

Ethylene responses and quality of antioxidant-rich stored barberry fruit (*Berberis microphylla*)



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ABSTRACT

In recent years there has been renewed interest in locally grown and underutilized wild species as sources of bioactive compounds. Barberry (*Berberis microphylla* G. Forst) is among the southernmost growing fruit bearing species worldwide. The aim of this study was to characterize this species. We evaluated the antioxidant capacity of ripe barberry fruits as compared to pear, orange, apple, strawberry and blueberry. In a second set of experiments ripe barberry fruit was harvested and treated with ethylene and 1-MCP and the changes in ethylene production and respiration rate during storage were assessed. Finally, ripe barberries were harvested and stored at 0, 5 or 10 °C for 0, 3, 7, 11 or 15 days. During this period we determined weight loss, respiration rate, the percentage of rotten and physiologically decayed berries, firmness, sugar content, acidity, pH, anthocyanins, phenolics and antiradical capacity. Barberries showed 10-fold higher antioxidant capacity than apple, orange and pear and superseded four times that of antioxidant-rich fruits such as blueberry. 1-MCP treatments increased ethylene production suggesting that the hormone auto inhibits its biosynthesis. The fruit stored best at 0 °C and this should be the recommended temperature to minimize deterioration and prevent losses of bio-active compounds. The fruits show a non-climacteric physiology, with ethylene biosynthesis being under negative feedback control. Barberries are an extraordinary high source of antioxidants among fruits.

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1. Introduction

Fruits are among the main components of a healthy diet (Vicente et al., 2009), and increasing their intake has been repeatedly associated with a reduced risk of chronic diseases (Liu, 2003). This has been linked with the presence of a number of bioactive compounds (Hooper and Cassidy, 2006). The beneficial effect of fruit consumption on disease prevention has been linked to a large extent with the presence of antioxidants (Wang et al., 1999). Berry fruits rank among the top in terms of antioxidant capacity and their consumption has been encouraged (Seeram, 2008). Strong evidence supports the benefits of berries in disease prevention (Zafra-Stone

et al., 2007). Dietary supplementation with berry extract significantly stimulated antioxidant defence enzymes in red blood cells, reduced inflammatory responses and inhibited the growth of cancerous cells (Kong et al., 2003).

A number of locally grown underutilized wild species may have been shown to accumulate greater content of bioactive compounds than the cultivated counterparts (Ruiz et al., 2010; Ruiz-Rodríguez et al., 2011). Several fruit bearing species historically used by local cultures as food or for medicinal purposes (Dominguez Diaz, 2010) remain poorly characterized. *B. microphylla* commonly known as barberry or “calafate” is a spiny evergreen shrub widely distributed in Patagonia. It is among the fleshy fruit-producing plants growing farther South on the globe (Moore, 1983; Orsi, 1984; Landrum, 1999; Alonso and Desmarchelier, 2006). The purple berries are consumed either fresh or processed in marmalades, jams, non-alcoholic beverages and ice creams (Arena and Curvetto, 2008; Arena et al., 2012). Very limited information is available regarding the antioxidant capacity, physiology and postharvest performance

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of fresh barberry fruit. Given the little information available in this work we characterized the antioxidant capacity, ripening physiology and postharvest behavior of barberry fruit

2. Experimental

2.1. Plant material

Barberry fruit having full surface deep purple color and free from decay blemishes and other defects was harvested from a natural population located near Ushuaia city, 54° 48' S, 68° 19' W (Tierra del Fuego, Argentina). Fruit was handled carefully to avoid damage, placed in shallow 6-cm deep clamshells to prevent crushing of fruit in the bottom layer and immediately sent to the laboratory.

2.2. Antioxidant capacity of barberry as compared to other fruit species

Samples (1.5 g) from purple barberries, ripe pear cv. Bartlett, orange cv Washington Navel, ripe Red Delicious apple and strawberry (cv. Camarosa) (100% red color) were ground in ethanol and centrifuged (10 min at 12,000 × g at 4 °C for 10 min). The supernatant was collected and brought to 100 mL with water. Antioxidants were measured according to Brand-Williams et al. (1995).

