Scientia Horticulturae 216 (2017) 169–176

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Impact of dynamic controlled atmospheres on reactive oxygen species, antioxidant capacity and phytochemical properties of apple peel (cv. Granny Smith)

Asanda Mditshwa[a], Olaniyi Amos Fawole[a], Filicity Vries[b], Kobus van der Merwe[b], Elke Crouch[c], Umezuruike Linus Opara[a,d,*]

[a] Postharvest Technology Research Laboratory, South African Research Chair in Postharvest Technology, Department of Horticultural Sciences, Faculty of AgriSciences, Stellenbosch University, P/Bag X1, Stellenbosch 7602, South Africa
[b] Postharvest & Wine Technology, ARC Infruitec-Nietvoorbij, P/Bag 5026, Stellenbosch, 7599, South Africa
[c] Department of Horticultural Sciences, Faculty of AgriSciences, Stellenbosch University, P/Bag X1, Stellenbosch 7602, South Africa
[d] Postharvest Technology Research Laboratory, South African Research Chair in Postharvest Technology, Department of Food Science, Faculty of AgriSciences, Stellenbosch University, P/Bag X1, Stellenbosch 7602, South Africa

ARTICLE INFO

Article history:
Received 2 November 2016
Received in revised form 3 January 2017
Accepted 6 January 2017
Available online 13 January 2017

Keywords:
Malus domestica
Ascorbic acid
Total phenolics
Antioxidant capacity
Reactive oxygen species
Lipid peroxidation

ABSTRACT

This study was conducted to investigate the effect of dynamic controlled atmospheres (DCA) on antioxidant capacity, reactive oxygen species (ROS) and phytochemicals in the apple peel of 'Granny Smith' harvested at pre-optimal and optimal maturity over two growing seasons. Fruit was stored in DCA (<0.5% O_2; 1% CO_2) for up to 20 w at 0 °C and regular air (RA) was used as a control treatment. Phytochemicals, antioxidant capacity and lipid peroxidation were spectrophotometrically measured. ROS were measured by confocal laser-scanning microscopy on apple peel treated with fluorescent probe 2′,7′-dichlorodihydrofluorescein diacetate. Principal component analysis (PCA) and Pearson's correlation were used to investigate major changes and relationship among the studied variables. Fruit stored in DCA were characterized by higher antioxidant capacity, ascorbic acid and total phenolics concentration. However, lipid peroxidation and ROS were significantly lower in DCA stored fruit. PCA displayed two clusters that could easily be identified as DCA and RA stored fruit. Positive correlation scores for lipid peroxidation and ROS corresponded with RA stored fruit whilst strong negative stores for antioxidant capacity, ascorbic acid and total phenolics corresponded with DCA stored fruit. Pearson's correlation showed a strong relationship between ascorbic acid, phenolics and antioxidant capacity. Lipid peroxidation and ROS also showed a significant ($p < 0.05$) positive correlation. This study showed that the high fruit quality in DCA correlates with higher antioxidant capacity, total phenolics and ascorbic acid concentrations in this fruit.

1. Introduction

Dynamic controlled atmospheres (DCA) have been shown as the potential technique to control superficial scald incidence and improve postharvest quality attributes in pome fruits. DCA has an ability to maintain fruit firmness, acidity and overall fruit quality (DeLong et al., 2007; Gabioud et al., 2009; Tran et al., 2015; Weber et al., 2015; Bekele et al., 2016; Bessemans et al., 2016). However, the biochemical mechanisms used by DCA in controlling superficial scald and improving postharvest quality of apples is not yet understood. Superficial scald is presumed to be an oxidative stress; this is attributed to the reduced incidence after the application of antioxidants such as diphenylamine (DPA) or low oxygen storage (Lurie and Watkins, 2012). Moreover, α-farnesene oxidation products such as conjugated trienes (CTs) and 6-methyl-5-hepten-2 one (MHO) are cited as the main causal agents of superficial scald (Rowan et al., 1995; Mir et al., 1999; Wang and Dilley, 2000).

