Anthocyanidins in Babica, Ljutun and Crljenak Kaštelanski grapes, and kinetics of their degradation under different storage conditions

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Summary

Profiles of anthocyanidins (delphinidin, cyanidin, malvidin, pelargonidin and peonidin concentrations) of the skin extracts from autochthonous Croatian (Dalmatian) red grape varieties Babica, Ljutun and Crljenak Kaštelanski were analysed using high-performance liquid chromatography. Identification and quantification of aglycones after acid hydrolysis of the anthocyanins was performed using anthocyanidin standards. In all extracts, malvidin was the major anthocyanidin (ranging from 0.2237 $mg \cdot l^{-1}$ to 0.2738 $mg \cdot l^{-1}$), while the extracts of Ljutun and Crljenak Kaštelanski also contained high amounts of delphinidin. Pelargonidin derivatives were detected in all samples and their concentration ranged from 0.0359 $mg \cdot l^{-1}$ to 0.0371 $mg \cdot l^{-1}$. The concentrations of total monomeric anthocyanins and their changes during 70 days of storage under different conditions (at room temperature exposed to the sunlight, at room temperature in the dark, and at +4 °C protected from light) were measured by the pH-differential method. Degradation rates of total monomeric anthocyanins in all extracts exposed to light followed the first order reaction kinetics, and after 70 days there were no monomeric forms in them. The highest degree of anthocyanins stability was recorded for the samples that were kept at +4 °C and protected from light, where only 5–8% of these compounds were degraded.

Keywords

anthocyanin degradation; light; temperature; kinetic reaction; high-performance liquid chromatography

The food production today is almost inconceivable without use of additives, during some phase of food processing, treatment, packaging, transportation and/or storage, due to consumer's expectations that it should taste good and look delightful [1]. Additives are used to recover or to emphasize the original features, to ensure uniformity and to guarantee the quality of food products [2]. They perform various functions in food, e.g. ensure and improve its safety, freshness, taste and texture, prevent oxidation of food components and development of off-flavours, enhance its nutritional quality, etc. Among different groups of additives, the most commonly used are colour additives, which compensate the colour loss due to exposure to different environmental conditions or enhance natural colour of food products. Over the years, the safety of many colour additives has come into question, so there is a growing tendency to use natural colour compounds instead of synthetic ones [3–5].

Anthocyanins belong to the most utilized natural colorants in the food industry, being the widely spread water-soluble pigments of vascular plants. They are distributed in leaves, flowers, berries and/or roots of fruits and vegetables, revealing

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colours from orange and red to various shades of blue and purple [6–12].

In the last few years, interest in use of anthocyanins as an alternative to artificial colorants has increased, due to their health benefits and positive pharmacological properties, like antioxidative, anticancer, anti-inflammatory and anti-obesity properties, vasoprotective effects or their role in the reduction of coronary heart diseases [13–17]. In addition, anthocyanins of red grapes/wines became very important as chemical markers for differentiation of grape cultivars, for detection of hybrid grapes as well as for revealing adulteration of red wines [2, 7, 10, 18–20].

However, these compounds are very susceptible to degradation, what is their major disadvantage because they play a critical role in the colour quality of many fresh and processed foods. Their stability is affected by different factors, such as their structure and concentration, pH of the medium, water activity, presence of different solvents, oxygen, enzymes, sugars, proteins, metallic ions, processing and storage conditions. The knowledge of anthocyanins chemistry, their stability and factors affecting it, as well as of their possible stabilization, is extremely important for the food industry. It should enable minimal degradation of anthocyanins by choosing the appropriate food processing treatment and storage conditions of the products that will overcome the problem of their limited application. The prediction of the kinetics of degradation and changes of anthocyanins quality during the storage are also a matter of a great concern [4, 9, 12, 20–23]. The anthocyanidin profiles of some Croatian red grape cultivars have been described by different authors [24–27], however, as we know, to date, there is no information on their degradation kinetics.

The aim of this study was to investigate anthocyanidin profiles of grape skin extracts from three autochthonous Dalmatian cultivars and the kinetics of their degradation under different storage conditions during 70 days. The established mathematical model enables the prediction of their degradation and could be used in further studies dealing with the potential application of these extracts as natural colorants in food industry.

