Investigation of Physicochemical Properties of Grape Juice and Apple Juice Containing Anthocyanin Pigment Extracted from *Roselle* (*Hibiscussabdariffa*) Petals

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ABSTRACT: *Colors are used in foods to make them more appealing. Roselle petals contain functional compounds such as anthocyanins and polyphenols in addition to natural pigments. The objective of this study was to investigate the physicochemical properties of grape and apple juice-based drinks containing anthocyanin extracted from roselle petals. To do this, the extracted pigments were added to the apple juice and the grape juice at concentrations of 5 and 10% w/w. pH, acidity, total phenolic content, anthocyanin, degradation index, hydroxymethylfurfural (HMF), as well as the sensory parameters, were measured for the drink samples stored at 4 and 25°C for 30 days. The results showed that with increasing storage time, pH decreased and acidity increased. The anthocyanin content of the samples ranged from 3.530 to 9.55 mg/L and their total phenol content ranged from 0.180 to 0.630 mg/mL and the degradation index of the samples ranged from 1.510 to 2.980 and their HMF content ranged from 5.560 to 10.260 ppm. With increasing temperature and storage time anthocyanin, total phenolic content decreased and the degradation index, HMF content increased. Apple juice drinks containing 10% anthocyanin stored at 4°C had the highest polyphenolic and anthocyanin content. The results of the sensory evaluation revealed that the apple juice treatment containing 10% anthocyanin stored at 4°C was the superior treatment due to its highest bioactive compounds and sensory score.*

KEYWORDS: *Anthocyanin; Apple Juice; Grape Juice; Roselle.*

INTRODUCTION

Epidemiological studies have shown that the beneficial effects of fruits which are associated with reduced incidence of chronic heart disease and cancer are the result of their antioxidant activity [9]. Recently the consumption of fruits in the juice form has increased. Healthy drinks have become very popular among consumers in recent years.

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Production of juices based on preservation, diversification and creation of more markets for fruitshas been considered by food industry [5].

Grapes are one of the world'slargest fruit crop. The composition of grape juice is very similar to that of grapes except for fibers and oils which are removed during the preparation process. Grape juice contains fructose, organic acids, phenolic compounds, nitrogenous compounds, aromatic substances, minerals and *pecticsubstances* and its antioxidant activity is more than twicehigher thanthat of oranges, apples, grapefruits and tomatoes [35]. Grape juice has a large amount of water (81-86%) and high acidity due to the presence of tartaric, malic and citric acids. These acids cause low pH value and develop sweet-sour taste in this product [29].

Apple juice is among the most important products in juice industry. Apples and apple juice are healthy due to their bioactive components such as polyphenols,pectin's and organic acids [10]. Apples contain 85% water, 12-14% carbohydrate, ~ 0.3% protein, minerals and vitamins [2]. Food colorings play a crucial role in improving the properties and appearance of foods. Color as one of factors affecting the quality control influences the acceptance of juice by the consumer [6].

Until the advent of the first synthetic dye by Willliam Perkin in 1856, only pigments extracted from plants, animals and minerals were used as food coloring [31]. The reasons for using natural dyes in foods depend on several factors including the consumer's desire for colored foods, the availability and stability of natural dyes against different factors as well as lack of toxicity, nature and effects of other colors.

Therefore, extraction is one of the most widely used processes in food industry by which the desired compounds are separated from the foods.

Anthocyanins are a class of natural water-soluble pigments widely found in plant cell fluid and are responsible for the red, blue and purple colors of many fruits and vegetables [4]. Anthocyanins can increasethe nutritional value of foods through preventing the oxidation of lipids and proteins in food products. Anthocyanin content is affected by environmental and agronomic factors. They are unstable and their stability is largely influenced by processing parameters including pH, temperature, light, oxygen, enzymes, ascorbic acid, flavonoids and metal ions. They are extracted by using aqueous and alcoholic solvents such as ethanol and methanol often at low temperature (<30°C) preferably under vacuum and acidic conditions [4,34]. Anthocyanins have a great potential to be a healthy and effective edible coloring in food industries.

