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Extraction and Evaluation the Effect of Environmental Destructive Factors on Stability of Siyahe Sardasht Grape Anthocyanins

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ABSTRACT: The color of red juice of *Vitis vinifera* is mainly due to the presence of anthocyanins that most important group of water soluble plant pigments. In the stage of producing fruit juice it is great important to obtain good color and clearness of juice and to possibly keep them for a prolonged period of time. On the other hand the juice of black grape (*Vitis vinifera*) is very susceptible to browning and overall of color quality during storage. In this research the effects of four temperatures (5, 20, 30 and 40°C), four pH levels (1.5,3 ,4.5 and 6) and three light levels (dark, 400 Lux, 800Lux) on color and stability of anthocyanin during storage of three months were studied. Samples were analyzed for total anthocyanin pigment, color density, percentage of anthocyanin degradation. High pH decreased color density and increased browning during storage time. Low pH indicated lowest color less. Higher storage temperature (40°C) greatly reduced color less and increased anthocyanin degradation during storage time. Samples which stored at (30°C, 40°C) had unacceptable color after three months. The higher levels of light (800Lux) induced decrease of color density. Samples which were in dark conditions indicated low anthocyanin loss during three months of storage.

Key words: Anthocyanin, Light, Temperature, pH, Siyahe Sardasht.

INTRODUCTION

Today we are in the stage of globalization of the use of natural resources as food colors instead of artificial colors. Anthocyanins are a great group of natural pigments soluble in water which belong to flavonoids. They are responsible for red, amethystine and blue colors in a variety of flowers, fruits and vegetables and play an important role in pollination and protection against plant stresses (Harbon and Williams, 1992). They are responsible for the pretty red color of *Vitis vinifera* extract of Anthocyanins. In the producing stage of the extract, it is very important to obtain a pretty color and transparent extract and also to store it in a long period of time without degradation of its transparency. However Anthocyanins are instable and susceptible to instant destruction under temperature and light and pH conditions and turn into colorless forms (Thakur and Arya, 1989).

A variety of efforts were conducted for studying stabilized Anthocyanins in fruits, vegetables and Ornamental plants and numerous studies conducted in order to increase the stability of Anthocyanins (Robinson and Robinson, 1931). Production of Anthocyanins from cell culturing was studied (Callebaut *et al.*, 1993). On the other hand, the medical usage of Anthocyanins including the strong anti oxidant properties of red grape is important as well (1996b Hollman *et al.*, 1996a and Jackman *et al.*, 1987). Anthocyanins are an important element of producing grape and its red extract (Liao, 1970). There are plenty of Anthocyanin resources which apparently can be used as food color. The oldest and most abundant extract containing Anthocyanin are Enocianra and Enocyanin colors produced from red grape which are known as a general color.

It is certainly determined that Anthocyanin features such as its color are strongly influenced by the structure of Anthocyanin and pH (Bakowska *et al.*, 2003; Knekt, 2002, Mazza and Brouillard, 1990). Anthocyanins in low pH solutions are more stabilized than in solutions with high pH. However it is determined that Anthocyanins show a wide range of colors from 1 to 14 pH. The ionized nature of Anthocyanins is able to change the molecule structure according to pH format of the solution and cause creation of different colors in various pH levels (Lee and Nagys 1988).

Various studies (Williams and Hrazdina 1979; Van *et al.,* 1974; Timberlake and Bridle 1967; Sondheimer 1953; De 1999; Brouillar *et al* 1991) demonstrate that pH has a major influence on grape extract's color. In a normal situation the colorless form of Anthocyanin increases in comparison to the ionized form of red Carbonium as soon as pH level increases from 3 to 4. The colorless form of Anthocyanin increasingly rises in comparison to ionized for of red Carbonium. As pH approaches a normal condition the Pseudobase anhidrosis form of blue-purple color of Anthocyanin begin to dominate. Storage time is one of the factors that have a sensible effect on the color of Vtis vinifera's extract. Increasing the storage time causes formation of Anthocyanin polymers and Tanins whereas the number of free anthocyanins decreases. (Ballinger *et al.,* 1973, Lee and Nagys 1988, Morris *et al.,* 1986, Sondheimer 1953). During the storage time, typically the extract turn into a red color with low red transparency and finally it result in stabilization of the extract. The Anthocyanins are sensitive against temperature of storage environment and it result in decreasing desired color of extract and more brown color which is due to pigment destruction and polymerization (Morris *et al.,* 1986). The destruction speed of anthocyanins increases with temperature rising during storage time and preparation process of the extract (Dyrby *et al*., 2001; Morris *et al*., 1986, Sims and Morris 1984). Temperature raising in pH 2-4 results in glycolyze reduction in anthocyanins and hydrolyzing the band of glycoside (Lee HS and Nagys 1988). This results in more color reduction in anthocyanins since Aglycons are less stabilized than their glycoside forms (Dao *et al.,* 1998). It is obvious that formation of Chalcone is the first step in temperature destruction of anthocyanins (Liao, 1970). Finally temperature destruction results in creation of brown color productions especially in presence of oxygen (Mazza and Brouikkard, 1990). Light affects anthocyanins in two different ways: light is effective in biosynthesis of anthocyanins, light facilitates their destructions (Dyrby *et al*., 2001). When anthocyanins are in a dark situation they keep their color so much better. After 24 hours, difference in colors is determined when anthocyanins are exposed to light. When grape extract is kept in darkness, only 30% of color of anthocyanins is lost while if it is exposed to light with the same conditions 50 % of pigments is destructed. The most destruction happens when they are exposed to florescent light and the temperature of environment gradually decreases (Markakis, 1982).