MATERIALS AND METHODS

Anthocyanidin standards: cyanidin chloride (3,3',4',5,7-pentahydroxyflavylium chloride), delphinidin chloride (3,3',4',5,5',7-hexahydroxyflavylium chloride), malvidin chloride (3,4',5,7-tetrahydroxy-3',5'-dimethoxyflavylium chloride), pelargonidin chloride (3,4',5,7-tetrahydroxyflavylium chloride), high-performance liquid chromatography (HPLC) grade, ≥96 %, were purchased from Extrasynthese (Genay, France). Methanol (HPLC grade, ≥99.9 %) was supplied by Sigma-Aldrich (St. Louis, Missouri, USA); formic and hydrochloric acid from Kemika (Zagreb, Croatia), sodium acetate and potassium chloride were obtained from Alkaloid AD (Skopje, Macedonia). Water was prepared by purification with a Millli-Q water purification system (Millipore, Bedford, Massachusetts, USA). Spectrophotometric measurements were performed on Specord 200 spectrometer (Analytik Jena, Jena, Germany). The HPLC system used was HP 1090 Series II, equipped with UV/Vis photodiode array detector (Agilent Technologies, Santa Clara, California, USA).

Plant materials and extraction of anthocyanins

The wholesome grapes of *Vitis vinifera* L. varieties Babica, Ljutun and Crljenak Kaštelanski, were harvested at the stage of their technological maturity (October, 2013) from the same vineyard located in Kaštela, region of Dalmatia (Croatia). All vines from the vineyard are of the same age (approx. 10 years old) and the identity of the varieties has been confirmed. After the harvest, grapes were transported to the laboratory where berry skins of approximately 500 g of grape berries were manually separated from the pulp. The skins were washed out with cold distilled water and dried by setting between two sheets of filter paper. Ten grams of ground skins homogenized for 1 min in a high speed grinder (Moulinex, Paris, France) were added 150 ml of acidified methanol (methanol : hydrochloric acid = 99.99 : 0.01, v/v). The suspension was stirred (5 Hz) for 15 min with a shaker Vibromix 313 (Tehtnica, Železniki, Slovenia), left for 15 min at room temperature and than filtered through Whatman qualitative filter paper, Grade 4 (Whatman, Maidstone, United Kingdom). The procedure of extraction was performed in triplicate, and the obtained extracts from the same grape variety were combined in total extract. Into each of three tubes (19 × 150 mm), about 25 ml of the total extract were transferred. The tubes were capped tightly and stored under different conditions (at room temperature in the dark, at room temperature exposed to sunlight, and at +4 °C in the refrigerator) for 70 days.

Monomeric anthocyanins

The content of total monomeric anthocyanins was determined by the pH-differential method, as decribed by Giusti and Wrolstad [28]. The

method is based on the structural transformation of the anthocyanin chromophore as a function of pH, and it allows the measurements of total anthocyanins even in the presence of the interfering compounds or degraded pigments. The concentration of total monomeric anthocyanin (c_{MA}) pigments was calculated using the Eq. 1 [28]:

$$c_{MA} = (A \times MW \times DF \times 1\,000) / (\varepsilon \times 1) \quad (1)$$

where A is absorbance of the diluted sample calculated as in Eq. 2, MW is molecular weight of cyanidin-3-glucoside (MW = 449.2 g·mol^{-1}), DF is dilution factor and ε is molar absorptivity (ε = 26.900 l·mol^{-1}·cm^{-1}). Number 1 000 represents a factor for conversion from grams to milligrams, while number 1 represents path length in centimetres [28].

$$A = \Delta A_{1.0} - \Delta A_{4.5} \quad (2)$$

where $\Delta A_{1.0}$ is difference of absorbances ($A_{520} - A_{700}$) at pH 1.0, $\Delta A_{4.5}$ is difference of absorbances ($A_{520} - A_{700}$) at pH 4.5, A_{520} is absorbance measured at 520 nm, A_{700} is absorbance measured at 700 nm.

