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Influence of sucrose and high temperature on grape anthocyanin stability and furfural formation

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ABSTRACT

Anthocyanins are a group of compounds that belong to flavonoid family and these are of great interest in the food industry, mainly due to their coloring properties. The aim of this study was to examine the effect of high concentration of sucrose and high temperature on the stability of Rish¬ baba and Ghare ghezel uzom varieties of Urmia grape anthocyanins at low pH (2 and 3) and different time periods and to measure furfural content after 20 h heating at 90 ℃ by HPLC. After extraction of anthocyanins, the pH value was adjusted on 2 and 3 by citrate buffer (0.1 M). The extraction was exposed to different concentration of sucrose (40 and 60%) at 90℃ for 52 h. Degradation of anthocyanin was evaluated according to absorbance at 520 nm. The extraction of furfural was accomplished by 1, 2 dicholoromethane. The results showed that the absorbance on 520 nm from 0 to 4 hours decreased and then increased because of brown pigment replaced instead of anthocyanin from 4 h. Furfural content was higher in samples with anthocyanin+sucrose than samples without sucrose. The results indicated that browning depends on the pH and sucrose concentration.

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1. Introduction

Grape (Vitis Vinifera L.) is one of the most important plant species. This fruit is rich in phenolic compounds such as phenolic acids, resveratrol, and flavonoids (flavanols, anthocyanins and procyanidins) (1).The antioxidant activity of grape is associated with these compounds (2).There are a large number of anthocyanins in skin of grapes. The amount of anthocyanins is different in various varieties and cultivar, season and environmental factors can affect it. The most anthocyanins that are found in red grapes are malvidin (Mv), peonidin (Pn), delphinidin (Dp), petunidin (Pt) and cyanidin (Cy) (3).

Grape consumption is important in terms of beneficial effects on humans like increasing of the antioxidant proteins oxidation, reduction of low density lipoproteins (LDL) and improvement of cardio-vascular oxidation and neurocognitive function (4). Anthocyanins are the most important group of natural pigments after chlorophylls that are non-toxic and water soluble and are found in many plants (5). This flavonoid pigments are responsible for the colors of red, purple and blue in many fruits, vegetable and flower's petals (6). These compound are considered in food industry because of their color attributes and are a good alternative to synthetic colors (7). In addition, interest in these compounds in recent years has increased due to the antioxidant, antivirus, antibacterial and anticancer properties of these compounds (8). Anthocyanins are different based on the location and number of hydroxyl and methoxyl groups on the basic structure of anthocyanins, the type,

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number and position of sugar units connected, the number of acetylated sugars and the type of acetylation (9).

Anthocyanins in terms of stability are weak and susceptible to degradation easily. Stability of anthocyanins depends on several physical and chemical factors such as light, temperature, pH, metals, oxygen, ascorbic acid, sugar, enzyme, co-pigments (10). Anthocyanin destruction and polymerization occurs during heating that may discolor the anthocyanins (11). The effect of sugar on stability of anthocyanins depends on type, concentration and structure of sugar. According to Wrolstad et al. (1990), the stability of anthocyanins from strawberry increased by increasing the sucrose concentration to 20% (12). On the other hand, the degradation of anthocyanins from red cabbage and black currant extracts increased when the concentration of sucrose was low, while the results from grape extracts were opposite (13).

The studies of Daravind and Cian (1968), different sugars (sucrose, fructose, glucose and xylose) increased the degradation of anthocyanins. In general, most of anthocyanins extracts show low stability in the presence of sugar (14). Brown polymeric pigments are one of the effects of anthocyanin reaction with product of sugar degradation (15). Thermal degradation products of sucrose include furfural, caramel and maillard reaction product (MRP) which are also known as a browning agent (16) and it is known that furfural is a factor that causes the degradation of anthocyanins (17). Millard reaction is a kind of chemical reaction between amino acid and reducing sugars. It is usually done in the presence of heat. This reaction results in the production of molecules that their nature is not clear (18). Temperature, pH and duration of heating are factors that affect the browning reaction and degradation of anthocyanins (19).

In this study, the effect of high concentration of sucrose and high temperature on the stability of anthocyanins of Rish baba and Ghare ghezel uzom varieties of Urmia grape at low pH (2 and 3) at different time periods to measure furfural content after 20 h heating at 90 ℃ was aimed to investigate.

