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Hot water treatments performed in the base of the broccoli stem reduce postharvest senescence of broccoli (*Brassica oleracea* L. Var italic) heads stored at 20 °C

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ABSTRACT

Heat treatments cause a moderate and reversible stress that interrupts the normal metabolism (senescence or fruit ripening) of the product. Although there is a large number of reports about heat treatments on broccoli heads, the effect of subjecting only the stems to thermal treatments has not been studied yet. One of the main reasons to analyze this approach is that the hormone ethylene is actively produced in the stem cutting area.

Different hot water treatments were performed on the first 5 cm of broccoli stems with various combinations of time-temperature. Treatment carried out at 50 °C for 3 min was chosen for further analysis of different quality and senescence parameters, taking into account that broccoli heads presented a delayed change in Hue and L values when compared with controls during storage. While control heads looked yellow, heat-treated samples retained most of their green colour. Furthermore, control heads presented higher weight loss, lower total and soluble protein, and lower total soluble sugar after 3 or 5 storage days, giving evidence of the fact that a heat treatment just on the stem contributes to the delay of broccoli senescence and to the maintenance of the overall quality of the product during storage.

1. Introduction

Broccoli (*Brassica oleracea* L. Italica group) is a worldwide used vegetable with high nutritional value and health benefits. Broccoli inflorescences are harvested while the floral heads, branchlets and florets are totally immature. Such harvesting causes an important stress and a depletion of water and hormones, which leads to rapid senescence (King and Morris, 1994). During the postharvest period, inflorescences lose their green colour, turn yellow and decrease their nutritional and nutraceutical quality, diminishing the concentration of proteins, sugars, ascorbic acid and glucosinolates (Jia et al., 2009; Page, Griffiths, & Buchanan-Wollaston, 2001). Diverse methodologies have then been utilized in order to extend the postharvest life of broccoli, including refrigeration (Gillies & Toivonen, 1995), modified atmosphere (Eason et al., 2007; Fernández-León, Fernández-León, Lozano, Ayuso, & González-Gómez, 2013), UV-C and UV-B (Aiamla-or, Kaewsuksaeng, Shigyo, & Yamauchi, 2010; Costa, Vicente, Civello, Chaves, & Martínez, 2006), 1-MCP (Gong & Mattheis, 2003; Gómez-Lobato, Hasperué, Civello, Chaves, & Martínez, 2012; Ma et al., 2010), and heat treatments (Costa, Civello, Chaves, & Martínez, 2005).

The use of heat treatments as a methodology to extend postharvest life of different products has been studied and their beneficial effects have been well documented (Lu, Vigneault', Charles, & Raghavan, 2007). Heat treatments cause a moderate and reversible stress that interrupts the normal metabolism (senescence or fruit ripening) of the product (Lu, Vigneault, Charles, & Raghavan, 2007). As that is a momentary stress, after certain time the product continues with its regular metabolism. Postharvest heat treatments can control insect pests, prevent fungal rots and affect the ripening and senescence of fruits and vegetables (Lurie, Jemric, Weksler, Akiva, & Gazit, 2004; Porat, Pavoncello, Peretz, Ben-Yehoshua, & Lurie, 2000). In the cases of ripening and senescence, heat

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treatments induce a momentary stress that reduces the expression of genes related to these processes, which in turn delays the normal ripening and senescence (Büchert, Civello, & Martínez, 2011; Gómez-Lobato et al., 2012; Martínez & Civello, 2008).

In broccoli, it was largely documented that both hot water (Tian, Woolf, Bowen, & Ferguson, 1996) and hot air (Costa et al., 2005; Funamoto, Yamauchi, Shigenaga, & Shigyo, 2002) treatments can effectively delay postharvest senescence.

Broccoli senescence is accelerated by the gaseous hormone ethylene. It has been shown that treatments with exogenous ethylene or ethephon (an ethylene releasing agent) can accelerate degreening and promote protein degradation and loss of sugars (Gapper et al., 2005). Endogenous ethylene biosynthesis is triggered by harvesting. Surprisingly, Kato et al. (2002) demonstrated that ACC (1-aminocyclopropane-1-carboxylic acid) synthase (ACS), one of the main enzymes involved in ethylene biosynthesis, is mainly induced in the cut surface of stem tissue, in the first 2 mm thick and the basal portions of curds soon after harvesting.

In this work we performed a heat treatment with hot water in the first 5 cm of the cut zone of the stem and analyzed several senescence and quality parameters of broccoli heads during postharvest storage.

2. Materials and methods

2.1. Plant material

Broccoli (*Brassica oleracea* var. Italica) heads were obtained from a commercial grower in La Plata, Argentina (34° 59′S and 58° 3′W) during the spring season of the southern hemisphere. Broccoli heads, healthy and of marketable size, were harvested at 8 a.m. and immediately transported to the laboratory to be processed.

