Postharvest Biology and Technology 133 (2017) 72–80

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Combination of nitric oxide and 1-MCP on postharvest life of the blueberry (*Vaccinium* spp.) fruit

Gustavo Esteban Gergoff Grozeff[a,b,c,*], Matías Leonel Alegre[a], María Eugenia Senn[a], Alicia Raquel Chaves[d], Marcela Simontacchi[a], Carlos Guillermo Bartoli[a]

[a] Instituto de Fisiología Vegetal CCT CONICET La Plata, Universidad Nacional de La Plata, Facultad de Ciencias Agrarias y Forestales, Facultad de Ciencias Naturales y Museo, Diagonal 113 Nº 495, 1900 La Plata, Argentina
[b] Laboratorio de Investigación en Productos Agroindustriales (LIPA), Facultad de Ciencias Agrarias y Forestales-UNLP, Calle 60 y 119 s/n, 1900 La Plata, Argentina
[c] Cursos de Fruticultura y Fisiología Vegetal, Facultad de Ciencias Agrarias y Forestales – UNLP, Calle 60 y 119 s/n, 1900 La Plata, Argentina
[d] Centro de Investigación y Desarrollo de Criotecnología de Alimentos CCT CONICET La Plata, Facultad de Ciencias Exactas (UNLP), 47 y 116, 1900 La Plata, Argentina

ARTICLE INFO

Keywords:
Ascorbic acid
Berries
Citric acid
Dehydroascorbic acid
Glutathione
Malic acid

ABSTRACT

Blueberries are a perishable fruit that loses its firmness and weight rapidly after harvest. High concentrations of ascorbic acid (AA) and phenolic compounds such as anthocyanins are important attributes contributing to the quality of the fruit. The aim of this work is to extend the postharvest life of three blueberries cultivars; 'Mistý and 'Blue Cuineś, firm and soft cultivars respectively and 'Blue Chiṕ with intermediate firmness. The fruit was treated separately or in combination with a nitric oxide donor, S-nitrosoglutathione (GSNO) and 1-methylcyclopropene (1-MCP) and stored at 4 °C for 14 d. The best treatment for 'Blue Cuineś was the combination of 1-MCP and GSNO, while 'Mistý softened slower when treated with 1-MCP, and 'Blue Chiṕ was not affected by treatment. The 1-MCP + GSNO treatment maintained higher concentrations of AA and GSH in 'Blue Cuineś. AA concentrations declined in 'Mistý but did not respond to GSNO. The combination of 1-MCP and GSNO extended the postharvest life of 'Blue Cuineś by improving the concentrations of AA and glutathione in the berries but not for 'Mistý. These results demonstrate that blueberry cultivars respond differentially to 1-MCP and nitric oxide, and that the sequence of application could be used to slow fruit softening and maintain antioxidant properties.

1. Introduction

Blueberries are a type of fruit commonly named "berries" or "fruit of the forest". Fruit of this heterogeneous group of species has high levels of antioxidants but are extremely perishable (Kader, 2002; Perkins-Veazi, 2016).

Blueberries are classified as climacteric fruit that show a moderate respiration rate and a low ethylene production compared with other fruit and with an average potential storage life of two weeks (Kader, 2002). To minimize damage and to improve postharvest life, blueberry fruit should be handled carefully and packed directly into small containers (Miller and McDonald, 1988).

Many studies related to blueberry's postharvest life extension have focused on the effects of treatments like cold temperature (Connor et al., 2002), high oxygen atmospheres (Zheng et al., 2003), edible coatings (Duan et al., 2011), ultraviolet radiation (Perkins-Veazi et al., 2008), 1-methylcyclopropene (1-MCP) (DeLong et al., 2003; Chiabrando and Giacalone, 2011) and many others.

The production of ethylene in blueberries depend on the ripening stage: when fruit turns a pink color, there is a peak of ethylene production and when the fruit develops to full color, the production of this hormone returns to the basal level (El-Agamy et al., 1982). The application of an inhibitor of the action of ethylene (aminoethoxyvinylglycine) retards the ripening process of the blueberry fruit (Dekazos, 1979), while ethephon accelerates ripening, stimulating fruit softening and decreasing the titratable acidity (Ban et al., 2007).

