

## Distribution, stability and fate of phenolic compounds in white and purple eggplants (*Solanum melongena* L.)



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### ARTICLE INFO

#### Article history:

Received 24 May 2013

Accepted 13 January 2014

#### Keywords:

Quality

Antioxidants

Peroxidase

Polyphenol oxidase

Hydrogen peroxide

### ABSTRACT

Eggplants rank among the vegetables richest in antioxidants, but little is known about the allocation, stability, and turnover of these metabolites. The distribution, accumulation and degradation of phenolic antioxidants in the inner and outer pulp of two commercially important eggplant types (white and dark purple), at harvest and after 14 and 30 d of refrigerated storage under non-chilling conditions (10 °C and 90% RH) were determined in this study. Chlorogenic acid (ChA) was histolocalized by fluorescence with 2-aminoethyl-diphenylborinate and the activity of phenolic compounds oxidizing enzymes (polyphenoloxidase, PPO and peroxidase, POD) as well as H<sub>2</sub>O<sub>2</sub> concentration in both fruit regions was determined. During storage, dark purple fruit were more susceptible to dehydration and showed greater deterioration than white eggplants. Both genotypes accumulated higher sugar content in the inner pulp as opposed to acids, which were more concentrated in the outer region. At harvest, pulp antioxidant capacity was similar in both eggplant types. TEAC and DPPH\* assays and *in situ* localization, showed greater total antioxidants and ChA content in the core than in the outer pulp in both white and dark purple fruit. The stability of ChA was markedly different between genotypes. In white fruit, antioxidants increased during the first two weeks of storage, remaining stable afterwards. In contrast, in dark purple eggplants, phenolic compounds declined after an initial stage at which they accumulated. PPO and POD *in vitro* activities, associated mainly with fruit seeds, fibers, and vascular bundles did not correlate with pulp browning or loss of phenolic antioxidants. Instead, the reduction of ChA in the core of dark purple fruit was associated with increased production of H<sub>2</sub>O<sub>2</sub>. Results indicate that antioxidants are predominantly located in the inner pulp of eggplants regardless of the genotype, but are more stable in white fruit. Rather than being the result of browning reactions, substantial losses of phenolic antioxidants in whole eggplants under the recommended storage conditions likely result from seed coat development and vascular lignification in the immature fruit.

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### 1. Introduction

Fruits and vegetables are a good source of a variety of non-nutritive bioactive compounds that can provide a number of health benefits including the activation of human defenses and the reduction of inflammatory responses (Landete, 2012). Phenolics are one of the largest groups of plant bioactives and the interest in the identification of their mode of action, distribution, fate and

stability in fresh produce is increasing (El-Seedi et al., 2012; Sivakumar et al., 2012). Eggplants are among the richest vegetables in terms of antioxidant capacity (Cao et al., 1996; Hanson et al., 2006), with 5-caffeoylquinic acid, known as chlorogenic acid (ChA), being the main contributor (Gajewski et al., 2009; Whitaker and Stommel, 2003).

Previous studies established that the steady state content of eggplant antioxidants can be largely affected by genotype (Ma et al., 2010), crop management practices (Luthria et al., 2010), postharvest storage regime, as well as by the extent of processing (Singh et al., 2009; Lo Scalzo et al., 2010). Almost all studies conducted to date in eggplant have analyzed global antioxidant contents and only a few investigations attempted to determine the pattern of accumulation within the pericarp. Whitaker and Stommel (2003) evaluated the longitudinal distribution of phenolic compounds and

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found that caffeoylquinic acid isomers are located in the midsection in all genotypes and also in the blossom end of dark purple-black pigmented varieties. Despite the large phenotypic variation found in eggplants, most research conducted to date has focused on dark purple varieties, while variegated and white types have received very little attention. The different genotypes may show large variations not only in terms of their total concentrations, but also in their stability. However, this remains poorly characterized. Degradation of phenolic compounds has been repeatedly associated with tissue browning, resulting from the action of polyphenol oxidases (PPOs) and peroxidases (PODs) (Flick et al., 1977). While extensive browning is rapidly induced in eggplant upon cutting and at chilling temperatures (Concellón et al., 2004; Prohens et al., 2007), it is not clear if this is the main determinant of antioxidant loss under recommended storage conditions (10 °C and 90% RH). To gain further insight regarding the distribution, stability and fate of phenolic compounds in eggplant fruit, we evaluated the distribution of antioxidants and phenolic compounds in the inner (I) and outer (O) zones of the pulp of white and purple fruit as well as their stability during storage at non-chilling temperatures. We also measured H<sub>2</sub>O<sub>2</sub> and histolocalized polyphenol oxidase (PPO) and peroxidase (POD) as well as ChA, their most abundant potential substrate within the fruit.

## 2. Materials and methods

### 2.1. Chemicals

DPPH• (2,2,-diphenyl-1-picrylhydrazyl) and ABTS•+ (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid), chlorogenic acid, gallic acid, catechol, pyrogallol, pyrocatechol, and horseradish peroxidase (HRP) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Ethanol was purchased from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA). Folin–Ciocalteu reagent, Na<sub>2</sub>CO<sub>3</sub>, and NaOH were purchased from Anedra (Bs. As., Argentina). Amplex Red® was obtained from Molecular Probes (Eugene, OR USA). Aminoethyl-diphenylborinate was purchased from Fluka (Buchs, Switzerland). All other chemicals and solvents were of the highest commercial grade and used without further purification.