Roselle is one of the good sources of anthocyanin pigment. Roselle (*Hibiscus sabdariffa*) belonging to the *Malvaceae* family is a medicinal plant that is known as sour tea in Iran. In Iran, Sistan and Baluchestan province due to its climatic diversity, location and natural habitat is a large number of medicinal plants, including sour tea. Different parts of roselle including flowers, leaves and seeds can be used in food and pharmaceutical industries. It is rich in anthocyanins, flavonoids, ascorbic acid and many other invaluable components and has antibacterial activity [22]. The calyces of roselle are rich in anthocyanin, ascorbic acid, and other phenolic compounds. It has been observed that its components, such as vitamins (c and e), polyphenols acids and flavonoids, mainly anthocyanins, have functional properties. They contribute benefit to health as a good source of anti- oxidant as well as a natural food colorant. Anthocyanins present in roselle are delphinidin, 3-sambubioside, cyanidin 3- sambubioside, delphinidin 3- glucoside, and cyanidin 3- glucoside. Flavonoids gossypetin, hibiscetine, and sabdaretine. Pigment, formerly reported as hibiscine has been identified as daphniphylline [11].

Kirca et al. (2006) studied the stability of anthocyanins extracted from black carrot in juices (apple, grape, grapefruit, tangerine, and lemon) and different nectars (apricot, peach and pineapple). The results showed that anthocyanins were more stable in apple and grape juice at 70 and 80°C than in citrus and among the nectar samples they were more stable in peach and apricot nectars [14].

Fakhari et al. (2009) investigated the extraction of pigments from red beetroot and its stability conditions. The results revealed that the pigment extracted from red beetroot had a significant potential in food industry especially for coldfoods [8].

Recently many restrictions have been put in place on the use of synthetic edible colorings by international organizations and research institutes. Due to the widespread use of anthocyanin in various industries, especially in beverage industry its applications nowadays is increasing. Therefore, the aim of the present study was to optimize the conditions for using anthocyanins and their

Table 1: Treatments in current research.

Treatment	Drink	Roselle petal pigment (%w/v)	Temperature (℃)
1	Grape juice	5	4
2	Grape juice	10	4
3	Apple juice	5	4
4	Apple juice	10	4
5	Grape juice	5	25
6	Grape juice	10	25
7	Apple juice	5	25
8	Apple juice	10	25

stabilization in appleand grape juice-based drinkswhich are functional products due to the presence of flavonoids in a way so that they develop color in the beverages.

EXPERIMENTAL SECTION

Materials

Grape and apple juice were purchased from Sunich company (Iran) and refrigerated (4℃) Roselle petals were purchased from a grocery in Tehran. The chemical compounds including 0.1 N sodium chloride, potassium chloride, chloric acid, sodium acetate, hydrochloric acid, Folin-Ciocalteu reagent, ethanol, gallic acid, sodium carbonate, potassium *Ferro cyanide*, zinc acetate and diethyl ether were purchased from Merck company (Germany).

Samples preparation

Pigment extraction

Anthocyanin was extracted from roselle petals by maceration and solvent methods. First,to increase the contact surface between the solvent the dried petals were completely crushed the dry matter thereby increasing the extraction efficiency and then they were sieved with meshNo.16.

Methanol and water in the ratio of 0.5:1.5 were used as the solvent system for extraction and then, 100g of the crushed plant petals and 50mLof solvent were mixed in a 250 mL Erlenmeyer. The Erlenmeyer was capped with a polyethylene cover to prevent the solvent from evaporating. Then it was kept in an incubator shaker for 72 h. To achieve Brix 60 [15], the solution was filtered with *whatmanfilter*paper No.1 and concentrated by rotary evaporator at 60°C.

Use of rosellepetal pigment in grapeand apple juice-based drink

The extracted pigment from roselle petals at twoconcentrations of 5 and 10% w/v was added to the drinks containing 20% apple and grape juiceat 4 and 25 ℃ temperatures (Table 1) pH, acidity, total phenolic content, anthocyanindegradation index, HMF and total acceptance were measured after 1, 15 and 30 days [27].