2.3. Responses of barberry to exogenous ethylene 1-MCP

Fruit was randomized to provide three experimental units of 400 fruits per treatment. The experimental units were placed in plastic containers and kept as untreated controls or treated with 100 $\mu\text{L L}^{-1}$ ethylene or 1 $\mu\text{L L}^{-1}$ 1-MCP for 20 h. 1-MCP was released from 50 mL-capped test vials containing weighed amounts of SmartFresh™ powder (0.14% active ingredient; Rohm and Haas, Argentina) by adding warm water (40 °C) through a septum. Each vial was vortexed and placed in the container. The vial was then opened followed by placement of the container lid within 10 s. The lid of each container was also taped to ensure a tight seal. After 20 h at 20 ± 2 °C, the containers were vented. The experimental units were then stored in air at 20 °C and 95% RH. The experiment lasted 5 d, since barberries were severely deteriorated after 6 d, with external signs of fungal attack or physiological decay. We determined fruit respiration rate and ethylene production as described in sections below.

2.4. Effect of storage temperature on barberry quality

Fruit was put in 45 PET perforated clamshells (120 fruits each and stored for 0, 3, 7, 11 or 15 d at 0, 5 or 10 °C. Three trays were evaluated for each temperature and storage time. Samples were taken during the storage period and fruit was immediately analyzed or otherwise frozen in liquid N₂ and stored at –20 °C until use.

2.5. Analytical determinations

2.5.1. Ethylene production

Fruit was confined into tightly sealed flasks and incubated at 20 °C. One millilitre of the head-space gas was extracted after 1 h and ethylene was quantified on a gas chromatograph (Hewlett Packard 5890 Series II) fitted with a FID and a stainless steel Porapak N column (3.2 mm × 2 m; 80/100 mesh) as described elsewhere (Trincherò et al., 1999). The injector, oven and detector temperatures were 110, 90, and 250 °C respectively. N₂ was used as the carrier gas at a flow rate of 22 mL min⁻¹. Three independent replicates per treatment and date were evaluated.

2.5.2. Respiration rate

The respiration rate of fruit treated with ethylene or 1-MCP stored at 20 °C was measured using an Agilent 4890 gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA) fitted with a TCD and equipped with a Carboxplot™ (Chrompack) column (0.53 mm × 25 m, 25 μm thick), as previously described (Concellón et al., 2005). Analysis was performed isothermally at 100 °C, with the injector and the detector temperatures held at 80 and 200 °C respectively. Helium was used as the carrier gas at a flow rate of 9 mL min⁻¹. Three independent replicates per treatment and date were evaluated. Gas samples for ethylene and CO₂ analyses were collected from the same jars.

The respiration rate of fruit stored at 0, 5 or 10 °C was measured with a CO₂ IR sensor (Alnor Compu-flow, Model 8650, Alnor USA). Fruit trays were transferred from cold storage and maintained at room temperature until reaching 25 °C. Fruit (100 g) were enclosed in tightly-sealed flasks and gases were allowed to accumulate for 20 min. Oxygen levels never dropped below 18% and CO₂ levels remained below 0.5% in all treatments and perfect linearity was observed during 1-h confinement. The sensor was introduced in the flask to perform CO₂ measurements and the respiration rate was calculated. Three measurements were done for each treatment analyzed. Results were expressed as milliliters of CO₂ per kilogram of fruit produced in per hour.

2.5.3. Weight loss

Fruit trays were weighed at the beginning of the experiment, and during storage. Weight loss (WL) was calculated as: $WL = 100 \times (W_i - W_f) / W_i$, being W_i and W_f the initial and final sample weight, respectively. Results were expressed as percentage of weight loss.

2.5.4. Firmness

Fruit firmness was determined in a Texture Analyzer (TA.XT2, Stable Micro Systems Texture Technologies, Scarsdale, NY) equipped with a 3-mm diameter flat probe. Fruit was compressed 2 mm at a rate of 0.5 mm s⁻¹ and the maximum force developed during the test was recorded. Sixty measurements were done for each temperature and storage time. Results were expressed in Newton.