A_{520} was determined experimentally by recording the sample UV-Vis spectra (from 200 nm to 800 nm) and it presents the wavelength of maximum absorption.

The appropriate DF was determined by diluting the samples with pH 1.0 potassium chloride buffer until the absorbance at 520 nm was within the linear range of the spectrophotometer. DF is calculated as a ratio between the final volume of the sample and the initial one. The sample dilutions, one with potassium chloride buffer, pH 1.0, and the other with sodium acetate buffer, pH 4.5, were prepared using the calculated DF factors.

All absorbance readings were made against distilled water blanks. The samples were measured in triplicate and the results were expressed as average values, in milligrams of cyanidin-3-glucoside equivalents (C-3-gl) per litre of extract.

HPLC analysis

The present cyanidin, delphinidin, malvidin and pelargonidin in extracts were separated, quantified and identified by HPLC using Ultra Aqueous C_{18} column (250 mm × 4.6 mm, 5 μm particle size; Restek, Bellefonte, Pennsylvania, USA) maintained at 30 °C, using the method proposed by the anthocyanidins producer (Extrasynthese). The flow rate was set to 1.0 ml·min^{-1}, while the signal was monitored at 520 nm. A gradient elution with mobile phase consisting of solvent A (water : formic acid, 90 : 10, v/v) and solvent B (methanol) was applied as follows: from 95 % A and 5 % B at 0 min, to 40 % A and 60 % B at 20 min, to 60 % A and 40 % B at 25 min, to 0 % A and 100 % B at 30 min. The compounds were quantified from the areas of their peaks using the multiple point external standard curves, and identified by comparing their retention times and absorption spectra with those acquired for the standards. Sample spiking was used to increase confidence in peak identification. Each sample was injected twice in HPLC system. The obtained data for the calibration curves of standards established by a multi-point calibration method are presented in Tab. 1. The results are expressed in milligrams per litre of extract.

Acid hydrolysis of anthocyanins

Acid hydrolysis to liberate aglycons from anthocyanins was performed according to the modified procedure described by Qin et al. [11]. Test-tube containing 1 ml of grape berry skin extract and 5 ml of 2 mol·l^{-1} HCl was immersed into boiling water bath, held there for 2 h and, after that, immediately cooled in an ice bath. After the cooling, the prepared hydrolysates were analysed by HPLC.

Kinetics of anthocyanin degradation

The data obtained for changes of anthocyanins concentration in grape skin extracts during storage under different conditions were used in modelling

Tab. 1. Chemical structures, high performance liquid chromatography calibration curves and spectral characteristics of delphinidin, cyanidin, pelargonidin and malvidin.

Anthocyanin	Substitution pattern		Retention time [min]	Maximum wavelength [nm]	Equation	r^2
	R1	R2				
Delphinidin	OH	OH	18.9	536	$y = 69587x - 368.41$	0.9988
Cyanidin	OH	H	20.8	527	$y = 59505x - 331.88$	0.9986
Pelargonidin	H	H	22.4	517	$y = 81874x - 420.64$	0.9989
Malvidin	OCH_3	OCH_3	23.4	540	$y = 13661x - 83.494$	0.9982

The concentration of injected standard anthocyanidins solutions ranged from 0.025 mg·l^{-1} to 0.25 mg·l^{-1}.
x - concentration expressed in milligrams per litre; y - peak area in milliabsorbance units (mAU); r^2 - determination coefficient.

of kinetics of anthocyanin degradation. According to the previous studies, anthocyanin degradation follows a first-order reaction kinetics, which could be expressed by Eq. 3 [4, 12]:

$$-\frac{dC}{dt} = kC \quad (3)$$

where C is concentration of anthocyanins expressed in milligrams per litre, t is storage time (in days) and k is the first order degradation rate constant (expressed as reciprocal days).