2.2. Hot water treatments

Broccoli stems (5 cm, approximately) were carefully immersed, so as not to immerse their heads, in 25 L water baths with a temperature controller and a water circulation system. Six different hot water treatments were performed (3 min at 20 °C; 5 min at 47 °C; 3 and 5 min at 50 °C; 3 and 5 min at 53 °C) and nine heads per treatment were used. The treatment for 3 min at 20 °C was considered as control. Immediately after the treatments, stems were dried with domestic blotting paper and group heads were processed and labelled as day 0. The remaining broccoli heads were placed in plastic trays, wrapped with perforated PVC and stored at 20 °C in darkness. At initials, and after 3 and 5 days of storage, samples were segmented and the inflorescences were frozen using liquid nitrogen and stored at −20 °C until their use.

2.3. Superficial colour

Superficial colour of broccoli heads was evaluated with a chromameter (CR300, Minolta, Osaka, Japan), measuring the parameters L*, a* and b*, and calculating the Hue angle (HUE°) using the following formulas: $HUE^\circ = \tan^{-1}(b/a)$ when $a > 0$ and $b > 0$; $HUE^\circ = 180^\circ + \tan^{-1}(b/a)$ when $a < 0$ and $b > 0$. Five heads were used per treatment condition and five measurements were performed on each assayed head.

2.4. Weight loss

Broccoli head initial weight was recorded immediately after harvesting. The same heads were then weighted 3 and 5 days after the treatment. Weight loss was evaluated as the percentage of the initial weight and labelled as weight loss (%). Five heads per treatment were assayed.

2.5. Determination of chlorophyll content

Frozen broccoli florets were ground in liquid nitrogen, and 0.1 g of the resulting powder was mixed with 1 mL of acetone and stored for 4 h in darkness. The suspension was centrifuged at 10,000 × g for 10 min at 4 °C. The chlorophyll a, chlorophyll b and xanthophylls and carotenes content in the supernatant were measured by spectrophotometry according to Lichtenthaler (1987). All measurements were performed in quintuplicate and expressed as grams of chlorophyll a, chlorophyll b, xanthophylls and carotenes per kg of fresh tissue.

2.6. Reducing and total sugars content

Frozen broccoli florets were ground in liquid nitrogen, and 1 g of the resulting powder was suspended in 6 mL of ethanol. The mixture was centrifuged at 10,000 × g for 10 min at 4 °C and 1 mL of the supernatant was diluted to 5 mL using distilled water. This extract was utilized to determine the content of reducing sugars by using the Somogyi–Nelson method (Southgate, 1977).

For total sugar determination, 0.1 mL of the same ethanol extract was mixed with 1 mL of 2 g L^{-1} anthrone at 66% w/w H_2SO_4. The mixture was incubated at 100 °C for 12 min, cooled in ice-water bath per 20 min in darkness and the sugar content was measured spectrophotometrically at 625 nm.

Measurements were performed in triplicate and results were expressed as g glucose per kg fresh tissue.

2.7. Total and soluble protein content

For soluble protein content measurement, frozen broccoli florets were ground in liquid nitrogen and 0.5 g of the resulting powder was mixed with 5 mL of a buffer solution [50 mM Tris-HCl, 0.4 mL L^{-1} β-mercaptoethanol and 2 mM ethylenediaminetetraacetic acid, pH 7.5]. The mixture was centrifuged at 10,000 × g for 10 min at 4 °C and the soluble protein content was determined in the supernatant, according to Bradford (1976), using bovine serum albumin (Sigma, St Louis, MO, USA) as standard.

For total protein content measurement, 0.3 g of frozen broccoli powder was homogenized with 10 mL of extraction buffer [0.1 mM NaOH and 10 g L^{-1} sodium dodecyl sulfate (SDS)] and heated at 100 °C for 10 min. Samples were centrifuged at 10,000 × g for 20 min at 4 °C. In order to precipitate proteins, 5 vol of acetone were added to the supernatant, which was then incubated at - 20 °C for 12 h and centrifuged at 13,000 × g for 10 min at 4 °C. The obtained precipitate was dissolved in 0.2 mL of 0.1 mM NaOH and 10 g L^{-1} SDS and the protein content was measured according to Lowry, Rosebrough, Lewis Farr, and Randall (1951) using bovine serum albumin as standard. All measurements were performed in triplicate and soluble as well as total protein content was expressed as gram per kg of fresh tissue.

2.8. Total phenolic compounds

Frozen broccoli florets were ground in liquid nitrogen and 1 g of the resulting powder was suspended in 6 mL of ethanol. The mixture was centrifuged at 9000 × g for 10 min at 4 °C and the supernatant was used to determine total phenolic compounds according to Zieslin and Ben-Zaken (1992). A 50 μL aliquot of the extract was added to 500 mL of water and 100 μL of 0.5 mM Folin–Ciocalteu reagent. After 3 min of incubation at 25 °C, 500 μL of 10% w/v Na_2CO_3 solution was added and the reaction mixture was incubated for 1 h in darkness at the same temperature. The

samples were centrifuged at 4500 × g for 10 min and the absorbance was measured at 760 nm. The total phenolic content was calculated using gallic acid as standard. Measurements were performed in triplicate and results were expressed as mg galic acid per kg of fresh tissue.

2.9. Statistical analysis

Data for surface colour, Hue angle value and L value, were analyzed by ANOVA and the means were compared by Tukey test ($p < 0.05$). Data for chlorophyll a, chlorophyll b, xanthophylls and carotenes, soluble and reducing sugars, total protein, soluble protein, total phenolic and weight loss compounds were analyzed by ANOVA and the means were compared by Dunnett test with two tails ($p < 0.05$).

