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Postharvest senescence of florets from primary and secondary broccoli inflorescences

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ABSTRACT

The shelf-life of fresh-cut vegetables may be markedly affected by the type and quality of the raw materials. In this work we evaluated the influence of the type of inflorescence used for processing (primary and lateral) on the postharvest senescence of refrigerated fresh-cut broccoli. Florets from primary and lateral heads were cut and washed with chlorinated water, rapidly cooled to 4 °C, packed in plastic trays covered with perforated PVC and stored at 4 °C for 0, 14 or 21 d. During storage we evaluated floret deterioration, respiration rate, weight loss, color, chlorophyll content, sugars (glucose, fructose and sucrose), antioxidant capacity against $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals, ascorbic acid and Folin–Ciocalteu-reacting substances. Florets from lateral inflorescences were more perishable than fresh-cut broccoli obtained from primary heads. Terminal florets retained higher chlorophyll levels and showed delayed yellowing. Already at harvest primary-broccoli showed lower respiration rate. Florets form terminal heads showed lower weight and sugar loss during storage and maintained higher visual quality throughout the storage period at 4 °C. The inflorescence type also had large impact in the initial level of antioxidants as well as in their metabolism during storage. This information may be useful for vegetable processors.

1. Introduction

Changing life-styles in the last decades have raised the demand for healthy, convenient, additive-free, safe and nutritious foods (James and Ngarmsak, 2010). The fresh-cut sector has responded to these demands and has consequently been playing an increasing role in fruit and vegetable marketing in the United States, Europe and Asia (Cook, 2009; James and Ngarmsak, 2010). Apart from offering consumers a range of options in a single package, fresh-cut vegetables may reduce food preparation time and wastage at the household level.

It is accepted that the quality and shelf-life of fresh-cut vegetables may be markedly affected by the type and quality of the raw materials. The quality of green vegetables is determined by a number of preharvest conditions ranging from cultural practices, irrigation and fertilization to environmental conditions (Kader, 2008; Colelli and Elia, 2009). Water salinity and plant density were shown to affect the quality of fresh-cut lettuce (Scuderi et al., 2009; Luna et al., 2013). Nutrient supply has also been shown to play a crucial role for fresh-cut quality and shelf-life (Konstantopoulou et al., 2010; Bonasia et al., 2013). The genotype, harvest time and organ location plant have also been found to determine vegetables postharvest responses (Singh et al., 1992; Clarkson et al., 2005).

Broccoli has been traditionally marketed in the form of whole inflorescences. The shelf-life of intact broccoli may vary from 12 to 25 d at 4 °C depending on cultivar (Cantwell and Suslow, 1997). Broccoli has gained popularity due to the association of its consumption with the prevention of some types of cancer (Jeffery et al., 2003). In recent years minimally-processed broccoli florets have been offered as a "healthy snack" (Lemoine et al., 2010; Lemoine et al., 2010). Broccoli primary heads are harvested in an immature stage, before flower opening, when still dark green and firm (Hasperué et al., 2011). However, after harvest secondary lateral heads are induced. These smaller lateral inflorescences can

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account for as much as 30% of total yield and could be used for fresh consumption or processing (Rosa et al., 2002). Some studies have started to characterize primary and lateral broccoli. Calcium and sulfur levels were higher in the secondary inflorescences (Rosa et al., 2002). In contrast, the levels of sugars in terminal and lateral heads varied depending on the cultivar (Rosa et al., 2001). A survey of 11 broccoli cultivars produced in winter and summer showed that in most cases lateral heads were richer in total glucosinolate contents (Rosa and Gomes, 2001). Whether or not the floret source could also impact fresh-cut product postharvest responses has not been studied. The objective of this work was to perform a comparative analysis of the postharvest senescence of fresh-cut broccoli obtained from terminal and lateral inflorescences.