2.5.5. Percentage of rotten and physiologically decayed fruit

Rotten fruit and berries showing symptoms of physiological decay (loose of tissue integrity without visible signs of fungal growth) were evaluated by visual inspection. Results were expressed in percentage of rotten and physiologically decayed berries.

2.5.6. Anthocyanin

Frozen fruit pulp was ground and approximately 50 mg of the resulting powder were poured into 20 mL of 1% (v/v) hydrochloric acid containing methanol (1%, v/v). The slurry was stirred for 5 min and centrifuged at 12,000 × g for 10 min at 4 °C. The supernatant was saved and 1 mL aliquots were brought to 5 mL with of methanol–HCl (1%, v/v). The absorbance of the supernatant was measured at 515 nm (Pan et al., 2004) in a UV-Vis spectrophotometer (Beckman Model 1200, USA). Two independent extracts were prepared for each temperature and storage time tested measurements were performed in duplicate. Results were expressed as milligrams of cyanidin-3-glucoside per 100 g of fresh weight using the extinction coefficient $\epsilon = 29,600 \text{ M}^{-1} \text{ cm}^{-1}$.

2.5.7. Sugars, acidity and pH

For sugar measurements, frozen pulp tissue was processed in a refrigerated mill, and 1.5 g of the resulting powder was extracted with 5 mL of ethanol. The mixture was centrifuged at 9000 × g for

10 min at 4 °C and the supernatant was brought to 100 mL with water. Three independent extractions were done for each temperature and storage time. Total sugars were measured with the anthrone reagent (Yemm and Willis, 1954). Briefly, aliquots (20 μ L) of the ethanolic extracts were taken and brought to 500 mL with water. One milliliter of 2 g L⁻¹ anthrone in 98% (w/w) H₂SO₄ was added and held at 100 °C in a water bath for 10 min. The test tubes were cooled in water and the absorbance at 620 nm was measured. Results were expressed as grams of glucose per 100 g of fresh weight.

For acidity and pH measurements 10 g of processed fruits were added to 100 mL of water. Fruit pH was measured potentiometrically and acidity was determined titrimetrically with 0.1 M NaOH to pH 8.2 (AOAC, 1980). Three measurements were done for each temperature and storage time analyzed. Results were expressed as mmol. H⁺ per kilogram of fresh weight.

2.5.8. Phenolics

Approximately 1.5 g of fruit pulp was ground in a refrigerated mill with 5 mL 95% (v/v) ethanol. The mixture was then centrifuged at 9000 \times g for 10 min at 4 °C. The supernatant was collected and the pellet was re-extracted with 5 mL of 95% (v/v) ethanol and centrifuged as described above. The supernatants were pooled and brought to 100 mL with water. The extracts were used for total phenol measurements with the Folin reagent (Singleton et al., 1999). Two hundred microliters of 1:1 Folin–Ciocalteu reagent were added to 1.5 mL of the crude extract. After 3 min, 50 μ L of a solution containing 2% (w/v) Na₂CO₃ and 0.1 M NaOH were added, and the reaction mixture was incubated at the same temperature for 1 h. Absorbance was measured at 760 nm. The total phenolic content was calculated using phenol as the standard. Three extracts were prepared for each temperature and storage time tested and samples were measured in triplicate. Results were expressed as grams of phenol per kg of fresh weight.

2.5.9. Antioxidants

The changes in antioxidant capacity of *Berberis* fruit during storage was measured with DPPH^{*} as mentioned above. Three extracts were done for each storage temperature and time. Results were expressed as 1/EC₅₀ (mg⁻¹).

2.5.10. Statistical analysis

Data was analyzed by one way analysis of variance (ANOVA), and means were then compared by a Tukey multiple range test at $P < 0.05$.