Little contribution of the ethylene receptor inhibitor, 1-MCP, has been found in blueberries (DeLong et al., 2003; Chiabrando and Giacalone, 2011) and MacLean and NeSmith (2011) found that 1-MCP stimulated ethylene production in three different cultivars and accelerated the rate of firmness loss. Ethylene synthesis in blueberries is still not completely understood.

Nitric oxide (NO) is a bioactive molecule synthesized by plants that has important physiological functions. It is also involved in responses to stress conditions (Kopyra and Gwóźdź, 2003; Hung et al., 2002; Simontacchi et al., 2015). NO is a highly reactive molecule and it is assumed to react with a variety of targets including protein modifications (S-nitrosylation), tyrosine nitration and metal nitrosylation.

* Corresponding author.
E-mail address: gergoff.gustavo@agro.unlp.edu.ar (G.E. Gergoff Grozeff).

http://dx.doi.org/10.1016/j.postharvbio.2017.06.012
Received 14 November 2016; Received in revised form 30 June 2017; Accepted 30 June 2017

Ethylene and NO have antagonistic effects in climacteric fruit (Liu et al., 2007), since NO decreases ethylene synthesis, during ripening and fruit senescence. Possibly this interaction could be at the hormone receptors level: ethylene requires a transitions metal cofactor, copper, to bind to the receptors (Rodriguez et al., 1999) and it is possible that NO could affect this binding process. NO increases the activity of antioxidant enzymes (Flores et al., 2008; Kopyra and Gwóźdź, 2003), and also has been suggested as an antisenescent molecule (Jasid et al., 2009). NO has been used to extend postharvest life in strawberries (Wills and Leshem, 2000), delaying ripening and alleviating chilling injury in mangos (Zaharah and Zora, 2011) and Japanese plums (Singh et al., 2009). A complete list of these studies is mentioned in Manjunatha et al. (2010). The application of sodium nitroprusiate (a NO donor) to a non-climacteric fruit, such as strawberry, decreases the synthesis of ethylene, reducing the content of ACC (1-Aminocyclopropane-1-carboxylic acid) synthase (Zhu and Zhou, 2007); meanwhile in peach, a climacteric fruit, the effect was similar, but the target was the inhibition of the ACC oxidase (Zhu et al., 2006). Additionally, NO has been involved in reducing softening in bananas, affecting the enzymes for the synthesis pathway of ethylene and cell-wall disabling, such as polygalacturonase and pectin methylesterase (Cheng et al., 2009).

The characterization of quality parameters in different cultivars is an important starting point for studying the postharvest biology of blueberries. Within the quality parameters, fruit firmness is an important economic trait for fresh blueberries for the market (Ehlenfeldt and Martin, 2002). The cultivars of blueberries studied in this work were chosen from the previous work of Ehlenfeldt and Martin (2002). 'Blue Chip' has intermediate firmness, while 'Misty' is presented as one the firmest fruits. 'Blue Cuinex' was chosen due to its importance in the concert of cultivars produced in Argentina and for its quick loss of firmness during postharvest.

The objective of this work was to improve the postharvest life of three different cultivars of blueberries by the application of an ethylene response suppressor (1-MCP) in combination with a NO donor [S-nitrosoglutathione (GSNO)].

2. Materials and methods

2.1. Plant material, experimental setup and treatments

2.1.1. Experiment I

Blueberry fruit (*Vaccinium corymbosum* cvs. 'Blue Cuinex' and 'Blue Chip' and *V. corymbosum x darrowii* cv. 'Misty') was harvested from the orchard of the Experimental Station Julio A. Hirschhorn (National University of La Plata), located in Los Hornos, La Plata, Argentina (34° 59′ S, 57° 59′ W). The experiments were started in the last week of November 2012. The fruit was harvested when color reached 100% of the surface, placed in perforated clamshells containing 125 g each (approximately 30 fruit) and immediately transported to the laboratory. Twelve clamshells per treatment were prepared for each treatment and cultivar (48 clamshells in total for each cultivar). Clamshells containing fruit of the three cultivars were first divided into two groups and placed in a 40 L air tight chamber for treatment with 0 μL L^{-1} or 1 μL L^{-1} of 1-MCP (Smart Fresh ^{SM}) for 12 h at 23 °C. The two groups of clamshells were then divided and treated with a 10 mL of water or 10 mL of 1 mmol L^{-1} GSNO solution. In a 25 L closed chamber connected to a nebulizer to produce extremely small drops of solution that ensured the even distribution of the liquid for 30 min. The fruit was then carefully placed again in their respectively clamshells and stored at 4 °C for 14 d. Samples were taken at 0, 7 and 14 d after treatment. For each sample, at least ten fruit were taken from the clamshells. Measurements of weight loss, color and firmness were taken with at least six fruits from each of the six clamshells. The rest of the fruit was frozen in liquid nitrogen and stored at −80 °C until used for the rest of the measurements. Each sample consisted of ten fruit from each of the six clamshells.

