Postharvest Biology and Technology 66 (2012) 35–41

Contents lists available at SciVerse ScienceDirect

Postharvest Biology and Technology

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

Changes in quality and phenolic antioxidants in dark purple American eggplant (*Solanum melongena* L. cv. Lucía) as affected by storage at 0 °C and 10 °C

Analía Concellón[a,b,*], María J. Zaro[a], Alicia R. Chaves[a], Ariel R. Vicente[a,c]

[a] Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) (CCT La Plata CONICET-UNLP), 47 esq. 116, (1900) La Plata, Argentina
[b] Comisión de Investigaciones Científicas Pcia. de Buenos Aires (CIC-PBA), Argentina
[c] Cátedra de Agroindustrias, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata Calle 60 y 119, CP 1900, La Plata, Argentina

ARTICLE INFO

Article history:
Received 13 January 2011
Accepted 12 December 2011

Keywords:
Refrigeration
Postharvest
Chilling
Chlorogenic acid
Browning

ABSTRACT

Many beneficial effects associated with fruit and vegetable consumption are related to the presence of antioxidants, which could be greatly affected by postharvest storage conditions. Eggplants or aubergines (*Solanum melongena* L.) are among the top vegetables in terms of antioxidant content. In this work, we evaluated the effect of two postharvest temperature regimes on deterioration and antioxidants of dark purple American eggplants (cv. Lucía). Fruit were stored at 0 or 10 °C for 0, 3, 5, 10 or 14 d and weight loss, electrolyte leakage, chilling injury, and pulp browning were evaluated. We also followed DPPH• and Folin–Ciocalteu reacting substances and the content of chlorogenic and quinic acid by HPLC. Although weight loss was reduced in fruit held at 0 °C, higher electrolyte leakage and chilling injury manifested as surface scalds and pulp browning were found. Antioxidants (AOX) measured with the DPPH• radical and with the Folin–Ciocalteu reagent increased during the first 3 d of storage at 0 °C, but afterwards significant degradation was found. In contrast, a gradual but continuous accumulation of AOX was detected in fruit stored at 10 °C. The slow rate in the reaction between DPPH• and eggplant samples suggested that the main changes during postharvest storage were due to modifications in phenolic compounds. The major phenolic detected by HPLC was chlorogenic acid (ChA), an ester between caffeic (CA) and quinic acids (QA), which accumulated in fruit maintained at 10 °C, increasing by 60% after 14 d of storage. No free CA was found at any storage temperature or time, suggesting that its biosynthesis is activated simultaneously with the production of ChA. Free QA showed minor changes at 0 °C as pulp lightness decreased, indicating that ChA rather than CA may be the main substrate for browning reactions. Changes in eggplant fruit antioxidants during storage at chilling and non-chilling temperatures are discussed.

1. Introduction

Eggplant or aubergine (*Solanum melongena* L.) is a common annual vegetable crop grown in the sub-tropics and tropics. It is popular in Asia and some Mediterranean countries such as Greece, Italy and regions with similar cultural traditions. Eggplants are particularly rich in antioxidant compounds (Singh et al., 2009), which have been linked to various health benefits (Ames et al., 1993; Hung et al., 2004). They are known to have hepatoprotective properties (Akanitapichat et al., 2010) and have been shown to inhibit protein-activated receptor 2 inflammation associated with atherosclerosis (Han et al., 2003). In the last decades there have been great efforts oriented to understand the factors affecting the production, accumulation, and/or degradation of food antioxidants. In addition to the nutritional interest of this area in a broad sense, studies in some specific families such as phenolics is also of great value because they can potentially affect the susceptibility to browning after cutting or preparation before cooking.

Large natural variation in antioxidant capacity has been found in eggplant genotypes (Stommel and Whitaker, 2003; Hanson et al., 2006; Mennella et al., 2010). Raigón et al. (2010) reported that organic management and fertilization increased the accumulation of phenolic compounds. In contrast, Luthria et al. (2010) did not observe a consistent trend in the phenolic content of organically or conventionally grown eggplants. Though various works evaluated the performance of eggplant fruit during storage (Kozukue et al., 1978, 1979; Fallik et al., 1995; Concellón et al., 2004, 2005, 2007), only few studies have looked at influence of postharvest practices on antioxidant compounds. Lo Scalzo et al. (2010) reported higher antioxidant capacity on a dry weight basis in cooked eggplant fruit as compared to raw fruit. 1-MCP treatments reduced the degradation of phenolic compounds in purple eggplant stored at 10 °C

* Corresponding author at: CIDCA (CCT La Plata, CONICET–Facultad de Ciencias Exactas-UNLP), Calle 47 esq. 116, La Plata, Buenos Aires, CP 1900, Argentina.
Tel.: +54 221 4249287; fax: +54 221 4254853.
E-mail address: aconcell@quimica.unlp.edu.ar (A. Concellón).

0925-5214/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.postharvbio.2011.12.003

(Massolo et al., 2011). Gajewski et al. (2009) found that total phenolics in eggplant skin increased and were not affected in the pulp during storage at 20 °C. However, they did not evaluate the modifications occurring either at the recommended temperatures for storing the fruit (10 °C) or at chilling temperatures.

Hydroxycinamic acid derivatives such as chlorogenic acid (ChA) are the most common antioxidants in eggplant (Whitaker and Stommel, 2003; Singh et al., 2009). ChA is also the major soluble phenolic in other popular species such as potato, tomato, and coffee, making this compound one of the most abundant phenolics in the human diet (Niggeweg et al., 2004). Preharvest and postharvest conditions can greatly affect the ChA pool, but the developmental and environmental regulation of its metabolism is poorly understood (Joët et al., 2010). The aim of this study was to investigate the influence of postharvest storage conditions (time and temperature) on quality and phenolic antioxidants content in dark purple American eggplant.

