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# End of day harvest delays postharvest senescence of broccoli florets

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ABSTRACT

Broccoli heads were harvested at different times of the day (sunrise, midday and sunset) and then stored at 20 °C for 5 d. The samples harvested at sunset showed the lowest loss of colour and chlorophyll degradation, those picked at dawn had the highest degreening, whereas heads harvested at midday showed an intermediate behaviour. As broccoli shelf life is mainly determined by external appearance and surface colour, harvesting heads at the end of the day instead of in early morning can extend their shelf life by 1 d, when stored at 20 °C. Samples harvested at sunset also had the highest levels of total soluble and reducing sugars, antioxidants and phenolic compounds during storage. However, at harvest, samples obtained at different moments had the same levels of chlorophylls, soluble sugars, antioxidant and phenolics; whereas the starch content was clearly different among samples. Those harvested at dusk had higher starch levels than those harvested at sunset.

We hypothesize that starch degradation produces single sugars whose presence delays senescence in samples harvested at the end of the day. This fact was corroborated by providing an exogenous supply of glucose or sucrose to samples harvested in early morning, which also showed a delayed senescence by maintaining higher levels of chlorophylls, antioxidants and phenolics. Our results suggest that starch accumulated during daylight delays senescence in broccoli and helps to maintain better postharvest quality and nutritional parameters.



## 1. Introduction

Fresh broccoli (*Brassica oleracea* L.) is a vegetable with high nutritional value due to its low caloric content, high dietary fiber and ascorbic acid levels and wide range of anticarcinogenic and antioxidant compounds (King and Morris, 1994; Chen et al., 2008). Harvested broccoli consists of hundreds of immature floral buds and thick, fleshy flower branchlets attached to the central plant stem, which is collectively named the head.

Broccoli heads are harvested when florets are still immature, with the sepals completely surrounding the flower. Immature organs require a continuous supply of water, nutrients and hormones to maintain their homeostasis. After harvesting, these kinds of organs suffer a severe stress which leads to the appearance of senescence symptoms. Within 24 h of harvest, the expression of many broccoli senescence related genes is induced (Page et al., 2001; Coupe et al., 2004; Eason et al., 2005; Chen et al., 2008), previous to most of the visual changes detected afterwards. Accelerated senescence determines that broccoli becomes highly perishable, with a storage life of 2–3 d in air at 20 °C (King and Morris, 1994). During postharvest, a loss of the superficial green colour of the product is observed, which decreases the commercial acceptance of the broccoli florets. Moreover, senescence leads to loss of sugars, proteins and lipid peroxidation resulting in lower nutritional quality (King and Morris, 1994; Page et al., 2001).

Techniques to maintain commercial and nutritional quality of broccoli have focused on postharvest treatments such as refrigeration, thermal shock treatments (Ferguson et al., 2000; Tosun & Yücecan, 2008; Viña and Chaves, 2008), modified atmospheres (Vicente et al., 2003), UV-C (Pan et al., 2004; Costa et al., 2006; Lemoine et al., 2007) and 1-MCP (Able et al., 2002; Ma et al., 2009), among others. However, postharvest life of horticultural products is also affected by a range of preharvest factors such as climate, soils, plant stress and general crop and plant management. Another potentially important factor is the time of the day at which the samples are harvested. The diurnal cycle strongly influences many plant metabolic and physiological processes (Bläsing et al., 2005). For example, diurnal fluctuations were detected in the chlorophyll precursors in *Phaseolus vulgaris* (Argyroudi-Akoyunoglou and Prombona, 1996) and in the ascorbic acid in *Spinacea oleracea* (Kiyota et al., 2006). However, probably the most pronounced change influenced by the circadian rhythm is found in carbohydrate metabolism. Diurnal fluctuations in solar radiation substantially affect endogenous carbohydrate levels and carbohydrate reserves

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that accumulate during exposure to light are completely remobilized by the end of the dark period (Sicher et al., 1984). Starch is a polymeric compound that is utilized as a storage carbohydrate, whereas sucrose and other soluble sugars act as signalling molecules. Therefore, plants need to control the cellular content of soluble sugars and changes only occur when there is a stress like senescence. In these cases, homeostasis is broken and concentration of single sugars can vary.

These changes in plant diurnal metabolism, particularly carbohydrates, could impact the postharvest life of products, although limited information is available to corroborate this point.

The objective of this study was to determine the effect of harvesting time of day together with sugar levels on the postharvest life of broccoli heads.

## 2. Materials and methods

### 2.1. Plant material and storage conditions

Broccoli (*Brassica oleracea* var. *italica* cv. Iron) heads were obtained from a commercial grower in La Plata, Argentina (34°59′S and 58°3′W) during autumn of the southern hemisphere (May–June) with a10 h and 16 min average length of day. Fifty broccolis heads, healthy and of marketable size, were harvested at different hours of the day during the month of September, 2008. Samples were obtained at different hours of the day: 8 AM, 1 PM and 6 PM, and carried immediately to the lab. Heads were placed in plastic trays, wrapped with perforated PVC and stored in darkness at 20 °C with 95% humidity and no air movement for a period of 5 d. Samples were taken periodically, analyzed in fresh or processed by separating stem from florets. The latter were frozen in liquid nitrogen and stored at −20 °C until used.

