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Changes in bioactive compounds and response to postharvest storage conditions in purple eggplants as affected by fruit developmental stage

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ABSTRACT

Fruit maturity stage at harvest influences the response to postharvest storage conditions and bioactive compounds content. In this work fruit from two purple eggplant cultivars (Monarca and Perla Negra) were harvested at 12, 15, 18, 20 and 23 d after fruit set (designated as stages I through V) and changes in size, dry weight, calyx area, cell wall material (AIR, alcohol insoluble residue), firmness, respiration, and antioxidants (peel anthocyanins and pulp carotenoids, ascorbic acid, phenolics and chlorogenic acid) were determined. In a second set of experiments the postharvest performance of fruit harvested at stages I ("baby" eggplants), III and IV (traditional harvest stages) during storage at 0 or 10 °C was assessed. Fruit growth continued until late ripening in contrast to calyx expansion and peel anthocyanin accumulation, which were relatively earlier events. Fruit dry weight decreased between stages I and III, remaining constant afterwards. "Baby" eggplants had higher antioxidant capacity, chlorogenic acid (ChA), carotenoids and ascorbic acid contents than late-harvested fruit. ChA predominated in pulp placental tissues at stage I, spreading throughout the fruit core at as ripening progressed. No marked differences in dry mass, antioxidant capacity or responses to postharvest storage regimes were found between fruit harvested at stages III and IV. Late pickings increased yields and led to less dense fruit, which had lower respiration rates. Within this harvest window, storage at 10 °C maximized quality maintenance. In contrast "baby" eggplants stored better at 0 °C. Understanding the developmental changes in bioactive compounds and postharvest performance may help in the maximization of fruit antioxidant properties as well as in the selection of the optimal handling conditions for each ontogenic stage.

1. Introduction

Ranking among the top 10 in terms of antioxidants (Lo Scalzo et al., 2010; Whitaker and Stommel, 2003) eggplants are, together with tomato and pepper, the most widely known solanaceous fruit crops (Doganlar et al., 2002). Although they show diverse shapes (elongated, ovoid or slender types) and colors (purple, white, green or variegated), dark ovoid American type cultivars are by far the most popular (Muñoz-Falcón et al., 2008).

Eggplants are harvested at immature stages, before full seed development and mainly based on size (Gajewski and Arasimowicz, 2004; Jha and Matsuoka, 2002). However, there is a range of ontogenic stages at which they could be marketed. For traditional distribution channels fruit are mostly picked at intermediate developmental phases, which prevent bitterness and spongy texture but do not compromise yields markedly. Once limited to the fine dining sector, consumption of "baby" vegetables has started to spread to the general public (Shaw and Cantliffe, 2005). Miniature eggplants have several appeals to consumers; they have delicate taste and are attractive and tender. In addition, they can be directly incorporated into salads, side dishes and appetizers without extensive preparation.

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Harvest maturity is known to have major influence on fruit response to postharvest storage (Kader, 1996). Full-sized eggplants are chilling sensitive and storage at 10 °C is recommended to maximize postharvest life (Concellón et al., 2007). However, whether or not there are differences in chilling susceptibility within the normal eggplant harvest window is not clear. The optimal conditions for keeping highly perishable "baby" eggplants have not been determined either. While less mature fruit may also be more sensitive to chilling (Boonsiri et al., 2007; Mohammed and Brecht, 2002), this sensitivity still needs to be established.

Fruit maturity stage at harvest may be also a main determinant of the levels of bioactive compounds (Deepa et al., 2007; Vallejo et al., 2003). In eggplant, changes in antioxidants accompanying development have been studied, but some discrepancies are found in the literature. (Esteban et al., 1992) indicated that phenolic compounds peaked at intermediate ripening stages. In contrast, (Mennella et al., 2012) in a detailed characterization of phenolic compounds in different eggplant genotypes and allied species showed that phenolics dropped during development. Unfortunately this work was conducted only at three ripening stages, starting at intermediate development and "baby" eggplants were not evaluated. In addition, the last ripening stage tested corresponded to non-commercial, senescent fruit. Consequently, the aims of the present study were to evaluate the changes in antioxidants in purple eggplants at all commercially relevant stages as well as the influence of harvest maturity on fruit response to different storage regimes.

2. Materials and methods

2.1. Physico-chemical characterization of eggplant fruit at different developmental stages

Eggplants *cv*. Monarca (M) and Perla Negra (PN) were cultivated in a greenhouse (110 m × 25 m) in La Plata, Argentina. Seven rows per cultivar located in the central zone of the greenhouse were used for fruit tagging. Two hundred and fifty fruit were tagged immediately after set. At days 12, 15, 18, 20 and 23 after set (DAFS; defined as stages I, II, III, IV, V; respectively) 50 fruit were harvested and transported immediately to the laboratory. Individual fruit size (length and equatorial diameter), weight, bulk density and calyx area were determined. Fruit were then used to analyze respiration rate, firmness, dry weight for cell wall isolation and to perform chlorogenic acid histolocalization. Peel and pulp samples were frozen in liquid N_2 and stored at −80 °C until analysis of carotenoids, total phenolics, hydroxycynamic acids, total flavonoids, anthocyanins and chlorogenic acid as described in Section 2.3. The whole experiment was repeated three times during October–December 2011.

2.2. Postharvest evaluation of eggplant fruit at different developmental stages

Eggplants *cv*. Monarca (M) and Perla Negra (PN) cultivated and tagged as indicated in Section 2.1 were harvested after 12, 18 and 20 DAFS. Stage I represented "baby" eggplants. Stages III and IV were selected for being the most common harvest maturities used commercially for early and late harvests respectively. Fruit were packed in plastic trays, covered with perforated PVC (wrap film) and stored at 0 or 10 °C (85–90% RH) for 0 or 12 d. Upon removal from the cold storage samples were subjected to a 2 d shelf life period at 20 °C (12 + 2 d). Fruit deterioration index was determined and surface color and respiration rate were evaluated as indicated in Section 2.3. Forty fruit were used for each developmental stage and temperature analyzed.

