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## The Effect of Light, Temperature, pH and Species on Stability of Anthocyanin Pigments in Four *Berberis* Species

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**Abstract:** The anthocyanin pigment was extracted from the four different *Berberis* plant (*B. khorasanica*, *B. integerrima*, *B. orthobotrys*, and *B. vulgaris*) using the soaking and wetting in Ethanol (1% acidified). The extracted anthocyanin pigments then were exposed to number of environmental conditions, which could destabilize the anthocyanin molecules. These environmental conditions were included three different pHs (0, 1.5 and 3), various temperatures (5°C, 15°C, 25°C and 35°C) and presence or absence of light. The results of the study showed that increasing in pH, temperature or exposure to light is able to spoil the anthocyanin molecule. Another factor affecting the tolerance of anthocyanin towards the environmental condition is the role of different species. Among the various *Berberis* species anthocyanin pigment in *B. khorasanica*, showed the greatest resistance to destruction by environmental conditions followed by *B. vulgaris*, *B. orthobotrys*, and *B. integerrima*.

**Key words:** Color stability, pH, temperature, light, *berberis* species, anthocyanin

### Introduction

In the last decade, there has been a great increase in utilization of natural plant pigments in the food industries, replacing the implementation of artificial coloring agents, in order to provide a healthier food for consumers. However, in comparison with the natural coloring agents, the artificial coloring agents show greater resistance and stability when exposed to oxidation, changes in temperature, pH and other factors (Francis, 1989; Hong and Wrolstad 1990; She *et al.*, 1992; Fabre *et al.*, 1993).

Although anthocyanins are less stable in various environmental condition, they include varieties of colors such as orange, red, maroon and blue which make them an attractive alternative as coloring agents in food industries (Markakis, 1982; Francis, 1989). The intensity and stability of the anthocyanin pigments is dependent on various factors including structure and concentration of the pigments, pH, temperature, light intensity, quality and presence of other pigments together, metal ions, enzymes, oxygen, ascorbic acid, sugar and sugar metabolites, sulfur oxide etc (Mazza and Minitiati, 1993; Francis, 1989).

Anthocyanins have four different structures, which are in equilibrium and include flavylum cation, quinoidal base, carbinol pseudobase and chalcon. The relative amounts of these structures in equilibrium are varied and depend on the pH and anthocyanin structure (Mazz and Minitiati, 1993). Some anthocyanins are more stable than other depends on their molecular structure. The example of this is the Malvidin glycosides, the major anthocyanin in grape, which due to dimethyloxylolation of the molecules are more stable than other anthocyanins

(Bridle and Timberlake, 1997). Moreover, hydroxylation of organic acids results in more stable molecules in most cases (Bassa and Francis, 1987; Francis, 1989).

Another factor in increasing the stability of the anthocyanin is the co-pigmentation (Francis, 1989; Malien-Aubert *et al.*, 2001). There are also more stables color pigments exist in the fruits and vegetables, which their phyto-chemical structure and anthocyanin properties need to be investigated.

*Berberis* fruit is also among the plants, which contains large amount of anthocyanin. Transformation of these pigments to other forms by enzymes, oxidation, light, temperature etc, during storage cause color change from red to brown in *Berberis* fruits, which has a negative impact on appearance of the product.

### Materials and Methods

**Sample preparation:** Samples of *Berberis* were obtained locally. *Berberis khorasanica* and *Berberis vulgaris* gathered of Khorasan area in the east of Iran. *Berberis orthobotrys* and *Berberis integerrima* so gathered Azerbeyjan area in the west of Iran. *Berberis* fruits were washed with distilled water and kept frozen at -18°C till use.

**Methods:** We used the methods of Chiriboga and Francis (1970) using ethanol acidified with 0.1% hydrochloric acid 1%.

Samples were taken out of the freezer, left at room temperature for 30 minutes to defrost. 500 gram of each *Berberis* sub-species were put in a mixer, solvent added and a mixed for 10 minutes. The products then filtered in vacuum using Bochner funnel and wattman filter (grade

1), the remain of the mixture on the filter paper was washed again with the solvent and filter again to get a clear liquid. The filtered product then placed in a balloon container in a vacuum evaporator at 35°C to separate the ethanol-acid solvent. The balloon container was separated from the vacuum evaporator, and distilled water was added to dissolve the powder, which was formed at the bottom of the balloon container. The product then transferred to a 500ml container and brought the volume to 500 ml using distilled water. The product then centrifuged at 8000 rpm, the supernatant was separated and kept for further analysis. The same method used for all other *Berberis* species.

The anthocyanin content of the extracts was measured by spectrophotometer. The following two buffers were used : (a) 0.13 M HCl -0.05 M KCl, pH=1.0; and (b) 0.05M HCl -0.5M CH<sub>3</sub>COONa, pH=5. The mixture of buffer and samples were equilibrated in darkness for 1 hr and their absorbance was measured at 520 nm in a Beckman DU spectrophotometer. The pigment concentration was expressed in Encyanin equivalents (EE), obtained from a reference curve based on differential absorbance at 520 nm vs. Commercial Encyanin concentration (mg/ml).

## Results and Discussion

**The role of *Berberis* species on the stability of anthocyanin:** There are great inter-genus and inter-species variations between the level of phenolic compounds in fruits.