The reaction rate constant (k) was calculated using Mathcad (PTC, EAG Centar, Zagreb, Croatia) built-in function – Odesolve (Adams/BDF method). The half-life ($t_{1/2}$) of the reaction, i.e. the time needed for 50% degradation of anthocyanins, was calculated using Eq. 4 [4, 12].

$$t_{1/2} = -\ln(0.5)\, k^{-1} \quad (4)$$

RESULTS AND DISCUSSION

Anthocyanidins in Babica, Ljutun and Crljenak Kaštelanski grape skins extracts

The anthocyanidin profiles of prepared acidified methanolic extracts of the skins of autochthonous Dalmatian grape cultivars Babica, Ljutun and Crljenak Kaštelanski were determined by means of HPLC. The chromatograms were recorded at 520 nm because anthocyanins have a typical absorption band in the region of 490–550 nm [28]. The calibration curves and spectral characteristics obtained for standard anthocyanidin compounds (delphinidin, cyanidin, pelargonidin and malvidin) are given in Tab. 1. The anthocyanidins (aglycons) are the basic chemical structures of the anthocyanins (glycoside form, bound to a sugar). They are flavonoids with cationic flavylium structure, consist of an aromatic ring (A) bound to a heterocyclic ring that contains oxygen and is bound by a carbon-carbon bond to the second aromatic ring (B). Anthocyanins have diverse molecular structures, with the differences between the individual anthocyanins related to the number and position of hydroxyl and methoxyl groups in the aromatic ring B, as well as sugar molecules bound to a heterocyclic ring. The most common anthocyanins are usually conjugated to sugars (usually glucose), hydroxycinnamates and organic acids (malic or acetic acid). Although conjugation can take place on carbons 3, 5, 7, 3', and 5', it occurs usually at the C3 position [7]. More than 500 different anthocyanins and 23 anthocyanidins have been reported, but only six of them are common in vascular plants. Their elution, at an already well known order, was also confirmed by this study: delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin (Tab. 1), and it can be predicted on the basis of the number of hydrophilic phenolic and hydrophobic methoxyl groups in the aromatic ring B [6].

As there are commonly six anthocyanidins that occur in nature, the HPLC analysis of the complex samples could be simplified by the acid hydrolysis of the anthocyanins. This treatment removes sugars and acyl-groups from the anthocyanins and form an aglycon – anthocyanidin, which can be detected and confirmed as the parent compound [28]. The HPLC chromatograms for investigated grape skin extracts before and after hydrolysis are shown on Fig. 1. Prior to hydrolysis of grape skin extracts, nine significant peaks were detected in all samples (Fig. 1A–1C). Upon the hydrolysis, the aglycons were formed, hence only five peaks in the chromatograms appeared (Fig. 1D–1E). The small peak at a retention time (*RT*) of 17 min was probably the residue of the non-hydrolysed malvidin-3-glucoside, which is known to be the dominant anthocyanidin compound in red wine/grapes [19]. The anthocyanidins in the investigated samples were identified by comparison of their retention times, elution order and UV-Vis spectroscopic data. In this study, we used only four (of total six) anthocyanidins as standards and their elution order on chromatograms was: delphinidin, cyanidin, pelargonidin and malvidin. According to the literature data for elution order and by comparison of the HPLC retention times, it could be deduced that the peak at *RT* of approx. 20.9 min was peonidin [6, 9].

Amounts of distinct anthocyanidins presented in Tab. 2 are expressed as relative peak area (in percent), where the sum of the areas was considered as 100%, and also as concentrations (in milligrams per litre) calculated by the corresponding equation given in Tab. 1. The dominant anthocyanidin peak of malvidin in all three samples (Fig. 1D–1E) represented more than 50% of the total peaks area revealed at 520 nm (in Babica extract its share was even 60%). Maletić et al. [19] also confirmed the domination of malvidin derivatives in Babica variety, while their concentration in Ljutun and Crljenak viški variety was lower. The extremely high concentrations of malvidin derivatives (over 67%), particularly the predominant anthocyanin malvidin-3-glycoside, were also confirmed in other grape varieties [4, 26, 27]. The concentrations of malvidins were more than 5-times higher than those of other compounds and ranged from 0.2237 $mg \cdot l^{-1}$ (Crljenak Kaštelanski extract) to 0.2738 $mg \cdot l^{-1}$ (Ljutun extract). The share of the investigated anthocyanidins in