### 2.2. Plant material storage conditions and sampling

Purple and white eggplants (*Solanum melongena* L.) cvs Lucía and Cloud Nine, respectively were produced in greenhouses in La Plata, Buenos Aires-Argentina. Fruit was harvested at commercial maturity and transported to the laboratory. After eliminating the fruit having defects, the eggplants were washed by immersion for 3 min in water containing 100 mg L<sup>-1</sup> NaClO (pH 6.5) for 2 min and subsequently the calyxes were sprayed with 0.45 g L<sup>-1</sup> prochloraz (Sportak®, Bayer Crops Science, Brasil). The eggplants were then packed in polyethylene terephthalate (PET) trays, covered with 50 μm thick polyvinyl chloride (PVC, wrap film) and stored at 10 °C (90–95% RH) for 0, 14 or 30 d. Respiration rate and weight loss measurements were performed on whole fruit. After that the eggplants were peeled and three slices (10 mm thick) from the midsection of each fruit were taken and immediately used for pulp color determinations and histochemical analysis of chlorogenic acid, polyphenoloxidase (PPO), and peroxidase (POD) activities. Tissue sampling for further chemical analysis was performed also by taking three slices from the midsection region of the fruit (10 mm thick). The discs were peeled and divided into I and O zones. The O zone was obtained by separating the external 4 mm of the pulp, while the remaining central tissue was considered to be the inner region zone. Samples from each fruit zone were immediately processed or frozen in liquid N<sub>2</sub> and stored at –80 °C

until use. Thirty fruit were used for each genotype and storage time. The experiment was repeated twice.

### 2.3. Weight loss

Individual fruit were weighed at the beginning of the experiment, and during storage. Weight loss (WL) was calculated as:  $WL = 100 \times (W_i - W_f)/W_i$ , with  $W_i$  and  $W_f$  being the initial and final sample weights, respectively. Results were expressed as percentage of weight loss. Thirty fruit were evaluated for each genotype and storage time.

### 2.4. Respiration rate

Two fruit were put into each 3-L glass flask. The flasks were tightly sealed and incubated at 10 °C for 30 min in order to prevent CO<sub>2</sub> concentrations over 0.5% that may inhibit respiration. Carbon dioxide was continuously monitored with an infrared sensor (ALNOR Compu-flow, Model 8650, Argentine). Fruit respiration was calculated and expressed as CO<sub>2</sub> production in mg kg<sup>-1</sup> s<sup>-1</sup>. Four replicates were done for each genotype and storage time.

### 2.5. Soluble sugars

One gram of the frozen powder of fruit pulp from I or O zones was transferred to a Corex® tube containing 5 mL ethanol. The suspension was vortexed and then centrifuged at 14,000 × 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 further analysis. Two replicates were performed for each fruit zone, genotype, and storage time. Sugars were measured with the anthrone reagent (Yemm and Willis, 1954). Briefly, aliquots (50 μL) of the ethanolic extracts were taken and brought to 500 μL with water. One milliliter of 2 g L<sup>-1</sup> anthrone prepared in 98% (w/w) H<sub>2</sub>SO<sub>4</sub> was added and held at 100 °C in a water bath for 10 min. The test tubes were cooled in water, and the absorbance at 620 nm was measured (UV-Mini 1240 model, Shimadzu Corporation, Japan). Glucose was used as a standard and results were expressed as mass of glucose per kilogram of fresh weight (g kg<sup>-1</sup>). Measurements were done in triplicate.

### 2.6. Acidity

Frozen fruit zone (I or O) was processed in a mill (Model A11, IKA Works Inc., SP Brazil) and 15 g of the resulting powder were weighed in a beaker. One hundred milliliters of water were added and acidity was determined by titrating with 0.1 mol L<sup>-1</sup> NaOH until pH 8.2 (AOAC, 1980). Four replicates were evaluated for each fruit zone, genotype and storage time. Results were expressed as [H<sup>+</sup>], millimoles per kilogram of fresh weight (mmol kg<sup>-1</sup>).

### 2.7. Pulp color

A Minolta colorimeter model CR-400 (Minolta, Osaka, Japan) was used to determine the lightness (L\*) of pulp tissue (0 = black and 100 = white) and the a\* and b\* chromaticity values which were used to calculate the hue angle ( $180 - \tan^{-1} b^*/a^*$ ). A 1 cm wide cross section was excised from the fruit midsection and pulp color was immediately measured. Thirty measurements were done for each eggplant genotype, zone, and storage time.

### 2.8. Antioxidant capacity against DPPH• and ABTS•+ radicals

Eggplant ethanolic extracts were prepared as described in Section 2.5 and the supernatants were used to measure antioxidant

capacity with DPPH• and ABTS•+ radicals. The DPPH• assay was done according to the method described by Brand-Williams et al. (1995) with minor modifications. Test tubes containing 0, 5, 10, 20, 30, 40 and 50  $\mu\text{L}$  of sample and ethanol to a final volume of 125  $\mu\text{L}$  were prepared. After that 500  $\mu\text{L}$  of a 60  $\text{mg L}^{-1}$  solution of the radical DPPH• in ethanol were added. Samples were vortexed and incubated at 20 °C for 60 min. The absorbance at 515 nm was measured and the equivalent mass of fruit tissue required to consume 50% of the initial DPPH• was calculated ( $\text{EC}_{50}$ ). The antioxidant capacity was expressed as  $\text{EC}_{50}^{-1}$ . Three measurements were done for each eggplant zone, genotype and storage time.

The ABTS•+ assay was performed as described by Arnao et al. (2001). The ABTS•+ stock solution was prepared by weighing 7 mmol of ABTS ammonium salt and 2.45 mmol of  $\text{K}_2\text{S}_2\text{O}_8$ , which were added to water to make 1 L and allowed to react overnight at 20 °C in darkness. ABTS•+ working solutions were prepared by diluting the stock solution to an absorbance of  $0.700 \pm 0.03$  at 734 nm. Fifty microlitres of ethanolic fruit extracts, prepared as described above, were added to 1 mL of ABTS•+ working solution, vortexed, and incubated for 6 min. The absorbance at 734 nm was measured. Corresponding blanks without fruit extract were used to determine the stability of the ABTS•+. Samples were measured in triplicate. Trolox® was used as antioxidant standard and results were expressed as Trolox equivalents per mass, fresh weight ( $\text{mmol kg}^{-1}$ ).