3. Results and discussion

3.1. Antioxidant capacity of barberry as compared to other fruits

Barberry antioxidant capacity was extremely high: more than 10-fold higher to that of pear, orange, and apple. Among berries barberry displays the highest antioxidant capacity. Strawberries showed seven times less antioxidants than barberry and even blueberries which have been shown to rank on top among fruits in their ORAC values (Ehlenfeldt and Prior, 2010) has only 25% of the antioxidant capacity of barberry (Fig. 1A). Barberry extracts have shown beneficial effects preventing oxidative stress (Albrecht et al., 2010). Kinetics of the reaction between DPPH^{*} and barberry extracts was similar to that of blueberry (Fig. 1B). DPPH^{*} consumption was less rapid than that of orange a fruit in which ascorbic acid represents the main antioxidant. The reaction between phenolics and DPPH^{*} had a slow rate of reaction (Sánchez-Moreno et al., 1998). Interestingly, antioxidants showed a weak correlation with anthocyanins ($r = 0.04$), but a higher correlation with phenolics ($r = 0.61$) suggesting that other aromatic compounds are contributing substantially

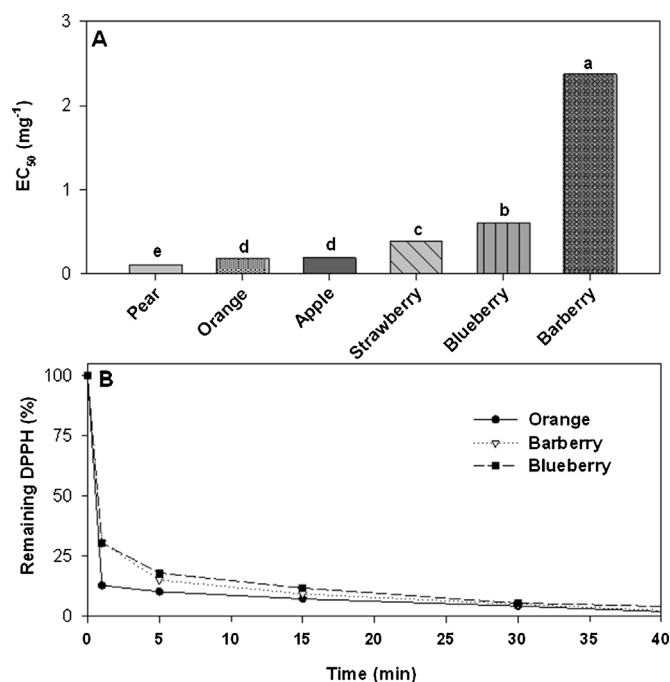


Fig. 1. (A) Antioxidant capacity of barberry as compared to other fruit species and (B) kinetics of the reaction of orange, blueberry or barberry fruit extracts with the radical DPPH^{*}. Different letters indicate significant differences on a Tukey test at a level of significance of $P < 0.05$.

to total antioxidant activity. Overall, results show that barberry is rich source of phenolic antioxidants.

3.2. Responses to exogenous ethylene and 1-MCP

Barberries are rounded, deep purple, small berries with a weight close to 0.5 g. Most berries are non-climacteric, blueberries show a climacteric ripening pattern (Kader, 2002). Respiration rate changed throughout the experimental period, but no differences were detected among treatments (Fig. 2A). Respiration rate was ~ 36 mg kg⁻¹ h⁻¹ at day 1 and dropped to 24 mg kg⁻¹ h⁻¹ after 2 d of storage according to a non-climacteric behavior. Subsequently fruit respiration increased reaching at the end of the experiment similar to those of day 1. The increase in respiration after the second day of storage at 20 °C may be associated with senescence and over ripening rather than with a climacteric response. In climacteric fruits, exogenous ethylene advances the timing of the climacteric and autocatalytic ethylene production is invariably associated with increased respiration rates (Abeles et al., 1992). The fact that the treatments with 1-MCP and ethylene did not cause any modification in the timing of the increase in CO₂ production supports a non-climacteric physiology.