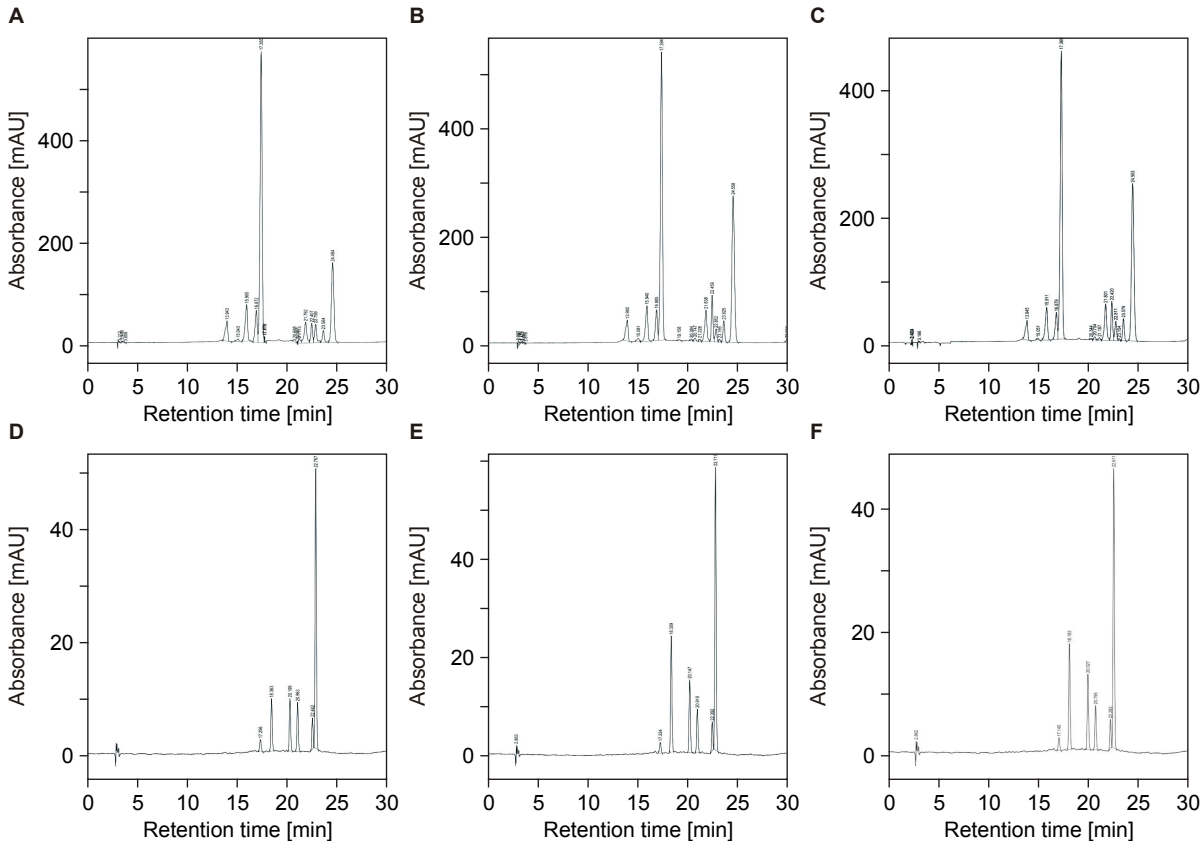

Fig. 1. Chromatograms of the grape skin extracts before and after the acid hydrolysis.

A, B, C – extracts from Babica, Ljutun and Crljenak Kaštelanski varieties, respectively before acid hydrolysis; D, E, F - extracts from Babica, Ljutun and Crljenak Kaštelanski varieties, respectively, after acid hydrolysis.
Absorbance was measured at 520 nm. mAU – milliabsorbance units.

