

Changes in Anthocyanin and Fruit Quality Attributes of Barberry (*Berberis vulgaris* L.) Grown in Different Altitude During Growth and Maturation

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Received: 18th July 2017 / Accepted: 5th December 2018

ABSTRACT

Purpose: Seedless barberry is one of the economical sources of anthocyanin pigment. However, short harvesting period creates many difficulties in harvesting and drying processes, leading to low quality. Therefore, it was very important to study different barberry growing areas and also evaluating fruit quality in these regions, to propose sooner harvesting in some regions.

Research Method: The study was conducted in two regions (Marack and Amirabad) of Birjand suburbs, South Khorasan, Iran, during two growing seasons (2014-2015), to investigate the major physicochemical changes of barberry fruit during different growth and development stages (89, 121, 152, 183 and 214 days after full bloom (DAFB)).

Findings: The results showed that fruit fresh weight and volume, pH, total soluble solids (TSS), maturity index and anthocyanin significantly increased, as harvest season progressed. The region conditions also influenced the above-mentioned parameters. Data indicated that lower night temperatures in the Marack led to better fruit indices compared with Amirabad, which may be resulted from a higher altitude as compared to Amirabad. The better fruit maturity index and anthocyanin content obtained in Marack at the fourth harvest date (183 DAFB), compared with Amirabad, which creates a sooner harvest potential. With regard to data obtained and especially higher anthocyanin accumulation, we propose mountainous regions for seedless barberry cultivation, compared to lands as it leads to sooner harvesting and better quality of fruit for fresh market.

Research Limitations: It was better to study the physicochemical changes of barberry fruits in more important regions and with different altitude.

Originality/Value: This study provides an insight into mountains with high altitude as main regions for barberry cultivation to increase the nutritional quality of fruit.

Keywords: altitude, anthocyanin, chemical properties, seedless barberry, temperature

INTRODUCTION

Barberry (*Berberis vulgaris* var. *asperma*) belongs to Berberidaceae family and is an important endemic plant in Southern Khorasan province of Iran (Rezvani Moghaddam *et al.*, 2007; Aghbashlo *et al.*, 2008; Fallahi *et al.*, 2010). Barberry fruit is highly valued for its potential health benefits, and many medicinal properties have been reported for all parts of the plant such as tonic, antimicrobial, antiemetic, antipyretic, and antipruritic and cholagogues actions. It has been used in some cases like

cholecystitis, cholelithiasis, jaundice, dysentery, leishmaniasis, malaria and gall stones (Zargari, 1983; Aynehchi, 1986). The observed health benefits of barberry fruit are mainly due to presence of anthocyanin pigments. The roles of anthocyanin pigments as medicinal agents have been well-accepted dogma in folk medicine throughout the world. Anthocyanins belong to

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flavonoids, a group predominant in teas, honey, fruits, vegetables, nuts, olive oil, cocoa, and cereals. Daily intake is estimated from 500 to 1000 mg (Lila, 2004).

Recently, there has been a great increase in the application of anthocyanin in the food industries, replacing synthetic coloring agents (Laleh *et al.*, 2006; Fan *et al.*, 2008) as the colorful anthocyanins are the most recognized visible members of the bioflavonoid phytochemicals. Many studies showed that qualitative indices of berries such as anthocyanins and soluble solids are affected by many factors such as temperature and light intensity (Bergqvist *et al.*, 2001; Yamane *et al.*, 2006; Laleh *et al.*, 2006), harvest time, drying and harvest methods (Chandra and Todaria, 1983; Arena and Curvetto, 2008; Fallahi *et al.*, 2010; Rezvani Moghaddam *et al.*, 2011). Temperature is an important factor influencing flowering, fruit set and ripening in strawberries, tomatoes, (Ledesma *et al.*, 2008; Kositsup *et al.*, 2009; Adams *et al.*, 2001). Anthocyanin accumulation in plants is sensitive to environmental conditions (Oren-Shamir, 2009); low temperatures enhance its accumulation (Oren-Shamir, 2009; Tarara *et al.*, 2008), whereas at high temperatures the pigment concentration is reduced (Mori *et al.*, 2007; Tarara *et al.*, 2008), which reflects concurrent decrease in synthesis rate and accelerated degradation (Oren-Shamir, 2009).