2. Materials and methods

2.1. Plant material and storage conditions

Broccoli (*Brassica oleracea* var. *Italica* cv. Legacy) heads were harvested at commercial maturity from a commercial grower in La Plata, Argentina (34° 59′S and 58° 3′W). Thirty primary (terminal) and 30 secondary heads were harvested at 8:00 AM and carried immediately to the laboratory. The inflorescences were separated into florets with stems and immersed in chlorinated water (sodium hypochlorite, 150 mg L^{-1}) for 5 min. Approximately 110–140 g of broccoli florets were placed in plastic trays (around 15 florets per tray), wrapped with perforated PVC and stored in darkness at 4 °C for 0, 14 and 21 d. Four trays were prepared for each inflorescence type and sampling date. Samples were taken at the aforementioned times and immediately evaluated or frozen in liquid nitrogen and stored at −80 °C until analysis. The whole experiment was repeated twice with similar results.

2.2. Surface color

Color (L^* a^* b^* system) was evaluated with a colorimeter (Minolta CR-400, Osaka, Japan) using D65 illuminant lighting conditions during the period of storage. Ten measurements were done per tray, inflorescence type and storage time. The Hue angle (h°) was calculated as $h^\circ = \tan^{-1}(b/a)$ when a and $b > 0$ or $h^\circ = 180^\circ + \tan^{-1}(b/a)$ when $a < 0$ and $b >$ 0; and chroma was calculated as $(a^2 + b^2)^{1/2}$.

2.3. Chlorophylls

Frozen samples were processed in a mill and approximately 0.4 g of the obtained powder was added to 2.5 mL of acetone/water (80/20) and homogenized. The homogenate was vortexed for 15 s and centrifuged at 5500 × g for 5 min. The supernatant was collected and the extraction procedure was repeated with the addition of 2.5 mL of acetone/water (80/20). Chlorophyll content was determined spectrophotometrically according to Lichtenthaler (1987) and expressed as mg kg^{-1} of chlorophyll on a fresh weight basis. Three independent samples consisting of five florets each were ground and used for analysis.

2.4. Weight loss and respiration rate

The broccoli trays were weighed during storage period and weight loss (WL) was calculated from initial (IW) and final weights (FW) as $WL(\%) = (IW - FW/IW) \times 100$.

For respiration measurements, broccoli florets held at 20 °C for 2 h until reaching room temperature, weighing approximately 150 g were placed in a 3 L flask, sealed and incubated for 15 min at 20 °C (to prevent CO_2 levels over 1% which would affect the respiration rate). CO_2 concentration in the headspace was determined using an infrared analyzer (Alnor, CompuFlow Model 8650 MN, USA). Results were expressed as rate of CO_2 evolution in mg kg^{-1} s^{-1}. Three samples from different florets were analyzed for each inflorescence type and sampling date.

2.5. Deterioration index

Samples were visually evaluated on individual trays based on surface yellowing, dehydration symptoms, and decay by using an intensity scale (0 = excellent quality without changes relative to day 0; 1 = good quality with minor changes relative to day 0; 2 = samples with up to 1 senescent, dehydrated or yellow floret per tray, 3 = samples with up to 3 senescent, dehydrated or yellow florets per tray, 4 = samples with up to 5 senescent, dehydrated or yellow florets per tray, 5 = unfit for being marketed, 6 = unfit for being marketed and with fungal decay. The deterioration index (DI) was calculated as follows:

DI = Σ (Injury level × Number of trays in this level)/Total number of trays. Four trays of primary and secondary inflorescences for each sampling time were taken.

2.6. Ascorbic acid

Frozen samples were processed in a mill and approximately 0.6 g of the obtained powder was homogenized with 2.5 mL of 5% m/v metaphosphoric acid. The mixture was vortexed for 1 min and then centrifuged at 12,000 × g for 10 min at 4 °C. A high performance liquid chromatograph (Waters 1525 Binary HPLC Pump) fitted with a photo diode array detector and a C_{18} column (4.6 × 150 mm, 5 μm, Waters Corp., USA) was used for ascorbic acid (AA) determination. The mobile phase was 0.5% m/v metaphosphoric acid/acetonitrile (93/7), at an isocratic flow rate of 16.7 μL s^{-1}. Detection was performed at 245 nm. For identification and quantitation a standard ascorbic acid solution was employed. Results were expressed as mass of AA on a fresh weight basis, mg kg^{-1}. Three extracts per sample and storage time were obtained and measurements were done in triplicate.