2. Materials and methods

2.1. Chemicals

DPPH• (2,2,-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulphonic acid), mannitol, chlorogenic acid, caffeic acid and quinic acid were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Acetone, ethanol, methanol and formic acid were purchased from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA). Folin–Ciocalteu reagent, Na_2CO_3 and NaOH were purchased from Anedra Bs. As., Argentina. All other chemicals and solvents were of the highest commercial grade and used without further purification.

2.2. Plant material and storage conditions

Eggplants (*S. melongena* L.) cv. Lucía were grown in La Plata (Argentina). Fruit were harvested in the summer (December) between 20 and 25 d post flowering, after reaching a mass of 150–200 g, when the pulp was firm, the surface glossy and before complete seed development. After eliminating defective fruit, the eggplants were washed, air dried and randomly packed in groups of two in plastic (PET) trays and covered with perforated PVC (50 μm thick). Packed fruit were stored at 0 °C or 10 °C (85–90% RH) for 0, 3, 5, 10 and 14 d. Each sampling day, 20 fruit were used for each temperature evaluated. Electrolyte leakage, weight loss, pulp color, and chilling injury measurements were performed on fresh fruit immediately after removal from cold storage. Slices from the fruit equatorial zone were taken, peeled, frozen in liquid nitrogen, and stored at −80 °C until use. The experiment was repeated three times (three independent harvests).

2.3. Weight loss

Individual fruit 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 the initial sample weight and W_f the final sample weight. Results were expressed as percentage of weight loss. Twenty fruit were evaluated for each temperature and storage time.

2.4. Electrolyte leakage

Samples for electrolyte leakage analysis were taken from the equatorial region of 6 random fruit for each treatment and storage time and analyzed as described in a previous work (Concellón et al., 2005). Discs (3 mm × 10 mm) from the pulp tissues, weighing approximately 2 g, were obtained with a cork borer and incubated in 20 mL of 0.6 mol L^{-1} mannitol at 20 °C. The conductivity of the bathing solution at 20 °C was measured with a conductimeter (Oakton Model 510, IL USA) after 0 h (C_i) and after 2 h at 20 °C (C_f). Afterwards the tissue was homogenized and centrifuged at 17,500 × *g* for 15 min at 20 °C and the conductivity of the supernatant was measured to determine total electrolytes (C_t). Electrolyte leakage (EL) was calculated as: $EL = 100 \times (C_f - C_i)/C_t$. Results were expressed as a percentage of total electrolytes that leaked out of the tissue in the incubation time. Meaurements were done in triplicate.

2.5. Chilling injury index

On each sampling day, both internal and external chilling injury (CI) symptoms were visually analyzed. CI was determined according to the following scale: 1 = no damage, 2 = low damage, 3 = regular damage, 4 = moderate damage, 5 = severe damage. Observations were made on 20 fruit for each temperature and storage time. The CI index was calculated according to the following equation:

$$CI = \sum \frac{\text{Injury level} \times \text{No. of fruit at that level}}{\text{Total no. of fruit}}$$

2.6. Browning of pulp tissue

A 0.5 cm wide cross section was excised from the fruit central section and pulp lightness (L^*) was immediately measured with a colorimeter (Minolta, CR-400, Osaka, Japan). Twenty fruit were evaluated for each temperature and storage time and two measurements were done on each fruit.

2.7. Tissue extraction and sample preparation

For sample preparation, approximately 1 g of frozen fruit tissue was ground in a mill and the resultant powder was transferred to a tube containing 5 mL ethanol. The suspension was vortexed and then centrifuged at 17,000 × *g* for 10 min at 4 °C. The supernatant was collected and the pellet was re-extracted with 5 mL ethanol and centrifuged as described above. The supernatants were pooled and used for determinations of DPPH• and Folin–Ciocalteu reacting substances. Three extracts were done for each temperature and storage time. For HPLC analysis, the same tissue extraction was done but the ethanolic extract was evaporated on a rotary evaporator (model R-124, Büchi Labortechnik AG, Flawil, Switzerland) at 40 °C. The residue was suspended in 2 mL of formic acid:methanol:water (1:10:89) and filtered through a 0.45 μm nylon filter (Osmonics Inc., Minnesota, USA) prior to HPLC analysis. Three extracts were done for each temperature and storage time analyzed.

2.8. Folin–Ciocalteu and DPPH• reacting substances

Folin–Ciocalteu (FC) reacting substances were measured according to Singleton and Rossi (1965). Fifty microlitres of FC reagent (diluted 1:1 with distilled water) were added to 350 μL of the extract and 500 μL of distilled water. After 3 min, 100 μL of a solution containing 1.88 mol L^{-1} Na_2CO_3 in 0.1 mol L^{-1} NaOH were added and the mixture was brought to 2.5 mL with water and incubated at 20 °C for 90 min. The absorbance at 760 nm was measured and total phenolics content was calculated by using ChA as standard. Samples were measured in triplicate and results were expressed as milligrams of chlorogenic acid per kilogram of fresh weight. Although phenolic compounds react with the FC reagent at alkaline pH generating a blue complex (Singleton and Rossi, 1965) other reducing agents such as ascorbic acid (AA) also react with FC.