Physiochemical methods

pH and acidity measurement

Acidity was measured by titration using 0.1% sodium hydroxide and pH value was measured by pH meter (Model Mettler Toledo-M A235, Switzerland) according to Iranian national standard No. 2685[12].

Anthocyanin measurement

The anthocyanin content was determined using the pH differential method. The color of anthocyanin varies with the pH, being reddish at pH 1 and colorless at pH 4.5, so samples were diluted in buffers at pH 1 (prepared using HCl and sodium acetate) and pH 4.5(prepared using HCl and KCl). A 0.1-mL aliquot of each sample was added to1.4 mL of each buffer and vortexed. The absorbance was then measured at 510 and 700 nm with a spectrophotometer to predict the anthocyanin content using the following formula:

$$\text{Anthocyanin (mg/L)} = \text{A} \times 1000 \times \text{MW} \times \text{DF}/\varepsilon \times 1 \quad (1)$$

Where A = (A510 nm − A700 nm at pH 1) − (A510 nm − A700 nm at pH 4.5), MW = molecular weight of standard anthocyanin, DF = dilution factor, l = path length (in cm),

ε= molar extinction coefficient (in L/mol/cm), and 1000 = factor for converting from grams to milligrams [17].

Total phenolic measurement

Total phenolic content was measured by using Folin-Ciocalteu reagent. 10 mL of ethanol, 100mg/L of standard gallic acid solution and 100 mL of *Folin-Ciocaiteu* reagent were added to 110 mL of each sample. After 5 min, 750mL of 6% sodium carbonate solution were added in other to induce the reduction reaction and to intensify the color. It was kept in the dark at room temperature for 60 min and then the absorbance was read at 780 nm by spectrophotometer (HACH-DR/4000U, USA). Gallic acid was used as the standard for drawing the calibration curve. Total phenolic content was calculated based on the equivalent amount (mg/L gallic acid/mL sample) according to equation 2:

$$\mathrm{Tp} = \frac{\mathrm{A2} \times \mathrm{C2}}{\mathrm{A1}} \qquad (2)$$

Where *TP* is the concentration of total polyphenols (mg/L), C_2 is the concentration of gallic acid (mg/L), A_1 is the absorbance of standard gallic acid and A_2 is the absorbance of sample [7].

Anthocyanin degradation index (DI) measurement

DI of anthocyanin was measured by spectrophotometer (Model Biochrom S200, UK) and by dividing the absorbance at 420 nm by the absorbance at 520 nm. By absorbing the samples in Wavelengths of 520 and 420 nm were measured and from0.1 M citrate buffer to zero the device was used. Increase in DI after pigment degradation usually indicates a decrease in red (A520) and an increase the color is brown (A420) [7].

$$\mathrm{DI} = \frac{\mathrm{A420}}{\mathrm{A520}} \qquad (3)$$

HMF measurement

HMF content was measured by HPLC (Model Infinity 1200, Agilent grap, (Germany). To do so, Column 18C (25cm long, I.D. 4.6μm), particle size of 4.5μm and temperature of 20°C were used. 4 mL of distilled water, 15% potassium *ferrocyanide* and 30% zinc acetate were added to 1mL of each drink. They were stirred and centrifuged at 5000 rpm. This procedure was repeated two more times. After each centrifugation, the supernatant was removed and added to the solution and its volume reached 10mL by adding distilled water. 5mL of the sample were poured into a separator flask to which 5 mL of diethyl ether were added. It was thoroughly stirred and then the lower solution was discarded and the upper solution was kept and this process was repeated3 times.

Finally, both solutions were mixed and 5 mL of distilled water were added to the resulting solution. The samples were kept at 40°C to remove diethyl ether. The sample then was filtered with a 4.5 μm filter paper, and then injected into HPLC and HMF content (ppm) was measured by using the equation derived from the standard curve [32].

Sensory evaluation

Sensory properties were measured by 10 trained panelists by using 5-point hedonic scale as 5, 4, 3, 2 and 1 represented very good, good, intermediate, bad and very bad, respectively [18].