## 2.9. Phenolic compounds

Aliquots of the ethanolic extracts prepared as described in Section 2.5 were used to determine phenolic compounds with the Folin–Ciocalteu reagent (Singleton et al., 1999). Briefly 50  $\mu\text{L}$  of 1:1 diluted Folin–Ciocalteu reagent was added to 350  $\mu\text{L}$  of the extract and 500  $\mu\text{L}$  of distilled water. After 3 min, 100  $\mu\text{L}$  of a solution containing 20% (w/v)  $\text{Na}_2\text{CO}_3$  in 0.1  $\text{mol L}^{-1}$  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 phenolics content was calculated by using ChA acid as standard. Samples were measured in triplicate and results were expressed as mass of ChA per fresh weight mass of fruit ( $\text{mg kg}^{-1}$ ). ChA HPLC analysis was performed on ethanolic extracts of both white and purple eggplants to determine the correlation between spectrophotometric and chromatographic assays. The ethanolic extracts were 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  $\mu\text{m}$  nylon filter (Osmonics Inc., Minnesota, USA) prior to HPLC analysis. Phenolics were separated and quantified by HPLC as previously reported (Concellón et al., 2012).

## 2.10. Histochemical localization of chlorogenic acid

ChA *in situ* localization was done with Neu' reagent as described by Mondolot et al. (2006) with minor modifications. ChA was visualized by a light green fluorescence when excited under UV light. To start the staining, eggplant slices (10 mm thick) from the midsection were dipped for 30 s in 10 mL of 1% (w/v) 2-amino-ethyl-diphenylborinate in absolute methanol. Samples were immediately examined in a light stereomicroscope (Modular Stereomicroscope Leica MZ10 F, Leica Microsystems Ltd., Germany). Samples were excited at 425 nm and emission at 480 nm was evaluated. Images were obtained with a digital color camera Leica DFC490 (Leica Microsystems Ltd., Germany, 8 megapixels). Negative controls were obtained by analyzing slices dipped in methanol.

## 2.11. Enzyme assays

Polyphenol oxidase (PPO) extraction and activity were determined according to Concellón et al. (2004) with minor modifications. Approximately 5 g of frozen pulp tissue were ground in a mill and homogenized with 20 mL of phosphate buffer (50  $\text{mmol L}^{-1}$ , pH 6.5; 10  $\text{g L}^{-1}$  polyvinylpyrrolidone; and 0.1% (v/v) Triton X-100). The suspension was centrifuged at  $14,000 \times g$  for 15 min at 4 °C, and the supernatant was used to assay PPO activity. The reaction mixture contained phosphate buffer (100  $\text{mmol L}^{-1}$ , pH 6.5); 1 mL of 20  $\text{mmol L}^{-1}$  pyrocatechol and 50  $\mu\text{L}$  of extract in a total volume of 3 mL. The reaction mixture was incubated at 25 °C and the enzymatic activity was determined spectrophotometrically at 430 nm. One unit of enzymatic activity (UEA) was defined as the amount of extract producing a variation of absorbance of 0.1 units per min under the assay conditions. Results were expressed as UEA per kilogram of fresh weight. Three extracts were prepared for each treatment and storage time analyzed and each one was measured in triplicate.

Pyrogallol peroxidase (POD) extraction and activity were determined according to Massolo et al. (2011). Approximately 5 g of ground pulp was added to 20 mL of phosphate buffer (50  $\text{mmol L}^{-1}$ , pH 6.5; 10  $\text{g L}^{-1}$  PVPP, 1  $\text{mmol L}^{-1}$  phenylmethylsulfonyl fluoride (PMSF) and 1  $\text{mol L}^{-1}$  NaCl). The suspension was centrifuged at  $14,000 \times g$  for 10 min at 4 °C and the supernatant was used to assay POD activity. The reaction mixture contained 300  $\mu\text{L}$  of phosphate buffer (50  $\text{mmol L}^{-1}$ , pH 6.5); 300  $\mu\text{L}$  of 8  $\text{mmol L}^{-1}$   $\text{H}_2\text{O}_2$ ; 200  $\mu\text{L}$  of 45  $\text{mmol L}^{-1}$  pyrogallol and 50  $\mu\text{L}$  of enzymatic extract. The reaction mixture was incubated at 25 °C and the enzymatic activity was determined spectrophotometrically at 430 nm. One unit of enzymatic activity (UEA) was defined as the amount of extract producing a variation of absorbance of 0.1 units per min under the assay conditions. Results were expressed as UEA per kilogram of fresh weight. Three extracts were prepared for each treatment and storage time analyzed and each one was measured in triplicate.

## 2.12. Histochemical localization of PPO and POD

The histochemical detection of PPO was carried out according to Onsa et al. (2007) by incubating the fruit transversal sections from the midsection zone for 2 h in 15 mL of phosphate buffer (100  $\text{mmol L}^{-1}$ , pH 6.0) containing 0.5 mL of 50  $\text{mmol L}^{-1}$  catechol and 0.25 mL of catalase (2  $\text{g L}^{-1}$ ). Corresponding controls were performed by dipping the samples as previously described, but without adding catechol. After 2 h of incubation at room temperature the samples were observed in a Modular Stereomicroscope Leica MZ10 F (Leica Microsystems Ltd., Germany). Pictures were taken with a digital camera Leica Model DFC490 (Leica Microsystems Ltd., Germany).

POD was histochemically localized following the procedure described by McInnis et al. (2006). A slice (10 mm thick) was taken from the midsection of the fruit and then incubated with 15 mL of phosphate buffer (100  $\text{mmol L}^{-1}$ , pH 7.4) containing 0.5 mL of 0.25% (v/v) guaiacol and 0.5 mL of 100  $\text{mmol L}^{-1}$   $\text{H}_2\text{O}_2$ . After 2 h at 20 °C the samples were observed and images were acquired as previously described for PPO. Corresponding controls were performed by immersing the samples in the same conditions previously described but without  $\text{H}_2\text{O}_2$ .