2.3. Analytical measurements

2.3.1. Calyx area

The fruit calyxes were detached from the pericarp and digitized using a Hewlett–Packard model C4480 scanner. The areas were calculated by using AutoCAD® 2014. Ten fruit were used per cultivar and developmental stage and results were expressed in cm^2.

2.3.2. Dry weight

Three grams of pulp tissue (Iw) were cut with a razor blade into small cubes and dried at 70 °C in a vacuum oven (2.5 kPa) until constant weight (Fw). Fruit pulp dry weight (DW) was calculated as:

$$DW(\%) = \frac{100 - (100 \times (Iw - Fw))}{Iw}$$

Measurements were done in triplicate for each cultivar and developmental stage. Results were expressed as percentage of fresh weight.

2.3.3. Bulk density

Individual fruit were weighed and volume was subsequently determined by water displacement in graduated jars containing stoppers that allowed total fruit immersion. Twenty fruit were evaluated for each cultivar and developmental stage.

2.3.4. Respiration rate

Carbon dioxide production was measured by incubating two fruit in 3 L hermetic jars. After 15 min the gas concentration was obtained using an IR sensor (Alnor Compu-Flow, Model 8650, United States). Three measurements were done for each cultivar and developmental stage. Results were expressed in μmol kg^{-1} s^{-1}.

2.3.5. Surface color

Peel color was evaluated with a colorimeter (Minolta, Model CR-400, Osaka, Japan) by measuring the parameters L* a* b*. Thirty fruit were analyzed for each cultivar and storage condition.

2.3.6. Firmness

Firmness was evaluated in a texture analyzer (TA.XT2, Stable Micro Systems Texture Technologies, Scarsdale, NY, USA) fitted with 3 mm probe. Each sample was compressed for 8 mm distance at the equatorial position, at the rate of 0.5 mm s^{-1}. Thirty fruit were analyzed and two measurements were done on opposite sides of each fruit. Results were calculated as the initial slope of the force deformation curve and expressed in kN m^{-1}.

2.3.7. Cell wall isolation

Fruit cell walls were isolated according to (Vicente et al., 2007). Frozen pulp tissue was ground in a mill and 7.5 g of resultant powder was added to 50 mL of ethanol and boiled for 20 min. The insoluble material was filtered and sequentially washed with 50 mL of ethanol, chloroform and methanol (1:1), and acetone, yielding the crude cell wall extract (alcohol insoluble residue, AIR). The AIR was dried overnight at 37 °C and weighed. Results were expressed as grams per kilogram on fresh weight basis. Measurements were done in triplicate for each cultivar and developmental stage.

2.3.8. Anthocyanins

Anthocyanins were extracted from the peel of at least six eggplants. The peel was frozen in liquid N_2, powdered and 0.5 g were extracted 4 times with HCl and methanol (1:99, v/v). After centrifugation (12,000 × *g* for 5 min) the supernatant was taken to 50 mL with HCl and methanol (1:99, v/v). Samples were vacuum concentrated to 2 mL, filtered through a 0.45 μm nylon membrane and 10 μL were injected into a liquid chromatograph (Model HP 1100

Agilent Technologies, CA, USA) equipped with a Symmetry C18 column (150 × 3.9 mm, Waters, USA) and a photodiode array detector (DAD). Samples were eluted according to (Wu et al., 2004)), using as mobile phases A (5% formic acid aqueous solution) and B (methanol). The gradient selected was: 0–2 min: 5% B; 2–10 min: 5–20% B; 10–15 min: 20% B; 15–30 min: 20–30% B; 30–35 min: 30% B; 35–45 min: 5%. The flow rate was set at 8.3 μL s^{-1} and sample detection was performed at 520 nm. The peaks were integrated and results were expressed on fresh weight basis. Measurements were done in triplicate for each cultivar and developmental stage. To further identify anthocyanins samples were run in an HPLC Agilent 1100 LC (Agilent Technologies Inc., USA) equipped with a binary pump connected directly to a mass spectrometer detector (MS-VL quadrupole, Agilent Technologies, USA). The MSD was operated with an electrospray ionization interface in the positive mode (ESI^+) with the following settings: capillary temperature and voltage, 350 °C and 3.0 kV, respectively; nebulizer gas (N_2) flow rate 0.2 mL s^{-1}; nebulizer pressure, 0.3 MPa; fragmenter voltage, 200 V. Mass spectronic data were acquired in the full scan mode to follow the representative fragments for delphinidin-derived as delphinidin-3-rutinoside (D3R, *m*/*z* 303–611) which was the main anthocyanin. The same column and chromatographic conditions as for the HPLC-DAD analyses were used here, except that the mobile phase was A (0.2% trifluoroacetic acid-TFA-aqueous solution) and B (methanol).

2.3.9. Carotenoids

Frozen pulp was ground in a mill and 6 g of the resulting powder was extracted with 10 mL of hexane:acetone:ethanol (2:1:1, v/v). Samples were vortexed and 2 mL of water were added. Samples were then left at room temperature for 5 min to allow phase separation. The absorbance of the hexane layer (upper phase) was measured in a spectrophotometer at 470 nm and the carotene content was calculated using β-carotene as standard. Measurements were performed in triplicate. Results were expressed as mg kg^{-1} on fresh weight basis.