Data analysis

In this study, four time-independent variables (first day, Fifteenth and thirtieth), storage temperature (4 and 25 degrees C), the percentage of pigment (5 and 10% Weight / volume) and type of the drink (apple juice and grape juice) were designed. In order to data analysis one-way analysis of variance (Duncan)was used by Minitab 16 with 95% Reliability.

RESULTS AND DISCUSSION

The pH and Acidity measurement

Acidity and alkalinity are two chemical characteristics of different compounds which are measured by an index called ph. The pH variations may cause instability of anthocyanins and their color changes [19]. Changes in pH and acidity of the apple and grape juice-based drink containing different concentrations of roselle pigment stored at 4 and 25°C are reported in Table 2. pH value of all samples dropped slightly which was not statistically significant ($p>0.05$). pH increased on day 15 and again decreased on day 30. The increased was likely caused by copigmentation of anthocyanins on day 15 and when this unstable association was disappeared it decreased.

Yaghoobi (2015) reported that the addition of green tea extract to lime juice within the first two months of storage at 4°C has no significant effects on pH [37].

Table 2: Effect of different concentrations of roselle pigment and temperatures on pH and the acidity changes (% citric acid g/100g) in grape and apple juice.

Treatment	pH			Acidity (% citric acid g/100g)		
	Day 1	Day 15	Day 30	Day 1	Day 15	Day 30
1	2.600 ± 0.026^{aA}	2.580 ± 0.025^{aA}	2.570 ± 0.025^{aA}	0.260 ± 0.010^{bA}	0.270 ± 0.010^{Ba}	0.280 ± 0.011^{cA}
2	2.600 ± 0.104^{aA}	2.560 ± 0.102^{aA}	2.540 ± 0.101^{aA}	0.280 ± 0.005^{bB}	0.290 ± 0.005^{bAB}	0.300 ± 0.006^{cA}
3	2.600 ± 0.052^{aA}	2.540 ± 0.050^{aA}	2.530 ± 0.050^{aA}	0.330 ± 0.016^{aA}	0.340 ± 0.017^{aA}	0.350 ± 0.017^{bA}
4	2.600 ± 0.078^{aA}	2.520 ± 0.075^{aA}	2.520 ± 0.075^{aA}	0.350 ± 0.014^{aA}	0.360 ± 0.014^{aA}	0.370 ± 0.014^{abA}
5	2.600 ± 0.026^{aA}	2.560 ± 0.025^{aA}	2.560 ± 0.025^{aA}	0.260 ± 0.005^{bB}	0.280 ± 0.005^{Ba}	0.290 ± 0.005^{cA}
6	2.600 ± 0.052^{aA}	2.540 ± 0.050^{aA}	2.540 ± 0.050^{aA}	0.280 ± 0.008^{bB}	0.300 ± 0.009^{bAB}	0.310 ± 0.009^{cA}
7	2.600 ± 0.052^{aA}	2.520 ± 0.050^{aA}	2.530 ± 0.050^{aA}	0.330 ± 0.013^{aA}	0.350 ± 0.014^{Aa}	0.360 ± 0.014^{abA}
8	2.600 ± 0.078^{aA}	2.490 ± 0.074^{aA}	2.470 ± 0.074^{aA}	0.350 ± 0.010^{aB}	0.370 ± 0.011^{Aab}	0.390 ± 0.011^{aA}

Results are presented as mean±SD.
Different small letters in each column represent significant difference.
Different capital letters in each row represents significant difference.

The acidity of drinks during 30-day storage increased significantly ($p\leq0.05$). The effect of storage time on decreasing pH and increasing the acidity was less significant for the samples stored at 4℃. Also, in apple juice-based containing 10% concentration of roselle pigment and stored at 25 ° C, showed a greater decrease in pH and the acidity increased. In fact, the predominant acid in apple juice was mallic acid [28] and in grape juice was tartaric acid [28] which can affect the changes in acidity over thetime. High temperature and reactions occurred in the drinks could reduce pH and acidify of the drink. *Yoosefi et al*. (2018) examined the pH changes and the acidity of fruit juice samples based on apple and aloe vera for 15 days of storage at 4 and 25 ° C with a concentration of 5 and 10% EchiumAmoenum petals. With time and temperature increase, the pH decreases, and the acidity increases. Also Because of the nature of the aloe vera juice compared to the Apple juice had a lower pH and higher acidity the changes were clearer [38].

