Agrotechniques in Industrial Crops

Journal Homepage: https://atic.razi.ac.ir

Investigating Some Physicochemical Characteristics of Seedless Barberry (*Berberis vulgaris*) Fruits in Three Geographical Regions of Iran

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ARTICLE INFO

Original paper

Article history:
Received: 6 Jul 2024
Revised: 8 Nov 2024
Accepted: 26 Jan 2025

Keywords:
Altitude
Anthocyanin
Flowering
Qualitative traits
Soluble solids

ABSTRACT

Water deficiency and poor soils are the most critical limiting factors for agriculture in Iran. Therefore, it is very important to cultivate plants such as barberry that tolerate these conditions. In this study, we investigated some physicochemical characteristics of barberry fruits in three different regions in Qazvin province. The seedless barberry plants were obtained from the Ghaenat orchards in South Khorasan, and cultivated in the Esmailabad, Yezbar and Alamut located research stations in the Qazvin, Abyek and Moalem Kelayeh cities, respectively. The phenological and fruit characteristics of plants were evaluated in different research areas. According to the results, the earliest times of flowering and fruit set occurred in the Yezbar station with a lower altitude (1170 m), followed by Esmailabad (1329 m) and Alamut (1450 m) regions. Fruit ripening date in the Alamut with a higher altitude was earlier than in other regions. The highest fresh (1.5 g) and dry weights (0.31 g) of 10 fruits were also observed in the Alamut region. The highest and the lowest fruit pH (2.96-2.6) and soluble solids (20.48-17.18%) were observed in the stations Yezbar, and Alamut, respectively. The highest and the lowest amounts of anthocyanin (432.67-273.1 mg.g^{-1} dry weight), titratable acid (3.84 -1.5 %) and ascorbic acid (794.1-573.6 mg.100 g $^{-1}$ dry weight) were observed in Alamut and Yezbar regions, respectively. The highest amounts of anthocyanin, titratable acid, and ascorbic acid belonged to the fruits harvested from the Alamut with a higher altitude. Based on the results, the quality of the fruit depends on the altitude of the region, and increasing the altitude improves the quality of the fruit. It can be concluded that barberry fruits in the Alamut region with better nutritional value and the examined fruit indices in this region were better than the other regions.

DOI: 10.22126/ATIC.2024.10821.1151

1. Introduction

The barberry plant (*Berberis vulgaris*) is one of the important horticultural crops of Iran, which is very valuable due to its good resistance to soil and climatic conditions as well as medicinal, ornamental and food applications (Alemardan *et al*., 2013). Iran is the largest producer of seedless barberry with a production quantity of more than 22,322 tons of dried berries in around 19,220 ha (Ahmadi *et al*., 2021). This plant originated in South Khorasan Province and has since spread to other regions. Many species of this plant have been identified and reported by Iranian botanists, among which the seedless barberry, is the most important variety and cultivated in many orchards. It is well documented that the fruit and plant display medicinal properties. Fruit anthocyanin has recently been given much attention and is considered a useful additive (i.e. as a natural coloring agent) in food industries (Alemardan *et al*., 2013). Barberry has been used for many centuries due to its highly nutritious

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benefits, ornamental value, and medicinal properties (Kamiab *et al*., 2023). Iranian seedless barberries are resistant to drought and can be grown and developed in any soil with acceptable production. For these reasons, compared to many other crops, their cultivation has higher economic benefits in regions with a dry climate and frequent water shortages (Alemardan *et al*., 2013).

Various studies on the quantitative and qualitative characteristics of barberry fruit, as well as its flowering and fruiting characteristics in order to evaluate the compatibility of this plant in different regions of the world have been conducted. The quality of barberry fruit depends on various factors such as the variety, altitude, the length of the growing season, the time of harvest, and the temperature of the region. Rezvani Moghaddam et al. (2013) studied the most suitable harvest time of seedless barberry fruit in the Ghaenat region in South Khorasan province. The results showed that the best time to harvest fruit in the study area was the third decade of November. The highest fruit length (9.91 mm), 100-berries fresh weight (11.13 g), 100-berries dry weight (3.42 g), and yield per shrub (13.71 kg fresh fruit and 4.56 kg dry fruit) were obtained on this date. Considering that the lack of water and poor soil are the most important limiting factors for Iran's agriculture, it is very important to cultivate tolerant crops such as barberry in these conditions. On the other hand, summer heat in low-lying plains and hot winds are limiting factors for growth and barberry production (Alemardan *et al*., 2013). Therefore, we investigated some fruit traits of a commercial clone of seedless barberry at three different altitudes in Qazvin province to determine which area is suitable for its cultivation.