2.7. Sucrose, glucose and fructose

Frozen broccoli florets were ground in a refrigerated mill and 0.6 g of the obtained powder were homogenized with 5 mL of ethanol and vortexed for 1 min. The mixture was centrifuged at 5580 × g for 10 min at 4 °C; the supernatant was recovered and filtered through 0.2 μm RC membrane (Cole-Parmer, USA). Soluble sugars were determined by using a high-pressure liquid chromatography system equipped with a refractive index detector (Waters, IR 2414) and a Hypersil Gold Amino column (4.6 × 250, 5 μm, Thermo Sci. USA). An isocratic flow rate of 16.7 μL s^{-1} of acetonitrile/water (70/30) was used for analysis. Results were expressed on a fresh weight basis as mg kg^{-1}. Three extracts per sample and storage time were obtained and measurements were done in triplicate.

2.8. Folin–Ciocalteu-reacting substances

Approximately 50 g of frozen broccoli florets were ground in a mill and 0.6 g of the resultant powder was homogenized in 5 mL of ethanol as described above. A sample of the crude extract (50 μL) was added to 950 μL of distilled water and 50 μL of 1:1 diluted Folin–Ciocalteu reagent. After 3 min, 100 μL of a solution containing 20% (m/v) Na_2CO_3 in 0.1 mol L^{-1} NaOH were added, and incubated at 25 °C for 90 min (Singleton et al., 1999). The absorbance was measured at 760 nm and the total phenolic content was calculated using gallic acid (GA) as standard. Results

were expressed as mass of GA equivalents on a fresh weight basis, mg kg^{-1}. Four replicates were analyzed per type of inflorescence and storage time.

2.9. Antioxidant capacity against DPPH• and ABTS•+ radicals

Broccoli ethanolic extracts were prepared as described in Section 2.7 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, 50, 75, 100 and 150 μL of sample and ethanol to a final volume of 150 μL were prepared. After that, 1000 μL of a 59.2 mg L^{-1} solution of the radical DPPH• dissolved in ethanol solution were added. Samples were vortexed and incubated at 20 °C for 90 min. The absorbance was measured at 515 nm (UV-Mini 1240 model, Shimadzu Corporation, Japan) and the equivalent mass of florets tissue required to consume 50% of the initial DPPH• was calculated (EC_{50}). The antioxidant capacity was expressed as EC_{50}^{-1} per kilogram. Four replicates were analyzed per type of inflorescence 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 $K_2S_2O_8$, 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 ± 0.03 at 734 nm. Twenty microlitres of ethanolic extract, 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 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 antioxidant capacity (TEAC) on a fresh weight basis (mmol kg^{-1}).

2.10. Experimental design and statistical analysis

The experiments were designed according to a factorial design, the factors being the type of inflorescence (primary, secondary) and the storage time at 4 °C. Data were subjected to analysis of variance (ANOVA), and means were compared by a Fisher test by using the software InfoStat (Di Rienzo et al., 2012) at a significance level of $P < 0.05$.

3. Results and discussion

3.1. Deterioration index, weight loss and respiration rate

The deterioration index increased during storage for both primary and secondary florets (Fig. 1B). However, broccoli from primary heads showed already lower deterioration than lateral florets after 14 days at 4 °C. The differences were even higher at the end of the storage period (Fig. 1A. Secondary broccoli florets also were more susceptible to dehydration. After 14 d of storage the weight loss was 2.5 and 5.5% for primary and secondary broccoli respectively (Fig. 1C).

Primary and secondary heads were different at harvest in regards to their respiration rate (RR) (Fig. 1D). Florets form terminal inflorescences presented a RR 50% lower than lateral florets. During cold storage the RR decreased, but at all sampling dates primary broccoli still maintained higher values than the corresponding secondary florets. The higher weight loss of the secondary inflorescences could be due to a greater surface of sepal, given that the flowers in the branchlets seemed to be larger than those of primary inflorescences.