3. Results

3.1. Heat treatment selection

The first 5 cm of broccoli stems were immersed in hot water at different combinations of time-temperature. Then, samples were stored for five days at 20 °C in order to accelerate senescence.

Hue values varied from 139.8 to 144.6 at initial time with no difference among treatments, and decreased in all samples during storage at 20 °C (Table 1). In non-treated heads, Hue decreased to until 108 after three days and then reached values near to 87, indicating an intense yellowing. Treatment at 47 °C for 3 min caused a lower decrement of Hue value after three days but not after five days. Treatments at 50 °C reduced the yellowing, particularly after three days. Also, the reduction of Hue values was

Table 1
Changes in Hue values of broccoli heads during storage at 20 °C.

Treatments		Day 0*	Day 3*	Day 5*
20 °C	3 min	139.8 ± 1.4^{a}	108.4 ± 2.3^{a}	86.9 ± 0.9^{a}
47 °C	5 min	144.3 ± 1.6^{a}	120.8 ± 2.1^{b,c}	95.1 ± 1.3^{a,b}
50 °C	3 min	143.6 ± 3.5^{a}	133.5 ± 1.3^{d}	108.2 ± 5.6^{b,c}
	5 min	139.4 ± 1.1^{a}	135.4 ± 2.0^{d}	115.3 ± 6.3^{c}
53 °C	3 min	144.6 ± 1.5^{a}	128.0 ± 1.9^{c,d}	99.4 ± 3.0^{a,b}
	5 min	140.7 ± 1.1^{a}	119.3 ± 2.2^{b}	88.7 ± 1.4^{a}

*Data correspond to HUE angle means ± SE of five independent broccoli heads. Results were analyzed by ANOVA and means were compared by Tukey test at $p < 0.05$. Different letters indicate significant statistical differences between treatments at the same storage time.

Table 2
Changes in *L values of broccoli heads during storage at 20 °C.

Treatments		Day 0*	Day 3*	Day 5*
20 °C	3 min	37.7 ± 0.3^{a}	47.5 ± 0.5^{d}	54.9 ± 1.6^{a}
47 °C	5 min	38.9 ± 0.3^{a}	45.0 ± 0.4^{a,c}	53.2 ± 0.8^{a,b}
50°C	3 min	38.6 ± 0.3^{a}	43.1 ± 0.4^{b,c}	50.9 ± 0.9 ^{a,b}
	5 min	38.8 ± 0.4^{a}	42.2 ± 0.4^{b}	49.2 ± 1.0^{b}
53°C	3 min	38.0 ± 1.2^{a}	45.2 ± 0.5^{a}	53.2 ± 0.6 ^{a,b}
	5 min	39.3 ± 0.4^{a}	46.2 ± 0.5^{a,d}	54.6 ± 0.9^{a}

*Data correspond to *L value means ± SE of five independent broccoli heads. Results were analyzed by ANOVA and means were compared by Tukey test at $p < 0.05$. Different letters indicate significant statistical differences between treatments at the same storage time.

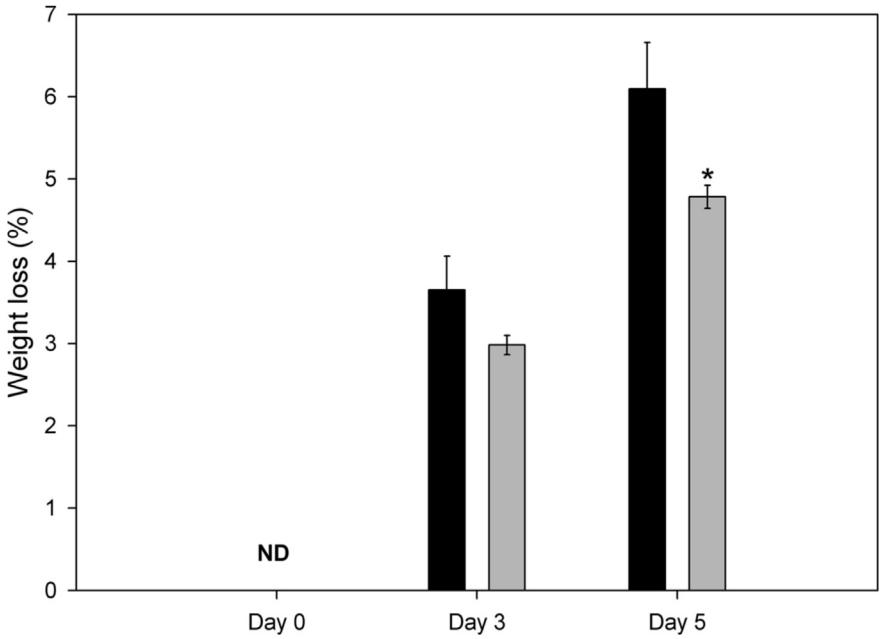

Fig. 1. Weight loss of broccoli heads stored during 5 days at 20 °C. Control broccoli heads (20 °C 3 min; ■); treated broccoli heads (50 °C 3 min; ■). Non determinate (ND). Asterisks indicate significant statistical differences at the same storage time between control (20 °C) and treated (50 °C) samples.

significantly lower than controls after five days. Finally, samples treated at 53 °C showed a lower reduction of Hue values in comparison to controls after three days, but these differences did not remain after five days.