2.3.10. Ascorbic acid

Frozen eggplant pulp was ground in a mill and 8 g of the resulting powder were extracted with 12 mL of 6% (w/v) trichloroacetic acid (TCA) for 30 min at 0 °C. The homogenate was centrifuged at 12,000 × *g* for 10 min at 4 °C. The supernatant was used for ascorbic acid content according to (Lemoine et al., 2010), with minor modifications. Aliquots (900 μL) from each extract were pipetted into test tube containing 600 μL 0.2 mol L^{-1} phosphate buffer (pH 7.4). Then 1500 μL 10% (w/v) TCA, 1200 μL 42% (v/v) H_3PO_4 and 1200 μL 4% (w/v) 2,2′-dipyridyl dissolved in 70% (v/v) ethanol were sequentially added. Finally 0.6 mL of 3% (w/v) $FeCl_3$ prepared fresh daily were pipetted into the reaction mixture. The test tubes were incubated in a water bath at 42 °C for 40 min and the absorbance at 525 nm was determined in a Shimadzu UV-mini 1240 spectrophotometer (Shimadzu, Japan). Ascorbic acid was used as a standard. Three replicates were used for each cultivar and developmental stage and results were expressed as mg kg^{-1} on fresh weight basis.

2.3.11. Phenolic compound classes

Samples were analyzed according to (Obied et al., 2005). Fruit pulp was frozen in liquid N_2, ground in a mill and 4 g of the resultant powder were extracted with 10 mL 70% (v/v) ethanol for 30 min and centrifuged at 12,000 × *g* for 10 min at 4 °C. Five hundred microlitres aliquots were added to 1 mL 96% (v/v) ethanol acidified with 0.1% HCl. Samples were taken to 10 mL with 2% HCl. The absorbance was measured at 320 nm to determine hydroxycinnamic acid (HCA) derivatives using ChA as a standard, and at 360 nm to estimate flavonoids (FL) using quercetin as a standard. Two extracts were prepared for each cultivar and developmental stage and measured in triplicate. Results were expressed in relative molar concentration as follows:

$$\text{HCA or FL molar fraction} = \frac{\text{HCA or FL mol}}{\text{HCA mol+FL mol}}$$

2.3.12. Total phenolic compounds

One gram of frozen pulp tissue was ground in a mill and added to 20 mL ethanol. The suspension was vortexed, extracted during 30 min and then centrifuged at 12,000 × *g* for 10 min at 4 °C. For total phenolics determination 50 μL of Folin–Ciocalteu reagent diluted 1:1 in water were pipetted into test tubes containing 350 μL ethanolic extracts and 500 μL water. After 3 min, 100 μL 20% Na_2CO_3 in NaOH 0.1 mol L^{-1} and 1.5 mL distillated water was added. The reaction mixture was vortexed and then incubated for 90 min (Singleton et al., 1999). The absorbance was measured at 760 nm and phenolic compounds content was calculated using ChA as a standard. Two extracts were prepared for each cultivar and developmental stage and measured in triplicate. Results were expressed as mg kg^{-1} on fresh weight basis.

2.3.13. Antioxidant capacity

The ABTS assay was performed as described by (Arnao et al., 2001). Fifty microlitres of ethanolic fruit extracts, prepared as described for phenolic compounds were added to 1 mL of $ABTS^{\bullet+}$ working solution (absorbance of 0.700 ± 0.03 at 734 nm), incubated for 6 min and the absorbance at 734 nm was measured. Trolox® was used as antioxidant standard and results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) in mg kg^{-1} on fresh weight basis. Samples were measured in triplicate.

2.3.14. Chlorogenic acid

Sample preparation was performed as described in Section 2.3.12. Chromatographic analyses were carried out on an HPLC (HP 1100 Agilent Technologies, CA, USA). A reverse phase XSelect CSH C18 column (750 × 4.6 mm, Waters, USA) was used. The elution was performed using as mobile phase mixtures of A (0.4% formic acid) and B (methanol). The gradient used was 0–15 min: 5–35% B; 15–35 min: 35–65% B. The flow rate was set at 8.3 μL s^{-1}, the injection volume was 20 μL and the detection was set at 320 nm. ChA was quantified by an external standard method. Results were expressed as mg kg^{-1} on fresh weight basis. Measurements were done in triplicate for each cultivar and developmental stage.

2.3.15. Histochemical localization of chlorogenic acid

ChA *in situ* localization was done with Neu' reagent as described by (Mondolot et al., 2006). ChA was visualized as a light green fluorescence when excited under UV light. Eggplant slices (0.5 cm thick) from the mid-section were dipped 30 s in 10 mL of 1% (w/v) 2-amino-ethyl-diphenylborinate in absolute methanol. Samples were immediately examined in a UV light stereomicroscope (Modular Stereomicroscope Leica MZ10 F, Leica Microsystems Ltd., Germany). Sample excitation and emission wavelengths were set at 425 and 480 nm respectively. The images were obtained with a digital color camera Leica DFC490 (Leica Microsystems Ltd., Germany, 8 megapixel). Negative controls were obtained by analyzing samples without 2-amino-ethyl-diphenylborinate addition.

2.3.16. Deterioration index

Fruit was visually inspected as previously reported (Concellón et al., 2012) with slight modifications. A hedonic rating scale from 0 (no damage) to 4 (severe damage), based on surface dehydration, decay and surface pitting was used to estimate fruit deterioration. Forty fruit were evaluated for each cultivar, developmental stage