Barberry fruits display higher ethylene production levels (~ 3 nL g⁻¹ h⁻¹) than other harvested berries such as blackberry, blueberry, cranberry and raspberry (Kader, 2002). Ethylene production did not change significantly during storage in control fruit and was not affected by ethylene treatment (Fig. 2B). The rate of ethylene production remained low to moderate (2–4 nL kg⁻¹ h⁻¹ at 20 °C) in the absence of physiological or pathological injury. To further investigate barberry behavior during storage, fruit was exposed to the strained alkene 1-MCP, one of the most effective antagonists of ethylene action in plants (Watkins, 2006). 1-MCP elicited a clear stimulatory effect on ethylene biosynthesis during barberry storage. Fruit treated with 1-MCP showed higher ethylene production than control and ethylene-treated fruit throughout the experiment. Ethylene production was 72–125% higher in

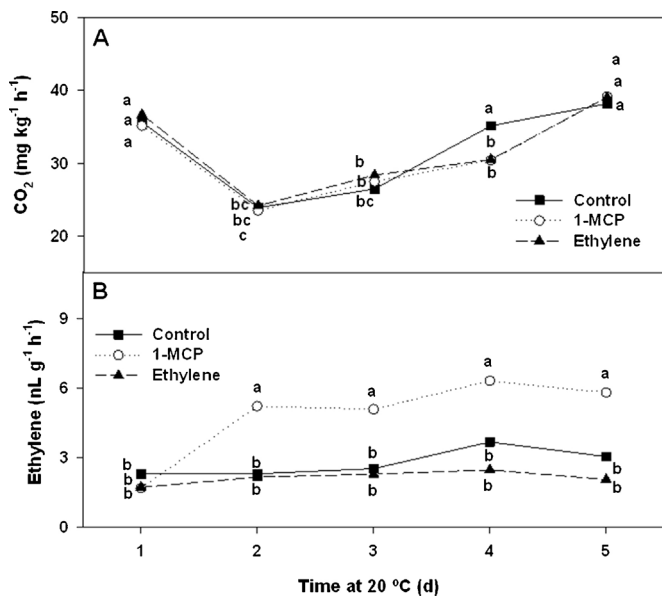


Fig. 2. (A) Respiration rate ($\text{mg kg}^{-1} \text{h}^{-1}$) and (B) ethylene production ($\text{nL g}^{-1} \text{h}^{-1}$) of control, ethylene-treated (ethylene) and 1-MCP-treated (1-MCP) barberry fruit, stored at 20 °C and 90% RH for 5 days. Different letters indicate significant differences on a Tukey test at a level of significance of $P < 0.05$.

1-MCP-treated fruit than in control fruit between days 2 and 5. 1-MCP is capable of inactivating ethylene signaling in different plant tissues for a varying number of days by single exposure to relatively low dose levels (Watkins, 2006). Thus, these results strongly suggest that ethylene production in ripe barberry fruit is under a negative feedback regulatory mechanism. Similar results have been reported for different non-climacteric fruits, among them, cherries (Gong et al., 2002) and grapefruit (Mullins et al., 2000). Increased ethylene biosynthesis upon treatments with 1-MCP was detected in a few climacteric fruit species such as banana (Pelayo et al., 2003; Inaba et al., 2007) and fig (Sozzi et al., 2005), indicating a negative feed-back regulation in ethylene biosynthesis after harvest.

3.3. Effect of storage temperature on barberry visual, organoleptic and nutritional quality

3.3.1. Weight loss and respiration rate

Over-ripening, excessive softening and pathogen attack mainly by the necrotroph *Botrytis cinerea* are the main determinants of berry fruit postharvest losses (Mitcham et al., 2006). Preventing deterioration and extending their storage capacity have been the main challenges in the distribution of superior quality berries. Fruits stored at 0, 5 and 10 °C maintained acceptable quality for 15, 11 and 7 d, respectively. As expected, fruit weight loss increased with higher temperatures and with longer storage periods (Fig. 3A). Fruit kept at 0 and 5 °C had weight losses of 2.8 and 3.9%, while fruit maintained at 10 °C attained a weight loss close to 3.9% already at day 7. Fruit weight loss can be caused by both respiration and dehydration (Kader, 2002). However, based on the respiration measurements performed and the theoretical loss CO₂ should not exceed 1% even at 25 °C. Fruit respiration rate increased the higher temperatures and the longer the storage (Fig. 3B). The berries maintained at 10 °C attained a maximum respiration rate close to after 7 d. Since all the measurements of CO₂ production were done in all cases after transfer and equilibration at 25 °C the greater values in fruit stored at 10 °C may be the result of higher damage.