## 2.13. Hydrogen peroxide

Measurements were performed using the method described by Gomez et al. (2008) based on the enzymatic oxidation of Amplex Red® at pH 7.4 by hydrogen peroxide in the presence of horseradish peroxidase (HRP). Samples were taken from I and O zones of fruit midsection slices. Discs were obtained with a cork borer (6 mm

diameter  $\times$  2 mm thick). Fifteen discs weighing approximately 0.1 g were incubated at 20 °C in a beaker containing 993  $\mu$ L of phosphate buffer (50 mmol L<sup>-1</sup>, pH 7.4), 5  $\mu$ L of 5 mmol L<sup>-1</sup> Amplex Red® and 2  $\mu$ L of HRP (10 IU mL<sup>-1</sup>). After 180 min the absorbance at 560 nm was measured. A standard curve with H<sub>2</sub>O<sub>2</sub> was performed and results were expressed as mmol of H<sub>2</sub>O<sub>2</sub> released after 180 min per kilogram of fresh fruit. Measurements were done in triplicate.

#### 2.14. Statistical analysis

Experiments were performed in a factorial design. The factors were the genotype (white and purple) and storage time at 10 °C (0, 14 and 30 d) for respiration rate and weight loss evaluations. The pericarp zone (inner and outer) was added as a factor for all other measurements. Data were analyzed by ANOVA and means were compared by a Fisher test at  $P < 0.05$ .

### 3. Results and discussion

#### 3.1. Fruit appearance, weight loss, respiration, color, sugar, and acid distribution

White eggplants showed less deterioration than purple fruit after 30 d of storage (Fig. 1A). The main factors reducing fruit quality were surface dehydration, epidermal scalds and wilting of the calyx tips. Even though browning is usually indicated as one of the most important deterioration factors in eggplant, in the present study even after one month of refrigerated storage at non-chilling temperatures (10 °C) no marked changes in pulp

color occurred (Fig. 1A). The respiration rate for both eggplant types was moderate (10–12  $\mu$ g kg<sup>-1</sup> s<sup>-1</sup>) (Fig. 1B). CO<sub>2</sub> production was comparable in both genotypes and decreased during storage, showing a non-climacteric pattern. Purple eggplants are more susceptible to dehydration than white fruit (Fig. 1C). After 30 d at 10 °C, weight losses were 7.3% and 4.8% in purple and white fruit, respectively.

Purple eggplants showed a higher level of ethanol soluble carbohydrates than white fruit ( $P < 0.05$ ) (Table 1). Both eggplant varieties showed higher sugar content (ca. 20%) in the inner zone and manifested no changes during storage. In both pulp zones purple fruit also showed higher acidity than white eggplants ( $P < 0.05$ ). Contrary to sugars, acids were greater in the O zone of the pulp. The main change occurring during postharvest storage was a reduction of fruit acidity in the O region of dark fruit (Table 1).

Fruit pulp color also differed between genotypes. The O pulp of purple fruit showed a light green ring (Fig. 1A) associated with the presence of residual chlorophylls, while the central region was light yellow (Table 1). The hue angle decreased in both pericarp zones of dark fruit after 14 d of storage, remaining unchanged afterwards. White fruit had lighter pulp (Fig. 1A) and showed slight color variations between the O and I zones (Table 1). Minor changes in pulp lightness occurred throughout storage. Overall, white eggplants had higher storage capacity than purple fruit. The genotypes showed differences in acidity and sugar content, though their relative distribution was similar with acids being more concentrated in the periphery and sugars in the inner zone. Interestingly, internal browning was not a major deterioration factor in either white or purple fruit stored at 10 °C.

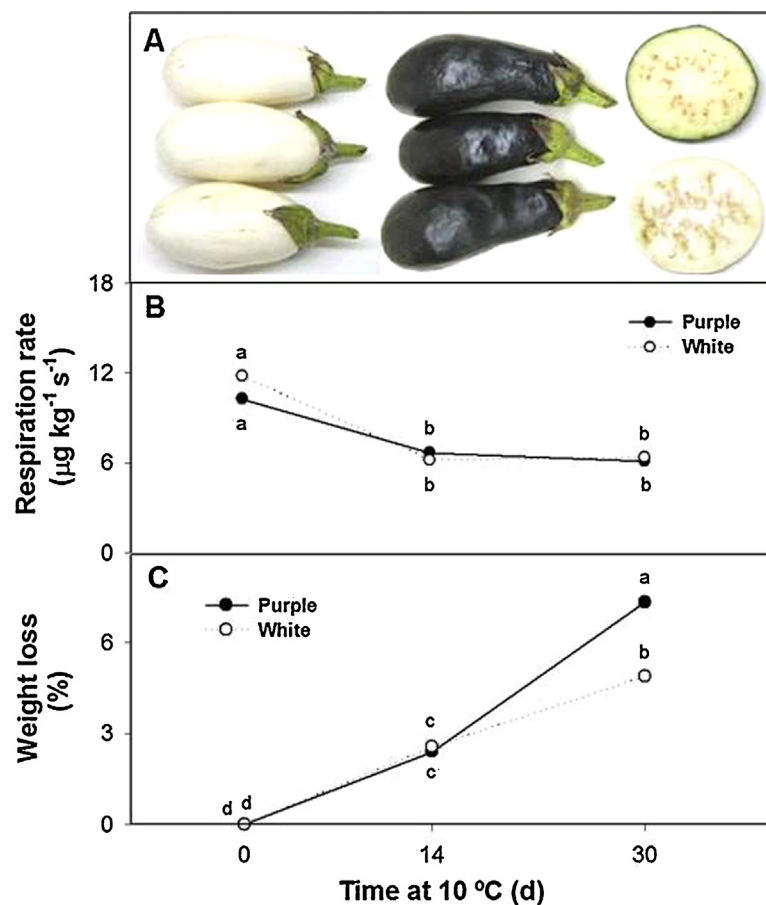


Fig. 1. (A) Appearance of eggplant fruit stored at 10 °C for 30 d. (B) Respiration rate and (C) weight loss of white and purple eggplants stored for 0, 14 or 30 d at 10 °C. Values with different letters indicate significant differences based on a Fisher test at a level of significance of  $P < 0.05$ .

