature data [6]) and the degradation process of these compounds during storage at 25^{0}C was investigated. The degradation followed first order reaction model and the kinetic parameters values (k and $t_{1/2}$) were determined.

The extract is less stable than the concentrate juices obtained from sour cherry fruits.

EXPERIMENTAL SECTION

The dried sour cherry fruits were crushed in a mortar. 2 g of fruits were transferred to an Erlenmeyer flask, 50 ml of 96% ethanol and 0.1 ml of concentrated HCl were added and the mixture was stirred for 1 h at room temperature and then filtered. The filtrate was quantitatively transferred to a 100 ml volumetric flask and made up to 100 ml with 96% ethanol. The extract was kept away from light in a thermostated water bath, preheated to 25^{0}C, well capped to avoid evaporation. At regular time intervals, samples were taken and analysis was conducted immediatly.

5 ml of ethanolic extract were transferred to a 10 ml volumetric flask and made up to 10 ml with corresponding buffer (potassium chloride buffer, 0.025 M, pH = 1 and sodium acetate buffer, 0.4 M, pH = 4.5) and allowed to equilibrate for 15 minutes.

The absorbance of each solution was then measured at 525 ($\lambda_{VIS\ max}$) and 700 nm (for haze correction), using a Perkin Elmer Lambda 35 UV-VIS double beam spectrophotometer.

Pigment content was calculated as equivalents of cyanidin-3-glucoside (MW = 442.9 g/mol, ε = 26900 l/mol/cm) [12]. Visible spectra of samples were recorded by scanning the absorbance between 400 and 700 nm. Quartz cuvettes of 1 cm pathlength were used, absorbance readings were made against distilled water as a blanck. All the measurements were carried out at room temperature (~22^{0}C).

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