2. Materials and methods

Samples of Vitis varieties such as Rishbaba and Ghare ghezel uzom were prepared in September 2015 from Department of agricultural economics. Then fruits were washed with distilled water and kept frozen at 8℃ until use.

2.1. Extraction of anthocyanins

The methods of Chirboga and Francis (1970) were used to study the effects of high temperature and concentration of sucrose on stability of anthocyanins (20). Eight hundred gram from each varieties was put into a mixer and after adding ethanol solvent (acidified with 0.1% hydrochloric acid) was mixed for 10 minutes. Then the products were filtered in Whatman filter (grade 1). Filtering was performed 2 times. The extracts were concentrated under vacuum in an evaporator (Buchi-German) at 35℃ to be dried out. Concentrated extracts were transferred to a 1000 ml container and brought the volume to 1000 ml using distilled water and centrifuged at 8000 g, the supernatant was separated and kept for further analysis. Also, the methods of Chen et al. (2013) to measure the amount of furfural was used. In this method, 30 g of both varieties was required and the solvent was a mixture of methanol: water: formic acid with ratio of 70: 28: 2. The extraction process was similar to the above procedure (21).

2.2. Determination of total monomeric anthocyanin content

The total monomeric anthocyanin content was determined by using a pH differential method (22). First, the extracted anthocyanins were adjusted to pH 1.0 and 4.5 with buffers. WPA biowave S2100 Diode Array and 1 cm pathlenght cell were used for spectral measurement at 520 and 700 nm (MW= molecular weight, DF= dilution factor, A= ($A_{520\ nm}$- $A_{700\ nm}$) pH1.0- ($A_{520\ nm}$- $A_{700\ nm}$) pH4.5, ε= molar extinction coefficient, 1= pathlength in cm and 10^3= factor for conversion from g to mg).

$$\text{Anthocyanin content (mg/L)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1}$$

2.3. IR studies

The extracted anthocyanins were analyzed by Fourier transform infrared (NEXUS 670 FT-IR

2.4. Sugar treated under conditions of pH and temperature

In this study, the impacts of sucrose was evaluated on the stability of anthocyanins. To examine the effect of sucrose, two various levels of sucrose (40 and 60%) was selected (23). At first, the pH of 90 ml of anthocyanin extracts was adjusted by citrate buffer (0.1M) on 2 and 3 with pH meter and was spilled in

three groups of test tube with three repeats in each group. Then different concentration of sucrose was added to anthocyanin extracts. The samples were placed in water bath at 90℃ and the absorption of samples was measured in 520 nm after 2, 4, 20, 26, 46 and 52 hours with spectrophotometer (WPA biowave S2100 Diode Array-UK).

2.5. Treatment for formation of furfural

The examined samples included anthocyanin solution, 60% sucrose solution and anthocyanin with 60% sucrose solution at pH 2. These samples were placed in water bath at 90℃ for 20 h (23).

2.6. Furfural extraction

After heating the samples for 20 h, 1 ml of samples was taken and mixed with 1, 2 dichloromethane for 5 minutes. Two phases were formed which upper phase was pale yellow. This phase contained furfural and was used for injection to HPLC (24).

2.7. HPLC analysis

The amount of furfural was measured by HPLC (Knauer-Germany). Length of column was 25 cm and its inner diameter was 406 µm. Acetonitrile: water (8:92) used as a mobile phase. Flow rate of mobile phase was considered 1 ml/min. The detector was set on 280 nm (25).

2.8. Statistical analysis

For all experiments 3 repeats were considered. Results were expressed as the mean ± standard deviation (SD). One-way ANOVA and Tukey's test were conducted to determine the significant differences between the treatments. Values were considered statistically significant at $P < 0.05$. Excel and SPSS 23 were used for charting and statistical analysis.

3. Results

The most absorption of anthocyanins is at 520nm. Absorbance and reducing the absorption at 520nm showed the red color and degradation of anthocyanins, respectively.

3.1. The total monomeric anthocyanin content

Dominant anthocyanin in grapes is malvidin-3-glucoside so, the anthocyanin content was calculated as malvidin-3-glucoside, using a molar extinction coefficient (ε) of 28,000 $Lcm^{-1}mg^{-1}$ and molecular weight of 493.2 gL^{-1} (26). The following table shows the anthocyanin content of studied varieties.