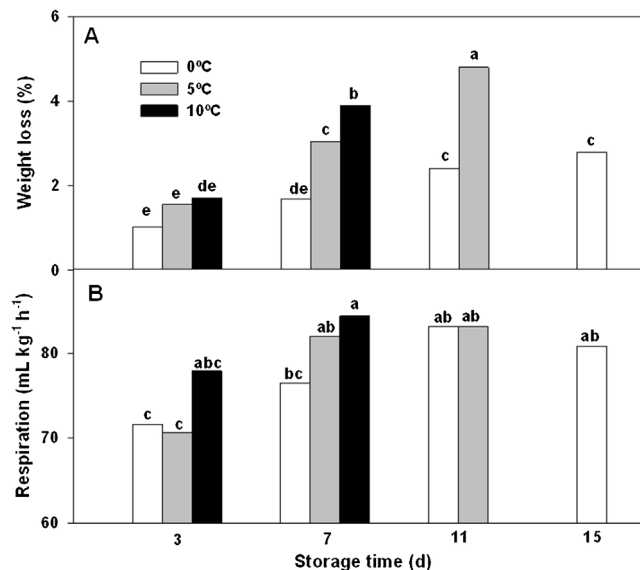


Fig. 3. (A) Weight loss (%) and (B) respiration rate of barberry fruit stored for 0, 3, 7, 11 or 15 days at 0, 5 or 10 °C. Different letters indicate significant differences on a Tukey test at a level of significance of $P < 0.05$.

3.3.2. Pathological and physiological decay and firmness

Preventing deterioration and extending their storage capacity have been the main challenges in the distribution of superior quality berries. The main factor contributing to deterioration during storage was physiological decay which was associated over ripening and senescence. In many cases no exudate was observed because the peel maintained its integrity, but the fruit surface turned clear and shrunk. Percentage of rotten fruit was lower than that of fruit showing physiological decay regardless of storage temperature and duration (Fig. 4A). Fruit stored at 0 and 5 °C attained a 36.8 and 35.3% of physiologically decayed berries at 15 and 11 days respectively, while in fruit kept at 10 °C this value was reached already a day 7 (Fig. 4B). Firmness decreased 60% in fruit stored at 10 °C for 1 week (Fig. 5A). Maintaining the berries at 0 °C prevented firmness changes for 15 d. Barberry fruit stored at 0 °C

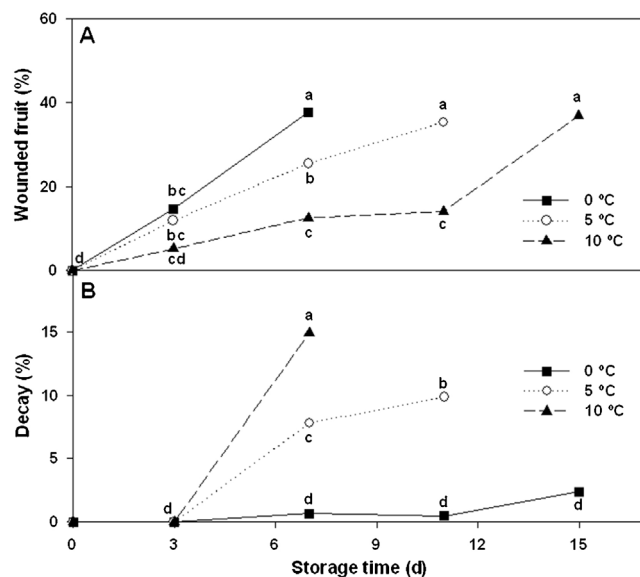


Fig. 4. (A) Physiological and (B) pathological decay (%) of barberry fruit stored for 0, 3, 7, 11 or 15 days at 0, 5 or 10 °C. Different letters indicate significant differences on a Tukey test at a level of significance of $P < 0.05$.