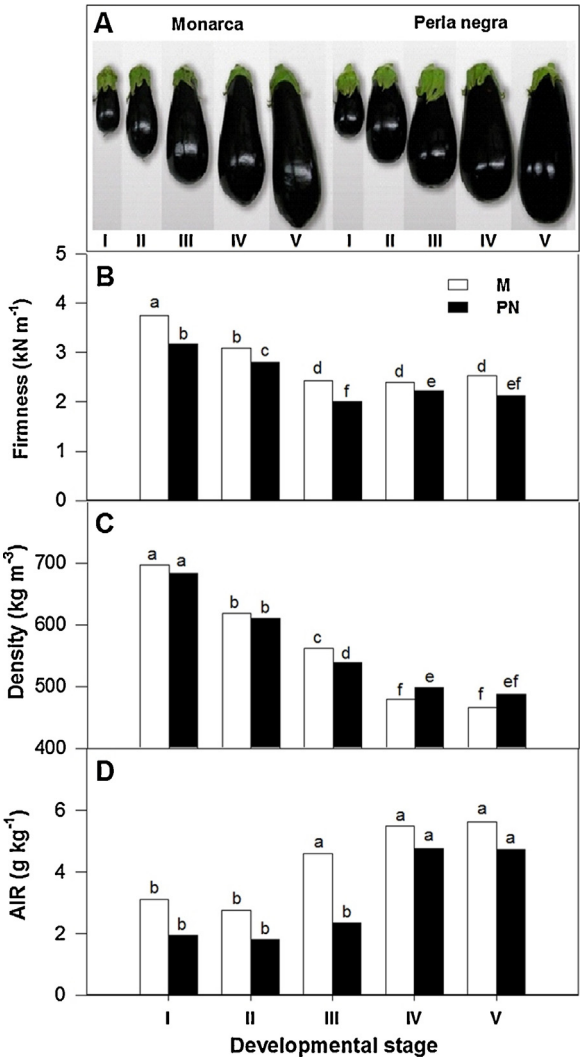

Fig. 1. (A) Appearance, (B) firmness, (C) density and (D) alcohol insoluble residue (AIR) in Monarca (M) and Perla Negra (PN) eggplants at 12, 15, 18, 20 and 23 DAFS (Stages I through V, respectively). Values with different letters indicate significant differences according to Fisher's test ($P < 0.05$).

and storage temperature. The deterioration index (DI) was calculated according to the following equation:

$$DI = \frac{\Sigma(\text{Injury level} \times \text{N}^\circ \text{ of fruit on the level})}{\text{Total N}^\circ \text{ of fruit}}$$

2.4. Statistical analysis

The experiments were carried out in a completely randomized design. Results were subjected to analysis by ANOVA and means were compared by a Fisher test at $P < 0.05$.

3. Results and discusion

3.1. Fruit appearance, growth, dry weight and respiration

Fig. 1A shows purple eggplants *cv*. Monarca (M) and Perla Negra (PN) during ontogeny. PN eggplants showed higher equatorial diameter (15%) and were less elongated than M (Table 1). For a given stage, fruit weight was 20% higher in PN than in M (Table 1). Developmental stage I represented "baby" fruit and stages III and IV were selected corresponding to the normal range of maturities used for harvesting full-sized eggplants. "Baby" eggplants were less rounded than fully developed fruit; they had 40–50% of their full length and diameter but only 13% of the final weight (Table 1). Fruit expanded rapidly between stages I and III (*ca.* 25 g per day), but both cultivars showed their maximum growth rate (*ca.* 40–60 g per day) in the transition to stage IV. Compared to the pericarp, fruit calyx expansion took place relatively early. In both cultivars, already at stage I the calyx had *ca.* 70% of the full area while the fruit only presented 13% of their final mass (Table 1). Whole fruit mass continued increasing until stage V, indicating that anticipated harvests would reduce yields. However, picking beyond stage V is not recommended since it would result in spongy fruit showing excessive seed development and bitter taste (Chen and Li, 1996).

In contrast to other reports indicating that color intensity increased as fruit approached the optimal picking maturity (Esteban et al., 1992), peel final color was reached already at stage II (data no shown). Eggplant harvest decisions are normally based on fruit size. Given the rapid growth between stages III and IV, for the studied cultivars, fruit could be marketed with weights ranging between 200 and 350 g (Table 1). Whether or not this dramatic difference in size is also associated with changes in antioxidant contents and postharvest responses has not been determined.

In accord with a previous reports (San José et al., 2013) eggplant dry weight varied during development between 6.5 and 7.5% (Table 1). Between stages I and III the dry weight decreased, suggesting that fruit growth was predominantly supported by water uptake. Subsequently, the dry weight remained stable (Table 1). This, together with the continuing growth pattern observed, indicated that assimilates are still being actively imported.

Like fruit from other crop species, the highest respiration rate (RR), measured as the rate of evolution of CO_2, was recorded at stage I (Table 1) (Fawole and Opara, 2013). "Baby" eggplants from both cultivars had comparable RR (0.39–0.42 μmol kg^{-1} s^{-1}). Similar to Rodriguez et al. (1999) and consistent with a non-climacteric ripening pattern, a decreasing trend in RR was found during development. Fruit picked at stage IV had 30% lower RR than those harvested just two days earlier (stage III).

3.2. Firmness, density and alcohol insoluble residue (AIR)

For both cultivars highest firmness was found at stage I (Fig. 1B). The fruit softened markedly in the transition between stages I and II with no changes detected afterwards. The structural and biochemical modifications associated with eggplant softening have received almost no attention to date. Contrary to what has been reported for most fruits, (Esteban et al., 1993) found that pectin content increased during eggplant development, indicating that new cell wall material is being deposited. Although softening has been usually associated with changes in cell wall composition, tissue architecture may also lead to marked changes in mechanical properties (Vicente et al., 2007). Interestingly, fruit bulk density decreased from 700 to 500 kg m^{-3} during development (Fig. 1C) and showed, high correlation with firmness ($r = 0.84$ and 0.89 for M and PN, respectively). This indicates that changes in fruit intercellular spaces may be related to eggplant softening occurring as ripening progresses. Anyhow the fact that at stages IV and V softer PN eggplants presented higher density than M fruit, suggests that other factors determine texture of late ripening eggplants. Further studies to address this would be useful.