Depends on the *Berberis* subspecies the stability of anthocyanin varies. Stability of anthocyanin extracted from four different *Berberis* subspecies in a fixed temperature of 25°C and pH =2 measured. The results show that *B. Khorasanica* contains the most stable form of anthocyanin and *B. Integerrima* has the least stable form of anthocyanin. The level of anthocyanin destruction after 84 days in *Berberis* plant tested was 27%, 51%, 67% and 91% for *B. khorasanica*, *B. vulgaris*, *B. orthobotrys* and *B. integerrima* consecutively. B.R Cordenusi *et al.* (2003) have shown the importance of Strawberry Cultivar as a particular species of strawberry that determines the quality of the harvested product and increase the shelf life.

**The effect of pH on the stability of anthocyanin:** Another factor which affects the stability of anthocyanin is the pHs (0, 1.5, 3). Our results show that increasing pH cause greater destruction of anthocyanin in samples.

Flavylium salts are stable only in highly acidic conditions. These salts loose the proton in higher pH and transform into quinoidal base, which is an unstable pigment, and immediately bond to water and form colourless compound called chromenol. All the experiments were performed in a fixed temperature of 25°C in an 84 days period and total of 5 separate

measurements for each category.

The result of our study shows that the percentage of anthocyanin destruction in pHs of 0, 1.5, and 5 in various *Berberis* as shown in the Table 1.

Table 1: The percentage of anthocyanin destruction in pHs of 0, 1.5, and 5 in species of *Berberis*

<i>Berberis</i> species	pH= 0	pH = 1.5	pH= 3
<i>B. integerrima</i>	67%	68%	94%
<i>B. vulgaris</i>	33%	44.87%	64%
<i>B. khorasanica</i>	10.27%	27%	81.69%
<i>B. orthobotrys</i>	28.26%	69.11%	88.94%

As the results show, the degree of anthocyanin destruction between pH=1.5 and 3 is considerably greater than that of pH =0 to pH = 1.5.

Morris *et al.* (1986) have reported that in warm agricultural area, high pH of the grapes at the time of harvest could cause problem for the juice making industry. Higher pH in grapes can cause fading the colour and decrease in stability of the products.

Angela and Little (1977) has studied the combination of the colour pigments in Strawberry jam and packaged Strawberries at the 37.7°C during time. She has recorded the data for pH of 2, 3 and <1 and showed that destruction of anthocyanin pigments increases with increase in pH.

**The effect of temperature on the destruction of anthocyanin:** Temperature also is another factor, which has a role in destabilising the anthocyanin molecular structure; with increase in temperature we see a greater degree in anthocyanin destruction.

We suggest that the speedy destruction of anthocyanin in higher temperatures could be due to hydrolyzation of 3-Glycoside structure, which has a protective effect in unstable anthocyanin.

The other suggestion is that the hydrolyzation of the pyrilium ring resulted in production of chalkons, which are responsible for brown colour developed in food containing anthocyanin. In a fixed pH of 2, the effect of four different temperatures of 5°C, 15°C, 25°C and 35°C on level of anthocyanin extracted from four different *Berberis* species during an 84 days period were measured in 5 separate instances. The results show that percentage of destruction of anthocyanin in 5°C, 15°C, 25°C and 35°C which are presented in the Table 2.

Palamidis and Markakis (1975) has studied the effect of temperature on the stability of anthocyanin in soft drinks and have shown that increase in the storage temperature greatly accelerate the destruction of pigments in soft drinks.

Maccarone *et al.* (1985) have studied the stability of anthocyanin in red orange juice in 15°C, 25°C and 35°C during a 15 day period and found that the increase in

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Table 2: The percentage of anthocyanin destruction in temperature of 35, 25, 15 and 5 in species of *Berberis*

<i>Berberis</i> subspecies	5°C	15°C	25°C	35°C
<i>B. integerrima</i>	41.05%	51.09%	62.33%	89.42%
<i>B. vulgaris</i>	10.22%	32.70%	77.87%	28.09%
<i>B. khorasanica</i>	25.37%	47.67%	77.64%	84.42%
<i>B. orthobotrys</i>	57.81%	60.28%	65.62%	83.05%

Table 3: Effect of presence or absence of light the percentage of destruction of anthocyanin in four *Berberis* species

	Presence of light	Absence of light
<i>B. integerrima</i>	79.04%	72.06%
<i>B. vulgaris</i>	85.22%	59.22%
<i>B. khorasanica</i>	26.4%	21.23%
<i>B. orthobotrys</i>	96.61%	75.24%

temperature accelerates the destruction of anthocyanins. This studies verify our results.

**The effect of presence or absence of light on the stability if anthocyanin:** Light is another factor, which affects the stability of anthocyanin. The effect of light on accelerating the destruction of anthocyanin in the four *Berberis* species has been presented in graphs 10-13. pH in all samples was 2 and in temperature kept at 25°C. The period of experiments was 84 days and data was reordered in 5 separate instances. In the presence or absence of light the percentage of destruction of anthocyanin is shown in Table 3 for four *Berberis* species

Palamidis and Markakis (1975) have also studied the role of light on the stability of anthocyanin in grape juice and showed that exposure of the pigments to light accelerates their destruction. Their experiments showed that after placing the juice samples containing anthocyanin in dark for 135 days at 20°C, almost 30% of the pigments were destroyed, but placing the same samples in the same temperature and same period of time in the presence of light destroyed more than 50% of total pigments.

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