L values ranged from 37.7 to 39.3 at the beginning of the assay and increased during storage at 20 °C, indicating an increase in colour brightness, due to the characteristic change from dark green to yellow during senescence of broccoli heads. The highest increments were detected in the control and the 53 °C treated samples, while the lowest L values were those of samples treated at 50 °C (Table 2)

Based on results obtained with Hue and L values, treatment performed at 50 °C during 3 min was chosen for further analysis of different quality and senescence parameters.

3.2. Weight loss

Immediately after treatments, heated samples did not show weight loss. During storage at 20 °C, an average weight loss of 3–4% was measured after three days with no difference between control and treated samples. However, the weight loss was higher in controls (6.0%) when compared with heated samples (4.8%) after five days (Fig. 1).

3.3. Chlorophyll content

The content of chlorophylls *a* and *b* decreased during storage at 20 °C. Control samples showed a rapid decline in chlorophylls, losing more than 60% of the initial content after three days of storage. In contrast, heat treated samples presented higher levels of chlorophylls, losing only 22% of the chlorophyll *a* and 40% of the chlorophyll *b* at the same storage period (Fig. 2). Levels of chlorophylls were similar in both control and heated samples after five days. Delaying of chlorophyll degradation correlated with a similar delay in Hue an L changes after three days of storage, when control heads looked yellow, while heat-treated samples retained most of

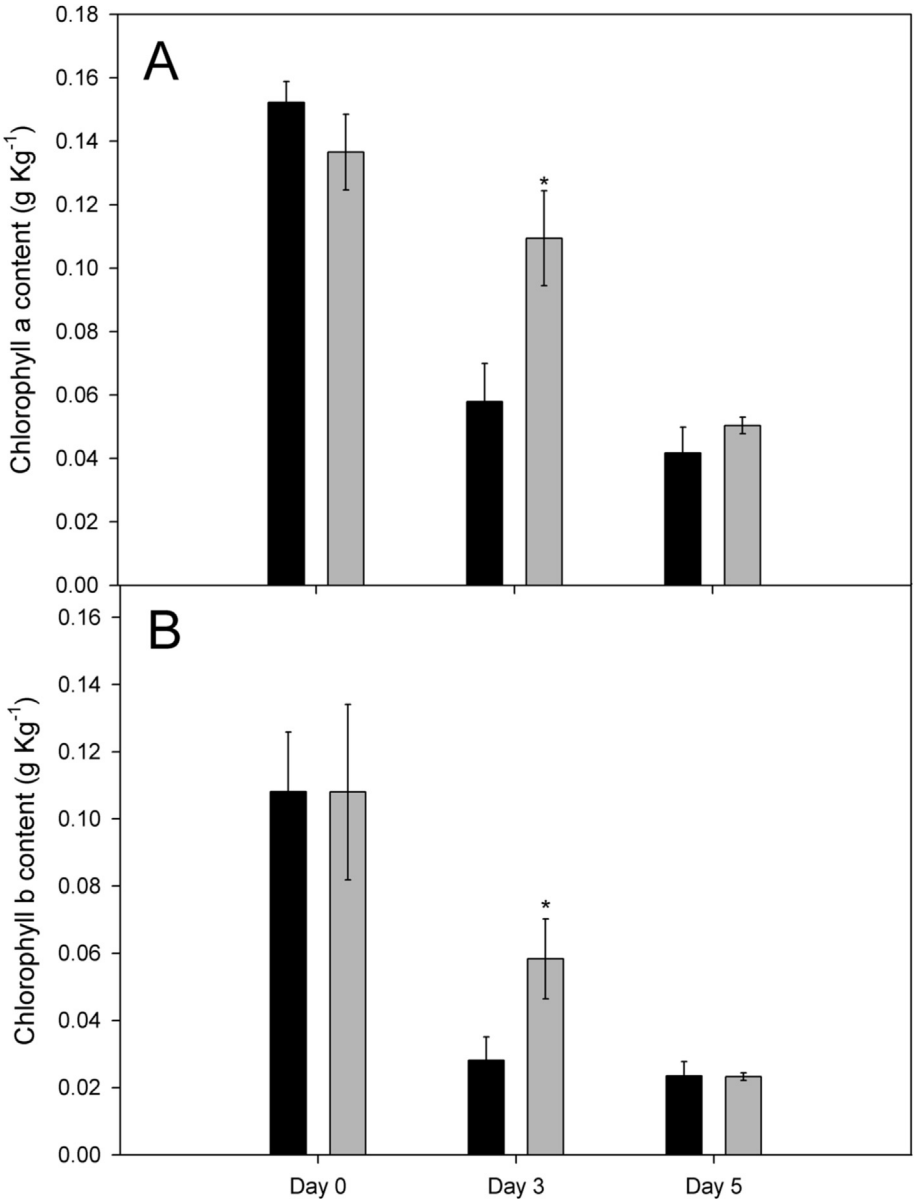

Fig. 2. Changes in chlorophyll a (A) and chlorophyll b (B) content of broccoli heads stored during 5 days at 20 °C. Control broccoli heads (20 °C 3 min; ■); treated broccoli heads (50 °C 3 min; ■). Asterisks indicate significant statistical differences at the same storage time between control (20 °C) and treated (50 °C) samples.

their green colour.