Apart from α-farnesene hypothesis, superficial scald susceptibility or resistance could also be influenced by the antioxidant pool of the fruit during storage (Anet, 1972). For instance, Barden and Bramlage (1994a) reported high antioxidant pool to be highly associated with low α-farnesene and consequently reduced scald incidence in apples. Meir and Bramlage (1988) also showed that

* Corresponding author at: Postharvest Technology Research Laboratory, South African Research Chair in Postharvest Technology, Department of Horticultural Sciences, Faculty of AgriSciences, Stellenbosch University, P/Bag X1, Stellenbosch 7602, South Africa.
E-mail address: opara@sun.ac.za (U.L. Opara).

http://dx.doi.org/10.1016/j.scienta.2017.01.011
0304-4238/

the high storage potential of optimally harvested 'Cortland' apples is attributed increasing antioxidant capacity in the cuticle during maturation. A strong relationship between scald symptoms and phenol concentration has also been reported in 'Delicious' apples (Ju et al., 1996). Moreover, a scald resistant 'Golden Delicious' is reportedly having a higher phenol concentration compared to scald susceptible 'Cortland' apples (Ju and Bramlage, 1999). A recent investigation has compared the reactive oxygen species (ROS) accumulation with activities of enzymes that scavenge toxic compounds on scald susceptible tissues of 'Granny Smith' apples (Sabban-Amin et al., 2011). Interestingly, low ROS concentration corresponded with a higher enzyme activity and lower scald incidence. Lipid peroxidation is another biochemical disorder that often precedes the expression of chilling injury or superficial scald symptoms (Lyons, 1973). In fact, lipid peroxidation increases with scald incidence and severity in 'Fuji' apples (Lu et al., 2014). Rao et al. (1998) also found a relationship between lipid peroxidation and scald incidence in 'White Angel' x 'Rome Beauty' hybrid selections.

Most published research on superficial scald of apples has focused on the effect of treatments on scald associated volatiles such as α-farnesene, CTs and MHO. Furthermore, recent work on DCA has largely focused on its feasibility to control superficial scald. Very little information about the mechanisms used by DCA to maintain fruit quality is available. In this study, we hypothesize that DCA inhibits scald and maintains postharvest quality by retarding the loss antioxidants and phytochemical properties of the fruit tissue, thereby reducing ROS accumulation and lipid peroxidation. Therefore, the objective of this study was to assess the evolution of antioxidant capacity, phytochemical contents and reactive oxygen species in the peel of DCA stored 'Granny Smith' apples harvested at pre-optimal and optimal maturity over two growing seasons.

2. Materials and methods

2.1. Fruit source and treatments

The study was carried out over two seasons during 2013 and 2014 (referred to as season 1 and season 2, respectively) apple growing season. Seventeen-year old 'Granny Smith' apple trees grafted into M109 rootstock grown on a commercial orchard in Grabouw, South Africa (34° 12′12" S, 19° 02′35" E) were used in this study. The tree spacing was 4 × 1.5 m giving a total of 1667 trees per hectare. All trees were irrigated by micro sprinklers and pruned to a central leader. The trees received the same irrigation and fertilizer program. Fruit free from blemishes were hand-picked at 165 and 172 days after full bloom (DAFB) which are commonly considered in the fruit industry as pre-optimal (starch breakdown = 12.5%; firmness = 82 N; TSS = 10.52° Brix; TA = 1.46 mg/100 mL) and optimal maturity (starch breakdown = 36.3%; firmness = 79 N; TSS = 12.16° Brix; TA = 1.29 mg/100 mL), respectively. The fruit was thereafter transported to the laboratory in an air-conditioned car. Uniformly sized fruit with diameter of 70 ± 2 mm and mass of 160 ± 5 g were randomly divided into 3 replications, each comprised of 100 fruit in ventilated plastic carton. Fruit was thereafter stored in cold storage. The chlorophyll fluorescence non-destructive monitoring system (HarvestWatch, Satlantic Inc, Halifax, Canada) with an ability to predict and indicate the low oxygen limit (LOL) was used to determine DCA set points (Prange et al., 2011; Wright et al., 2012). In this study, the DCA was established within 48 h after harvest, using compressed air and CO_2 plus N_2 from a membrane generator (Isosep, Isolcell, Italy). Accordingly, the gas composition of the storage chamber was analysed at 90 min intervals and adjusted when necessary. Generally, the O_2 levels ranged between 0.3% to 0.5% whilst CO_2 was maintained at 1% and 95% RH. DCA storage regimes ranged between 5 d to 20 w. To avoid frequent opening of the storage rooms, Four DCA chambers with a volume of 15.65 m^3 were used to avoid frequent opening of the storage rooms. In each chamber, a sensor was installed in a plastic basket with a sample of 6 apples. For each treatment and storage time, 10 fruit per replicate were peeled under subdued light. The peel was immediately frozen with liquid nitrogen, freeze dried, pulverised and stored at −80 °C until use for extraction and measurement of total antioxidants, total phenolics, ascorbic acid and lipid peroxidation. ROS production was determined from fresh samples.