Table 1. The anthocyanin content of studied varieties. Numbers show the mean ± standard deviation.

Varieties	Anthocyanin content
Rish baba	119.22±13.67
Ghare ghezel uzom	111.70±7.05

3.2. The effect of sucrose concentrations under conditions of low pHs (2 and 3) and high temperature (90℃)

With the passing time, in samples that were without sucrose, absorption was reduced. Samples containing sucrose showed decreased absorption until 4. After 4, the absorption of them increased.

At pH 2, the absorption of samples containing sucrose 40% was higher than samples containing sucrose 60%. Results of pH 3 were in contrast with pH 2. At pH 3, the absorption of samples containing sucrose 60% was high. These results were similar in the two varieties.

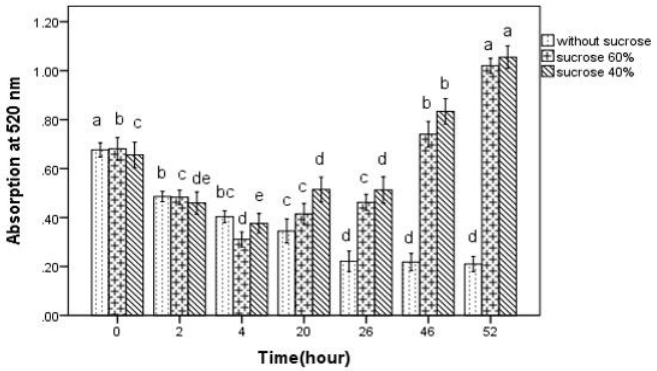

Figure 1. The effect of sucrose on stability of anthocyanins of Ghare ghezel uzom under condition 90℃ and pH 2. Point with different letters in each bars means significant difference (p≤0.05)

Figure 1 shows that in Ghare ghezel uzom at pH 2, there were significant differences between different concentrations (p≤0.05). At 2 h, there were no significant difference between different concentrations. Also, difference between sucrose 40 and 60% at 52 h was not significant. In Rish baba at pH 2 (figure 2), there were not significant difference between sucrose 40 and 60% at 0 and 2 h but at other times the differences can be seen between different concentrations (p≤0.05).

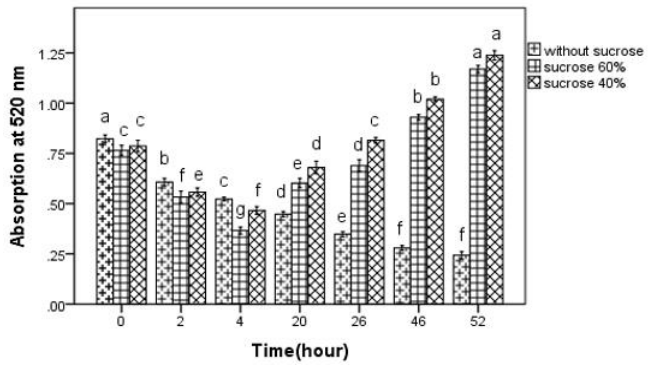

Figure 2. The effect of sucrose on stability of anthocyanins of Rish baba under condition 90℃ and pH 2. Point with different letters in each bars means significant difference (p≤0.05)

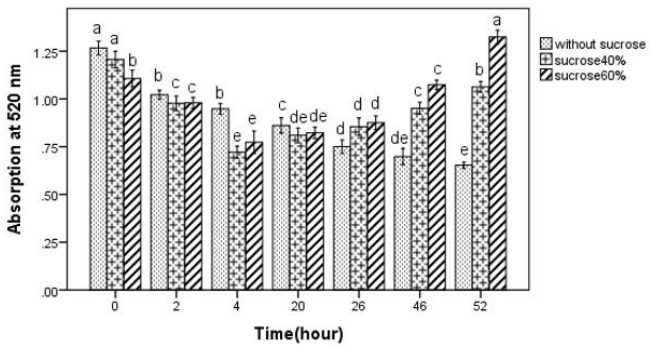

Figure 3. The effect of sucrose on stability of anthocyanins of Ghare ghezel uzom under condition 90℃ and pH 3. Point with different letters in each bars means significant difference (p≤0.05)