MATERIAL AND METHODS

The grape used in this study is the Black vaudeville of Sardasht which is a type of Vitis vinifera and is grown in wild nature in sardasht mountains located in south of western Azerbaijan province and also is cultured agriculturally in the south places of this province.
Samples were collected from Sardasht Mountains in the south of western Azerbaijan in the beginning of autumn and were transferred in a Coleman full of ice to the University of Oromieh. We did our best to collect some fully ripen samples. Samples were washed with warm and mild water and finally with distilled water and they were packed into plastic bags and finally kept in refrigerator in -18 °C until the date of experiment.

A. Anthocyanins extraction steps

The extraction of Anthocyanin was carried out using Francis and Chiriboga(1970) method. We led the grapes' ice melt down (5000 g) and then turned them into a solution inside a mixer with an extraction solvent which was acidic methanol (250 ml) containing 0.1 percent Chloridric acid. Samples crushed by number one whatman paper filter were filtered under the influence of vacuum. The anthocyanin of remaining dross was extracted using the same extraction acid and this process continued until some colorless dross was left on the filter paper. The acidic solvent (acidic methanol) was separated using a rotary device (Buchi Rotavapor-r) under pressure reduction and the resulting anthocyanin was concentrated. We set the water bath temperature on 35 °C in order to impede destruction of anthocyanins.

B. Preparing samples and spectrophotometer

To make absorption of samples readable in a specific range of wavelengths the sample concentrated extract was diluted with distilled water and the spectrophotometer device scanned at wavelength intervals of 350-600 nm. The prepared samples were filtered by using filter papers before application. The maximum amount of absorption for diluted grapes' extract was 530 nm. Distilled water was used as control solution throughout entire stages. The spectrophotometer device used for measurements was WAP biowave S2100 Diode Array.

C. Absorption range

Estimating the concentration of Anthocyanin. In order to determine the concentration of Anthocyanin, we measure the sample absorption in pH 1 and 5 with 520 nm wavelength and we use the following formula:

A = [(A λ_{vis} -A700nm) pH 1 - (A λvis - A 700nm) pH 5
We used the following formula to estimate Monomer Anthocyanin in one sample:
Anthocyanin (100 mg/ml) = (A×MW ×DF × 1000)/)ε × 1
MW=molecule weight
MW and ε used in this formula are taken from the dominating Anthocyanin in the sample. In this study cyanidin 3-glocosoid is the dominating Anthocyanin in sample which for it we have MW = 449.2 and ε = 26900 (Kopjar and Pilizota 2009).
DF- dilution factor

D. Statistical analyze

In this study the results were analyzed using Analysis of Variance and SPSS software and the mean difference of data was analyzed using Duncan test and probability of $P<0.05$.

RESULTS AND DISCUSSION

A. Study results of destruction by light

In this study, effects of light and darkness were tested on destruction level of Anthocyanin. In this experiment, grape's extract was exposed to three different conditions; 400 lux, 800 lux and darkness. As specified in Fig. 1, a significant difference can be observed based on Anthycianin content of samples in comparison to other samples and control samples and exposure of samples to different light conditions. Treatment of samples with 400 lux light resulted in reduction of Anthocyanin amount in extract. The grape's extract shows the most color reduction and the most Anthocyanin destruction in 800 lux environment such that at the end of three month storage of the extract in this condition, 82.960 percent of extract's Anthocyanin was destructed. While the least percentage of Anthocyanin destruction occurred in darkness conditions. During three months, the environment's temperature was 20 °C in the conditions mentioned above.

B. Study results of destruction by temperature

Four temperature levels 5, 20, 30 and 40 were tested in order to study the effect of temperature on stabilization of Anthocyanins. Results showed that temperature has a significant effect on destruction of grape extract's anthocyanin. It was determined in this evaluation that the Anthocyanin level decreases with temperature rising.

