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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 Barberries showed 10-fold higher antioxidant capacity than apple, orange and pear and superseded four times that of antioxidant-rich fruits such as blueberry. 1-MCP treatments increased ethylene production suggesting that the hormone auto inhibits it 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.

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 μL L^{-1} ethylene or 1 μL 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 N_2 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 (Trinchero et al., 1999). The injector, oven and detector temperatures were 110, 90, and 250 °C respectively. N_2 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 Carboplot™ (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 CO_2 analyses were collected from the same jars.

The respiration rate of fruit stored at 0, 5 or 10 °C was measured with a CO_2 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 CO_2 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 CO_2 measurements and the respiration rate was calculated. Three measurements were done for each treatment analyzed. Results were expressed as milliliters of CO_2 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 × (Wi − Wf)/Wi, being Wi and Wf 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 ε = 29,600 M^{-1} 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