Table 1

Sugars, acidity, pH, total phenolics and antioxidants of barberry fruit stored for 0, 3, 7, 11 or 15 days at 0, 5 or 10 °C. Values represent the means ± S.D. of three independent replicates.

		Time (days)				
		0	3	7	10	15
Sugars (g kg ⁻¹)	0 °C	10.6 ± 5.8e	124.9 ± 5.9abc	12.0 ± 3.8bcd	12.0 ± 3.7bcd	11.3 ± 3.3de
	5 °C	10.6 ± 5.8e	115.4 ± 7.2cde	12.3 ± 8.1bc	12.6 ± 13.7ab	Nd
	10 °C	10.6 ± 5.8e	12.1 ± 8.6bcd	13.4 ± 6.1a	Nd	Nd
Acidity (mmol. H ⁺ kg ⁻¹)	0 °C	244.5 ± 13.8ab	235.9 ± 5.8ab	223.4 ± 7.9ab	230.3 ± 9.3ab	230.1 ± 5.8ab
	5 °C	244.5 ± 13.8ab	251.7 ± 4.4a	228.5 ± 19ab	217.5 ± 7.0b	Nd
	10 °C	244.5 ± 13.8ab	243.8 ± 11.5ab	218.5 ± 0.9b	Nd	Nd
pH	0 °C	3.56 ± 0.06abc	3.57 ± 0.04abc	3.63 ± 0.08ab	3.57 ± 0.01abc	3.55 ± 0.02abc
	5 °C	3.56 ± 0.06abc	3.46 ± 0.08c	3.57 ± 0.05abc	3.49 ± 0.02bc	Nd
	10 °C	3.56 ± 0.06abc	3.56 ± 0.03abc	3.65 ± 0.06a	Nd	Nd
Phenols (g kg ⁻¹)	0 °C	11.4 ± 0.6abc	12.0 ± 0.2ab	11.2 ± 0.7bc	11.1 ± 0.6bc	9.90 ± 0.1c
	5 °C	11.4 ± 0.6abc	12.3 ± 0.9ab	13.2 ± 0.9a	10.6 ± 0.3bc	Nd
	10 °C	11.4 ± 0.6abc	11.8 ± 0.5abc	11.02 ± 0.7bc	Nd	Nd
Antioxidants (kg ⁻¹) × 10 ⁻⁵	0 °C	36.6 ± 0.2a	32.6 ± 0.8ab	32.5 ± 1.4ab	32.9 ± 0.1ab	28.3 ± 2.3b
	5 °C	36.6 ± 0.2a	34.9 ± 2.7a	34.5 ± 0.1a	29.3 ± 0.4b	Nd
	10 °C	36.6 ± 0.2a	34.5 ± 3.2a	36.9 ± 2.0a	Nd	Nd

Different letters indicate significant differences on a Tukey test at $P < 0.05$. ND: not determined.