Since AA has been found to form the blue molybdenum complex even at acidic pH it is possible to differentiate its contribution to that of phenolic compounds (Singleton et al., 1999). In order to do this in eggplant extracts two series of four test tubes containing either: (1) 500 μL of water (Control), (2) eggplant ethanolic extract (Fruit sample), (3) 500 μL of a standard solution of AA (50 mg L^{-1}) or (4) 500 μL of a standard solution of ChA (50 mg L^{-1}) were prepared. Then, 500 μL of water and 50 μL of FC reagent (diluted 1:1 in water) were added to each test tube. After 3 min, 100 μL of a solution containing 1.88 mol L^{-1} Na_2CO_3 in 0.1 mol L^{-1} NaOH were added to the first series of four tubes (FC, at high pH) and 100 μL of water were added to the second series of tubes (FC at low pH). The mixtures were brought to 2.5 mL with water, incubated at 20 °C for 90 min and the absorbance at 760 nm was measured. ChA plus AA were determined in the tube series in which the reaction was performed at high pH (FC at high pH) while AA acid was evaluated by measuring the absorbance (760 nm) of the test tubes in which the reaction was performed at low pH (FC at low pH) while. Standard curves for AA and ChA at both pH conditions were done, in order to make corrections for differences in the extinction coefficient of the molybdenum blue complex.

The antioxidant capacity with the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical was performed as reported by Brand-Williams et al. (1995). In this test, the extracts reduce the stable and purple radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•) to the yellow-colored diphenylpicrylhydrazine. Loss of purple color of the solution indicates the scavenging capacity of the samples. Aliquots of ethanolic extract (50, 70, 90, 100 and 120 μL) were added to test tubes containing 1 mL of 40 mg L^{-1} DPPH• in ethanol prepared daily and taken to a final volume of 1.25 mL with ethanol. The absorbance at 515 nm was measured at different times with a spectrophotometer (UV-Mini 1240 model, Shimadzu, Japan) until the reaction reached a plateau (60 min). The amount of fruit (milligrams) necessary to decrease the initial DPPH• concentration by 50%, was calculated and defined as EC_{50}. Results were expressed as $EC_{50}{}^{-1}$. In order to determine the contribution of AA and ChA to eggplant antioxidants kinetics of fruit extracts AA and ChA standards with DPPH• were performed by measuring at different times the absorbance (515 nm) of test tubes containing either 75 μL of fruit extract or 50 μL of 100 mg L^{-1} AA or ChA and 1 mL of 40 mg L^{-1} DPPH• in ethanol and taken to a final volume of 1.25 mL with ethanol.

2.9. RP-HPLC analysis

Phenolics of the eggplant fruit extracts were separated and quantified by RP-HPLC using a Waters Model 6000A LC system (Milford, MA, USA) coupled to a diode array detector (DAD). The chromatographic separations were performed on a C-18 Altex Ultrasphere™-ODS column (250 mm × 4.6 mm i.d., 5 μm particle size). The mobile phase flow rate was 8.3 μL s^{-1} and consisted of a gradient of 1% formic acid in water (A) and methanol (B). Total run time was 21 min and the gradient program was as follows: 0–30% B (5 min), 30–50% B (5 min), 50–70% B (4 min), 70% B isocratic (4 min), 70–100% B (2 min), 100–0% B (1 min). The UV–vis spectra were recorded in the 210–600 nm range and the chromatograms were acquired at 320 nm. The injection volume was 20 μL. A calibration curve was done with a solution (550 mg L^{-1}) of standard ChA. Three measurements were done for each temperature and storage time and results were expressed in milligrams of chlorogenic acid per kilogram of fruit on a fresh weight basis.

2.10. HPLC–MS analysis

An HPLC–MS was used to identify both quinic and chlorogenic acid and quantify quinic acid. The experiments were performed with an HPLC Agilent 1100 LC (Agilent Technologies Inc., USA) equipped with a binary pump connected directly to a mass spectrometer (MS-VL quadrupole, Agilent Technologies, USA). The MS was operated with an electrospray ionization interface in the negative 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, 140 V. Mass spectronic data were acquired in the full scan mode to follow the representative fragments for quinic (*m*/*z* 191) and chlorogenic (*m*/*z* 191-353-707) acids. Subsequently, negative mode (ESI^-, *m*/*z* 191) was used for identification. The sensitivity of the mass spectrometer was optimized using the QA and ChA standards. The column, mobile phase, solvent gradient, flow rate and injection volume were the same described in Section 2.9. A standard curve was prepared with a solution HPLC grade QA. Three measurements were done for each temperature and storage time analyzed and results were expressed in milligrams of quinic acid per kilogram of fruit on a fresh weight basis.

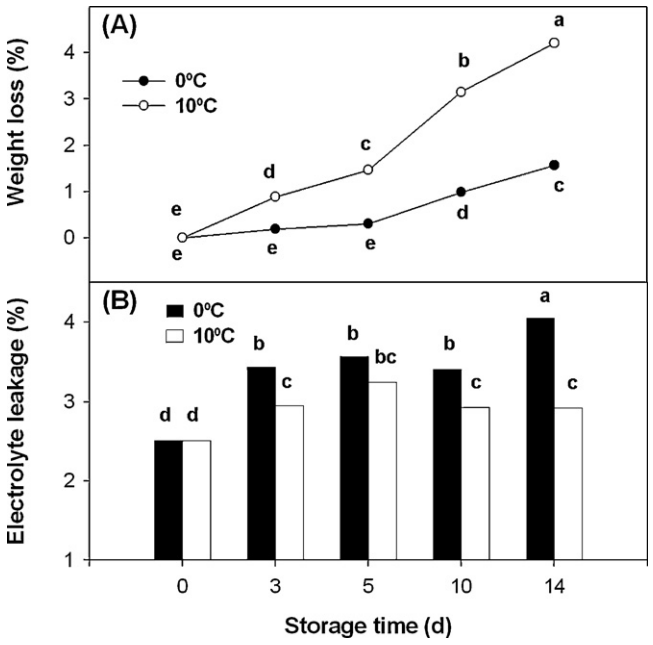

Fig. 1. (A) Weight loss and (B) electrolyte leakage of dark purple eggplant fruit (cv. Lucía) stored at 0 or 10 °C for 14 d. Values with different letters are significantly different ($P < 0.05$).