However, little information is available regarding the effect of environmental factors on quality and chemical attributes of barberry fruit, particularly in South Khorasan province as the major area of barberry production in Iran. Therefore, the aims of this research were to evaluate the effect of cumulative heat units

(growing degree days) on fruit physicochemical characteristics; and to find effects of altitude on fruit quality of barberry.

MATERIALS AND METHODS

Region characteristics

The experiment was carried out in two seedless barberry orchards located in Amirabad (1480 m altitude, 32° 56' N and 59° 13' E) and Marack (1648 m altitude, 32° 58.09' N and 59° 25.21' E) of Birjand, Iran, during the 2014 and 2015 growing seasons (Table 1).

Fruit harvesting times and calculation of growing degree days (GDDs)

Fruit sampling in each shrub consisted of 500 g fruit that was harvested at different times (July 6, August 6, September 6, October 6, November 6 and December 6) and transferred to the Horticultural laboratory for further studies. Growing degree days was calculated according to the method by Wien (1997). The temperature was recorded using datalogger (Extech Instruments, Model RHT20, Humidity and Temperature Datalogger, USA) during the experiment.

Physical properties

Physical properties were determined on 100 fruits randomly selected of shoots from each shrub. Fruit weight was measured by an electronic balance with an accuracy of 0.001 g. Fruit volume was measured by a liquid displacement method. Fruit density was estimated by employing the method described by Westwood (1993).

Table 01: Some characteristics of different studied regions

Region	FB (Day)	L	A	pH	EC _w (dS/m)		pH	EC (dS/m)
					Water	Soil		
Marack	April 9	32° 58.1N	1648	6.7	3.51	Loam	7.5	7.6
Amirabad	April 15	32° 56.0N	1480	6.8	4.51	Loam	7.8	7.6

FB: Full Bloom; L: Latitude; A: Altitude; EC: Electrical conductivity; Tex: Texture

Chemical composition

The pH was measured with a digital pH meter (Model 601, Metrohm, Herisau, Switzerland). Total soluble solid (TSS) was determined with a hand-held refractometer (RF 10, 0-32 °Brix, Extech, USA). Titratable acidity (TA) was determined by titration to pH 8.1 with 0.1 N NaOH and expressed as g of citric acid per 100 g of juice (AOAC, 1984). Finally, the total titratable acidity was calculated based on citric acid:

$$LV (\%) = \frac{W}{A \times N \times V \times 100}$$

Where V is the used volume of NaOH (ml), N is the NaOH normality, M is the molecular weight of citric acid and A is the dilution factor.

Maturity index was calculated by dividing TSS to TA. Determination of spectroscopy-based anthocyanins was performed based on the pH difference (Swain, 1965). The method consists of two buffer systems: potassium chloride and sodium acetate. A solution of 0.025 M of potassium chloride was produced and then pH 1 was adjusted using HCl. Sodium acetate buffer was produced using 0.4 M sodium acetate and pH 4.5 was adjusted using acetic acid. For each system, the sample was diluted 1:10 with each buffer solution.

Anthocyanin content was estimated as pelargonidin 3-glucoside at 510 nm, as the major anthocyanin in barberry fruit with maximum wave lengths of the visible ($\lambda_{vis-max}$) at 510 nm (Swain, 1965). The absorbency difference between the buffer systems was calculated according to the Eq. (1):

$$A = [(A_{max} - A_{700nm})_{pH1.0}] - [(A_{max} - A_{700nm})_{pH4.5}] \quad (1)$$

Then, the concentration of total anthocyanin (mg/L) was calculated according to the Eq. (2):

$$\text{Total Anthocyanin (mg/L)} = \frac{A \times MW \times DF \times 1000}{\epsilon \times d} \quad (2)$$

A: absorbance; A_{max} : absorbance at 510 nm;
 A_{700} : absorbance at 700 nm

MW: molecular weight of the pelargonidin 3-glucoside = 433.39 g/mol

DF: dilution factor = 10

ϵ : coefficient of molar absorptivity = 15600

d: pathlength (cm) = 1

Statistical analysis

The experiment was conducted in a Complete Randomized Block Design (CRBD) with three replications. Each treatment contained 9 shrubs (three shrubs per replicate). Data analysis was carried out using SAS 9.1 and means were compared by the Duncan Multiple Ranges Test at 5% level to distinguish the difference.