In fruit that do not accumulating starch the AIR represents mainly cell wall structural polymers including cellulose, hemicelluloses, pectin and lignin. In contrast to most fruits in which cell wall AIR decreases during ontogeny accompanying softening (Brummell, 2006) progressive accumulation of ethanol insoluble material was found in both cultivars (Fig. 1D). A marked increase in AIR was found at stage III in M. In PN the AIR rose more rapidly at stage IV. The rise in AIR could be in part to pectin deposition as

Table 1
Weight, length, diameter, calyx area, dry weight and respiration rate in Monarca (M) and Perla Negra (PN) eggplants at 12, 15, 18, 20 and 23 DAFS (Stages I to V, respectively). Each value represents the mean ± standard error. Values with different letters superscripts within a column indicate significant differences according to Fisher's test ($P < 0.05$).

cv. stage	Weight (g)	Length (cm)	Diameter (cm)	Dry weight (%)	Respiration (μmol kg^{-1} s^{-1})	Calyx area (cm^2)
M I	50.8 ± 4.9^{i}	8.6 ± 0.5^{g}	4.0 ± 0.3^{j}	7.5 ± 0.8^{a}	0.39 ± 0.03^{a}	2.0 ± 0.2^{f}
M II	111.1 ± 9.4^{h}	12.9 ± 0.6^{e}	5.0 ± 0.3^{h}	7.0 ± 0.3^{abc}	0.32 ± 0.02^{b}	2.3 ± 0.2^{ef}
M III	192.7 ± 15.4^{f}	15.6 ± 0.8^{d}	6.1 ± 0.4^{f}	6.8 ± 0.3^{cd}	0.25 ± 0.02^{cd}	2.7 ± 0.2^{de}
M IV	290.4 ± 19.3 d	17.7 ± 0.7^{c}	7.4 ± 0.3^{d}	6.8 ± 0.2^{bcd}	0.18 ± 0.01^{e}	3.1 ± 0.5^{cd}
M V	367.6 ± 27.3^{b}	21.1 ± 0.7^{a}	7.7 ± 0.4^{c}	6.8 ± 0.3^{bcd}	0.15 ± 0.01^{e}	3.3 ± 0.2^{c}
PN I	57.6 ± 4.6^{i}	7.8 ± 0.6^{h}	4.2 ± 0.2^{i}	7.3 ± 0.1^{ab}	0.42 ± 0.02^{a}	3.1 ± 0.2^{cd}
PN II	149.0 ± 9.8^{g}	12.5 ± 0.7^{f}	5.8 ± 0.3^{g}	6.6 ± 0.1^{cd}	0.27 ± 0.03^{c}	3.3 ± 0.2^{c}
PN III	225.0 ± 18.6^{e}	15.8 ± 0.6^{d}	6.9 ± 0.5^{c}	6.6 ± 0.3^{cd}	0.22 ± 0.01^{d}	3.5 ± 0.3^{c}
PN IV	354.1 ± 17.5^{c}	17.5 ± 0.7^{c}	8.2 ± 0.3^{b}	6.5 ± 0.2^{d}	0.16 ± 0.03^{e}	4.1 ± 0.6^{b}
PN V	444.8 ± 32.9^{a}	20.2 ± 1.1^{b}	8.8 ± 0.4^{a}	6.5 ± 0.3^{cd}	0.16 ± 0.01^{e}	5.0 ± 0.6^{a}

reported by Esteban et al. (1993). Progressive lignification of seeds, fibers and vascular tissues may likely contribute to the increase of ethanol insoluble material occurring during development.

3.3. Peel anthocyanins

Although eggplants could show a diversity of surface hues (Matsubara et al., 2005), purple fruit are by far the most popular. Peel color is determined by delphinidin-derived anthocyanins. Previous studies showed that dark cultivars may differ in the major anthocyanin: in some cases delphinidin-3-(p-coumaroylrutinoside)-5-glucoside (nasunin or NAS) predominates while in other genotypes delphinidin-3-rutinoside (D3R or tulipanin) is present at higher concentrations (Matsubara et al., 2005; Wu and Prior, 2005). In the present study NAS was absent and only D3R was identified as main anthocyanin in both cultivars, confirmed by HPLC-DAD-MSD (m/z 303–611). Similar to Wu and Prior (2005), we found other three minor anthocyanins with low retention time than D3R. Their MS profile indicated that they contained delphinidin structure. They probably were delphinidin 3-rutinoside-5-galactoside (D3R-5 Gal, peak 1), delphinidin 3-rutinoside-5-glucoside (D3R-5Glu, peak 2) and delphinidin 3-glucoside (D3Glu, peak 3) (Fig. 2A) as was suggested by Wu and Prior (2005). At all developmental stages, D3R was the main anthocyanin and their content was least at stage I, for both PN and M, being the 60–70% of the maximum concentration reached already at stage II (Fig. 2B). Afterwards, anthocyanin levels remained unchanged.

3.4. Pulp antioxidants

We then determined the levels of carotenoids and ascorbic acid and phenolic compounds within the fruit pulp. PN had higher carotenoid contents than M, with the largest difference observed in "baby" eggplants (Fig. 3A). Similarly to previous work (EL-Qudah, 2008)), carotenoids ranged between 4 and 10 mg kg^{-1}. In the present work carotenoid content was highest at early development. However, compared to carotenoid in rich-products (EL-Qudah, 2009; Aizawa and Inakuma, 2007) these levels are low. For fruit harvested at either stage III or IV no differences in carotenoid are found. (EL-Qudah, 2009)

As depicted in Fig. 3B, ascorbic acid ranged between 40 and 70 mg kg^{-1} and showed no marked differences between cultivars. AA levels are also low compared to those found in AA-rich commodities (Hanson et al., 2006; Lee and Kader, 2000). A slow decreasing trend in AA content was found during development. However significant reductions were only recorded at stage V in PN. This differs from the results reported by (Esteban et al., 1992) who found that AA peaked as fruit approached harvest maturity.