Anthocyanin measurement

Anthocyanins members of the flavonoid group. They are responsible for red, purple, and blue colors in many flowers, fruits, and vegetables and play an important role in pollination and protection against environmental tensions [36]. The changes in anthocyanin of the apple and the grape juice containing different concentrations of roselle pigment stored at 4 and 25℃ are presented in Table 3.

The results showed that the anthocyanin content of all treatments is decreased over the time, however, this decreasing trend statisticallywas not significant ($p>0.05$). The anthocyanin content of the samples stored at 25℃ was lower than that of the samples stored at 4℃ as on the last day of storage(day 30) The lowest anthocyanin content (3.530mg/L) was observed for the grape juice sample containing 5% roselle pigment stored at 25℃ and the highest anthocyanin content (9.550 mg/L) was found for the apple juice treatment containing 10% roselle pigment stored at 4℃. The anthocyanin variations in apple juice ranged from 3.77 to 9.69 mg/L and in grape juice ranged from 3.530 to 9.59 mg/L.

Increasing the pigment content from 5 to 10% increased the anthocyanin content and increasing the storage temperature from 4 to 25℃ decreased the amount of anthocyanin. In fact, the thermal stability of anthocyanin depends on its structure, the presence of oxygen, and its reaction with other compounds. There is a significant relationship between temperature and anthocyanin degradation.

Kirca et al. (2006) reported that the anthocyanin of black carrot added to apple and grape juice at 70 and 80℃ were more stable than in other citrus juices [14].

In general, grape juice samples had higher anthocyanin content than apple juice on the first day and the anthocyanin content decreased over time as on day 30 apple juice samples had higher anthocyanin content than

Table 3: Effect of different concentrations of roselle pigment and temperatureson anthocyanin changes (mg/L) in grape and apple juice treatments

Treatments	Type of drink	Pigment concentration (%)	Storage temperature (°C)	Day 1	Day 15	Day 30
1	Grape juice	5	4	4.160 ± 0.166^{bA}	4.010 ± 0.160^{cdA}	3.940 ± 0.157^{cdA}
2	Grape juice	10	4	9.590 ± 0.191^{aA}	9.420 ± 0.188^{abA}	9.330 ± 0.186^{abA}
3	Apple juice	5	4	4.290 ± 0.085^{bA}	4.220 ± 0.084^{cA}	4.180 ± 0.083^{cA}
4	Apple juice	10	4	9.690 ± 0.297^{aA}	9.600 ± 0.288^{aA}	9.550 ± 0.286^{aA}
5	Grape juice	5	25	3.800 ± 0.152^{bA}	3.630 ± 0.145^{dA}	3.530 ± 0.141^{dA}
6	Grape juice	10	25	9.250 ± 0.185^{aA}	9.050 ± 0.181^{bA}	8.940 ± 0.178^{bA}
7	Apple juice	5	25	3.900 ± 0.039^{bA}	3.820 ± 0.038^{cdAB}	3.770 ± 0.037^{cbA}
8	Apple juice	10	25	9.380 ± 0.281^{aA}	9.280 ± 0.278^{abA}	9.220 ± 0.276^{abA}

Results are presented as mean±SD.
Different small letters in each column represent a significant difference.
Different capital letters in each row represent a significant difference.

grape juice. The reason may be the higher pH value of the apple juice samples which caused less anthocyanin degradation during storage. *Inggrid et al*., 2017 Investigated the effect of pH (2, 7, and 12) and temperature (5°C, 30°C, and 55°C) on total anthocyanin activity of Roselle and stated that the highest amount of total anthocyanin was in 80.4 mg/l Roselle petals was at 5 °C and pH 2 [11].