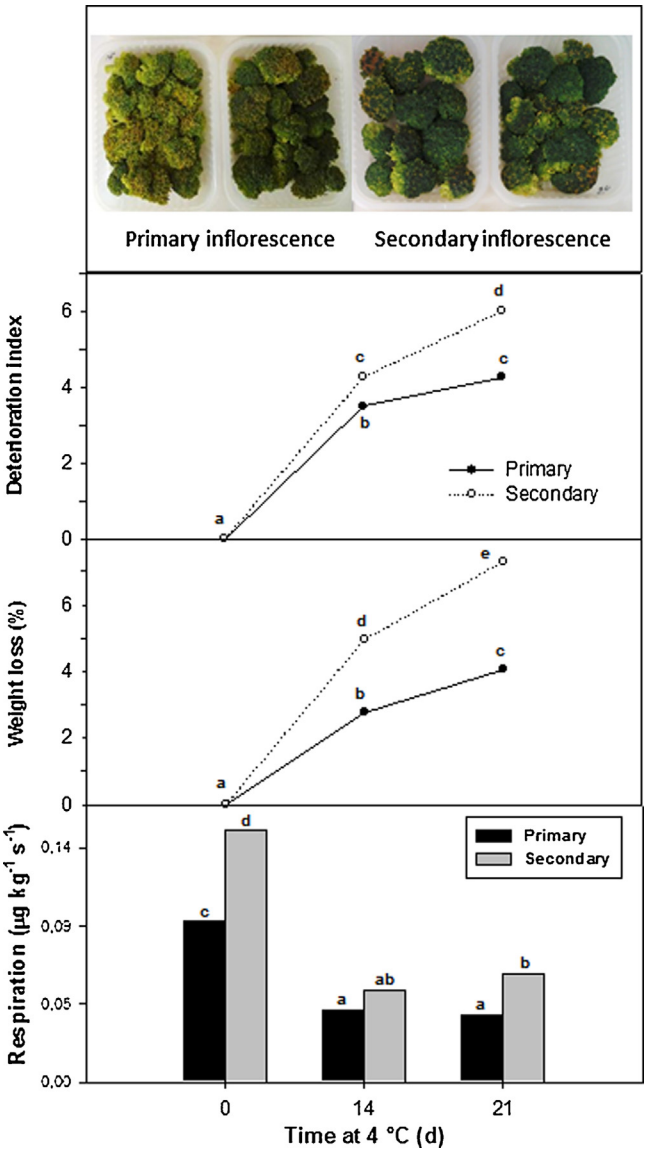

Fig. 1. A. Appearance of fresh-cut broccoli prepared from primary and secondary inflorescences stored for 14 d at 4 °C. B deterioration index, C. weight loss, and D. respiration rate of fresh cut broccoli from primary and secondary inflorescences during storage at 4 °C for 0, 14 and 21 d. Columns with different letters indicate differences based on a Fisher test at a level of significance of $P < 0.05$.

3.2. Color and chlorophylls

The most visible sign of senescence is the loss of chlorophyll from the sepals. During postharvest senescence, broccoli shows an important loss of chlorophylls (King and Morris, 1994), which can be delayed by a cold storage (Toivonen, 1997). The Hue angle and saturation (chroma) values of primary and secondary broccoli

Table 1
Changes in Hue (°), chroma and lightness (L^*) values of primary and secondary florets during postharvest storage at 4 °C.

	Hue		Chroma		Lightness	
	Primary	Secondary	Primary	Secondary	Primary	Secondary
0 d	124.35ab	121.50b	12.34a	15.28d	35.28a	37.14a
14 d	122.39b	112.50d	20.68b	26.89e	40.46b	45.48c
21 d	118.81c	103.48e	24.38c	32.62f	44.70c	52.13d

Different letters indicate differences based on a Fisher test at a level of significance of $P < 0.05$.

showed no significant variation at harvest (Table 1). However, during storage the changes in color were markedly different between primary and secondary heads. Terminal florets showed almost no variation in the Hue angle throughout the storage period as opposed to lateral broccoli which showed a rapid decrease of Hue values as the florets yellowed. The Hue angle is highly correlated with total chlorophyll content of broccoli florets (Tian et al., 1994). The loss of green color in lateral broccoli was also depicted from the large increase observed in lightness. Broccoli from secondary heads showed increased L^* values in storage as opposed to primary heads in which changes were limited (Table 1). Noteworthy, after 3 weeks, the fresh cut-product from primary heads were greener than florets from secondary heads stored only for 14 d. The concentration of chlorophyll in primary and secondary heads at harvest was similar (Table 2). During the refrigerated storage a rapid loss of chlorophylls was found in secondary florets. At the end of the storage period primary broccoli maintained higher chlorophyll levels (1.2 fold) than secondary florets. This was due to a decreased degradation of both Chl a and b (Table 2).