3.4. Carotenoids and xanthophylls content

The content of carotenoids and xantophylls increased almost 60% in control samples after five days of postharvest storage (Fig. 3a). Differently, heat treated samples did not change the level of carotenoids and xanthophylls through the same period. When the content of carotenoids and xanthophylls was expressed on chlorophyll content basis, an increase in both control and treated samples was observed, although this increment was more important in controls (Fig. 3b).

3.5. Soluble sugars

Immediately after treatments, the content of total and reducing soluble sugars increased approximately 35–40% (Fig. 4) in heat treated samples. During storage at 20 °C, the level of both total and reducing sugars increased after three days and then decreased after five days in controls. Differently, a continuous decrement of sugar content was observed in treated samples. Nevertheless, the content of both total and reducing soluble sugars maintained higher in treated samples when compared with the controls after five days.

3.6. Total and soluble protein content

Total protein content diminished during storage in both control and treated heads (Fig. 5a), with no significant difference between samples.

Content of soluble proteins represented approximately 40% of the total protein. Treated samples showed an increment of about 50% immediately after heating. During storage, the level of soluble proteins diminished continuously in control heads, while treated samples had a decrement during the first three days and then

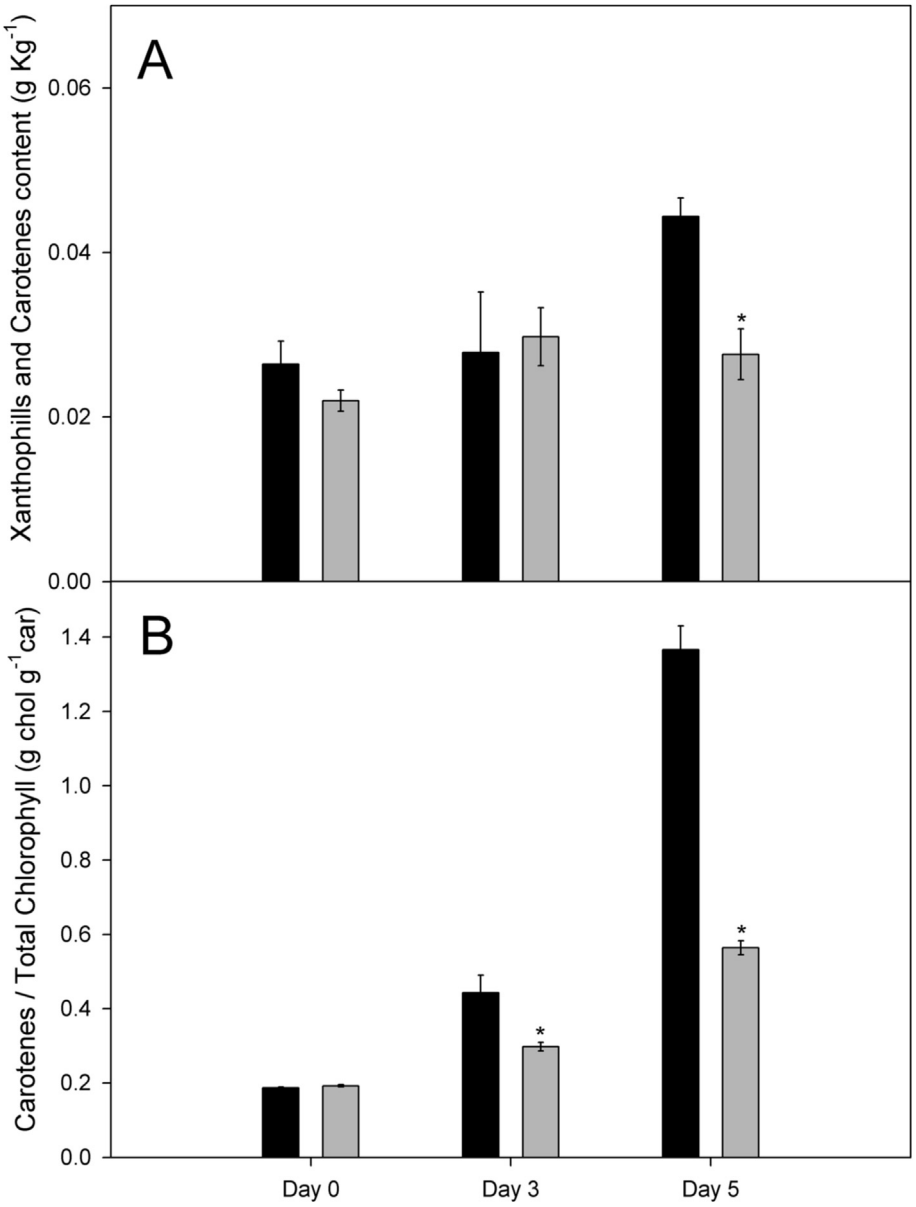

Fig. 3. Changes in xanthophylls and carotenes (A) and Carotenes/Total chorophyll (B) content of broccoli heads stored during 5 days at 20 °C. Control broccoli heads (20 °C 3 min; ■); treated broccoli heads (50 °C 3 min; ■). Asterisks indicate significant statistical differences at the same storage time between control (20 °C) and treated (50 °C) samples.