**Table 1**  
Sugars, acidity, color (hue) and lightness ( $L^*$ ) of the inner (I) and outer (O) pulp of purple and white eggplants stored at 10 °C for 0, 14 or 30 d.<sup>1</sup>

	Genotype and zone	Time at 10 °C (days)		
		0	14	30
Sugars (g kg <sup>-1</sup> )	Purple-I	41.2 <sup>a</sup>	44.2 <sup>a</sup>	42.4 <sup>a</sup>
	Purple-O	35.6 <sup>b</sup>	36.9 <sup>b</sup>	35.0 <sup>b</sup>
	White-I	34.0 <sup>b</sup>	36.9 <sup>b</sup>	34.5 <sup>b</sup>
	White-O	26.6 <sup>c</sup>	26.3 <sup>c</sup>	29.8 <sup>c</sup>
Titratable acidity ([H <sup>+</sup> ] mmol kg <sup>-1</sup> )	Purple-I	35.4 <sup>b</sup>	32.3 <sup>c</sup>	36.5 <sup>b</sup>
	Purple-O	46.9 <sup>a</sup>	45.5 <sup>a</sup>	37.0 <sup>b</sup>
	White-I	32.2 <sup>c</sup>	30.5 <sup>cd</sup>	28.3 <sup>d</sup>
	White-O	37.7 <sup>b</sup>	31.1 <sup>cd</sup>	37.5 <sup>b</sup>
Color (hue)	Purple-I	105.0 <sup>c</sup>	102.0 <sup>e</sup>	101.7 <sup>e</sup>
	Purple-O	114.3 <sup>a</sup>	112.3 <sup>b</sup>	113.2 <sup>b</sup>
	White-I	100.6 <sup>f</sup>	99.3 <sup>g</sup>	101.3 <sup>d</sup>
	White-O	103.2 <sup>d</sup>	104.1 <sup>cd</sup>	101.0 <sup>b</sup>
Lightness ( $L^*$ )	Purple-I	85.02 <sup>e</sup>	85.17 <sup>e</sup>	85.41 <sup>e</sup>
	Purple-O	78.76 <sup>f</sup>	78.94 <sup>f</sup>	76.70 <sup>f</sup>
	White-I	86.18 <sup>d</sup>	86.14 <sup>d</sup>	86.67 <sup>cd</sup>
	White-O	87.57 <sup>ab</sup>	88.14 <sup>a</sup>	87.22 <sup>bc</sup>

<sup>1</sup> Means with different letters indicate significant differences between fruit genotypes, storage times and pulp zones according to Fisher's least significant difference (LSD) test ( $P < 0.05$ ).

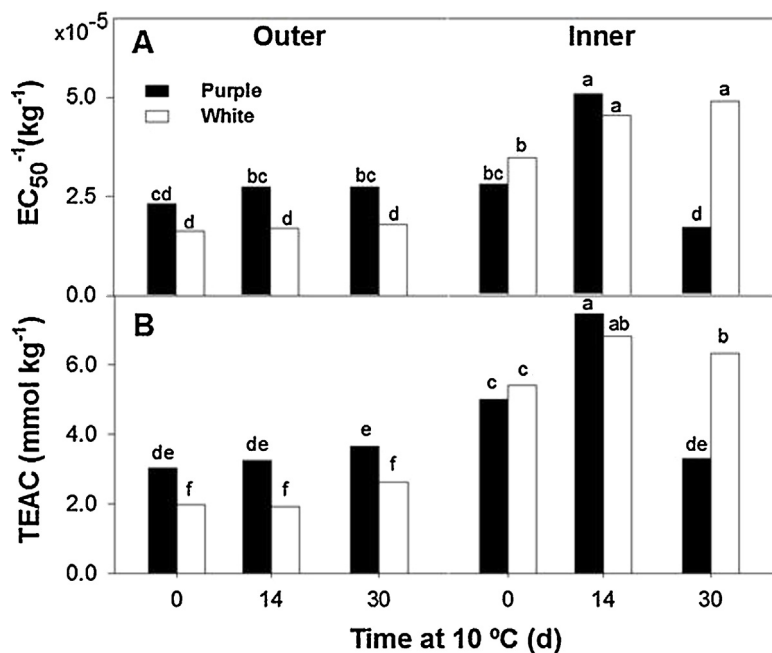
### 3.2. Antioxidant capacity, phenolic compounds, and chlorogenic acid

Eggplants are an excellent source of antioxidants (AOX), ranking among the vegetables richest in phenolic compounds. While total levels have been established the distribution of AOX within the fruit as well as their stability in different genotypes have received almost no attention (Whitaker and Stommel, 2003). The antioxidant capacity was evaluated by determining the ability of fruit ethanolic extracts to quench DPPH• (Fig. 2A) and ABTS•• radicals (Fig. 2B). At harvest, the antioxidant capacity of the pulp of white and purple fruit was similar. Antioxidants showed a preferential accumulation in the central pulp, which showed two to three-fold