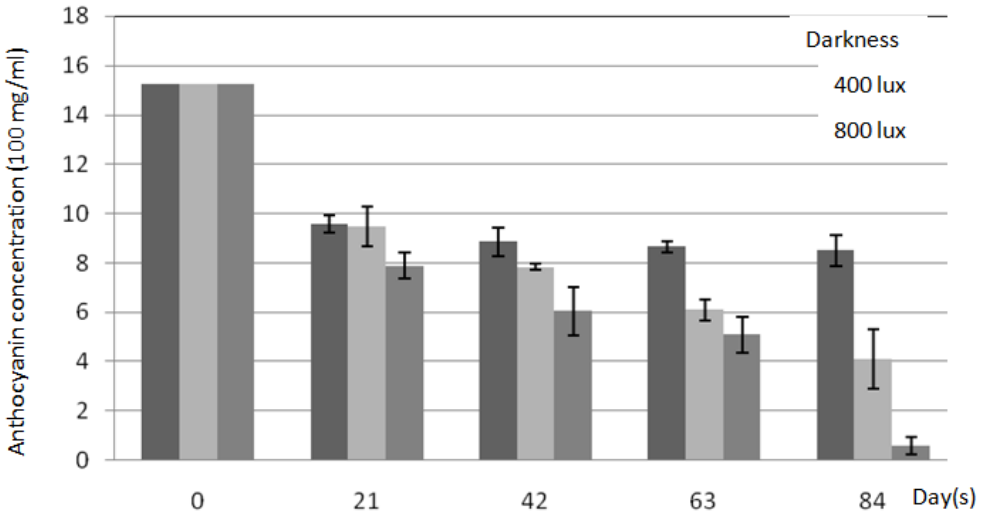

Fig. 1. Effect of light on Siyahe Sardasht grape anthocyanins.

As data curves show, the most destruction occurred in 40 °C such that 98.5 % of Anthocyanins were destroyed at the end of the three month storage period and the lowest destruction occurred in 20 °C (Fig. 2).

C. Study results of destruction by pH

Increase of pH is one of major factors involved in Anthocyanin destruction. In this test, effects of four pH domains (1.5, 3, 4.5, and 6) on Anthocyanin of grape's extract were measured.

Results obtain over storage period determined that high pH decreases the color intensity of extract and increases the brown color. As shown in Fig. 3 pH gradually increases, Anthocyanin absorption decreases at 530 nm and finally the Anthocyanin level decreases gradually such that this reduction was more obvious and clearer at pH-6 and the lowest destruction of Anthocyanin was for pH=1.5.

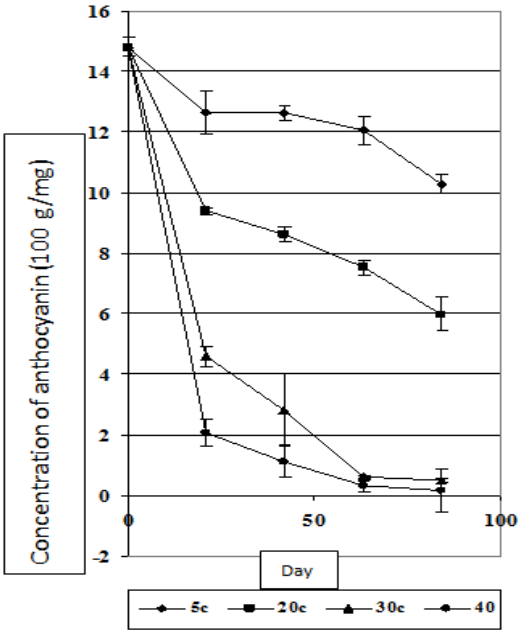

Fig. 2. Effect of temperature on siahe sardash grape's anthocyanin.

As mentioned previously, when Anthocyanins are exposed to a high temperature, the glycoside bonds are disconnected and cause separation of glycosides which the result of this is the reduction of color of extract under effect of temperature. On the hand, temperature destruction causes producing brown color productions which cause changing the original color of the extract themselves. Also, producing the products and derivatives of benzoic acids such as tri hydro benzalhyde as the last production of temperature destruction affects the color reduction in anthocyanins.

Lee and Nagy (1988) studied the effects of temperature and storing time on quality of grip fruit's extract. They found that as the temperature increases, concentration of 5 hydroxyl methyl furfural and furfural which are destruction products of anthocyanins increases.

Study of Timerlake (1966) proved that exposing the extract containing anthocanin to light results in facilitating formation of cation form of flavilium but in the absence of light, chalkun amount of extract is more than cation flavilium present in it. In 1993, Furtado specified that the final productions of light effect which causes destruction of anthocyanins are the same that exist in temperature destruction. Markakis and Palamidis (1975) studied the effect of light on stabilization of the anthocyanin extracted from grape. Exposing the extract to light increased the destruction of pigments. This experiment was conducted over a 135 days period in darkness and in the light.