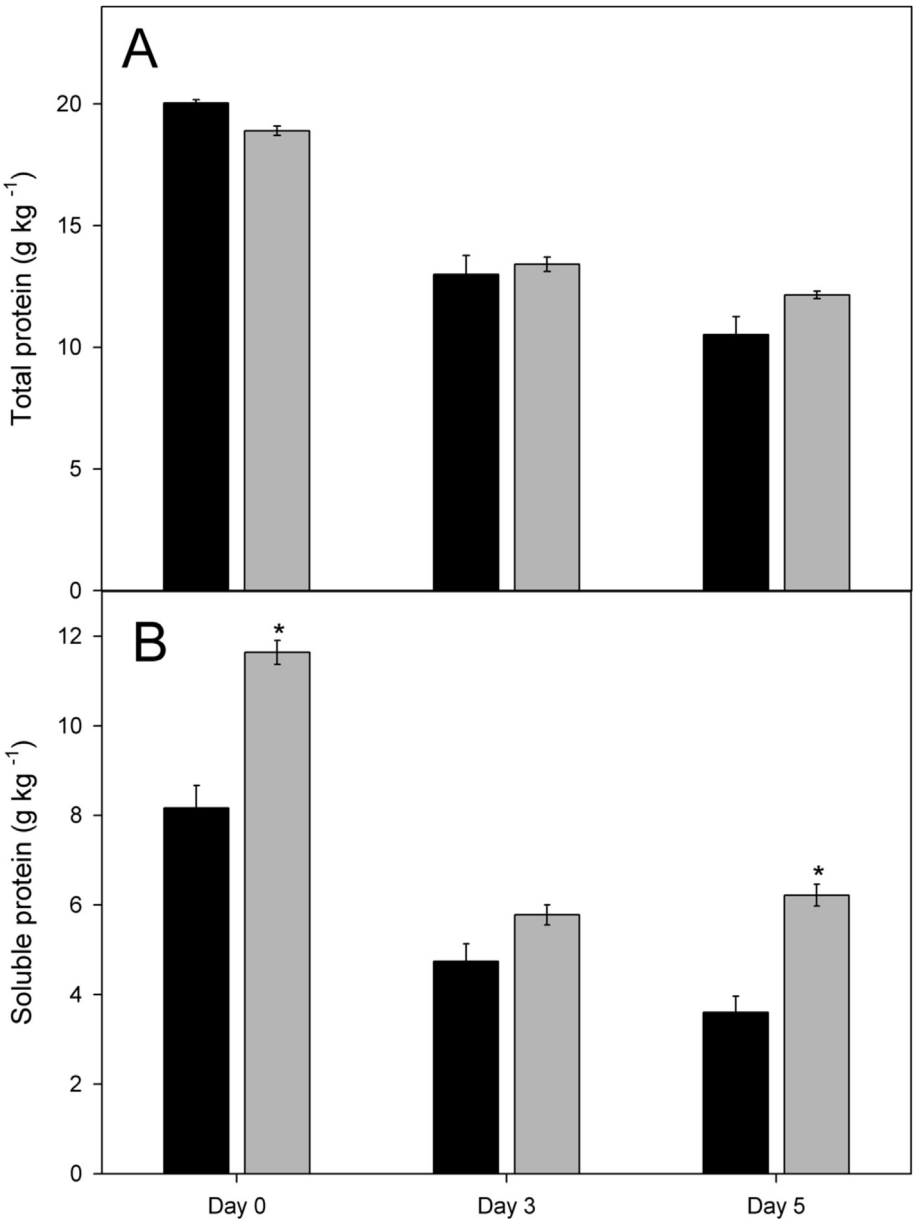

Fig. 4. Total protein (A) and soluble protein (B) content of broccoli heads stored during 5 days at 20 °C. Control broccoli heads (20 °C 3 min; ■); treated broccoli heads (50 °C 3 min; ■). Asterisks indicate significant statistical differences at the same storage time between control (20 °C) and treated (50 °C) samples.

remained constant until the end of the storage. As a consequence, the content of soluble proteins detected in treated samples was higher than that in controls after five days (Fig. 5b).

3.7. Phenolic compounds content

In control samples, the level of phenolics compounds decreased after three days and then increased at the end of storage (Fig. 6). Heating caused an increment in the level of phenolics at the beginning of the experiment. Then, the content of these compounds decreased during storage reaching lower values than controls.

4. Discussion

As previously mentioned, moderate heat treatments can delay postharvest senescence of different horticultural vegetables. In this work, we implement a new method to carry out the treatment by heating only 5 cm above the stem cutting area.

The reason for such treatment is the location of the main area of ethylene metabolism. The genes coding for enzymes responsible for the biosynthesis of this hormone are particularly expressed in the cutting zone (Kato et al., 2002). Having in mind that heat treatment could cause a temporary inhibition of the expression of these genes-, we decided to perform the treatment only in the area of higher gene expression. A similar treatment, performed only in cut stem of lily flowers, delayed leaf senescence and the onset of yellowing by 3–4 days (Woolf et al., 2012). The treatment maintained higher chlorophyll content and better values of chlorophyll fluorescence indicating lower chlorophyll degradation in lily leaves.