Total phenolics were already present at relatively high concentration (*ca.* 2000 mg kg^{-1}) at early development in both cultivars. Indeed, the maximum level of phenolic compounds was found at stage I (Fig. 3C). Phenolics dropped markedly at stage III, remaining subsequently unchanged. Differently, (Esteban et al., 1992) reported that phenolic compounds peaked at intermediate development. Our results are in accordance with those reported by (Mennella et al., 2012). However, in this study only three stages starting from intermediate development to overripe fruit were assessed. Overall, results clearly show that "baby" eggplants are richest in phenolics, carotenoids and ascorbic acid. Finally, fruit antioxidant capacity evaluated by TEAC showed a similar trend to that described for phenolics in both cultivars, decreasing during development and with a larger reduction between stages II and III (Fig. 3D). This confirms that at all developmental stages phenolics represent the predominant pulp antioxidant in eggplants.

3.5. Phenolic compounds sub-classes, chlorogenic acid content and distribution

We then determined the relative proportion of flavonoids (FL) and hydroxicinnamic acids (HCA) throughout development. In both cultivars at all stages HCAs represented 75 to 80% of total phenolics. Regardless of the ontogenic stage, FL contributed only with

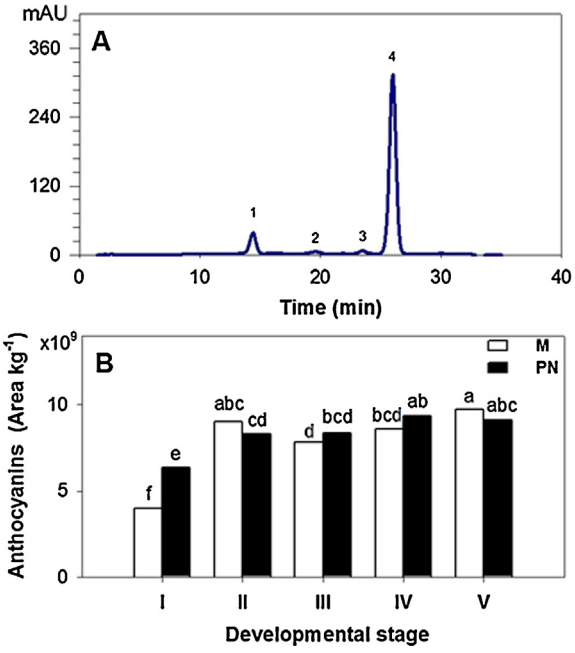

Fig. 2. (A) Representative HPLC profile of purple eggplant peel anthocyanins. Peak 1: delphinidin 3-rutinoside-5-galactoside, Peak 2: delphinidin 3-rutinoside-5-glucoside, Peak 3: delphinidin 3-glucoside and Peak 4: delphinidin 3-rutinoside. (B) Content of the mayor anthocyanin (peak 4) in Monarca (M) and Perla Negra (PN) eggplants during development. Values with different letters indicate significant differences according to Fisher's test ($P < 0.05$).

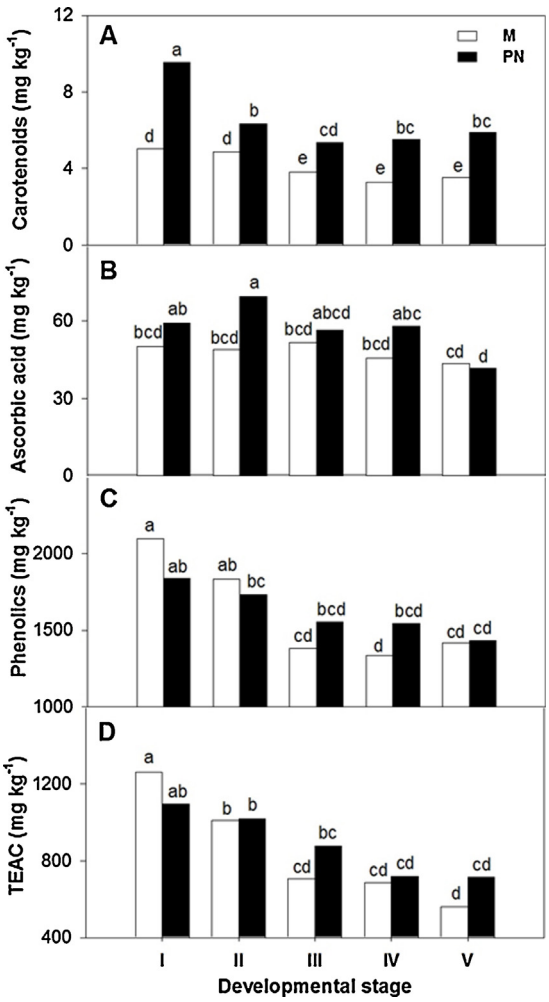

Fig. 3. (A) Carotenoids, (B) ascorbic acid, (C) phenolics and (D) antioxidant capacity (TEAC) in Monarca (M) and Perla Negra (PN) eggplants during development. Values with different letters indicate significant differences according to Fisher's test ($P < 0.05$).