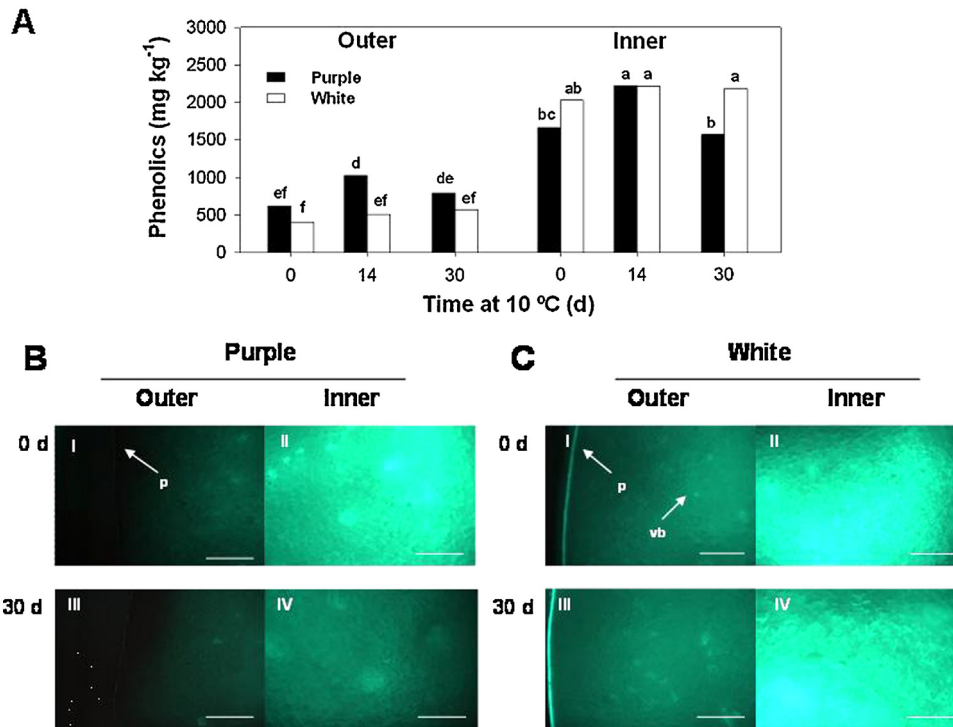
higher radical scavenging capacity than the periphery ( $P < 0.05$ ). After 14 d of storage, no changes on AOX were detected in the O zone of either white or purple fruit, but significant accumulation was recorded in the I region of both genotypes. Intriguingly, at the end of the storage period (30 d) the antioxidant capacity decreased markedly in I zone from purple eggplants, but remained stable in white fruit. Reported results on changes in eggplant antioxidant during storage have been controversial. Some works have shown no variations (Gajewski et al., 2009), while in other cases extensive turnover (Hu et al., 2010) or even steady increases have been measured (Mahmood, 2013). This may be related to differences among genotypes, storage conditions, or time points evaluated.

Prohens et al. (2007) reported significant variation in ascorbic acid content among eggplant varieties. However its content was 20–30 times lower than that of ChA. The analysis of phenolic compounds (Fig. 3A) paralleled, in general, the changes described for ethanol soluble DPPH• and ABTS•• scavenging capacity, suggesting that they represent the main antioxidants of both white and purple eggplants.

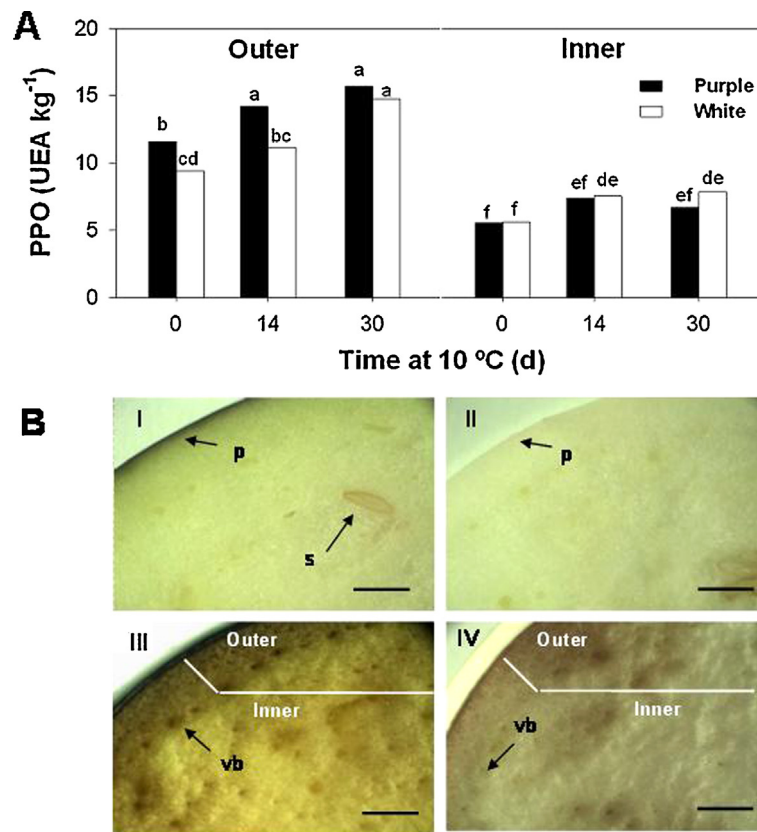
The HPLC profiles for both eggplant types showed a single major peak corresponding to ChA. The spectrophotometric determination of total phenolics with the Folin–Ciocalteu method showed high and positive correlations ( $r$  0.99) with ChA content analyzed by HPLC in both eggplant types studied (data not shown). Thus the assay may be a rapid screening method to select eggplant genotypes based on their ChA content. To get further direct evidence regarding the spatial distribution of ChA in both white and purple eggplant genotypes, we performed histochemical localizations with the Neu's reagent (Fig. 3B and C). Upon UV excitation 2-aminoethyl-diphenylborinate fluoresces in the presence of mono- and dicaffeoylquinic acids (Mondolot et al., 2006). Green fluorescent products indicate ChA or monocaffeoyl-quinic acid, while blue fluorescence suggests the prevalence of feruloylquinic-derivatives. Staining and visualization of whole purple eggplant slices confirmed that already at harvest ChA is less concentrated in the O zone (Fig. 3B-I) than in the core (Fig. 3B-II). The same trend was detected in white eggplants (Fig. 3C-I and II). The distribution throughout the slices showed an increasing accumulation of ChA near the core I



**Fig. 2.** Antioxidant capacity against (A) DPPH• and (B) ABTS•• radicals in the Outer and Inner pulp of white and purple eggplants stored for 0, 14 or 30 d at 10 °C. Values with different letters are significantly different on a Fisher test at a level of significance of  $P < 0.05$ . EC<sub>50</sub><sup>-1</sup>: (Equivalent concentration to decrease the initial DPPH• concentration to 50%)<sup>-1</sup>, TEAC: Trolox Equivalent Antioxidant Capacity.