2.11. Statistical analysis

Experiments were performed according to a factorial design. Data were analyzed using ANOVA, and the means were compared by the Tukey test at a significance level of 0.05 using the SYSTAT software.

3. Results and discussion

3.1. Weight loss, electrolyte leakage and chilling injury

Weight loss increased during storage at both storage temperatures, but was higher in fruit maintained at 10 °C. After 14 d WL was 4.2% in fruit stored at 10 °C as compared to 1.6% in fruit held at 0 °C (Fig. 1A). Electrolyte leakage also increased during storage at both temperatures. After 3 d higher EL was found in fruit stored at 0 °C although no visible symptoms of CI were detected yet (Fig. 1B). The values in the present study with American eggplants were lower than those reported in prior studies in Japanese

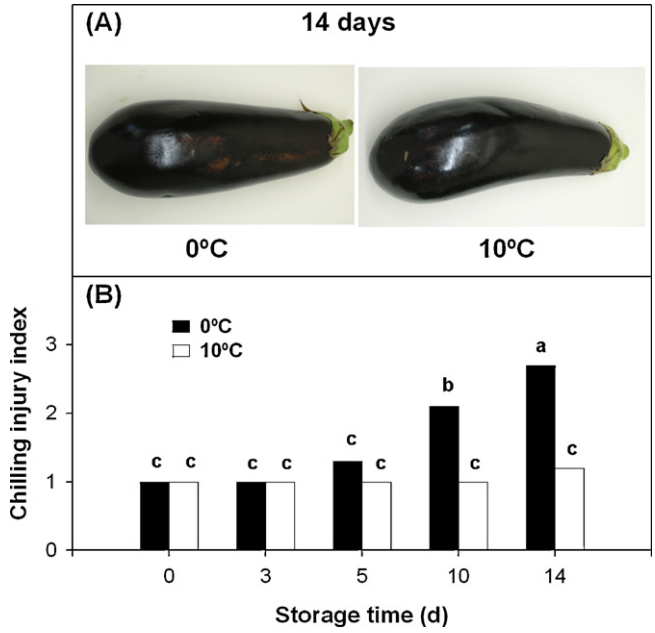

Fig. 2. (A) Appearance and (B) chilling injury index of dark purple eggplant fruit (cv. Lucía) stored at 0 or 10 °C for 14 d. Values with different letters are significantly different ($P < 0.05$).

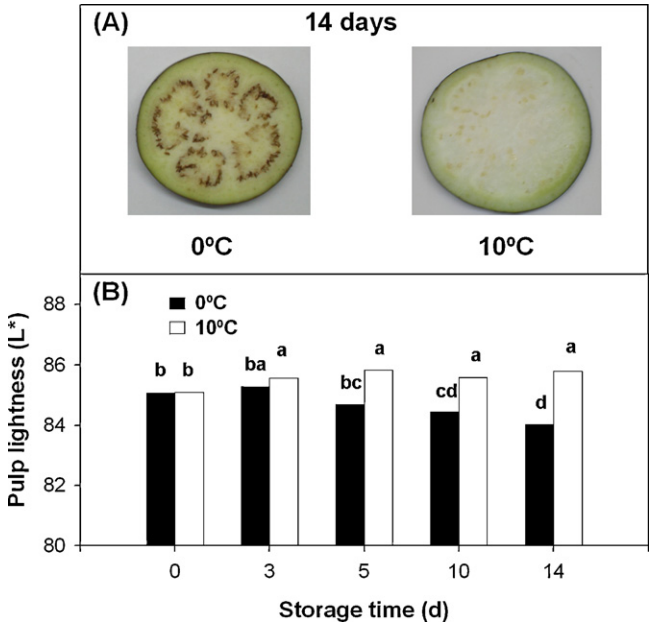

Fig. 3. (A) Pulp browning and (B) lightness (L^*) of dark purple eggplant fruit (cv. Lucía) stored at 0 or 10 °C for 14 d. Values with different letters are significantly different ($P < 0.05$).

eggplants, which are known to be highly susceptible to CI (Concellón et al., 2007). However, the differences could be also in part because in the present work measurements were done without a shelf life period after removal from cold storage. EL continued increasing in fruit held at 0 °C but remained unchanged in fruit stored at 10 °C. After both 10 and 14 d higher EL was found at 0 °C. During the first 5 d of storage at either 0 or 10 °C no damage symptoms were found, but afterwards internal browning and surface depressions were observed (Fig. 2A). After 10 d at 0 °C the CI index increased rapidly (Fig. 2B) and at the end of the storage period the differences were even more dramatic. Based on external appearance eggplants cv. Lucía maintain acceptable quality when stored at 10 °C for 14 d. In case of requirements of storage at lower temperatures dark purple eggplant fruit *cv.* Lucía should not be held at 0 °C for more than 5 d.