Total phenolic content measurement

Polyphenols are phenolic compounds of plants which is a specific group of secondary metabolites playing an important role in protecting tissues from oxygen or other active species radicals [13].

Changes in total phenolic content of apple and grape juice-based drink samples containing different concentrations of roselle pigment stored at 4 and 25°C are reported in Table 4. The total phenolic content of all treatments decreased significantly ($p\leq0.05$) with time as on day 30 the highest total phenolic content (0.630 mg/L) was found for apple juice treatment containing 10% pigment at 4°C and the lowest total phenolic content (0.180 mg/mL) was observed for the grape juice sample containing 5% pigment at 25°C.

The total phenolic content changes for apple juice treatments ranged from 0.680 to 0.230 mg/mL and for grape juice ranged from 0.650 to 0.180 mg/mL.

Increased amount of roselle pigment from 5 to 10% resulted in an increase in total phenolic content. However, increased storage time and temperature from 4 to 25°C decreased total phenolic content which indicates the degradative effect of temperature on total phenolic content [20].

Similarly, *Tsai et al.* (2005) reported that as the temperature increased, total phenolic content decreased likely due to the degradative effect of high temperature on the phenolic content [35].

The total phenolic content of apple juice-based drinks was higher than that of grape juice on day 30. The lower pH value of the grape juice sample compared to apple juice had a degradative effect on the total phenolic content. *Mohamad Ahmed et al.* (2020) reported phenolic compounds of the grape are chlorogenic acid, protocatechin, synergic acid, and ferulic acid [23]. *Özcan et al*. (2017) Stated that generally, the main phenolic compounds of all grapes were gallic acid, 3,4-dihydroxybenzoic acid, (+)-catechinve 1,2-dihydroxybenzene [24, 25].

Anthocyanin degradation index (DI) measurement

The degradation index (DI) is measured by calculating the absorbance at 420 nm and 520 nm [1]. Changes in DI of anthocyanin in apple and grape juice-based drinks containing different concentrations of reselle pigment stored at 4 and 25°C are shown in Table 5. DI increased significantly ($p\leq0.05$) over time as on day 30 the highest DI (2.980) was observed for the grape juice sample containing 5% roselle pigment at 25°C and the lowest DI (1.510) was related to the apple juice treatment containing

Table 4: Effect of different concentrations of roselle pigment and temperatures on total phenolic content (mg/mL) changes in grape and apple juice treatments.

Treatments	Type of drink	Pigment concentration (%)	Storage temperature (℃)	Day 1	Day 15	Day 30
1	Grape juice	5	4	0.310 ± 0.012^{cdA}	0.260 ± 0.010^{eB}	0.230 ± 0.009^{eC}
2	Grape juice	10	4	0.650 ± 0.013^{abA}	0.600 ± 0.012^{bcB}	0.570 ± 0.011^{bB}
3	Apple juice	5	4	0.350 ± 0.007^{cA}	0.320 ± 0.006^{dB}	0.300 ± 0.006^{dC}
4	Apple juice	10	4	0.680 ± 0.020^{aA}	0.650 ± 0.019^{aA}	0.630 ± 0.018^{aB}
5	Grape juice	5	25	0.280 ± 0.008^{dA}	0.220 ± 0.006^{eB}	0.180 ± 0.005^{fC}
6	Grape juice	10	25	0.620 ± 0.024^{Ba}	0.560 ± 0.022^{Cb}	0.520 ± 0.020^{cA}
7	Apple juice	5	25	0.310 ± 0.009^{cdA}	0.260 ± 0.007^{eb}	0.230 ± 0.006^{ec}
8	Apple juice	10	25	0.660 ± 0.026^{abA}	0.610 ± 0.024^{abA}	0.580 ± 0.023^{bB}

Results are presented as mean±SD.
Different small letters in each column represent a significant difference.
Different capital letters in each row represent a significant difference.

10% roselle pigment at 4°C. The increase in DI until day 30 indicates anthocyanin degradation.

The results showed that as the storage temperature increased from 4 to 25°C, DI of anthocyanin increased. With increasing temperature, a sharp increase occurred in browning and absorbance at 420 nm due to the degradation and polymerization of anthocyanins at high temperatures [21].