RESULTS AND DISCUSSION

Evaluation of different temperatures and calculation of growing degree days (GDDs)

Different parameters related to temperatures including monthly, day and night mean temperatures decreased as growth season progressed (Table 2). The day-night difference was higher in Marack compared with Amirabad (Table 2), which indicates cooler night. Moreover, lower night temperatures than 15°C observed in Marack, since September, and in Amirabad since November. So, cooler climate can be seen in Marack that may be resulted from a higher altitude (Tables 1 and 2). More cumulative heat units observed in Amirabad in comparison with Marack, up to July 6, and a reducing trend can also be seen monthly. Moreover, more than 1000 accumulated heat units can be seen in Amirabad, compared to Marack (Table 3).

Table 02: Monthly mean, day and night temperatures (°C) recorded in Marack and Amirabad regions, Birjand, Iran

Region	Temperature (°C)	April	May	June	July	August	September	October	November	Average
Marack	Monthly	15.4	19.5	26.6	28.2	27.7	24.0	21.9	12.3	21.1
	Day	22.8	27.5	35.6	36.5	36.3	34.1	31.0	20.2	29.4
	Night	8.0	11.5	17.7	19.8	19.2	13.9	12.7	4.4	12.8
	D-N D	14.8	16.0	17.9	16.7	17.1	20.2	18.3	15.8	16.6
Amirabad	Monthly	16.9	21.3	28.2	29.8	29.8	26.2	23.0	17.6	22.9
	Day	24.4	28.9	36.7	37.5	37.3	34.9	32.2	27.5	30.9
	Night	9.3	13.6	19.6	22.1	22.3	17.4	17.9	14.1	15.1
	D-N D	15.1	15.3	17.1	15.4	15.0	17.5	18.3	13.4	15.8

D-N D: day-night difference

Table 03: Cumulative heat unit (growing degree days = GDDs) from flowering up to 6 July and between all two harvesting times, and days after full bloom (DAFB) recorded in Marack and Amirabad regions, Birjand, Iran

Region	Monthly growing degree days (GDDs)					Total
	6 July	6 August	6 September	6 October	6 November	
Marack	560.90	347.00	238.80	227.10	44.70	1445.50
Amirabad	797.16	544.41	553.26	368.19	354.24	2617.26
DAFB	89	121	152	183	214	

DAFB: Days after full bloom, growing degree days = GDDs

Physical and chemical properties

Statistical analysis showed the significant effects of harvest time and region on quantitative and qualitative indices of seedless barberry. The lowest and highest berry fresh weight and volume obtained at 89 and 214 days after full bloom (DAFB), respectively, and then an increasing trend observed as the growing season progresses (Table 4), which was in agreement with findings of Rezvani Moghaddam *et al.*, (2013) on barberry. Moreover, the simple effect of the region also showed the highest values for both these traits in Marack (Table 4). Up to 152 DAFB, there was no significant difference between both regions in fruit fresh weight and volume. However, after this time, these traits significantly increased in Marack that may be attributed to lower temperatures,

especially cooler nights (Table 5), in agreement with findings of Sugiura *et al.*, (1991) and Candir *et al.*, (2009) on persimmon. A positive correlation was also shown between these two traits ($r=0.99$) (Table 6).

Total soluble solids (TSS) and total titratable acidity (TA) are the useful chemical criteria for defining the optimal harvest time (Arena and Curvetto, 2008; Fallahi *et al.*, 2010) in barberries. The highest pH and TSS/TA ratio were recorded in 214 DAFB that was in agreement with Rezvani Moghaddam *et al.*, (2013) on barberry. A significant increment in TSS was observed after 183 days, while TA was decreased significantly since this day (Table 7), in agreement with Candir *et al.*, (2009) on persimmon, Rezvani Moghaddam *et al.*, (2013) on barberry and Kulkarni and Aradhya (2005)

on pomegranate fruit. Biale (1960) suggested that increase in TSS attributed to starch hydrolysis into simple sugars that can be seen in barberry fruit. Reduction of TA coincided with TSS increment that was in agreement with Kulkarni and Aradhya (2005) on pomegranate fruit. Anthocyanin accumulation significantly

increased 183 DAFB, compared to 152 DAFB (Table 7) in accordance with findings of Rezvani Moghaddam *et al.*, (2013) on barberry. On the other hand, the lowest values for pH, TSS, TA, TSS/TA and anthocyanin content of barberry fruit were observed in Amirabad, compared with Marack (Table 7).