We performed several combinations of time/temperature and evaluated changes in superficial colour of heads during postharvest storage. The optimum combinations were 50 °C during 3 and 5 min, which produced the longer maintenance of green colour. Lower

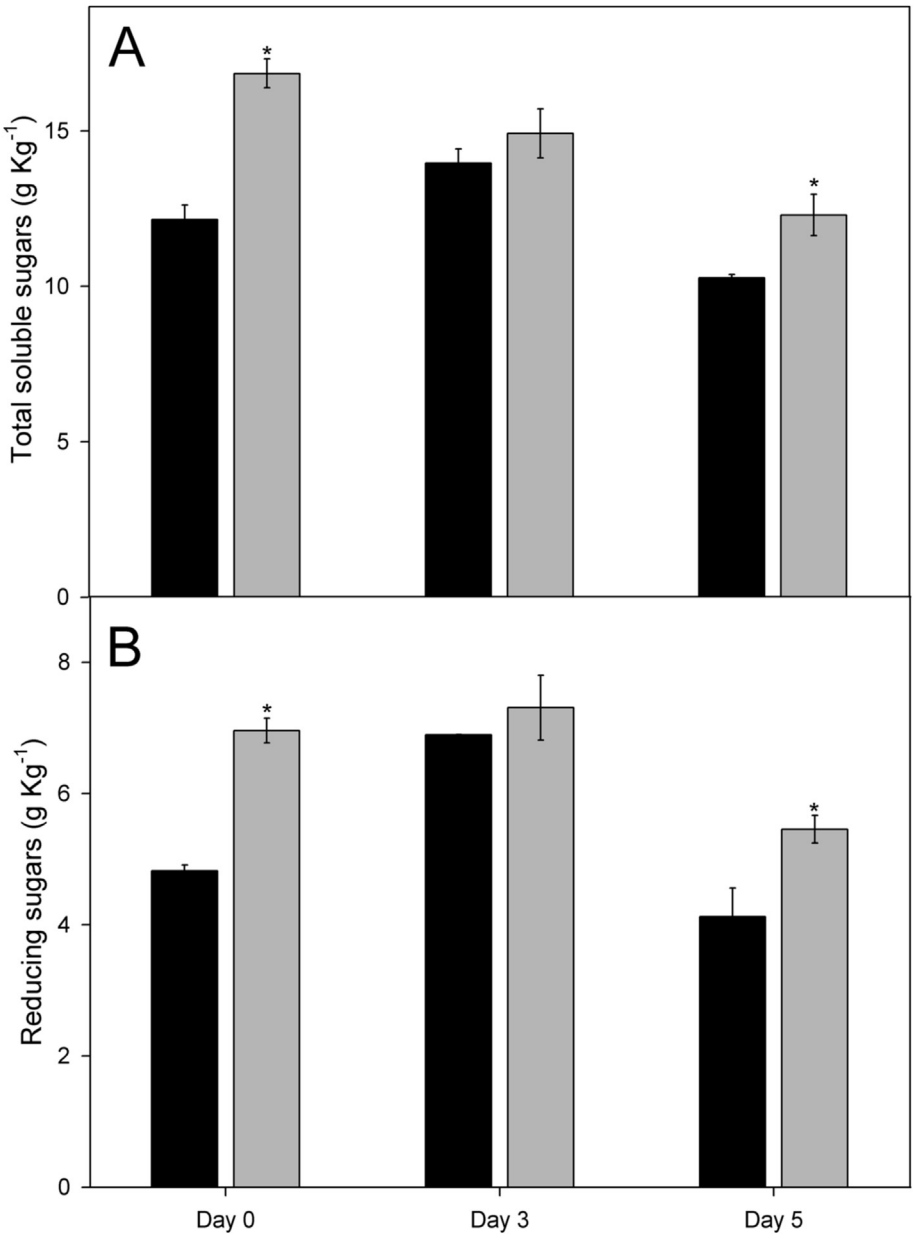

Fig. 5. Total soluble sugar (A) and reducing sugar (B) content of broccoli heads stored during 5 days at 20 °C. Control broccoli heads (20 °C 3 min; ■); treated broccoli heads (50 °C 3 min; ■). Asterisks indicate significant statistical differences at the same storage time between control (20 °C) and treated (50 °C) samples.

temperatures maintained the green colour during a longer period of time than controls but during a shorter period of time than the optimum treatment. Higher temperatures did not enhance the efficiency of treatment. Thus, we chose the treatment during 3 min at 50 °C due to lower time exposure.

It has been described that membranes suffer a disruption during senescence (Lim, Kim, & Nam, 2007) which in turn can enhance water loss. In the present work, the selected treatment caused a delay in senescence and a consequent reduction in weight loss during storage. Treated samples also showed a delay in the degradation of chlorophylls at least three days after treatment, similar to that described in other cases of heat treatments performed in broccoli (Costa et al., 2005). The case of xanthophylls and carotenes is peculiar, since the content of these components usually decreases during senescence (Biswal, 1995). However, broccoli is a particular tissue as it is an inflorescence and carotenes and xanthophylls accumulate in petals during flower development. Heat treatment can delay this process and the consequent accumulation of these pigments.