3.3. Sugars

Harvested broccoli florets usually show, concomitantly with the loss of green color, a rapid decline of soluble sugars (Nishikawa et al., 2005; Hasperué et al., 2014 Hasperué et al., 2014). Before storage, the total levels of soluble sugars in primary and secondary florets were similar (Fig. 2A). Glucose and fructose accounted for 85% of EtOH soluble sugars in both primary and secondary heads (Fig. 2B–D). Previous works also showed fructose and glucose as the major sugar in broccoli (Eason et al., 2007; Steindal et al., 2013; Hasperué et al., 2014). Secondary florets presented before storage higher levels of sucrose and fructose than primary broccoli but lower glucose content. Losses in all three sugars during storage occurred more rapidly in secondary than in primary heads. This is consistent with the higher metabolic activity found in lateral broccoli. After the 21 days storage period, primary heads had double levels of sugar with respect to secondary broccoli.

Some works have suggested that sugar starvation could be a main factor determining postharvest senescence of broccoli and other harvested commodities (Downs et al., 1997; Downs and Somerfield, 1997; Nishikawa et al., 2005; Hasperué et al., 2011). Results from this work show that both primary and secondary florets have similar sugar content upon harvest, but the main difference between the two types of inflorescences is their metabolic activity and consequently their carbohydrate utilization rate.

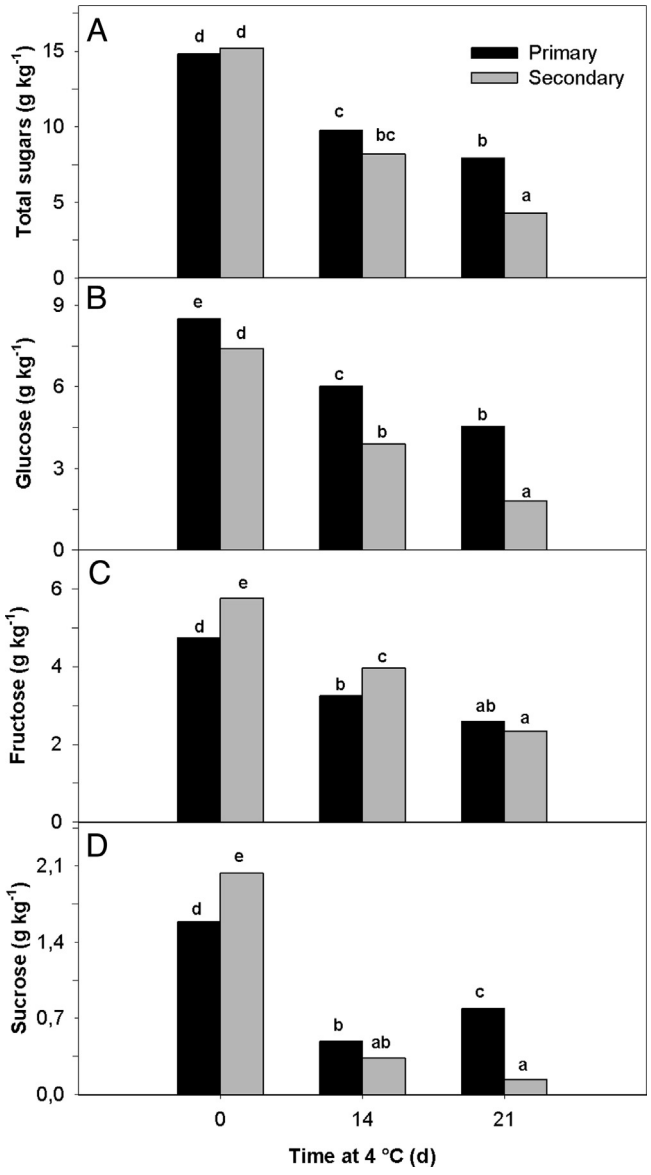

Fig. 2. A. Total soluble sugars, B. fructose, C. glucose and D. sucrose contents of broccoli from primary and secondary inflorescences stored for 0, 14 and 21 d at 4 °C. Columns with different letters indicate differences based on a Fisher test at a level of significance of $P < 0.05$.