Figure 5. Scanning of wavelength of anthocyanins of Ghare ghezel uzom at 0 and 20 h and pH 2

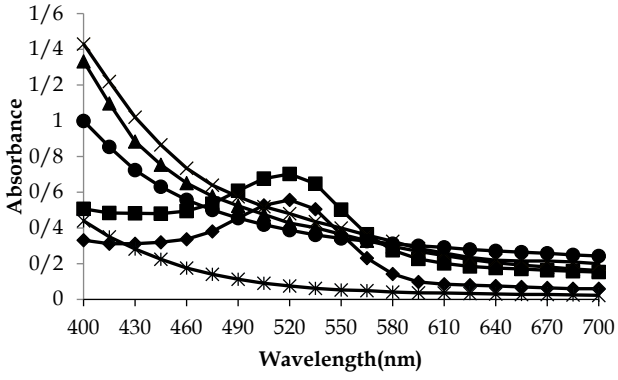

Figure 5. Scanning of wavelength of anthocyanins of Ghare ghezel uzom at 0 and 20 h and pH 2

According to figure 3 it is clear that there are significant difference between different concentrations (p≤0.05) in Ghare ghezel uzom at pH 3. At 2, 4, 20 and 26 h, there are not significant difference between sucrose 40 and 60% (p≤0.05). In other variety, Rish baba, there are significant difference between different concentrations (p≤0.05) at pH 3. At 0 h, there are no significant difference between different concentrations. Also, there are not significant difference between sucrose 40 and 60% at 2 and 4 h.

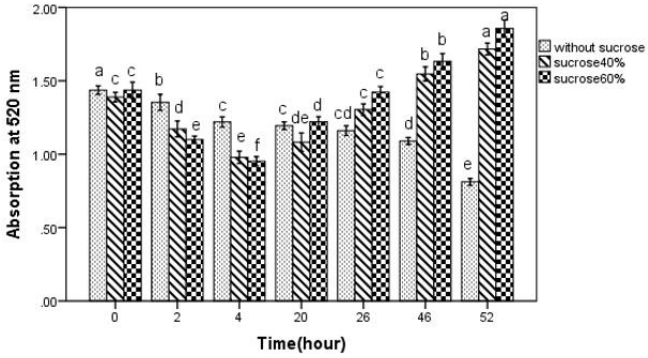

Figure 4. The effect of sucrose on stability of anthocyanins of Rish baba under condition 90℃ and pH 3. Point with different letters in each bars means significant difference (p≤0.05)

3.3. Scanning of wavelength

To study of browning process of anthocyanins, scanning of samples in a wide range of wavelengths (400-700) was done. The results showed that samples were not heated, had a peak at 520 nm. When the samples were heated for 20 h, they did not show any peak at 520 nm. These results were obtained from both varieties (Figures 5 and 6).

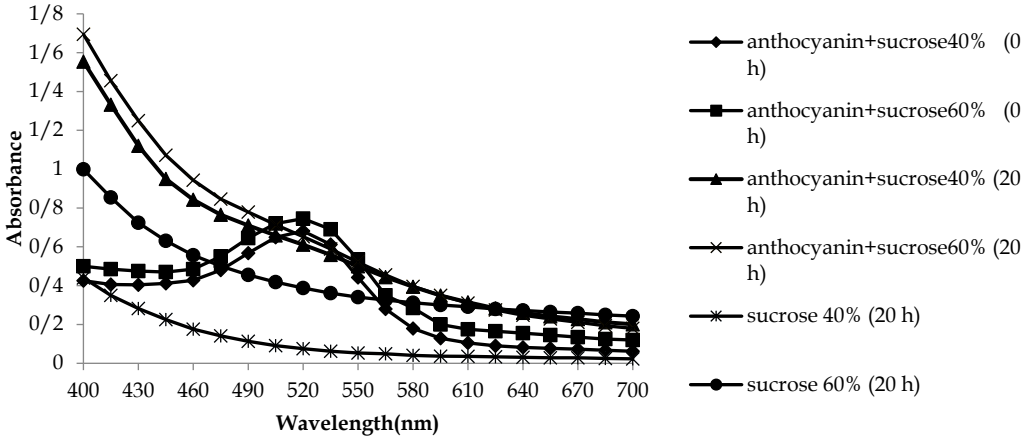

Figure 6. Scanning of wavelength of anthocyanins of Rish baba at 0 and 20 h and pH 2