3.2. Pulp browning

Browning is one of the main causes of postharvest quality loss of eggplant fruit (Pérez-Gilabert and García Carmona, 2000). In this work it was the main symptom of internal damage and occurred in the areas surrounding the seeds (Fig. 3A). The change in pulp lightness is shown in Fig. 3B. In fruit stored at 10 °C pulp lightness increased after 3 d due to completion of chlorophyll degradation and no changes were observed afterwards. Pulp lightness of fruit held at 0 °C showed no variation during the first 3 d of storage, but later on a continuous decrease associated with pulp browning was detected. After 5 d of storage the pulp of fruit maintained at 0 °C was already darker than fruit at 10 °C and the difference was higher at the end of the storage period.

3.3. Folin–Ciocalteu (FC) and DPPH• reacting substances

Eggplant fruit is a relatively good source of antioxidants ranked among the top vegetables in terms of oxygen radical absorbance capacity (Cao et al., 1996). We evaluated the influence of storage temperature on fruit phenolic compounds with the FC reagent (Fig. 4A). FC reacting substances accumulated during the first 3 d at 0 °C. Esteban et al. (1989) working with purple eggplant fruit also

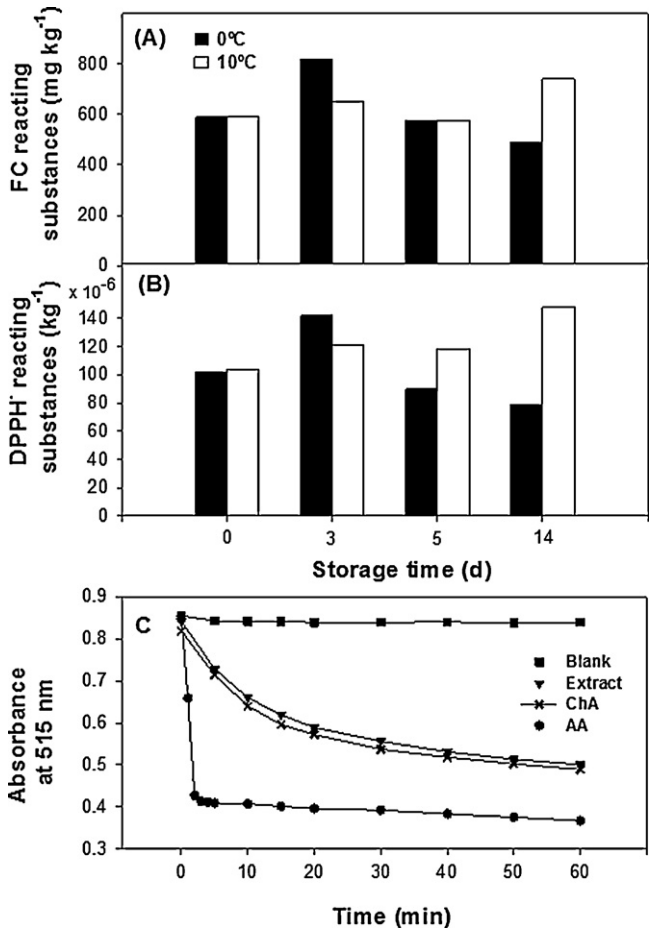

Fig. 4. (A) Folin–Ciocalteu (FC) reacting substances and (B) 2,2,-diphenyl-1-picrylhydrazyl (DPPH•) reacting substances of dark purple eggplant fruit (cv. Lucía) stored at 0 or 10 °C for 14 d. Values with different letters are significantly different ($P < 0.05$). (C) Time course of reactions of a typical eggplant extract (Extract), chlorogenic acid (ChA) and ascorbic acid (AA) with DPPH•.

found an initial increase in the content of phenolic compounds in fruit stored at 10 °C. After that a continuous decline was detected, resulting in a reduction from a maximum of 818 to 489 mg kg^{-1} after 14 d of cold storage. In contrast, in fruit stored at 10 °C the content of FC reacting substances at the end of storage was higher to harvest.

To further characterize the changes in antioxidants during postharvest storage we determined the content of DPPH• reacting substances (Fig. 4B). After 3 d at either 0 or 10 °C the content of compounds able to quench DPPH• increased, with the rise being higher in fruit maintained at 0 °C. Later on, the content of AOX continued increasing at 10 °C but in contrast a rapid degradation was found at 0 °C. At the end of storage fruit held at 0 °C presented lower level of antioxidants than at harvest as opposed to eggplants maintained at 10 °C, which showed a 60% increase. The antiradical capacity of some commodities has been reported to increase during postharvest storage (Ayala-Zavala et al., 2004).

AOX measured against the radical DPPH• showed a similar trend to that of FC reacting substances. Although the FC assay was developed for the estimation of total phenolics, AA reacts with polyphosphotungstate under acidic conditions (pH 3) (Singleton et al., 1999). By analyzing color development in FC assays at low or high pH we were able to test the contribution of AA and ChA to FC reacting substances in the fruit extracts. In the eggplant samples, color development upon reaction with the FC reagent occurred only at high pH (supplementary Fig. 1). This, together with the kinetics of fruit ethanolic samples with DPPH• which showed a profile resembling that of ChA (Fig. 4C) suggest that the main modifications during eggplant storage occurred in phenolics. Reduction in phenolic antioxidants during storage has been shown to occur associated with browning reactions (Massolo et al., 2011). Interestingly, in eggplants stored at 0 °C or 10 °C no correlation was found between browning and PPO or POD activity (Concellón et al., 2004; Massolo et al., 2011). The degradation of phenolic compounds in eggplants possibly might be limited by compartmentalization of the enzymes and substrates, which could be lost during storage at chilling temperatures. The rapid browning occurring upon cutting of eggplant tissues even prior to storage, suggests the enzymes and substrates are readily available. Besides this, the problem seems to be far more complex. Loss of soluble phenolics could occur in some commodities in association with lignification (Liu and Jiang, 2006). We have recently determined that in some cases after long term storage large losses (e.g. 50%) of soluble phenolic antioxidants in eggplant can occur in the abscense of browning (unpublished data). In this case, the fate of phenolic compounds could be, in part, due to lignin deposition in fibers, xylem vessels and seed coats. Despite the balance between these biological processes, results clearly show that the postharvest storage regime greatly affects the content of antioxidants of eggplant fruit. The rise observed at 10 °C increases significantly the nutritional value of the fruit and potentially the benefits associated with the consumption of antioxidants. Although the up regulation of pathways involved in the biosynthesis of phenolic compounds could be of interest from a nutritional point of view, this would also result in increased content of substrates for browning. Another aspect that could be a trade off of the accumulation of phenylpropanoids, is the build up of bitter compounds as has been shown in carrots (Lafuente et al., 1996).