3.4. Antioxidants

The inflorescence type also had large impact in the initial level of antioxidants as well as in their metabolism during storage. Broccoli is known to have a number of different of bioactive compounds including vitamin C, vitamin E, and phenolic compounds including flavonoids, carotenoids, and glucosinolates (Lin and Chang, 2005) presenting both antioxidant and anticancer activities (Podsedek, 2007). Large variation in the antioxidant capacity and contents has been reported depending on the species and even on the cultivar considered (Jeffery et al., 2003; Ferreres et al., 2006; Fernandes et al., 2007; Kaur et al., 2007). Also large variability has been found during storage (Leja et al., 2001; Starzyńska et al., 2003; Hasperué et al., 2011; Xu et al., 2012). In the present work we showed that the type of inflorescence is also an important factor determining the levels of AOX. Secondary broccoli florets showed higher AOX capacity than terminal florets at all sampling dates (Fig. 3A and B). The DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging capacity increased during storage, with higher values in secondary florets throughout the storage period. Previously, it was shown that some AOX compounds increase during early senescence and decline at advanced stages (Cavaiuolo et al., 2013). Senescence has been associated with increased production of reactive oxygen species (Overmyer et al., 2003) and antioxidants have been shown

Table 2
Changes in total chlorophylls, Chl a and Chl b (expressed as mg kg^{-1}) in primary and secondary florets during postharvest storage at 4 °C.

	Total chlorophylls		Chl a		Chl b	
	Primary	Secondary	Primary	Secondary	Primary	Secondary
0 d	375.6a	369.9a	265.2a	258.7ab	110.4a	111.3a
14 d	320.5b	264.2d	228.6b	184.5d	91.9b	79.7d
21 d	220.8c	153.4e	157.8c	101.4e	63.1c	51.9e

Different letters indicate differences based on a Fisher test at a level of significance of $P < 0.05$.