Table 04: Fresh weight and volume of barberry fruit harvested during different stages of growth and development in two regions Marack and Amirabad

Treatment		Fruit Fresh Weight of 100 berries (g)	Fruit Volume of 100 berries (cm ³)
Harvesting date (DAFB)	DAFB		
89	89	1.19c	3.30c
121	121	3.61c	8.20c
152	152	5.13c	9.20c
183	183	14.33b	38.90b
214	214	85.89a	23.80a
Region			
Marack		23.97a	63.80a
Amirabad		20.08b	54.70b

Means with the same letters in each column are not significantly different at the 0.05 level of probability.

DAFB: Days after full bloom

Fruit fresh weight and volume evaluated based on 100 number fruit

Table 05: Interactive effects of different regions and harvesting dates on fresh weight and volume of barberry fruit

Treatments	Fruit Fresh Weight of 100 berries (g)	Fruit Volume of 100 berries (cm ³)
Region×Harvesting date (DAFB)		
Marack×89	1.13e	3.00d
Marack×121	3.70e	8.60d
Marack×152	5.85e	9.70d
Marack×183	15.57c	44.50b
Marack×214	93.33a	99.00a
Amirabad×89	1.24e	3.52d
Amirabad×121	3.51e	7.66d
Amirabad×152	4.14e	8.22d
Amirabad×183	13.07d	33.22c
Amirabad×214	78.44b	73.00b

Means with the same letters in each column are not significantly different at the 0.05 level of probability.

DAFB: Days after full bloom

Table 06: Correlation coefficient of different harvesting dates in two regions on fruit fresh weight and volume of barberry

Character	Fruit Fresh Weight of 100 berries (g)	Fruit Volume of 100 berries (cm ³)
Fruit Fresh Weight (g)	1	
Fruit Volume (cm ³)	0.99**	1

* Significant at 1% level.

Table 07: Effects of harvesting dates on pH, total soluble solids (TSS), titratable acidity (TA), maturity index (TSS/TA) and anthocyanin content of barberry fruit under two regions

Treatment		pH	TSS (°Brix)	TA (mg/100g F.W.)	TSS/TA	Anthocyanin (mg/100g F.W.)
Harvesting date	(DAFB)					
H3	152	3.93b	13.27b	7.50a	2.03b	3.57b
H4	183	3.94b	15.25a	6.40b	2.07b	4.10a
H5	214	4.05a	15.88a	5.80b	2.73a	4.45a
Region						
Marack		4.11a	17.42a	7.60a	2.29a	4.57a
Amirabad		3.83b	13.25b	6.80b	1.94b	3.19b

Means with the same letters in each column are not significantly different at the 0.05 level of probability.

DAFB: Days after full bloom

Koshita *et al.*, (2007) found an increment of grape skin coloration under low night-time temperatures, which can be seen under Marack conditions, compared to Amirabad (Table 2) and may be related to lower night temperatures started from September. The interaction between location and harvesting times showed an increasing trend about pH and maturity index as the season progressed (Table 8) as reported by Chandra and Todaria (1983), Arena and Curvetto (2008) and Fallahi *et al.*, (2010). There was no significant difference in TSS and anthocyanin content among different harvesting times in Marack (152, 183 and 214 DAFB) that may be correlated with lower night temperatures started since September that was in agreement with findings of Mori *et al.*, (2007), Oren-Shamir (2009) and Tarara *et al.*, (2008), who observed that low temperature is effective on anthocyanin accumulation. Although an increasing trend was indicated about TSS and anthocyanin in Amirabad in agreement with Chandra and Tondria, (1983) and Rezvani Moghaddam *et al.*, (2013), however, the highest values of these