3.4. Phenolics by HPLC

Modifications of antioxidant phenolic compounds in eggplant were analyzed by HPLC-DAD. A major peak corresponding to a compound with the same retention time and spectrum as ChA was detected in all samples (Fig. 5A). The changes found for ChA during eggplant storage (Fig. 5B) showed a similar trend to those described for DPPH• and FC reacting substances. Previous works

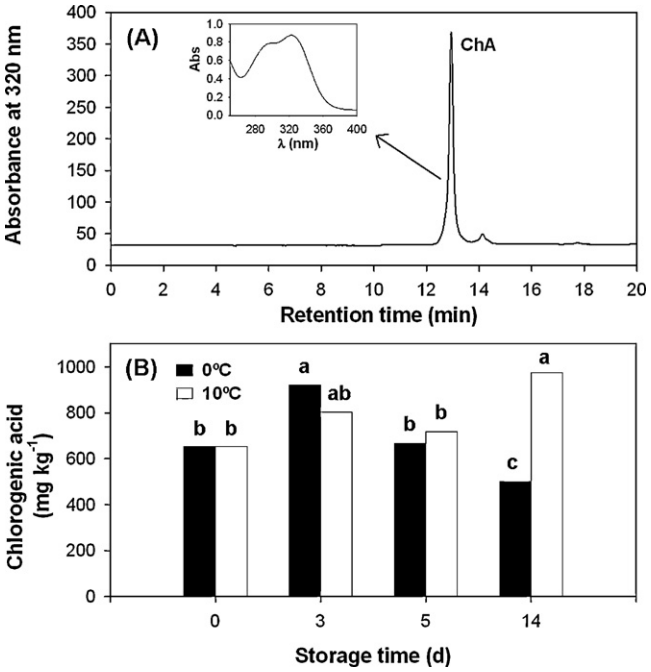

Fig. 5. (A) Main panel: typical HPLC-DAD data for eggplant extracts. The inner box shows the absorption spectrum of chlorogenic acid (ChA). (B) Chlorogenic acid content in eggplant fruit stored at 0 or 10 °C for 14 d. Values with different letters are significantly different ($P < 0.05$).

have shown that hydroxycinnamic acid conjugates (Whitaker and Stommel, 2003), and in particular ChA, typically accounts for 70% to 95% of total phenolics in eggplant fruit flesh (Stommel and Whitaker, 2003). Results found in the present work show that the accumulation of antioxidants observed in eggplants stored at 10 °C for 14 d and the reduction in fruit held at 0 °C (Fig. 4B) are mainly due to changes in the level of ChA (Fig. 5B). It has been shown that variations in temperature can modulate ChA accumulation (Joët et al., 2010). Phenolic compounds have been shown to accumulate in response to various stresses including low temperature (Clé et al., 2008; Massolo et al., 2011). In the present work, the rise of ChA occurred at both storage temperatures, but, at 10 °C, high ChA level was still found at the end of the storage period, whereas at 0 °C, chilling injury favored rapid turnover. Although ChA biosynthesis is not completely elucidated, recent works suggest that it might be formed from quinic acid and caffeoyl-CoA by the enzyme hydroxycinnamoyl-quinate hydroxycinnamate transferase (HQT) (Sonnante et al., 2010), which might be limiting in some cases. Clé et al. (2008) found low content of ChA in HQT-gene-silenced lines whereas an over expression of HQT in tomato resulted in a 70 fold increase in ChA (Niggeweg et al., 2004). The dramatic increase of ChA found at 10 °C with no detection of free caffeic suggests that this compound should be rapidly esterified with QA upon biosynthesis. In addition, CA production may increase as the biosynthetic pathway of derivatives such as ChA is activated. Previous works showing that phenylalanine ammonia-lyase (PAL), increases in eggplant at low temperatures (Massolo et al., 2011) and that ChA biosynthesis could increase upon chilling (Joët et al., 2010) support this. However, the availability of conjugated caffeic acid, which would be undetectable without sample hydrolysis, cannot be ruled out. In fruit stored at 0 °C the drop of ChA after long-term storage also occurred without accumulation of caffeic acid. This may be the result of either direct participation of ChA in PPO-mediated browning (Fig. 3B) or rapid oxidation of CA upon hydrolysis of ChA. In the latter case, a transient increase of free QA should be found.