Crljenak Kaštelanski and Ljutun extracts was almost the same, while Babica extract contained lower amounts of delphinidins and cyanidins. The concentrations of peonidins in all three grape samples were the lowest, which is in accordance with the results obtained by Maletić et al. [19]. Although previous studies on grape anthocyanins reported that *Vitaceae* family plants do not contain pelargonidin derivatives [4, 19], new reports confirmed their presence in berry skins of some most widely cultivated *Vitis vinifera* varieties in the world like Cabernet Sauvignon or Pinot Noir [7, 10], as well as in skins and pulp of Yan 73 grape hybrids [29]. These compounds were also detected in our samples and their concentrations ranged from 0.0359 mg·l^{-1} to 0.0371 mg·l^{-1}.

Tab. 2. Anthocyanidin profiles of grape skin extracts.

Anthocyanins	Babica		Ljutun		Crljenak Kaštelanski	
	A [%]	Concentration [mg·l^{-1}]	*A* [%]	Concentration [mg·l^{-1}]	*A* [%]	Concentration [mg·l^{-1}]
Delphinidin	11.5	0.0393	21.5	0.0511	19.8	0.0457
Cyanidin	11.4	0.0422	13.5	0.0476	14.5	0.0454
Pelargonidin	10.8	0.0368	8.2	0.0371	8.5	0.0359
Peonidin	6.2	nd	4.9	nd	5.1	nd
Malvidin	60.1	0.2368	51.8	0.2738	52.1	0.2237

A – compound peak area from the total peak area (100 %) revealed at 520 nm, nd – not determined.

Changes of anthocyanins concentration and kinetics of their degradation during storage

The changes of anthocyanins during storage of grape skin extracts under different conditions were monitored in two ways; first on the base of changes in concentration of total monomeric anthocyanins determined by pH differential method, and second on changes in the peak area of the dominant anthocyanidin in samples. The concentration of total monomeric anthocyanins in Babica, Ljutun and Crljenak Kaštelanski grape skin extracts was determined by spectrophotometric pH differential method. This is a fast, simple and non-destructive analytical technique based on reversible structural transformation of anthocyanins with a change in pH; (at pH = 1.0 the dominant form is coloured oxonium, while at pH = 4.5 it is colourless hemiketal form) [28].

The monomeric forms of anthocyanins are the most responsible for the red colour of grape and wines but also contribute to development of polymeric pigments during wine aging [8]. The concentration of total monomeric anthocyanins in the extract of grape Ljutun skins, expressed as milligrams of C-3-gl, was 315.6 mg·l^{-1}; in Babica extract it was 312.3 mg·l^{-1}, while in the extract of Crljenak Kaštelanski was significantly lower (272.2 mg·l^{-1}). MALETIĆ et al. [19] reported that total anthocyanins concentrations of Croatian autochtonous red wines ranged from 50 mg·l^{-1} to 315 mg·l^{-1}. In the wine from Crljenak viški variety they found the lowest concentration of anthocyanins, while in Babica and Ljutun wine, the detected concentrations were 3–4 fold higher. The contents of anthocyanidins in grape skins are influenced by many factors such as grape cultivar, maturity, climate factors, soil characteristics or agricultural activities [4, 7].

The changes in monomeric anthocyanin concentration during 70 days of storage under different conditions (light or dark, room temperature or at +4 °C) were monitored and are given in Fig. 2. It is well known that anthocyanins, due to their high reactivity, easily convert to colourless or undesirable brown compounds, and one of the most significant factors that influence their stability is temperature [19]. As can be seen from Fig. 2, the concentrations of monomeric anthocyanins in all samples stored in the dark or at +4 °C increased in first few days. The reason for this was probably the acid hydrolysis of the present polymeric forms. Anthocyanidin molecules are soluble in different solvents due to their polarity, but the most commonly used solvent for their extraction is methanol containing small amounts of organic acid (e.g. formic, acetic, citric or tartaric acid). METIVIER et al. [30] in their study found that extraction with methanol was by 20% more effective than with ethanol, and by 73% more effective than with pure water, while hydrochloric or formic acids are usually added to prevent degradation of the anthocyanins [3, 28]. These acidified solvents denature the membranes of cell tissues and dissolve pig-