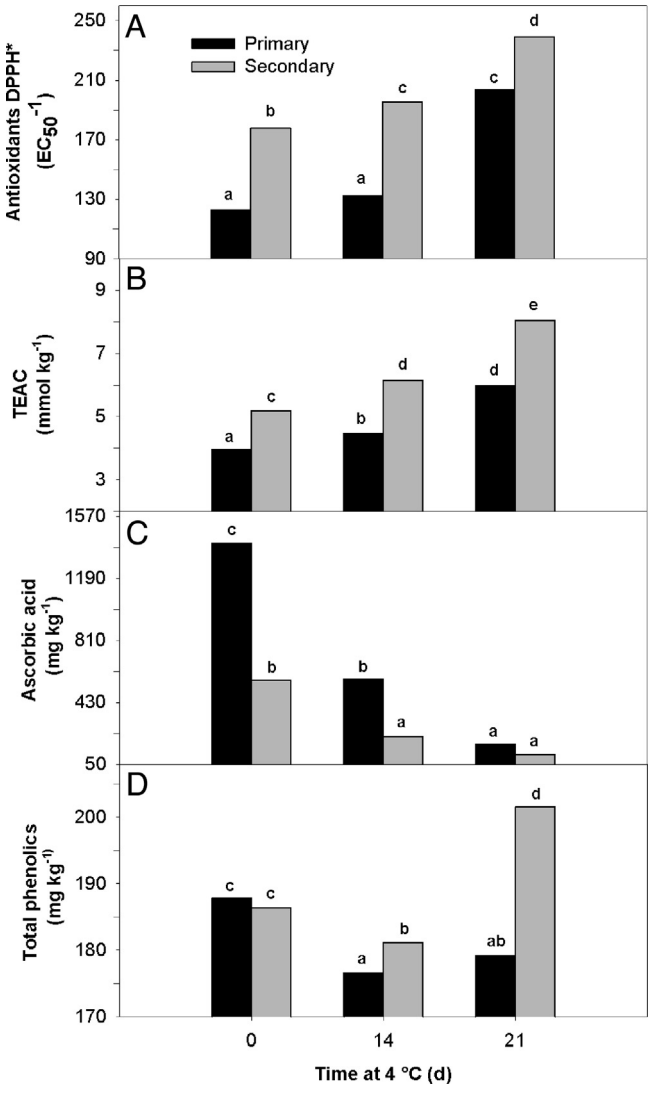

Fig. 3. A. DPPH• scavenging capacity, B. TEAC (trolox equivalents antioxidant capacity), C. Ascorbic acid and D. Folin–Ciocalteu reacting substances of broccoli florets from primary and secondary inflorescences stored for 0, 14 and 21 d at 4 °C. Columns with different letters indicate differences based on a Fisher test at a level of significance of $P < 0.05$.

to play a positive role in delaying organ aging (Munné-Bosch and Alegre, 2002). While this could be true for specific cellular compartments, the evaluation of tissue AOX capacity does not represents a good predictor of the aging potential of harvested green tissues since highly perishable secondary broccoli florets have higher AOX capacity than tissues from terminal inflorescences.

At harvest no differences in FC-reacting substances were found between primary and secondary broccoli despite the difference in antioxidant capacity (Fig. 3D). Phenolic compounds have been shown to be the major antioxidants in *Brassica* vegetables (Podsedek, 2007; Jahangir et al., 2009). During the initial 14 d of storage both types of broccoli florets showed a decreasing trend in the content of FC-reacting compounds. However, subsequently a marked increase was found in lateral florets. The accumulation of FC-reacting substances, as an index of post-harvest senescence, was observed in lettuce (Leja et al., 1994) and broccoli (Starzyńska et al., 2003).

As an enzyme cofactor and as a donor/acceptor in electron transport either at the plasma membrane or in the chloroplasts (Davey et al., 2000), ascorbic acid (AA) plays an essential role in scavenging active oxygen species produced under normal and stress conditions (Nishikawa et al., 2003). Temperature management after harvest is the most important factor to maintain vitamin C of fruits and vegetables (Lee and Kader, 2000). Primary heads had two fold higher levels of AA than secondary inflorescences before storage. After 14 d a rapid drop of AA was found in both types of florets. A continuous decrease of AA has been previously reported in broccoli (Vallejo et al., 2003; Zhan et al., 2012). Although the loss of AA was higher in primary broccoli still higher values were found in this group after 2 weeks of storage. At the last sampling date no differences in AA were found between primary and secondary broccoli. Overall results show that broccoli from terminal heads accumulates higher levels of AA but also has higher metabolizing capacity of this compound during storage. Terminal florets lost AA more rapidly during storage and no differences were detected after 21 d storage. In other studies, AA contents showed a relatively good correlation with broccoli senescence (Nishikawa et al., 2003; Shigenaga et al., 2005).

In this work the AOX capacity of the cultivar used did not correlate with the levels of ascorbic acid (AA) or FC-reacting substances. Velioglu et al. (1998) examined 28 plant products, and in many cases the high antioxidant activity was not correlated with the phenolics content. As in that case, other radical scavengers seem to be determinant of the hydrophilic AOX capacity. Overall, results indicate that primary and secondary inflorescence presented large differences in the level of antioxidants as well as in the prominence of the different AOX groups. Moreover the AOX dynamic also depends on the inflorescence type.

4. Conclusions

The quality and type of plant material are accepted to be major determinants of the storage capacity of fresh-cut vegetables. The degree at which this applies depends on the species considered. In this work we determined the differences in quality and postharvest senescence fresh-cut broccoli florets from primary and secondary heads. Terminal inflorescences retained higher chlorophyll levels and showed delayed yellowing, lower losses of sugars and dehydration than lateral heads. Florets from primary heads shave had significantly higher storage capacity than lateral broccoli inflorescences. The inflorescence type also had large impact in the initial level of antioxidants as well as in their metabolism during storage. This information may be useful for vegetable processors.

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