3.4. FT-IR spectra

The extracted anthocyanins from both varieties were confirmed by FT-IR spectroscopy (Figure 7 and 8). Both varieties had a similar spectral structure. Two peaks at 2929 and 2936 cm-1 were related to the –CH stretching modes. The peaks at 3426 and 3388 cm-1 were similar to the OH stretching vibration. The peaks in region between 1400-1650 cm-1 showed infrared absorption of C=C. Also, the peaks at 1635 and 1637 cm-1 can be related to the stretching of aromatic C=C in anthocyanins. This determined the presence of anthocyanins pigments in the extract of grapes (27).

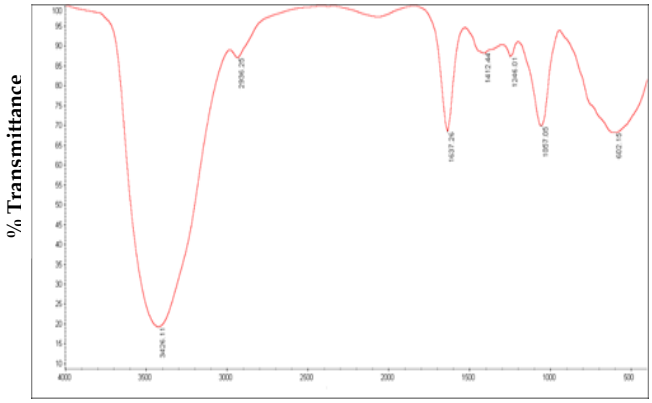

Figure 7. FT-IR spectra of the anthocyanin pigment extracted from Rish baba

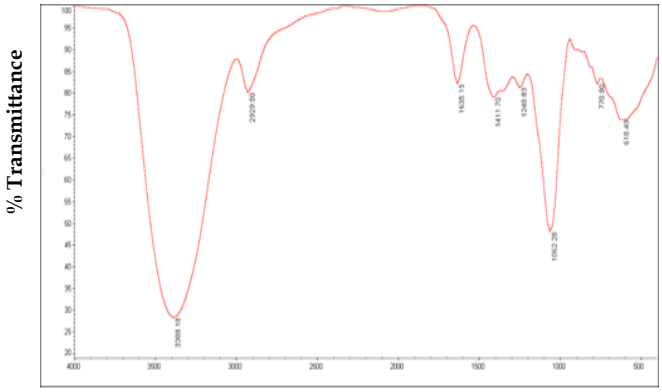

Figure 8. FT-IR spectra of the anthocyanin pigment extracted from Ghare ghezel uzom

3.5. Studies of furfural

In both varieties, samples with sucrose showed the highest amount of furfural and samples without sucrose had a small amount of furfural (Figure 9 and 10).