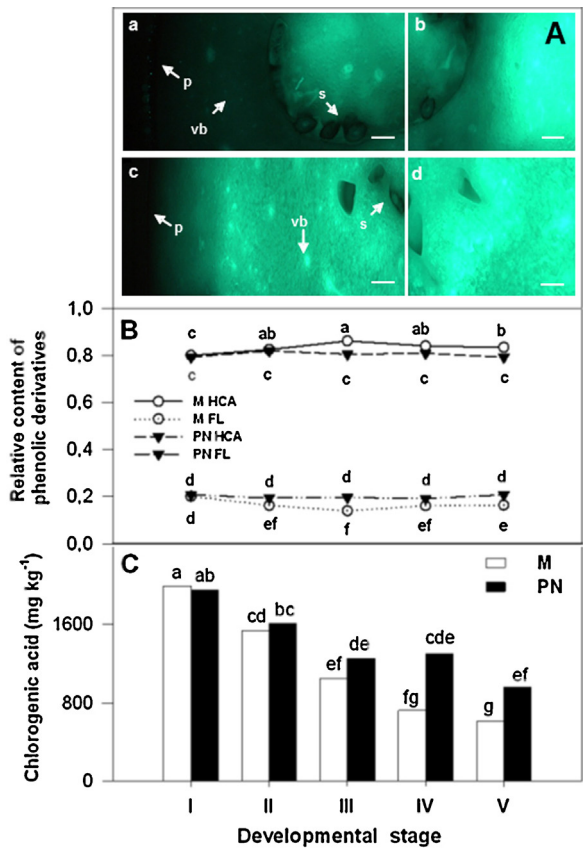

Fig. 4. (A) Histolocalization of chlorogenic acid detected by fluorescence with 2-aminoethyl diphenylborinate (light green signal) in Monarca eggplants. (a) Stage I, outer pulp; (b) stage I, fruit core; (c) stage IV, outer pulp; (d) stage IV, fruit core. Scale bar: 2 mm. p: peel; vb: vascular bundle. (B) Relative content of flavonoids (FL) and hydroxycinnamic acid (HCA) derivatives and (C) chlorogenic acid (ChA) content in Monarca (M) and Perla Negra (PN) eggplants during development. Values with different letters indicate significant differences according to Fisher's test ($P < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

20% of total phenolics (Fig. 4B). Both groups of phenolic compounds showed a decreasing trend during development (data not shown); HCA dropped from 6.2–7.0 to 2.3–3.1 mmol kg^{-1} for M and PN, respectively, whereas FL content decreased from 1.5–1.8 to 0.5–0.8 mmol kg^{-1} for M and PN, respectively.

Chlorogenic acid has been identified as the predominant HCA derivative found in eggplants from intermediate stages of development until late ripening (Mishra et al., 2012; Prohens et al., 2007; Raigoín et al., 2010; Stommel and Whitaker, 2003). In accordance with that, HPLC runs showed only a major peak corresponding to ChA for both cultivars and throughout development. Changes on ChA showed a similar trend to that described for eggplant pulp antioxidant capacity. ChA was higher in "baby" eggplant than in fruit harvested at stages III and IV. ChA content was near 2000 mg kg^{-1} in "baby" eggplant, decreasing at stage V to 900 and 600 mg kg^{-1} in PN and M, respectively (Fig. 4C). The distribution of ChA at different developmental stages was further tested by fluorescence with the 2-aminoethyl diphenylborinate, which may be used for *in situ* localization of caffeoyl-quinic acid derivatives by specific greenish fluorescence when excited under UV light (Mondolot et al., 2006). Both cultivars showed at stages I (Fig. 4A-a) and IV (Fig. 4A-c) lower ChA accumulation in the outer pulp (near the peel). In "baby" eggplants ChA-derived fluorescence predominated in the placenta and pulp core (Fig. 4A-a and b). At stage IV, ChA was still lower in the sub-epidermal region, but was more uniformly distributed in the inner pulp (Fig. 4A-c and d) compared to "baby" fruit (Fig. 4A-a and b). At stage IV fruit higher ChA was detected in the vasculature and core. This may be associated with fiber and vasculature lignification, which should be fed by aromatic monomers.

3.6. *Influence of fruit developmental stage on responses to different storage regimes*

Fruit developmental stage at harvest could also exert marked effects in the responses to different storage regimes. This has been clearly established in a number of fruit species (Getinet et al., 2008; Qian et al., 2013; Shin et al., 2008), but strikingly has received little attention in eggplant. The proper conditions for postharvest storage of "baby" eggplants have not been determined. The main factor contributing to quality loss in developed eggplants stored at 10 °C were calyx wilting and decay and peel browning (Fig. 5A and B). At 0 °C, fruit at stages III and IV developed chilling injury symptoms (pitting, and surface scalds). After 12 + 2 d and in both cultivars, fruit at stages III and IV showed lower deterioration at 10 °C than at 0 °C (Fig. 5C).

"Baby" eggplants showed extensive dehydration when held at 10 °C and this was indeed the main factor reducing quality (Fig. 5A and B). The high susceptibility of "baby" eggplants to dehydration may be due to their higher surface to volume ratio and incomplete cuticle wax deposition. Moreover, previous studies have shown

Table 2
Surface color (L*, a*, b*) and firmness in Monarca (M) and Perla Negra (PN) eggplants at stages I, III and IV (18, 20 and 23 DAFS, respectively). Each value represents the mean ± standard error. Values with different letters superscripts between storage temperatures indicate significant differences according to Fisher's test ($P < 0.05$).

cv. stage	L*		a*		b*		Firmness (kN m^{-1})	
	0 °C	10 °C	0 °C	10 °C	0 °C	10 °C	0 °C	10 °C
M I	25.2 ± 0.5^{bcde}	24.9 ± 0.58^{def}	4.0 ± 0.6^{bc}	4.3 ± 0.6^{b}	-0.4 ± 0.3^{d}	0.1 ± 0.2^{a}	1.4 ± 0.2^{a}	1.4 ± 0.2^{a}
M III	25.3 ± 1.3^{bcde}	25.0 ± 0.5^{cdef}	3.9 ± 0.8^{bcd}	3.6 ± 0.6^{cd}	-0.3 ± 0.3^{cd}	-0.3 ± 0.1^{cd}	1.0 ± 0.2^{cd}	1.0 ± 0.2^{de}
M IV	25.4 ± 1.0^{bc}	24.7 ± 0.8^{f}	4.2 ± 0.9^{b}	3.5 ± 0.6^{d}	-0.2 ± 0.4^{bc}	-0.3 ± 0.1^{cd}	1.2 ± 0.4^{bc}	0.9 ± 0.2^{e}
PN I	26.1 ± 0.4^{a}	25.6 ± 0.8^{b}	4.3 ± 1.0^{b}	3.7 ± 0.5^{cd}	-0.1 ± 0.4^{b}	0.2 ± 0.2^{a}	1.3 ± 0.2^{b}	1.4 ± 0.2^{a}
PN III	24.8 ± 0.6^{ef}	25.2 ± 0.6^{cde}	5.0 ± 1.1^{a}	3.9 ± 1.0^{bcd}	-0.2 ± 0.2^{bc}	-0.2 ± 0.2^{bc}	0.9 ± 0.3^{e}	1.0 ± 0.1^{d}
PN IV	24.5 ± 0.5^{f}	25.3 ± 0.8^{bcd}	5.4 ± 1.0^{a}	4.2 ± 1.0^{b}	-0.2 ± 0.1^{bc}	-0.3 ± 0.1^{cd}	1.0 ± 0.1^{de}	1.0 ± 0.1^{de}