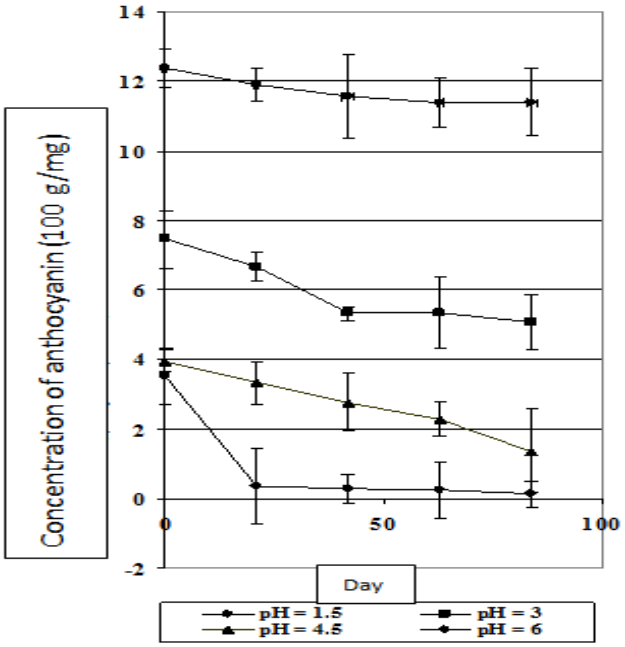

Fig. 3. Effect of pH on siahe sardash grape's anthocyanin.

Temperature of experiment's environment was 20°C and sunshine was used as the light for extract.50 % of pigments were eliminated in sunlight while in darkness and same temperature 30% of pigments were eliminated.

The color and stabilization of anthocyanins in solutions is strongly dependant of pH level of the solution. Anthocyanins are very stabilized and colorful in low pH levels and as soon as pH increases, their color gradually decreases. This color decrease is returnable and the red color of solution appears again by making it acidic. This feature causes limiting function of anthocyanins as a color ingredient for food products. In acidic solutions, anthocyanin exist 4 major forms.

A (Quinonidal base A), Flavylium cation, Carbinol or Pseudobase, Chalcone) in highly acidic conditions pH =0/5, red flavilium catyon is the dominating shape of anthocyanin. pH increasing induces both color intensity reduction and flavilium catyon' concentration reduction as anthocyanin is hydrated by nocleofillic attack, colorless form of carbinol is created and carbinol form loses its dual bond between loop A and loop B therefore no visible light is absorbed (Bolivia *et al*., 2003).

As soon as pH level increases, the flavilium catyon instantly loses protons and the colored keninonoidal shape is strengthened. When pH level increases more, crinol's form produces colorless chalcun by opening the loops. The amount of each form depends on pH level and specific structure of anthocyanin. As explained earlier, losing color of anthocyanin with equilibrium of four anthocyanin forms depends on pH level and AH+ flavilium catyon is the most stabilized and the most colorful form. The flavilium catyon that exists in acidic environments and forms that are dominating in alkali environments are destructed more easily and quicker (Belitz and Grosch 1999).

Many studies have shown the effect of pH on anthocyanins. In 1984, Morris and Sims came to this conclusion after their investigations on effects of three pH levels (2.9, 3.2 and 3.8) on stabilization of red muskadin extract's color that pH increase causes the extract to be lighter and reduces its red color. Based on their idea, reduction in color of extract is probably because of colorless Pseudobase from increase of anthocyanins that is dominating the colorful caryonim ion form. Eventually they concluded that low pH contribute to stabilization of color in red muskadin extract. Wegen Knecht *et al* (1960) found by studying the effect of pH on anthocyanins that they show various color under pH variations. They attributed this color changing to replacing effect in benzene loop. According to these researchers, anthocyanins in different pH suffer various replacements on benzene loop. According to them, 3-glocoside in 3.1-pH is as 3-acyle glycoside and in 4.2-pH is as 3.5-de glycoside and in 4.5-pH is as 3-acyle glycoside- 5-glycoside.

Bolivia *et al* (2003) in a study on red carrot anthocyanin, potato, red grape and red corn found that in pH-7 almost all anthocyanins in extracts are in form of koinoidal. After some experiments it was determined that the absorption strength in amethystine carrot and red potato in high pH is higher and this can be due to grinding of anthocyanins. In low pH, anthocyanins mainly exist in a red flavilium form. Statistical information indicates that pH increasing causes anthocyanin forms to be changed and results in increasing colorless forms in anthocyanins and these results are in agreement with the current study.

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