2. Materials and methods

2.1. Plant materials

Barberry-rooted suckers were prepared in early winter from South Khorasan Agricultural and Natural Resources Research and Education Center and planted in a 4×4 m distance. Agricultural practices on plants such as irrigation and fertilizing were applied equally in three stations.

2.2. Ecological conditions of planting areas

The present research was conducted in three research stations of Qazvin Agricultural and Natural Resources Research and Education Center, Qazvin province, in order to investigate the effect of climate on the fruit characteristics of a seedless barberry clone. Three research stations were included the Esmailabad, Yezbar and Alamut located in the Qazvin, Abyek and Moalem Kelayeh cities, respectively. The geographical, climatic and soil characteristics of three different stations are given in Table 1.

Table 1. Geographical, climatic, and soil characteristics of seedless barberry growing areas in Qazvin province

Name of the region	LA[1]	LO[2]	AL[3] (m)	AT[4] (℃)	MAT[5] (℃)	MIT[6] (℃)	ARH[7] (%)	AP[8] (mm)	ET_0 [9] (mm)	Soil texture	Soil pH	Soil EC (dS m^{-1})
Alamut	28°36′45′′ N	50°24′50′′ E	1450	13.92	36	-20	40	400	2135	Loamy sand	7.6	0.82
Yezbar	39°88′92′′ N	41°96′25′′ E	1170	15.87	40	-15	28	250	2783	Clay loam	7.5	4.4
Esmaeil abad	36°59′16′′ N	49°39′31′′ E	1329	15.31	39	-17	32	320	2110	Loam	7.8	1.5

LA[1]: Latitud, Lo [2]: Longitude, AL[3]: Altitude, AT[4]: Average annual temperature, MAT[5]: Annual maximum absolute temperature, MIT[6]: Annual minimum absolute temperature, ARH[7]: Average annual relative humidity, AP[8]: Average annual precipitation, ET_0 [9]: reference evapotranspiration.

2.3. Statistical analysis

A randomized complete blocks design (RCBD) with 9 replications was used and data analysis was done using MSTATC software and the comparison of means was done by LSD test.

2.4. Measurements

2.4.1. Phenological characteristics

Phenological characteristics such as full blooming time, end of flowering, fruit set and fruit harvest date were evaluated. Full blooming time was considered when 50% of the flowers on the plant opened. In addition, the end of blooming was determined when 20% of flowers faded and petals fell (Arena *et al*., 2013). Full blooming was considered as 50% of flowers in the anthesis phase and the end of blooming was registered when 20% of flowers were in petal fall (Arena *et al*., 2011).

2.4.2. Fruit characteristics

The berry fruit weight was measured using a digital scale (0.001 g). In order to measure the qualitative characteristics of the fruit, the extract was prepared. For this purpose, the fruit samples were dried in an oven at

50°C and then ground in a laboratory-type mill (Dessini Industrial mill model KD-250, Italy). Then, 25 g powdered sample was taken and mixed with 100 ml of distilled water and extracted using a cloth filter with a fine pore (50 microns). The total solids soluble in the extract were measured with a refractometer (ATAGO, Japan) at 25°C and expressed as Brix. The acidity of the extract was measured with a pH meter. To measure the titratable acidity (TA), five ml of the extract was poured into a container and adjusted to 100 ml by adding distilled water. Then the extract containing 2-3 drops of phenolphthalein (indicator) was titrated using 0.1 normal NaOH until reaching pH 8.23. Titratable acid (g acid per 100 ml of fruit extract) was reported based on malic acid (Equation 1) (Williams and Horowitz, 1984).

$$\text{Titrable acid} = \left(\frac{V \times N \times M}{2000}\right) \times A \quad (1)$$

Where V is the volume of NaOH consumed (ml), N is the normality of NaOH, M is the molarity of malic acid and A is the dilution factor. Malic acid is the dominant organic acid in ripe barberry fruits (Abudureheman *et al*., 2022). NaOH solution is used to neutralize the acidity of fruit juice.