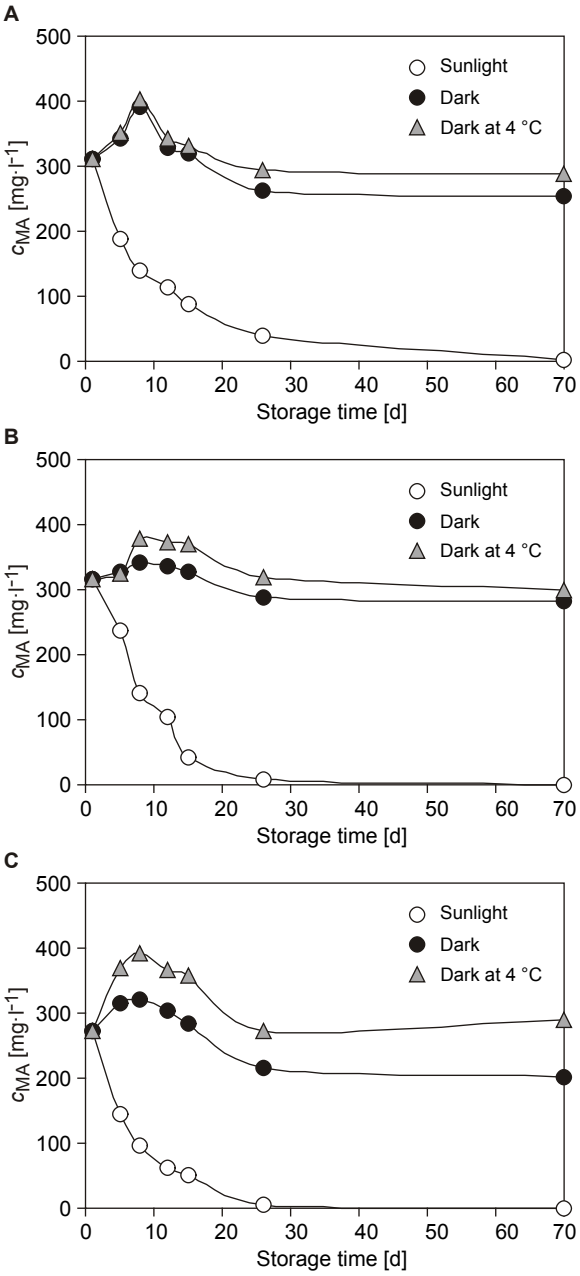

Fig. 2. Changes in monomeric anthocyanin concentration in grape skin extracts during 70 days of storage under different conditions.

A – Babica, B – Ljutun, C – Crljenak Kaštelanski.
c_{MA} – concentration of monomeric anthocyanins in milligrams of cyanidin-3-glucoside per litre of extract.

ments, but also can change their native forms (acid hydrolysis and breaking bonds with sugars, metals, co-pigments etc.). Acidification of extraction solvents (in our study methanol by HCl, at 0.1%, v/v) usually led to hydrolysis of anthocyanin glycosides into aglycone forms, and therefore it is not possible to detect the originally present compounds in the samples [3, 6, 9].

On the other hand, all extracts kept exposed to light showed very fast decrease of monomeric anthocyanin concentrations. After only five days, the concentration of monomeric anthocyanins in Crljenak Kaštelanski extract was by 47% lower, in Babica extract by 40% and in Ljutun extract it was by 25% lower. On 26th day of storage, the concentration of monomeric anthocyanins expressed as milligrams of C-3-gl was 40 $mg \cdot l^{-1}$ in Babica extract, while in Ljutun and Crljenak Kaštelanski extracts these concentrations were significantly lower, 8.3 $mg \cdot l^{-1}$ and 5.0 $mg \cdot l^{-1}$, respectively. On the 70th day of this study, there were no monomeric anthocyanin molecules present in the samples of all extracts exposed to light.

Tab. 3. Kinetic parameters for the degradation of anthocyanins in grape skin extracts exposed for 70 days to light.

Grape skin extract	k [d^{-1}]	$t_{1/2}$ [d]
Babica	0.088	7.877
Ljutun	0.096	7.220
Crljenak Kaštelanski	0.122	5.682

k – first order degradation rate constant, $t_{1/2}$ – half-life of the reaction.