Kirca et al. (2006) reported that storage temperature had a clear effect on anthocyanin degradation. Storage at 37°C resulted in faster degradation of anthocyanins of black carrot as compared to refrigerated storage (4°C) [14]. *Baghaei Amand et al.* (2013) reported that the addition of sucrose to sour cherry juice resulted in the highest DI of anthocyanin within 20 hr and then it decreased. DI was measured by using the absorbance at 420 and 520nm [1].

Hydroxymethyl Furfural (HMF) measurement

HMF is one of the products of sucrose degradation. It is used as one of the parameters of the browning reaction in juices [33].HMF as a product of the Millard reaction is an important parameter for assessing the quality of fruit juices. Changes in HMF of apple and grape-based drink samples containing different concentrations of roselle pigment stored at 4 and 25°C are reported in Table 6. As shown in the Table, HMF increased significantly ($p\leq0.05$) over time as on day 30, the highest HMF content (10.260 ppm) was found for the apple juice treatment containing 10% roselle pigment at 25°C and the lowest HMF (5.560 ppm) was observed for the grape juice containing 5% pigment at 4°C. Indeed, increased pigment concentration and storage temperatures resulted in a significant ($p\leq0.05$) increase in HMF content likely due to the degradation of sucrose over time which could increase the amount of HMF in the stored drinks. Since the apple juice drink inherently has 0.5% HMF, apple juice has higher HMF content than grape juice.

Lee and Nagys (1988) studied the effects of temperature on the quality of grapefruit extract and observed that as the temperature increased, the concentration of HMF as a product of anthocyanin degradation increased [16]. *Cao et al.* (2009) showed that the rate of formation of the products of thermal degradation of sugars at 90℃ was higher than that at 70 and 80 ℃ [3].

Sensory evaluation (Total acceptance)

Changes in the score of total acceptance of the apple and grape juice-based drink containing different concentrations of roselle pigment stored at 4 and 25°C are shown in Table 7.

The total acceptance score for all treatments decreased significantly ($p\leq0.05$)over time as on day 30 the highest sensory score (4.419) was related to the apple juice treatment containing 10% roselle pigment at 4°C and the lowest sensory score (3.102) was found for the apple juice containing 10% roselle pigment at 25°C. In fact, the increase in temperature from 4 to 25°C decreased the sensory score.

Table 5: Effect of different concentrations of roselle pigment and temperatures on anthocyanin degradation index changes in grape and apple juice treatments.

Treatments	Type of drink	Pigment concentration (%)	Storage temperature (°C)	Day 1	Day 15	Day 30
1	Grape juice	5	4	1.880 ± 0.037^{Db}	2.080 ± 0.041^{dA}	2.180 ± 0.043^{dA}
2	Grape juice	10	4	1.650 ± 0.066^{Eb}	1.840 ± 0.073^{eA}	1.930 ± 0.077^{eA}
3	Apple juice	5	4	1.440 ± 0.043^{fB}	1.610 ± 0.048^{fA}	1.690 ± 0.050^{fA}
4	Apple juice	10	4	1.350 ± 0.027^{Fb}	1.450 ± 0.029^{gA}	1.510 ± 0.030^{gA}
5	Grape juice	5	25	2.530 ± 0.050^{Ac}	2.830 ± 0.056^{aB}	2.980 ± 0.095^{aA}
6	Grape juice	10	25	2.300 ± 0.046^{bC}	2.590 ± 0.051^{bB}	2.430 ± 0.054^{bA}
7	Apple juice	5	25	2.090 ± 0.041^{Cb}	2.290 ± 0.045^{cA}	2.390 ± 0.47^{cA}
8	Apple juice	10	25	1.940 ± 0.058^{dB}	2.070 ± 0.062^{dB}	2.150 ± 0.064^{dA}

Results are presented as mean±SD.
Different small letters in each column represent significant difference.
Different capital letters in each row represent significant difference.