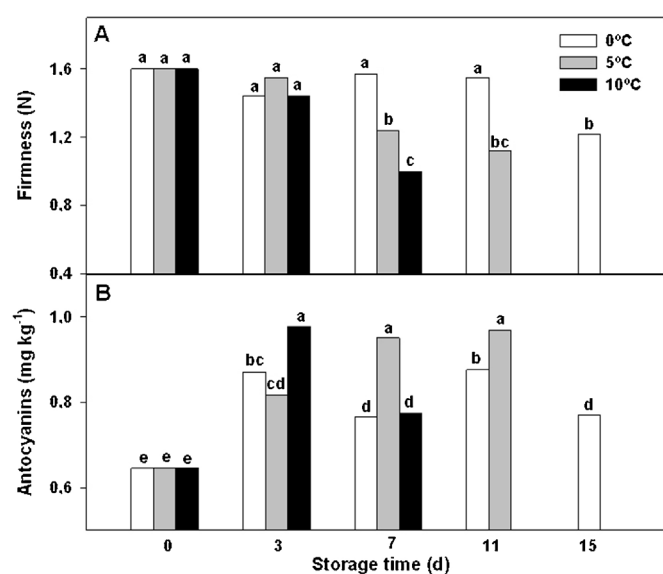


Fig. 5. (A) Firmness and (B) anthocyanins of barberry fruit stored for 0, 3, 7, 11 or 15 days at 0, 5 or 10 °C. Different letters indicate significant differences on a Tukey test at a level of significance of $P < 0.05$.

showed no chilling injury symptoms. Thus, to extend the maximum storage period without compromising fruit quality, barberry should be best stored near or at 0 °C.

3.3.3. Sugars, acidity and pH

Sugar content was 10.6% at day 0 and varied with the temperature and length of the storage (Table 1). Fruit stored at 0 and 5 °C attained the highest sugar contents (12.5 and 12.6%) at days 3 and 11, respectively, to then decrease, while fruits kept at higher temperature (10 °C) presented the maximum sugar content at day 7. The increase of sugar is not common in berry fruits after harvest since they usually do not accumulate starch (Manning, 1993). The higher values may be related to increased sugar extractability as fruit ripen. Acidity decreased during postharvest storage of barberry (Table 1), a typical behavior observed in several fruits during storage, resulting from the incorporation of acids in the TCA cycle as respiratory substrates (Kader, 2002). The greatest reduction in acidity was found in fruit stored at 10 °C. The pH of the fruit before

storage was 3.56. Berries stored at 0 °C or 5 °C did not show changes in pH during storage. pH of the fruit kept at 10 °C increased during storage in accordance to the reduction of titratable acidity. As found for decay and firmness the fruit stored at 0 °C showed the least changes in the attributes associated with fruit taste.

3.3.4. Anthocyanin, total phenolics and antioxidants during storage

Fruit anthocyanin content was 0.645 mg kg⁻¹ at day 0 and increased during storage (Fig. 5B). The accumulation of anthocyanin even during postharvest storage has been shown in other berries (Cordenunsi et al., 2005; Shin et al., 2007; Krüger et al., 2011). Barberries maintained at the highest temperature (10 °C) reached the maximum level of anthocyanins (0.978 mg kg⁻¹) already at day 3 and then decreased as fruit damage occurred. In fruit kept at 5 °C, anthocyanins peaked at day 11, while those stored at 0 °C showed an irregular behavior along the storage.

Total phenolics showed lower variations than anthocyanins (Table 1). The fruit stored at 0 or 5 °C presented lower levels of total phenolics the last sampling date. Barberries stored at 10 °C did not show changes in total phenolics after 7 d, but this fruit was not analyzed after 11 or 15 d due to high levels of damage. In other fruit species an increase in phenolic compounds has been observed during postharvest storage (Pineli et al., 2012). Antioxidants capacity was also affected by both the temperature and length of the storage period (Table 1). The antioxidants in fruits stored at 0 and 5 °C decreased to 28.3 and 29.3 DPPH• (kg⁻¹) × 10⁻⁵ at days 15 and 10, respectively, while in fruits kept at 10 °C, antioxidants stayed constant (39.9 DPPH• (kg⁻¹) × 10⁻⁵ at day 7).

4. Conclusions

Given its high respiration rate *B. microphylla* is a highly perishable fruit. The berries show a non-climacteric behavior with the absence of a respiratory rise and an autocatalytic ethylene production after harvest. The increase in respiration at long storage times is related to over ripening and senescence. Barberry is a chilling insensitive commodity, storing best at 0 °C, which should be the recommended temperature for postharvest handling. This Patagonian fruit has an extremely high level of antioxidants. The radical scavenging capacity is 10-fold higher than that of apple, orange and pear and supersedes four times the levels found in blueberry.

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