traits was observed in Marack (Table 8), which may be related to low night temperatures in this region. It has been reported that low temperature during fruit growth and development accelerates the coloration of the grape skin (Spayd *et al.*, 2002). Moreover, recently, crosstalk between a sugar-induced gene and an ABA signaling gene has been reported (Çakiret *et al.*, 2003). Koshita *et al.*, (2007) found that ABA level of grapevine fruit under lower night-time temperature is two times higher than higher night-time condition. It seems that night temperatures play a key role in coloration of barberry fruit, may be via ABA accumulation in fruit. Interactive effects of location and harvesting times showed that the total acidity significantly decreased in Marack since 183 DAFB, however, this reduction was observed in Amirabad since 152 DAFB (Table 8), which was in line with findings of Candir *et al.*, (2009) on persimmon.

Fresh barberry fruits are normally acidic and therefore, are not suitable to use afresh, although demand for fresh fruit in the market is increasing, particularly in Iran. However, fruits

harvested in Marack showed higher values of fresh weight, TSS, TA, TSS to TA ratio and pH compared with fruits harvested in Amirabad. So, it can be concluded that highly likely barberry fruit grown at higher altitudes are more suitable to use as fresh and also processed fruit. The sensorial quality of fruit taste that was evaluated by a panel of five assessors at each harvest time (H3, H4 and H5) revealed higher values of fruit taste in Marack than Amirabad in this regard (data not shown). In addition, positive correlation between pH with TSS, and also between TSS with anthocyanin was observed (Table 9). It seems that increment of TSS and anthocyanin content of fruit is coincident that was in agreement with Hunter *et al.*, (1991) in grapevine. On the other hand, a negative correlation was observed between TA and anthocyanin accumulation. Cabrita *et al.*, (2000) stated that anthocyanin pigments

undergo a reversible structural transformation with a change in acidity.

CONCLUSIONS

The results showed that quantitative and qualitative indices of seedless barberry were affected by harvest time and climate effects. The fruit fresh and dry weights were increased by delaying in harvest date. In addition, maturity index and anthocyanin content increased as the season progressed; however, low night temperatures significantly affected these significant traits in barberry fruits. The results suggests that higher altitude can be regarded for barberry production in Iran because of better quality indices and lower cost. Moreover, regarding anthocyanin accumulation and TSS, harvesting date of Marack may be soon, because of higher latitude, compared to Amirabad.

Table 08: Interactive effects of different regions and harvesting dates on pH, total soluble solids (TSS), titratable acidity (TA), maturity index (TSS/TA) and anthocyanin content of barberry fruit

Treatments (Location×DAFB)	pH	TSS (°Brix)	TA (mg/100g F.W.)	TSS/TA	Anthocyanin (mg/100g F.W.)
Marack×152	4.08b	15.00a	7.60a	1.97c	3.76a
Marack×183	4.09b	17.00a	7.50a	2.26b	4.87a
Marack×214	4.17a	19.00a	6.01b	3.16a	4.88a
Amirabad×152	3.71c	12.00c	7.34a	1.63c	1.74b
Amirabad×183	3.81c	12.00c	6.34b	1.88b	3.09b
Amirabad×214	3.93b	14.00b	5.94b	2.35b	4.50a

Means with the same letters in each column are not significantly different at the 0.05 level of probability; DAFB: Days after full bloom

Table 09: Correlation coefficient of different harvesting dates under two regions on pH, total soluble solids (TSS), titratable acidity (TA), maturity index (TSS/TA) and anthocyanin content of barberry fruit

Character	pH	TSS (°Brix)	TA (mg/100g F.W.)	TSS/TA	Anthocyanin (mg/100g F.W.)
pH	1				
TSS(°Brix)	0.89**	1			
TA(mg/100g F.W.)	0.24ns	0.28ns	1		
TSS/TA	0.72**	0.84**	0.21ns	1	
Anthocyanin(mg/100g F.W.)	0.53*	0.95**	-0.52*	0.09ns	1

ns not significant; * Significant in 5% level; **Significant in 1% level.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

ACKNOWLEDGEMENTS

The authors wish to thank the University of Birjand for their financial support in this research.

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