Table 6: Effect of different concentrations of roselle pigment and temperatures on HMF (ppm) changes in grape and apple juice treatments

Treatments	Type of drink	Pigment concentration (%)	Storage temperature (°C)	Day 1	Day 15	Day 30
1	Grape juice	5	4	4.650 ± 0.093^{aeC}	5.160 ± 0.103^{fB}	5.560 ± 0.111^{eA}
3	Apple juice	5	4	5.120 ± 0.204^{deB}	5.620 ± 0.225^{efAB}	6.040 ± 0.242^{eA}
4	Apple juice	10	4	7.980 ± 0.159^{bC}	8.430 ± 0.169^{bB}	8.860 ± 0.177^{bA}
5	Grape juice	5	25	5.580 ± 0.055^{dC}	6.120 ± 0.061^{deB}	6.710 ± 0.067^{dA}
6	Grape juice	10	25	8.850 ± 0.177^{aC}	9.390 ± 0.188^{aB}	9.980 ± 0.200^{aA}
7	Apple juice	5	25	5.630 ± 0.168^{dC}	2.230 ± 0.187^{dB}	7.420 ± 0.223^{cA}
8	Apple juice	10	25	9.240 ± 0.369^{Ab}	9.780 ± 0.391^{aAB}	10.260 ± 0.410^{aA}

Results are presented as mean±SD.
Different small letters in each column represent significant difference.
Different capital letters in each row represent significant difference.

Table 7: Effect of different concentrations of roselle pigment and temperatures on total acceptance changes in grape and apple juice treatments.

Treatments	Type of drink	Pigment concentration (%)	Storage temperature (°C)	Day 1	Day 15	Day 30
1	Grape juice	5	4	4.514 ± 0.090^{abA}	4.147 ± 0.082^{bcB}	3.963 ± 0.079^{bcB}
2	Grape juice	10	4	4.772 ± 0.143^{aA}	4.463 ± 0.133^{abAB}	4.308 ± 0.129^{aB}
3	Apple juice	5	4	4.669 ± 0.186^{aA}	4.330 ± 0.173^{abAB}	4.161 ± 0.166^{abB}
4	Apple juice	10	4	4.838 ± 0.145^{aA}	4.558 ± 0.136^{aA}	4.419 ± 0.132^{aA}
5	Grape juice	5	25	4.301 ± 0.086^{bcB}	3.882 ± 0.077^{cdA}	3.669 ± 0.073^{cdC}
6	Grape juice	10	25	4.110 ± 0.123^{cdA}	3.632 ± 0.109^{deA}	3.397 ± 0.101^{deB}
7	Apple juice	5	25	4.205 ± 0.084^{bcd}	3.757 ± 0.075^{dB}	3.529 ± 0.070^{dB}
8	Apple juice	10	25	3.860 ± 0.115^{dA}	3.352 ± 0.100^{eB}	3.101 ± 0.093^{eB}

Results are presented as mean±SD.
Different small letters in each column represent significant difference.
Different capital letters in each row represents significant difference.

Sohrabvandi et al. (2015) reported that the grape juice treatments stored under refrigerated conditions had higher sensory acceptance than those stored at ambient temperature [33].

CONCLUSIONS

In this study, 5 and 10% of anthocyanin pigment extracted from the petals of Roselle were added to two drinks of grape juice and apple juice, and at 4 and 25 °C and pH, acidity, anthocyanin content, total phenol, anthocyanin degradation index, HMF content, and sensory scores were measured during 30 days of storage. According to the results, increasing the temperature and storage time decreased pH and increased acidity. The amount of anthocyanins and total phenol compounds increased with decreasing the storage temperature from 4 to 25 ℃ and the time from 1 to 30 days in the samples. Anthocyanin degradation index and HMF content in apple juice samples were lower than grape juice samples and increasing temperature and storage time increased the mentioned parameters. The results of this study showed that apple juice drink stored at 4 ° C, which contains 10% anthocyanin extracted from Rosellepetal, due to having the highest amount of bioactive compounds and sensory evaluation points as an optimal treatment and product useful in terms of properties quality and health were introduced.

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