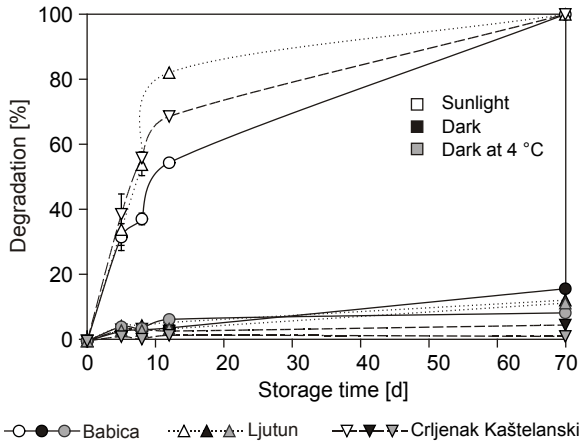

Fig. 3. Degradation of the dominant grape anthocyanin during 70 days of storage.

Dominant grape anthocyanin has peak at a retention time of 17.3 min.

In Tab. 3, the kinetic parameters for the degradation of anthocyanins in the samples exposed to light, k (degradation rate constant) and $t_{1/2}$ values (days for half degradation), are presented. Data for the degradation of the samples stored in dark and at +4 °C are not given because the results for degradation in these conditions did not follow first order kinetics. In the present study, the highest k value was found for Crljenak Kaštelanski grape variety and the lowest for Babica indicating that Babica extract was more stable than the other two. The same conclusion could be drawn by comparing $t_{1/2}$ values.

The highest degree of anthocyanidins stability was recorded for the samples stored at low temperature and protected from light. The concentrations of anthocyanins in samples kept at +4 °C were reduced only by 8% in Babica and by 5% in Ljutun extract, while in Crljenak Kaštelanski extract the final concentration of monomeric anthocyanins was a bit higher. The degree of anthocyanin degradation in samples stored at room temperature in the dark ranged from 10% to 25%.

Besides the changes of monomeric anthocyanins concentration in the samples during different conditions of storage, a decrease in the intensity (peak area) of the major anthocyanidin peak (peak at a retention time of 17.3 min in Fig. 1A, 1B and 1C) was recorded. According to the results obtained by HPLC after the sample hydrolysis, this peak corresponded to a malvidin derivative, malvidine-3-glucoside, which is the predominant anthocyanin in red grapes and wines [4, 7, 19]. The obtained results (Fig. 3) confirmed those obtained by UV-Vis method (Fig. 2). After 70 days of the experiment, the dominant peak totally disappeared from chromatograms of all samples that were exposed to light. On the 12th day, its degradation ranged from 55% (Babica extract) to 82% (Ljutun extract). Degradation of this compound was the lowest in extracts kept at +4 °C after 70 days of storage; 1.7% in Crljenak Kaštelanski, 3.7% in Ljutun, and 6.6% in Babica extract. The good stability was also recorded for extracts stored at room temperature in the dark. These data again confirmed that storage temperature has a strong influence on the degradation rate of individual anthocyanins. Similar to MOLDOVAN et al. [23], it could be concluded that storage at room temperature resulted in much faster degradation of anthocyanins compared to storage at low temperatures. This indicates that further increase of the temperature will result in a more accelerated degradation of the anthocyanins.

CONCLUSIONS

The present investigation provided information regarding the anthocyanidins present in the extracts of skins of autochthonous Dalmatian red grape cultivars: Babica, Ljutun and Crljenak Kaštelanski. It provided not only data on the chemical composition of investigated grape skin extracts, but also valuable information on their stability under different storage conditions. The changes of anthocyanins stability in the investigated grape skin extracts revealed a strong dependence on storage conditions. It was established that the degradation of anthocyanins from the samples stored exposed to light followed first order reaction kinetics. The investigated grape cultivars are rich in anthocyanidins, which makes them valuable raw materials for isolation of pigments that could be used in food industry as natural colorants. The established mathematical models enable the prediction of the anthocyanin degradation during the storage of the extracts. Finally, the attained conclusions could be used in further studies dealing with the addition of different substances in order to moderate/prevent the degradation of these valuable phytochemicals.

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