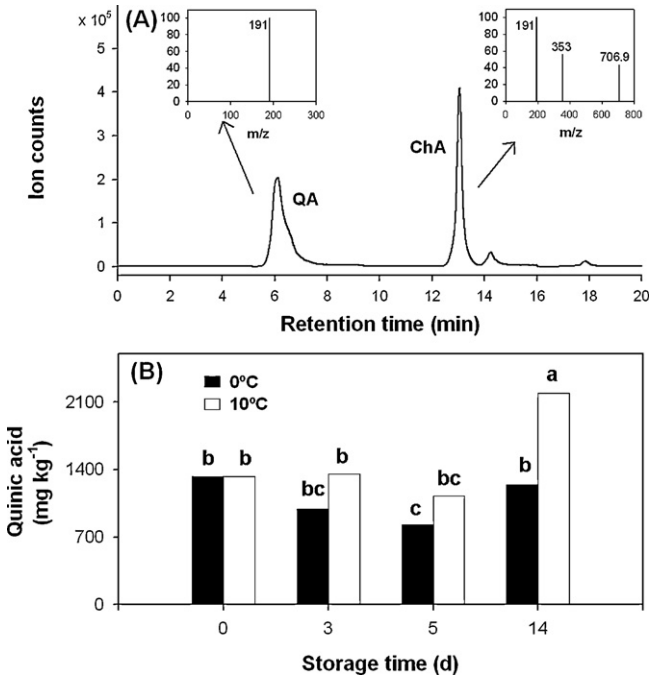

Fig. 6. (A) Main panel: typical HPLC–MS data for eggplant extracts. The boxes represent the mass spectra for quinic (QA) and chlorogenic acid (ChA) showing the most abundant fragments. (B) Quinic acid content in eggplant fruit stored at 0 or 10 °C for 14 d. Values with different letters are significantly different ($P < 0.05$).

To gain further insight regarding the changes of ChA metabolism in postharvest storage of eggplant we evaluated eggplant ethanolic extracts by HPLC–MS. Results of typical runs are shown in Fig. 6A. Two major peaks with retention times of 6.2 and 13.0 min were detected which were identified by their mass spectra as QA and ChA, respectively. Due to their chemical structure (abscence of double bonds) QA was detected by MS and not by DAD. Other minor peaks were observed at 14.2 and 17.8 min of retention times. Their mass spectra show a *m/z* 249 and 468 which are referred as *N*-caffeoylputrescine and *N,N′*-dicaffeoylspermidine by other authors working with eggplant (Whitaker and Stommel, 2003; Stommel and Whitaker, 2003; Singh et al., 2009). These two peaks will be not considered in further discussion due to their low concentration and little variation during eggplant storage. During storage QA increased one fold during 14 d storage at 10 °C and showed minor changes in fruit held at 0 °C (Fig. 6B). The build up of QA at 10 °C could contribute to increasing the pool of one of the precursors for ChA biosynthesis, which was quite active at this temperature. At 0 °C, the drop in ChA, together with the absence of free CA and the lack of accumulation of QA after 14 d supports the possibility that ChA might be the main substrate for browning reactions. However, some caffeic acid could be also recruited towards lignin biosynthesis and pulse chase experiments would be required to determine its fate univocally. In summary, the present work shows that the antioxidant pool of eggplant fruit is greatly affected by postharvest storage temperatures. While at 10 °C there is a continuous accumulation of ChA, fruit maintained at 0 °C showed a transient increase of antioxidants followed by a rapid decline together with pulp browning and chilling injury development.

Acknowledgments

This work was supported by the Agencia Nacional de Promoción Científica y Tecnológica (PICT 2006-00753; PICT 2009-0059 and 01120) and CONICET (PIP 2009-00353).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.postharvbio.2011.12.003.