**Fig. 3.** (A) Phenolic compounds in the Outer and Inner pulp of purple and white eggplants stored for 0, 14 or 30 d at 10 °C. Values with different letters are significantly different on a Fisher test at a level of significance of  $P < 0.05$ . (B) Histocalization of cholorgenic acid in purple (I, II, III and IV) and (C) white (I, II, III and IV) eggplants stored for 0 or 30 d at 10 °C. Scale bar: 2 mm (25×). p: peel; vb: vascular bundle.



**Fig. 4.** (A) Polyphenoloxidase (PPO) activity in the Outer and Inner pulp of purple and white eggplants stored for 0, 14 or 30 d at 10 °C. Values with different letters are significantly different on a Fisher test at a level of significance of  $P < 0.05$ . (B) Histocalization of PPO in purple (I, III) and white (II, IV) eggplants at harvest. The upper row shows the negative control. Scale bar: 4 mm (8×). p: peel; vb: vascular bundle; s: seed.

zone of the fruit (Supplementary video). The peel of white eggplants showed intense fluorescence indicating high ChA accumulation regions, too (Fig. 3C-I and III). Vascular bundles (vb) as well as immature seeds were specific sites of the pulp showing higher ChA accumulation. Similarly to what was found in *in vitro* evaluations (Fig. 3A), after 30 d no marked losses of ChA were found in either pulp zone of white eggplants (Fig. 3C-III and IV). In contrast, a clear reduction in ChA-derived fluorescence was detected in the I zone of purple fruit (Fig. 3B-III and IV). Taken together these results indicate that phenolic compounds, especially ChA, are the main antioxidants in both white and purple eggplants and the biosynthesis of ChA was highly active even under cold storage (10 °C). The increase of PAL, one of the regulatory steps of the phenylpropanoid pathway, reported in “Lucía” eggplant upon chilling, is in line with this conclusion (Massolo et al., 2011). In addition, ChA is unevenly distributed in the pulp, with a preferential accumulation in the core and in lignifying tissues. In contrast, ChA declined substantially in purple eggplants.

### 3.3. PPO and POD in vitro activity and localization

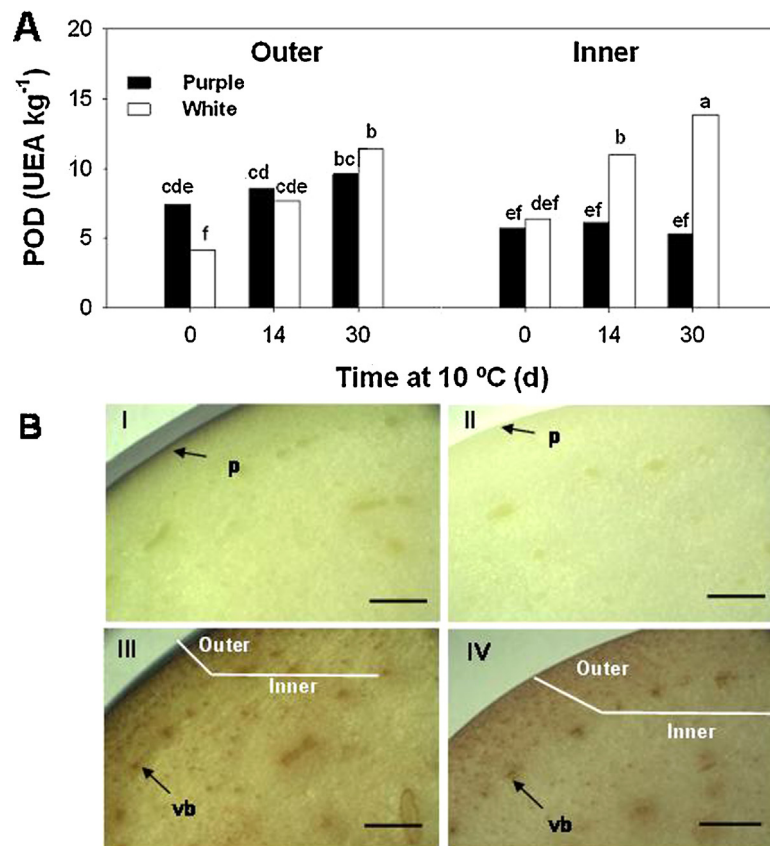
Pulp browning is a well-known problem contributing to the postharvest deterioration of many fruits and vegetables (Escalona et al., 2004; Das et al., 1997). PPO is one of the main enzymes catalyzing the oxidation of phenolic compounds. Analysis of PPO *in vitro* activity showed an increasing tendency during storage (Fig. 4A). Remarkably, for both genotypes, PPO was 2–3 fold higher in the O than in the I pulp showing no association with the losses of phenolics. Tissue histochemical analysis was performed to determine PPO spatial distribution within the pulp. The negative controls

showed almost no staining (Fig. 4B-I and II). In accordance with the *in vitro* evaluations, higher PPO staining was found in the outer pulp (Fig. 4B-III and IV). PPO staining was preferentially localized in vascular tissues, fibers, and seed coats. These results together with the lack of appreciable tissue browning (Fig. 1A) indicate that losses in phenolics and especially ChA (Fig. 3A and B), in stored purple eggplants were not due to PPO-mediated oxidation. Therefore, ChA should have been consumed in a different metabolic route.