The total anthocyanin was determined using the change in absorbance at two different pH. First, 2 ml extract was reached to a volume of 25 ml by a buffer solution including a mixture of 0.2 M potassium chloride and 0.2 M hydrochloric acid (pH=1). Then another 2 ml of extract was reached to a volume of 25 ml by a buffer solution including a mixture of 1 M sodium acetate and 1 M hydrochloric acid (pH=4.5). After 20 minutes, the absorbance of the samples was recorded at 520 and 700 nm wavelengths. The concentration of anthocyanins was calculated using the following equation (Hutabarat *et al*., 2019). Anthocyanin concentration was expressed as mg of cyanidin-3-glucoside in 100 ml (Equation 2).

$$\Delta abs=(A520-A700)\ pH_1-(A520-A700)\ pH_{4.5}$$
$$\text{Anthocyanin (mg. 100 ml}^{-1}) = (\Delta abs \times MW \times DF) \times 100/(\varepsilon \times L) \quad (2)$$

Where MW is molecular weight of anthocyanin cyanidin-3-glucoside (449.2 g mol^{-1}), DF is dilution factor of the extract, ε is molar absorption coefficient of cyanidin-3-glucoside (26900 L mol^{-1}cm^{-1}) and L is cell length cuvette (1 cm). The dilution factor was obtained by dividing the final volume of the sample by the initial volume.

The amount of total phenolic compounds was measured using the Folin-Ciocalceo (FC) method (Makkar *et al*., 1993). A volume of 450 µl of distilled water was added to 50 µl of the prepared extract. Then, 250 µl of Folin-Ciocalceo reagent was added, shaken moderately, and left to stand for 5 minutes. Then after, 1.25 ml of 20% sodium carbonate solution was added to the mixture and placed in the dark at room temperature for 40 minutes. Finally, absorbance was measured at 725 nm using a spectrophotometer. A calibration curve was prepared using different concentrations of Gallic acid and the results were expressed as mg of Gallic acid per 100 ml of extract.

3. Results and discussion

3.1. Phenological characteristics

The results showed a significant difference in barberry phenological characteristics in different areas. The flowering and fruit set dates were earlier in the Yezbar region with lower altitudes, followed by the Esmailabad and Alamut, respectively. Fruit set in the Yezbar occurred 12 days after full blooming time. However, the fruit ripening date in the Alamut region with higher altitude was earlier. Table 2 shows the time of full blooming, the end of flowering, fruit set and fruit harvest of barberry plants in the studied areas. The best harvest date for barberry is when the berries have ripened by autumnal cold and turned red (Fig. 1).

Fruit growth and maturity are also different in different regions. Therefore, the appropriate harvest date should be tested in a certain region. In mountainous regions with colder climates, barberry fruits turn red sooner than in warmer areas (Alemardan *et al*., 2013). Therefore, the better coloring of the fruit and sooner harvesting in the Alamut area can be due to the higher altitude and lower temperature in the area.

Table 2. The time of full blooming, the end of flowering, fruit set and fruit harvest of barberry plants in the different areas.

Name of the region	Full blooming date (opening of 50 percent of flowers)	End of flowering	fruit set date	Fruit harvest date
Alamut	April 28	May 4	May 12	September 12
Yezbar	April 23	April 29	May 6	September 28
Esmaeil abad	April 21	April 26	May 3	October 11

Figure 1. The time to harvest barberry fruits is when the berries are red.

3.2. Fruit characteristics

The results of variance analysis of the effect of evaluated regions on the characteristics of barberry fruit are shown in Table 3. All fruit characteristics except phenol were significantly affected by planting location.

3.2.1. Fresh and dry weight of ten fruits

The results showed that the average fresh and dry weight of 10 fruits (10 berries) were 1.3 g and 0.3 g, respectively. The highest fresh (1.5 g) and dry weight (0.31 g) of 10 fruits at the harvest date were observed in the Alamut region, which may be due to cool nights in this region (Table 4). Fresh and dry weight of fruit in the Yezbar and Esmailabad regions did not show any significant difference. Among climatic factors, temperature is critical and significantly affects some plant growth and development aspects (Sage and Kubien, 2007). Barberry is a cold-adapted plant (Montazeran *et al*., 2018) growing well in mountainous regions such as Alamut with relatively cold and long winters. In the low altitude regions, the intense heat of summer along with hot winds are the limiting factors in barberry climatic compatibility (Alemardan *et al*., 2013). Sugiura et al. (1991) and Candir et al. (2009) reported that cool nights are responsible for greater fruit growth and development in persimmons.

3.2.2. Fruit pH

The highest (2.96) and the lowest (2.6) fruit pH were observed in Yezbar and Alamut, respectively. A significant difference was observed between the three locations (Table 4). Farhadi Chitgar et al. (2017) reported the pH of seedless barberry cultivated in Iran was 2.30. Ali-Tavakoli-Kaghaz et al. (2023) evaluated barberry plants in five different habitats of Kerman province, Iran. In line with the present study's findings, their results showed the highest value (3.64) of pH was obtained in Anbarabad with the lowest altitude.