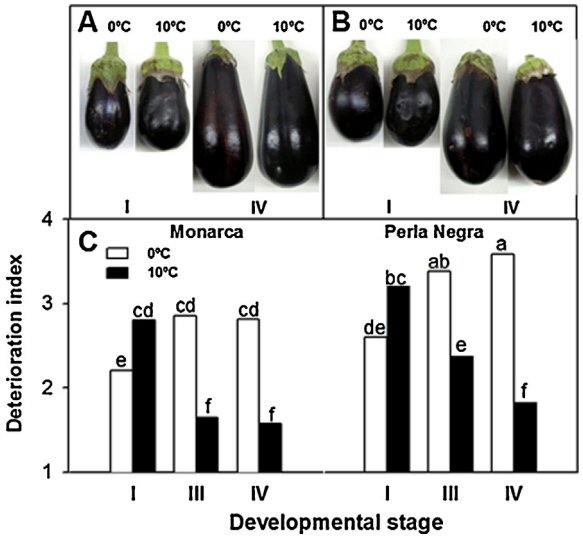

Fig. 5. (A) Appearance of Monarca eggplants, stages I and IV; (B) appearance of Perla Negra eggplants at stages I and IV (C) deterioration index M and PN eggplants stored for 12 d at 0 °C or 10 °C and subsequently transferred to 20 °C for 2 d (12 + 2 d). Developmental stages: I ("baby" eggplant), III (small commercial stage) and IV (large commercial stage). Values with different letters indicate significant differences according to Fisher's test ($P < 0.05$).

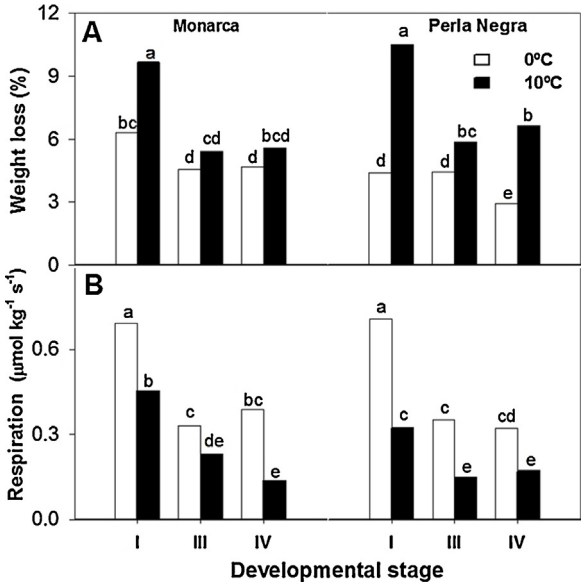

Fig. 6. (A) Weight loss and (B) respiration rate in Monarca (M) and Perla Negra (PN) eggplants stored for 12 d at 0 °C or 10 °C and subsequently transferred to 20 °C for 2 d (12 + 2 d). Developmental stages: I ("baby" eggplant), III (small commercial stage) and IV (large commercial stage). Values with different letters indicate significant differences according to Fisher's test ($P < 0.05$).

that most water loss of eggplants occurs through the calyx area (Díaz-Pérez, 1998) and the early development of this region may then also explain their high tendency to shrivel. For small eggplants storage at 0 °C and 90% RH was better; no marked chilling symptoms were observed and water loss was significantly reduced in both cultivars (Fig. 6A). Fruit postharvest regime did not result in differences in surface color (Table 2), but storage at 0 °C prevented softening of "baby eggplants" in both M and PN (Table 2).

"Baby" and "full-sized" fruit held at 0 °C showed, upon transfer to 20 °C, exacerbated RR (Fig. 6B). This indicates that all ontogenic stages evidenced chilling stress. It is worth noting that for both cultivars, fruit at stages III and IV presented similar responses to chilling in terms of their deterioration (Fig. 5B) and RR (Fig. 6B).

Overall, the present results show that in spite of their large size differences eggplants at stages III and IV do not show marked variation in dry weight, peel anthocyanin content, antioxidant capacity and susceptibility to chilling injury. The clearest difference between these two common harvest maturities is found in respiration rate and pulp density; delaying harvest within this period would result in a 25% reduction in RR and less dense eggplants.

4. Conclusions

In this study changes in growth and antioxidant content of purple eggplant fruit of two cultivars at all commercially relevant maturity stages, as well as the influence of fruit maturity at harvest on the response to different storage regimes were studied. "Baby" eggplants had higher antioxidant capacity than late harvested fruit and had higher concentrations of chlorogenic acid (ChA), carotenoids and ascorbic acid. ChA predominated in pulp placental tissues at stage I. Subsequently, it spread throughout fruit core especially in lignifying fibers and vasculature.

Harvesting at stages III and IV did not result in marked differences in antioxidant capacity or responses to postharvest storage regimes, but late pickings yielded spongy fruit with lower respiration rates. Within this harvest window, storage at 10 °C maximized quality maintenance. In contrast, antioxidant-rich "baby" eggplants were extremely susceptible to dehydration and stored better at 0 °C. Understanding the developmental-dependent changes in bioactive compounds and postharvest performance under different storage regimes of immature eggplants may aid in the selection of the optimal handling conditions for each ontogenic stage, as well as in the maximization of fruit antioxidant properties.

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