Apart from chlorophyll degradation, important compositional changes occur during the senescence of broccoli. A decrement in the content of proteins, sugars and fatty acid were previously described (Page et al., 2001; Pogson & Morris, 1997).

Protein catabolism in senescent tissues involves the disruption of the membranes, the release and solubilization of the anchored proteins and the subsequent degradation of soluble proteins (Gregersen, Holm, & Krupinska, 2008). The performed treatment did not affect the changes in the amount of total proteins but it did affect the levels of soluble proteins. Initially, the treated samples showed an increase of soluble proteins, probably due to a slight

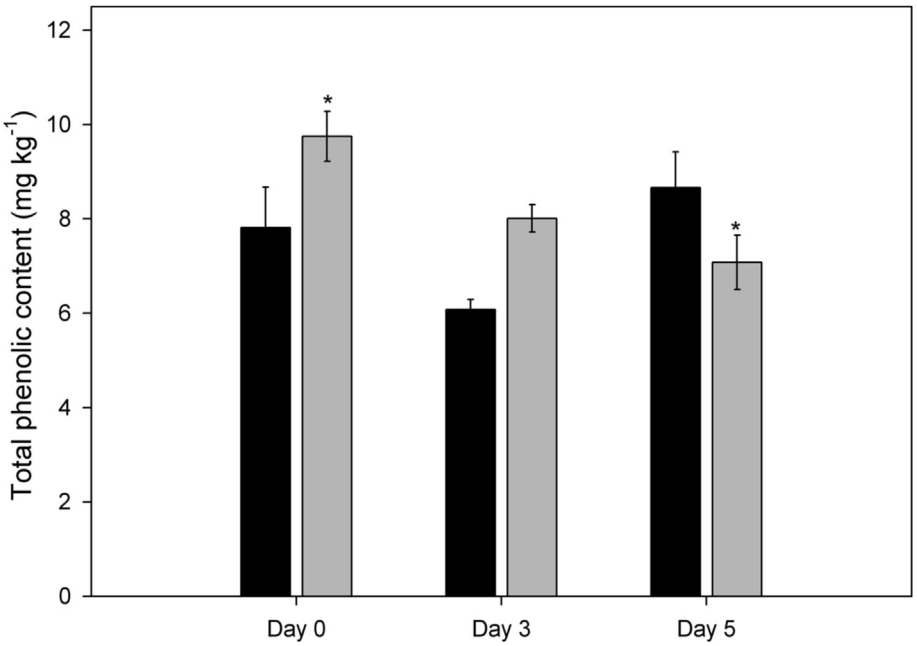

Fig. 6. Total phenolic content of broccoli heads stored during 5 days at 20 °C. Control broccoli heads (20 °C 3 min; ■); treated broccoli heads (50 °C 3 min; ■). Asterisks indicate significant statistical differences at the same storage time between control (20 °C) and treated (50 °C) samples.

increase in temperature caused by the treatment, which enhanced the solubilization of proteins. However, during storage, soluble protein degradation was delayed in the treated samples as it was described in broccoli that had other type of heat treatment (Costa et al., 2005).

During senescence of green tissues, starch is degraded while single sugars are translocated to other tissues or utilized as energy substrates. In our case, the increment of soluble (total and reducing) sugars detected by the third day in control samples could have been due to starch degradation (Büchert et al., 2011; Finger, Endres, Mosquim, & Puiatti, 1999); a fact that could compensate the consumption of single sugars. Once starch is completely degraded, no single sugars are incorporated and its content starts decreasing due to their use as energy substrate. In treated samples, it was detected a momentary increase in soluble sugars similar to the one previously described by Costa et al. (2005). This fact could probably be due to increased starch degradation by the slight increment in temperature of treated samples. However, as in the case of soluble proteins, sugar content was higher in treated samples as usually occurs in heat treatments (Costa et al., 2005).

Both an increment and a decrement in the content of phenolics were reported during postharvest senescence of broccoli (Costa et al., 2005; Starzyńska, Leja, & Mareczek, 2003). In our case, we detect a slight decrement in control samples after three days and an increment afterwards. As in the case of proteins and sugars, an increased level of phenolics was detected in treated heads immediately after heating. If head temperature increased after treatment, then the activity of enzymes associated with phenolic biosynthesis could also increase. An increment in phenylalanine ammonia-lyase was detected in strawberries immediately after a treatment at 42 °C for 3 h (Civello, Martínez, Chaves, Añon, & Añón, 1997) and in mandarins fruit exposed at 37 °C for 4 h (Sanchez-Ballesta, Zacarias, Granell, & Lafuente, 2000). However, at the end of storage (5 days) a lower accumulation of phenols was detected in treated samples. Taking into account that the performed heat treatment caused a delay in the occurrence of senescence, it could also have caused a delay in the accumulation of phenols.

5. Conclusions

Application of heat treatments causes a temporary inhibition of senescence during postharvest storage of broccoli (Costa et al., 2005; Tian et al., 1996). In this work, we performed an easier treatment for delaying broccoli senescence by only heating the base of the stem; and we obtained similar results to those previously described by Costa et al. (2005) in relation to this delay during storage.

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