2.2. Confocal microscopic analyses of ROS production

Determination of ROS in apple peel was carried out as described by Macarisin et al. (2007) and Sabban-Amin et al. (2011). The fluorescent probe 2,7-dichlorodihydrofluorescein diacetate in which dichlorodihydrofluorescein (DCF) fluorescence measurement quantifies general oxidative stress was used. 2,7-dichlorodihydrofluorescein enters cells in the diacetate form (H_2DCF-DA), and the acetate form (H_2DCF) is hydrolyzed by intracellular esterases and then reacts with oxidants, resulting in the highly fluorescent DCF. Acetate detects a broad range of oxidizing molecules rather than a single ROS form, and it is efficient in localizing ROS within plant cells (Joo et al., 2005). Immediately before microscopic analysis, slices of apple peel were cut from fruit and immediately immersed in a small Petri dish containing 10 mL of 10.0 μM H_2DCF-DA in loading buffer (50 mM MES buffer, pH 6.5). The H_2DCF-DA was freshly prepared from a 20 mM stock solution in dimethyl sulfoxide (DMSO). To prevent light-inducible oxidation, the slices were kept in the dark for 10 min and were thereafter transferred to a new Petri dish containing loading buffer to wash off excess dye. Model IX 81 inverted confocal laser-scanning microscope (FLUOVIEW 500, Olympus, Japan) equipped with a 488 nm argonion laser was used for sample examination and image acquisition. The fluorescent probe was excited with a 488 nm laser beam and the emission was collected through a BA 515–525 filter. For autofluorescence, a BA 660 IF emission filter was used. Magnification was increased by focusing the scanning laser beam onto a smaller area of the tissue. The transmitted-light images were obtained with Nomarski differential interference contrast (DIC) optics. The relative intensity of the fluorescence signal was estimated by calculating average pixel intensity from each successive focal plane of the apple peel slice, in 5 μm steps, with MICA software (Multi-Image Analysis, CytoView, Israel). The value of fluorescence intensity presented is the mean (±standard error (SE)).

2.3. Lipid peroxidation

Malondialdehyde (MDA), a suitable biomarker for lipid peroxidation in plant tissues (Katsuhara et al., 2005; Lu et al., 2014), was quantified according to Dhindsa et al. (1981) and Siboza and Bertling (2013) with slight modifications. Briefly, 0.1 g of freeze dried and pulverised apple peel was homogenised with 10 mL of ice cold 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 17764*g* for 15 min at 4 °C to precipitate particulates. In triplicates, 1 mL aliquot of the supernatant was thoroughly mixed with 4 mL of 20% TCA containing 0.5% thiobarbituric acid (TBA). The mixture was incubated at 95 °C for 30 min and thereafter quickly cooled in an ice bath. After centrifugation at 10000 for 15 min at 4 °C, the absorbance of the supernatant was read at 532 nm and corrected for nonspecific absorbance at 600 nm using UV–vis spectrophotometer (Thermo Scientific Technologies, Madison, Wisconsin). The concentration of MDA was calculated using an extinction coefficient (∈) of 155 mM^{-1} cm^{-1}.