Peroxidases (PODs) have been associated with browning, but are also implicated in the oxidative polymerization of phenolics, mainly monolignols, in the presence of H<sub>2</sub>O<sub>2</sub> to produce lignin. POD activity (Fig. 5A) and distribution (Fig. 5B) were monitored through *in vitro* and *in situ* incubation in guaiacol and H<sub>2</sub>O<sub>2</sub>. POD activity increased during storage in white fruit but remained unchanged in the I region of purple eggplants (Fig. 5A). POD *in vitro* activity showed no differences between pulp zones ( $P > 0.05$ ). Negative controls for histochemical evaluations showed almost no staining without the addition of the specific substrates during the experimental period (Fig. 5B-I and II). POD tissue staining was associated with lignifying tissues (Fig. 5B-III and IV).

### 3.4. Hydrogen peroxide

Hydrogen peroxide is produced in plants during normal metabolism (Karpinski et al., 1999). It participates in plant cells as a signal molecule in defense responses and as a co-substrate in several metabolic reactions including the polymerization of monolignols is well established (Neill et al., 2002a, 2002b). H<sub>2</sub>O<sub>2</sub> levels have been reported to increase during senescence, pathogen infection, and in response to common postharvest stress conditions such



**Fig. 5.** (A) Peroxidase (POD) activity in the Outer and Inner pulp of purple and white eggplants stored for 0, 14 or 30 d at 10 °C. Values with different letters are significantly different on a Fisher test at a level of significance of  $P < 0.05$ . (B) Histochemical localization of POD in purple (I, III) and white (II, IV) eggplants at harvest. The upper row shows the negative control. Scale bar: 4 mm (8×). p: peel; vb: vascular bundle.

**Table 2**  
Hydrogen peroxide in the Inner (I) and Outer (O) pulp of purple and white eggplants stored at 10 °C for 0, 14 or 30 d.<sup>1</sup>

	Genotype and zone	Time at 10 °C (days)		
		0	14	30
		H <sub>2</sub> O <sub>2</sub> (mmol kg <sup>-1</sup> )		
	Purple-I	3.33 <sup>ef</sup>	4.62 <sup>b</sup>	5.39 <sup>a</sup>
	Purple-O	2.90 <sup>fg</sup>	4.23 <sup>bcd</sup>	4.15 <sup>bcd</sup>
	White-I	4.49 <sup>bc</sup>	4.59 <sup>b</sup>	3.64 <sup>de</sup>
	White-O	2.31 <sup>g</sup>	3.91 <sup>cde</sup>	4.37 <sup>bc</sup>

<sup>1</sup> Means with different letters indicate significant differences between fruit genotypes, storage times and pulp zones according to Fisher's least significant difference (LSD) test ( $P < 0.05$ ).

as water deficit, nutrient deprivation, or chilling (Rogiers et al., 1998; Desikan et al., 2001). Reactive oxygen intermediates such as hydrogen peroxide, superoxide radicals, and hydroxyl radicals are also synthesized during lignification (Moïse et al., 2005). In the present study, both eggplant types showed higher H<sub>2</sub>O<sub>2</sub> content in the inner pulp (Table 2). In purple eggplants H<sub>2</sub>O<sub>2</sub> content increased during the initial 14 d. In white fruit H<sub>2</sub>O<sub>2</sub> accumulated in the outer pulp but remained unchanged in the I zone. At long storage times, the I zone of purple fruit showing the greatest loss of phenolics (Fig. 3A) also showed the highest concentration of H<sub>2</sub>O<sub>2</sub>. Based on pulp color measurements and determinations of H<sub>2</sub>O<sub>2</sub> and PPO and POD activities, the decrease of ChA in stored purple eggplants is not due to enzymatic browning. Instead results suggest that the loss of soluble phenolics in dark eggplants may be associated with lignification of seeds, fibers, and vascular vessels. This could occur if caffeoyl moieties from ChA are recycled (via caffeoyl-CoA) and reduced to monolignols. Previous works suggested that ChA may contribute to cell wall building (Schoch et al., 2001; Mondolot et al., 2006). Exogenously applied caffeic acid can be channeled into the phenylpropanoid pathway resulting in increased formation of lignin monomers (Bubna et al., 2011). In coffee plants marked drop of chlorogenic acid content coincided with an increase in the amount of cell wall-bound phenolic polymers supporting this (Aerts and Baumann, 1994).

#### 4. Conclusions

Eggplants accumulated sugars preferentially in the inner pulp, as opposed to acids and particularly AOX, which were two to threefold higher in the core. Fibers, vascular bundles and seed adjacent tissues were particularly rich in ChA, the main AOX, regardless of the eggplant type. Similar ethanol soluble AOX were found in white and purple fruit at harvest and both genotypes accumulated ChA during the first 2 weeks at 10 °C. However, purple eggplants were more susceptible to quality deterioration and showed a drastic reduction of phenolics at the end of the storage period. Conversely, white eggplants stored better on the basis of their visual quality and AOX stability. The decrease of ChA in the inner pulp of purple eggplants did not correlate with browning or *in vitro* PPO or POD activities. Instead the tissues exhibiting the greatest loss of ChA showed greater accumulation of H<sub>2</sub>O<sub>2</sub>. Substantial losses of phenolic antioxidants in whole eggplants under the recommended storage conditions might result from seed coat and vasculature development of the immature fruit rather than with pulp browning.

#### Acknowledgements

This work was supported by the Agencia Nacional de Promoción Científica y Tecnológica (PICT 2007-01116, 01120 and PICT 2009-0059) and CONICET (PIP 2009-00353).

#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.postharvbio.2014.01.016>.

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