Table 3. The mean square of the effect of different regions on the investigated characteristics of barberry fruit

Variation source	D.F.	Fruit fresh weight (g)	Fruit Dry weight (g)	TSS (%)	pH	Anthocyanin (mg per 100 g dry weight)	Titratable acid (%)	Ascorbic acid (mg per 100 g dry weight)	Phenol (mg gallic acid per 100 ml extract)
Location	2	0.287^{*}	0.018^{**}	25.477^{**}	0.275^{**}	61362.815^{**}	12.328^{**}	117177.481^{**}	556.491^{ns}
Replication	8	0.049^{ns}	0.003^{ns}	0.612^{ns}	0.005^{ns}	1287.146^{ns}	0.051^{ns}	1898.843^{ns}	236.257^{ns}
Error	16	0.054	0.003	0.147	0.010	1229.356	0.05	1229.981	1967.697
CV (%)		17.89	19.4	2.06	3.62	10.29	8.42	5.26	17.46

ns, * and ** are respectively meaningless, significant at the 5% level and significant at the 1% level.

Table 4. Mean (±Standard deviation) comparison of some traits of barberry fruits in three different regions of Qazvin province

Name of the region	Fresh weight of 10 fruits (g)	Dry weight of 10 fruits (g)	TSS (%)	pH	Anthocyanin (mg per 100 g dry weight)	Titratable acid (%)	Ascorbic acid (mg per 100 g dry weight)	Phenol (mg gallic acid per 100 ml extract)
Alamut	1.5 ± 0.263^{a}	0.31 ± 0.062^{a}	17.18 ± 0.545^{c}	2.6 ± 0.144^{c}	432.67 ± 18.27^{a}	3.84 ± 0.307^{a}	794.1 ± 45.42^{a}	245.37 ± 46.23^{a}
Yezbar	1.2 ± 0.222^{b}	0.23 ± 0.045^{b}	20.48 ± 0.385^{a}	2.95 ± 0.042^{a}	273.1 ± 27.32^{c}	1.5 ± 0.076^{c}	573.6 ± 26.53^{c}	260.0 ± 42.35^{a}
Esmaeil abad	1.17 ± 0.195^{b}	0.24 ± 0.046^{b}	18.3 ± 0.678^{b}	2.8 ± 0.049^{b}	316.0 ± 51.62^{b}	2.62 ± 0.224^{b}	632.7 ± 39.89^{b}	256.3 ± 15.49^{a}

Means with at least one letter in common have no significant difference at the 5% level using LSD test.

3.2.3. Anthocyanin

The highest fruit anthocyanin (432.67 mg g^{-1} dry weight) was observed in the Alamut region, followed by Esmail Abad (316 mg g^{-1} dry weight), and Yezbar (273.1 mg g^{-1} dry weight) regions. There was a significant difference among the three locations (Table 4). Moradinezhad et al. (2024) reported that the amount of anthocyanin in barberry fruit is an indicator of barberry quality. Koshita et al. (2007) and Choi et al. (2009) stated that low temperature induces anthocyanin synthesis in various plants. Marais et al. (2001) found that low temperature in autumn increases anthocyanin biosynthesis. In our study, it seems that the cool temperature of autumn in the Alamut region is the cause of more accumulation of anthocyanin and better coloring of the fruit. The barberry fruit is one of the rich sources of anthocyanin, which, besides having health-giving properties, can also be used as a natural dye (Ali-Tavakoli-Kaghaz *et al*., 2023). It has been reported that anthocyanin content increases significantly in barberry fruits with the progress of ripening (Rezvani Moghaddam *et al*., 2013). Anthocyanin content of *Berberis buxifolia* fruits significantly increased during the ripening phase, reaching a maximum by day 119 after full flowering phase. Anthocyanin production began by day 84 after full blooming and then markedly increased during the ripening period when fruit biomass and dry fruit weight were at their maximum values. (Arena *et al*., 2011). Khayyat et al. (2018) tried to find how the fruit quality of barberry is affected in three different locations around Birjand, Iran. They found that the highest anthocyanin content at harvest date accumulated in the Marvak region with higher altitudes (2079 m). Regarding the data and especially anthocyanin accumulation, they propose regions with higher altitudes for seedless barberry cultivation and production because of earlier harvests with more qualified fruit.