References

Akanitapichat, P., Phraibung, K., Nuchklang, K., Prompitakkul, S., 2010. Antioxidant and hepatoprotective activities of five eggplant varieties. Food Chem. Toxicol. 48, 3017–3021.
Ames, B.N., Shigenaga, M.K., Hagen, T.M., 1993. Oxidants, antioxidants, and the degenerative diseases of aging. Proc. Natl. Acad. Sci. U.S.A. 90, 7915–7922.
Ayala-Zavala, J.F., Wang, S.Y., Wang, C.Y., Gonzalez-Aguilar, G.A., 2004. Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit. Lebensm. Wiss. Technol. 37, 687–695.
Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol. 28, 25–30.
Cao, G., Sofic, E., Prior, R.L., 1996. Antioxidant capacity of tea and common vegetables. J. Agric. Food Chem. 44, 3426–3431.
Clé, C., Hill, L.M., Niggeweg, R., Martin, C.R., Guisez, Y., Prinsen, E., Jansen, M.A.K., 2008. Modulation of chlorogenic acid biosynthesis in *Solanum lycopersicum*; consequences for phenolic accumulation and UV-tolerance. Phytochemistry 69, 2149–2156.
Concellón, A., Añón, M.C., Chaves, A.R., 2004. Characterization and changes in polyphenol oxidase from eggplant fruit (*Solanum melongena* L.) during storage at low temperature. Food Chem. 88, 17–24.
Concellón, A., Añón, M.C., Chaves, A.R., 2005. Effect of chilling on ethylene production in eggplant fruit. Food Chem. 92, 63–69.
Concellón, A., Añón, M.C., Chaves, A.R., 2007. Effect of low temperature storage on physical and physiological characteristics of eggplant fruit (*Solanum melongena* L.). LWT – Food Sci. Technol. 40, 389–396.
Esteban, R.M., Molla, E., Villaroya, M.B., Lopez-Andreu, F.J., 1989. Changes in the chemical composition of eggplant fruits during storage. Sci. Hortic. 41, 19–25.
Fallik, E., Temkin-Gorodeiski, N., Grinberg, S., Davidson, H., 1995. Prolonged low-temperature storage of eggplants in polyethylene bags. Postharvest Biol. Technol. 5, 83–89.
Gajewski, M., Katarzyna, K., Bajer, M., 2009. The influence of postharvest storage on quality characteristics of fruit of eggplant cultivars. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 37, 200–205.
Han, S.W., Tae, J., Kim, J.A., Kim, D.K., Seo, G.S., Yun, K.J., Choi, S.C., Kim, T.H., Nah, Y.H., Lee, Y.M., 2003. The aqueous extract of *Solanum melongena* inhibits PAR2 agonist-induced inflammation. Clin. Chim. Acta 328, 39–44.
Hanson, P.M., Yang, R.Y., Samson Tsou, C.S., Ledesma, D., Engle, L., Lee, T.C., 2006. Diversity in eggplant (*Solanum melongena*) for superoxide scavenging activity, total phenolics, and ascorbic acid. J. Food Comp. Anal. 19, 594–600.
Hung, H.C., Joshipura, K.J., Jiang, R., Hu, F.B., Hunter, D., Smith-Warner, S.A., 2004. Fruit and vegetable intake and risk of major chronic disease. J. Nat. Cancer Inst. 96, 1577–1584.
Joët, T., Salmona, J., Laffargue, A., Descroix, F., Dussert, S., 2010. Use of the growing environment as a source of variation to identify the quantitative trait transcripts and modules of co-expressed genes that determine chlorogenic acid accumulation. Plant Cell Environ. 33, 1220–1233.
Kozukue, N., Kozukue, E., Kishiguchi, M., Lee, S.W., 1978. Studies on keeping-quality of vegetables and fruits. III. Changes in sugar and organic acid contents accompanying the chilling-injury of eggplant fruits. Sci. Hortic. 8, 19–26.
Kozukue, N., Kozukue, E., Kishiguchi, M., 1979. Changes in the contents of phenolic substances, phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) accompanying chilling-injury of eggplant fruit. Sci. Hortic. 11, 51–59.
Lafuente, M.T., Lopez-Galvez, G., Cantwell, M., Yang, S.F., 1996. Factors influencing ethylene-induced isocoumarin formation and increased respiration in carrots. J. Am. Soc. Hortic. Sci. 121, 537–542.
Liu, Z.Y., Jiang, W.B., 2006. Lignin deposition and effect of postharvest treatment on lignification of green asparagus (*Asparagus officinalis* L.). Plant Growth Reg. 48, 187–193.
Lo Scalzo, R., Fibiani, M., Mennella, G., Rotino, G.L., Dal Sasso, M., Culici, M., Spallino, A., Braga, P.C., 2010. Thermal treatment of eggplant (*Solanum melongena* L.) increases the antioxidant content and the inhibitory effect on human neutrophil burst. J. Agric. Food Chem. 58, 3371–3379.
Luthria, D., Singh, A.P., Wilson, T., Vorsa, N., Banuelos, G.S., Vinyard, B.T., 2010. Influence of conventional and organic agricultural practices on the phenolic content in eggplant pulp: plant-to-plant variation. Food Chem. 121, 406–411.
Massolo, J.F., Concellón, A., Chaves, A.R., Vicente, A.R., 2011. 1-Methylcyclopropene (1-MCP) delays senescence, maintains quality and reduces browning of non-climacteric eggplant (*Solanum melongena* L.) fruit. Postharvest Biol. Technol. 59, 10–15.
Mennella, G., Rotino, G.L., Fibiani, M., D'Alessandro, A., Francese, G., Toppino, L., Cavallanti, F., Acciarri, N., Lo Scalzo, R., 2010. Characterization of health-related compounds in eggplant (*Solanum melongena* L.) lines derived from introgression of allied species. J. Agric. Food Chem. 58, 7597–7603.
Niggeweg, R., Michael, A.J., Martin, C., 2004. Engineering plants with increased levels of the antioxidant chlorogenic acid. Nat. Biotechnol. 22, 746–754.
Pérez-Gilabert, M., García Carmona, F., 2000. Characterization of catecholase and cresolase activities of eggplant polyphenol oxidase. J. Agric. Food Chem. 48, 695–700.

Raigón, M.D., Rodriguez-Burruezo, A., Prohens, J., 2010. Effects of organic and conventional cultivation methods on composition of eggplant fruits. J. Agric. Food Chem. 58, 6833–6840.

Singh, A.P., Luthria, D., Wilson, T., Vorsa, N., Singh, V., Banuelos, G.S., Pasakdee, S., 2009. Polyphenols content and antioxidant capacity of eggplant pulp. Food Chem. 114, 955–961.

Singleton, V.L., Rossi Jr., J.A., 1965. Colorimetry of total phenolics with phosphomolybidic–phosphotungstic acid reagents. Am. J. Enol. Viticult. 16, 144–158.

Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods Enzymol. 299, 152–178.

Sonnante, G., D'Amore, R., Blanco, E., Pierri, C.L., de Palma, M., Luo, J., Tucci, M., Martin, C., 2010. Novel hydroxycinnamoyl-coenzyme a quinate transferase genes from artichoke are involved in the synthesis of chlorogenic acid. Plant Physiol. 153, 1224–1238.

Stommel, J.R., Whitaker, B.D., 2003. Phenolic acid content and composition of eggplant fruit in a germplasm core subset. J. Am. Soc. Hortic. Sci. 128, 704–710.

Whitaker, B.D., Stommel, J.R., 2003. Distribution of hydroxycinnamic acid conjugates in fruit of commercial eggplant (*Solanum melongena* L.) cultivars. J. Agric. Food Chem. 51, 3448–3454.