2.1.2. Experiment II

'Misty' and 'Blue Cuinex' fruit were harvested from the same orchard at the Experimental Station Julio A. Hirschhorn (National University of La Plata). Three independent experiments were performed starting in the last week of November 2012, 2014 and 2015, each consisting of three biological replicates. Fruit was harvested when color reached 100% of the surface, placed in perforated clamshells containing 125 g each (approximately 30 fruit) and immediately transported to the laboratory. Eighteen clamshells per treatment and harvest year were prepared for each cultivar. Treatments were performed as described in Experiment I, except that samples were taken at 0, 2, 7 and 14 d after harvest. Respiration rates were measured using three clamshells per treatment and sampling day (9 measurements in the three years). Once the measurements were finished, ten fruit were removed from each clamshell, frozen in liquid nitrogen and stored at −80 °C until use for the rest of the measurements. For each biochemical measurements, six samples of ten fruit each were taken from separate clamshells for each treatment, time of sampling and year of harvest.

2.1.3. Climatic data

Climatic data was recorded with a weather station (Davis Instruments Corporation model GroWeather Industrial, California, USA) located at 34° 59′ S, 57° 59′ W, 45 m above sea-level. The data was analyzed and kindly provided by Agronomist H. Martín Pardi from the Agrometeorology Section of the Experimental Station Julio A. Hirschhorn and Course of Agricultural Climatology and Phenology (Faculty of Agricultural and Forestry Sciences, National University of La Plata). Data was recorded daily during all the growing seasons of 2012, 2013, 2014 and 2015. Global radiation and mean, maximum and minimum temperatures were calculated as an average of these recordings for every month.

2.2. Weight loss

Weight loss was measured in 6 replicates, each consisting of 125 g fruit per clamshell per season per treatment. Fruit was weighed at 0, 7 and 14 d after harvest and fruit weight loss (WL) was calculated as follows: $WL = [(W_i - W_f)\ (W_i)^{-1}] \times 100$. W_i is the initial weight and W_f is the final weight of each sample of fruit. The results are expressed as percentage for each period of sampling.

2.3. Color and anthocyanins determinations

Fruit skin color was measured with a colorimeter (Minolta®, CR-400, Osaka, Japan) to obtain L*, a* and b* values. Thirty replicates taken from different clamshells were performed for each cultivar, harvest season and treatment at 0 and 14 d after harvest. Anthocyanins were extracted according to Angeletti et al. (2010) and measured at 515 nm with a UV–vis recording spectrophotometer (Shimadzu® UV-160A, Japan). Each measurement was made at harvest and after 2, 7 and 14 d of cold storage at 4 °C. The results were calculated as μmol of cyanidin-3-glucoside by using $\varepsilon = 29.000\ M^{-1}\ cm^{-1}$. Results are expressed in g of total anthocyanins per kg on a fresh weight basis.

2.4. Total soluble solids, titratable acidity and pH

For total soluble solids (TSS), 10 g of fruit was macerated using a mortar and pestle, and a few drops of the juice were placed in a refractometer (Milwaukee MA871, Rocky Mount, USA). Results are expressed in percentage (%). pH was potentiometrically measured (pH meter Hanna Edge®) and total titratable acidity (TTA) was determined titrimetrically with a 0.1 mol L^{-1} solution of NaOH until pH 8.2 was reached (AOAC, 1980). Results are expressed as g of citric acid per kg of fruit fresh weight basis.