3.2.4. Total soluble solids (TSS)

The mean of fruit TSS was 18.65%. The highest (20.48%) and the lowest (17.18%) fruit TSS contents at the harvest date were observed in Yezbar and Alamut regions, respectively (Table 4). It has been reported that with the ripening of barberry fruit, the amount of sugar and sweetness of the fruit, red color and anthocyanin increases, and the amount of sourness and berberine decreases (Rezvani Moghaddam *et al*., 2013). The increase in fruit-soluble solids is due to the hydrolysis of sucrose into simple sugars such as glucose and fructose (Khan *et al*., 2023). Arena et al. (2011) reported that soluble solids of *B. buxifolia* fruits significantly increased during the ripening phase, to reach a maximum by day 126 after full flowering. In the research of Ali-Tavakoli-Kaghaz et al. (2023) in three geographical regions in Kerman province, the highest fruit TSS was observed in the Anbarabad region with the lowest altitude, which is in line with the results of the present study. In the Yazbar area, which has a lower altitude, there is a higher TSS content. This can be attributed to the longer growing season experienced in low-altitude regions compared to their high-altitude counterparts.

3.2.5. Titratable acid

The mean of fruit titratable acid was 2.66%. The highest (3.84%) and the lowest (1.5%) titratable acid contents were obtained in the Alamut and Yezbar regions, respectively (Table 4). Farhadi Chitgar et al. (2017) reported the titratable acidity of seedless barberry cultivated in Iran was 5.63 %. The titratable acid is more in unripe barberry fruit and decreases as the fruit ripens (Rezvani Moghaddam *et al*., 2013). Total titratable acidity of *B. buxifolia* fruits significantly decreased during the ripening period (Arena *et al*., 2011). In Passion Fruit, it was reported that cool nights may be the reason for the increase in the titratable acid of the fruit (Kozai *et al*., 2007). Therefore, cool nights may be the reason for the higher titratable acid in Alamut than in Yezbar.

3.2.6. Ascorbic acid

A significant difference was observed between different locations in terms of ascorbic acid. The mean of fruit ascorbic acid was 666.852 mg. 100 g^{-1} dry weight. The highest (794.1 mg. 100 g^{-1} dry weight) and the lowest (573.6 mg. 100 g^{-1} dry weight) ascorbic acid contents were obtained in the Alamut and the Yezbar regions, respectively (Table 4). Ahmed et al. (2013) stated that not only the genotype but also the habitat of its growth is the main factor determining barberry fruits' growth, productivity, and food composition.

3.2.7. Phenol

The results showed that there was no significant difference between the three studied regions in terms of

fruit phenol content (Table 4). Due to their antioxidant properties, phenolic compounds play an essential role in eliminating free radicals and preventing the conversion of hydroperoxides into free radicals (Jimoh *et al*., 2008). Sasikumar et al. (2012) reported total phenolic compounds of fresh barberry fruits as 410 mg 100 g $^{-1}$. In the study of Akbulut et al. (2009), the total phenolic compounds of fresh barberry fruits was 789 mg 100 g^{-1}. Yildiz et al. (2014) stated that the total phenolic compounds of barberry fruits ranged from 2500 to 3720 mg gallic acid per liter of juice.

4. Conclusion

Based on the results, there was a significant difference in the phenological and fruit traits among different regions. This study showed that the altitude of the region affects the phenological characteristics of the barberry plant and the quantitative and qualitative characteristics of its fruit. The highest contents of anthocyanin (432.67 mg. g $^{-1}$ dry weight), titratable acid (3.84 percent), and ascorbic acid (794.1 mg.100 g $^{-1}$ dry weight) of the fruit were observed in the Alamut region with higher altitude. This can be due to the lower temperature of this region at the time of fruit ripening, which increases the biosynthesis of these compounds. In the three investigated regions, the fruits of plants cultivated in the Alamut region showed better indicators than other regions, and the quantity and quality of fruits were higher in this region. Therefore, seedless barberry cultivation is recommended in the Alamut region.

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No humans or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publications

All authors read and approved the final manuscript for publication

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis, and manuscript writing

Informed consent

The authors declare not to use any patients in this research.

Funding/Support

This study was supported by the Qazvin Agricultural and Natural Resources Research and Education Center, AREEO, Qazvin, Iran.

Acknowledgement

The authors of the article are grateful to the Qazvin Agricultural and Natural Resources Research and Education Center, AREEO, Qazvin, Iran for supporting this research.

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HOW TO CITE THIS ARTICLE

Ghasemi S., Ghasemi M., Zeraatgar H., Gharali B., Rasoli V. 2025. Investigating Some Physicochemical Characteristics of Seedless Barberry (*Berberis vulgaris*) Fruits in Three Geographical Regions of Iran. *Agrotechniques in Industrial Crops* 5(2): 81-87. 10.22126/ATIC.2024.10821.1151