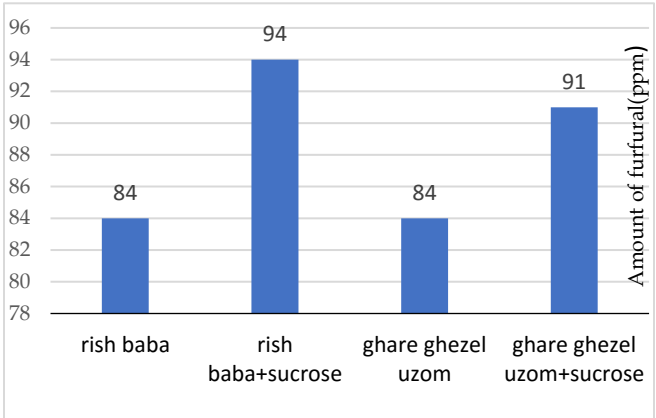

Figure 9. Comparing of the amount of furfural in studied varieties

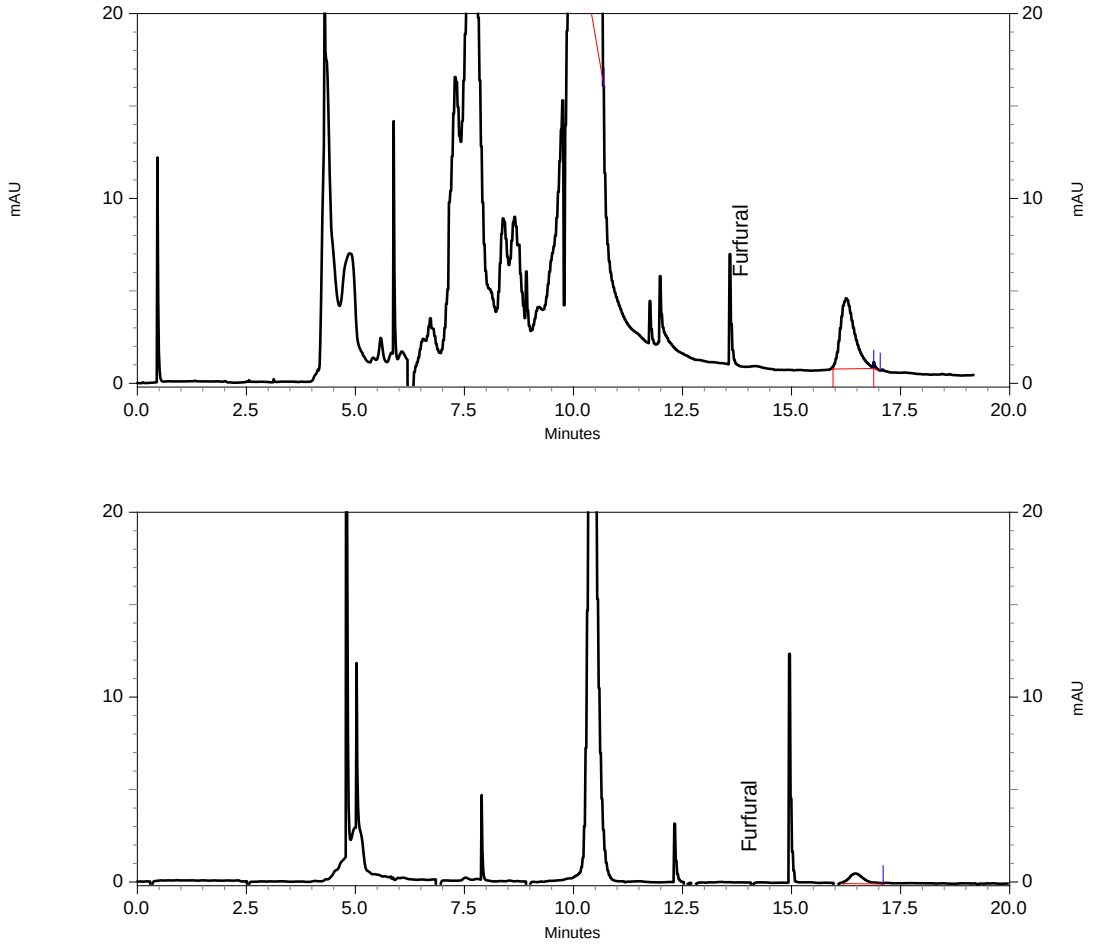

Figure 10. HPLC chromatograms of Rish baba. (A) Anthocyanin of Rish baba + sucrose (B) Anthocyanin of Rish baba without sucrose

4. Discussion

Acidic conditions led to stability of anthocyanins. These pigments are very sensitive and under the storage conditions they change to colorless forms and make brown pigments. Temperature and pH have the most important effect on stability of anthocyanins (28). There are hydroxyl groups in structure of anthocyanins. Anthocyanins are unstable to heat because of the presence of these groups (29). According to Turker et al. (2004) results, the reduction of black carrots anthocyanins and browning of those anthocyanins occurred after 24 days of being at 40°C (30). Albarici and Pessoa's work (2012) was to evalute the effect of heat treatment on anthocyanins of Açaí. They concluded that the temperature and time can affect the stability of anthocyanins wherease degradation of anthocyanins at 40°C was faster than at 0 and 25°C. In some cases, anthocyanins are stable at high temperatures when the heating time was low (31). Krica and Cemeroglu (2003) obtained similar results on anthocyanin of blood orange juice (32). Xiu-li et al. (2015) found that anthocyanins of purple sweet potato were unstable at pH 8 and the color of anthocyanins was changed because of instability during storage (29). In alkaline conditions, degradation of anthocyanins and phenolic hydroxyl oxidation were happened that cause instability of anthocyanins (33). Setareh et al. (2007) proved that the best pH for stability of anthocyanin is pH 3 (34). Bakhshayeshi et al. (2006) showed that increase of pH is damaged the anthocyanins (35). In both studied varieties, absorption at pH 2 was higher than pH 3 that has been confirmed by numerous studies. This is due to hydrolysis rate at pH 2 is higher than pH 3 so the hydrolysis of sucrose increases during the thermal treatment (36). The results show that in samples which were without sucrose, the absorption decreased over time because the

temperature was high and the degradation of anthocyanins occurred. According to Spayed et al (2002) results, destruction of anthocyanins accelerates by increasing the temperature (37). Amr and Al-Tamimi (2007) studied on Ranunculus asiaticus anthocyanin and found that 42% and 48% of anthocyanins were destroyed after 7 h under the influence of temperature at 50℃ and 80 ℃, respectively (38). Markasis and Palamadis (1975) were studied anthocyanins of grapes stability which were influenced by temperature and found that the increase temperature caused degradation of anthocyanins of grapes. When anthocyanins are destroyed, they will lose their color and nutritional quality (39). Furthermore, pasteurization temperatures cause degradation of anthocyanins (40). In other samples, the absorption decreased until 4 h then increased. Up to 4 h, destruction of anthocyanins occurred and after 4 h, some compounds were formed by special reactions and anthocyanins were replaced by these compounds because of that, the absorption was increased after 4 h. Rosso and Mercadante (2007) realized addition of sugars had a negative effect on stability of anthocyanins (41). Giusti and Wrolstad (2000) reported that hydrolisation of pyrilium ring of chalcone is responsible for brown color (22). Browning reaction is affected by some factors such as temperature, during of heating, pH and concentration of reactant (like sucrose) (19). Sucrose is destroyed during the heating and furfural is a one of the degradation products of sucrose which reacts with anthocyanins and form brown polymeric pigments (15, 16). Daravingas and Cain (1968) investigated the effect of different sugars (sucrose, glucose, xylose and fructose) on stability of anthocyanins and found that destruction of anthocyanins increased with all sugars which they studied (14). The impacts of sucrose, fructose and aspartame were studied by Rubinskience et al. (2005). The results of this work showed that aspartame and sucrose had similar effects on stability of anthocyanins. Also, they found that fructose has greater impact than glucose on the degradation of anthocyanin. According to this result, it is suggested that formation of furfural from ketohexose is more than aldohexose (42). Cao et al (2009) showed that the amount of products of thermal destruction of sucrose at 90℃ was more than 70 or 80℃. Furfural and hydroxymethylfurfural (HMF) were formed during the browning of sugars (43). These compounds may be destroyed the color of strawberry's anthocyanin (44). Karami et al. (2013) added sucrose to extracted anthocyanin of black cherries and found that the osmotic pressure increased with increscent of sucrose concentration that this caused water loss. When water lossing increases, anthocyanins are lost (45). Also, addition of sucrose has damaging effect on anthocyanins so, it can cause decresing of anthocyanins. FT-IR was used to demonstrate the presence of anthocyanins in our extractions. Sirvastava and Vankar (2010) worked on Canna indica and used FT-IR to determine the structure of anthocyanins in Canna indica (46). Also, Kim et al (2013) used this technique to confirm the presence of anthocyanins in extracts of Rhododendron flowers.

5. Conclusion

Stability of anthocyanins can affect by many factors such as pH, temperature, concentration of sugars and etc. In this study, results showed that high temperature destroyed the anthocyanins over time since the absorption at 520 nm decreased during the time. When the anthocyanin extracts were supplemented with sucrose, degradation and polymerization of anthocyanins occurred. This conclusion was confirmed by decreasing then increasing the absorption at 520 nm during the time. Scanning of wavelength is emphasized destruction of anthocyanin at high temperature. Also, we concluded that the amount of furfural in samples which have sucrose is higher than the samples which are without the sucrose.

Conflict of interest

The authors have no conflict of interest.

Acknowledgements

None.

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