### 2.2. Postharvest sugar treatments

Fifteen broccoli heads obtained at 8 AM were placed in containers having a solution of 2% or 10% glucose or 2%, 10% or 15% sucrose in a way that only the cut surface of the stem was in contact with the solution. Another 15 heads were placed in water and considered as controls. All heads were stored in darkness at 20 °C for a period of 5 d. Samples were taken periodically, analyzed in fresh or processed by separating stem from florets. The latter were frozen in liquid nitrogen and stored at −20 °C until used.

### 2.3. Superficial colour measurement

The superficial colour ($L^*a^*b^*$ system) was evaluated with a chromameter (Minolta CR300, Osaka, Japan) during the period of storage. Measurements were taken on days 0, 2, 3, 4 and 5 of postharvest, measuring 6 times per head. The hue angle ($h^\circ$) 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 $b > 0$.

### 2.4. Chlorophyll content

Samples were frozen in liquid nitrogen and milled with a grinder. Approximately 0.8 g of the obtained powder was added to 10 mL of acetone:water (80:20) and homogenized. This treatment allowed a completed extraction of chlorophylls. The homogenate was centrifuged at $5000 \times g$ for 10 min. The supernatant was extracted and chlorophyll content was determined according to Lichtenthaler (1987) and expressed as mass of chlorophyll per mass of fresh tissue, mg $kg^{-1}$.

### 2.5. Reducing and total sugars content

The content of total sugars was determined according to Lemoine et al. (2007). Frozen broccoli florets were ground in a refrigerated mill and 2 g of the obtained powder were homogenized with 12 mL of ethanol. The mixture was centrifuged at $12{,}000 \times g$ for 15 min at 4 °C. The supernatant was utilized to determine the content of reducing sugars using the Somogyi–Nelson method (Nelson, 1944). For total sugar determination, an aliquot of the supernatant was first hydrolyzed with 10% w/v HCl for 10 min and then processed as described above. For quantification, a standard glucose solution was employed. Results were expressed as mass of glucose per mass fresh tissue, g $kg^{-1}$.

### 2.6. Starch content

The starch content was measured by an enzymatic method adapted from Rose et al. (1991). Samples (0.6 g) were homogenized with 5 mL of a solvent mixture of varying polarity such as methanol, chloroform and water in order to remove soluble sugars and other interfering compounds. The homogenate was centrifuged at $2200 \times g$ for 10 min and the supernatant discarded. The procedure was repeated three times until the supernatant was clear and the solvents evaporated in a bath at 50 °C for 2 h. Residue was added with 0.1 M NaOH to solubilize the starch and then neutralized with 0.1N acetic acid to adjust the pH to 5.1. After that, 400 μL of the extract were mixed with 100 μL of enzyme solution (α-amylase 416,000 U/L and amyloglucosidase 2000 U/L) and placed in a bath at 50 °C for 23 h to perform enzyme digestion. After that, an aliquot of the sample solution was utilized to measure glucose using a kit of glucose oxidase (GOD) 10,000 U/L and peroxidase (POD) 1000 U/L (Wiener Lab, Argentina) by measuring absorbance at 505 nm. Starch content, based on dry weight, was expressed as g $kg^{-1}$.

### 2.7. Total antioxidant capacity and phenolic compounds content

Total antioxidant capacity was determined according to Lemoine et al. (2007). Frozen broccoli florets were crushed in a refrigerated mill and 2 g of the resultant powder was homogenized with 12 mL of ethanol. The mixture was centrifuged at $12{,}000 \times g$ for 15 min at 4 °C. Several aliquots of the ethanolic extracts were added to test tubes containing 3.9 mL of 0.025 g $L^{-1}$ 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol. Absorbance at 515 nm was measured at different times until the reaction reached a plateau. The percentage of remaining DPPH against the volume of extract was then plotted to obtain the amount of extract necessary to decrease the initial DPPH concentration by 50%, which was defined as $EC_{50}^{-1}$. The antioxidant capacity was expressed as $EC_{50}^{-1}$.

Phenolic compounds content was measured according to Lemoine et al. (2007). Frozen florets were crushed in a refrigerated mill and homogenized in ethanol and centrifuged as described above. A sample of the crude extract (100 μL) was added to 1110 μL of water and 200 μL of 1N Folin–Ciocalteau reagent. After 3 min at 25 °C, 1.5 mL solution of 2% w/v $Na_2CO_3$, 0.1N NaOH was added. The reaction mixture was incubated for 1 h at the same temperature. The absorbance was measured at 760 nm and total phenolic content was calculated using phenol as standard. Results were expressed as mass of phenol per mass of fresh tissue, g $kg^{-1}$.

### 2.8. Experimental design and statistical analysis

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

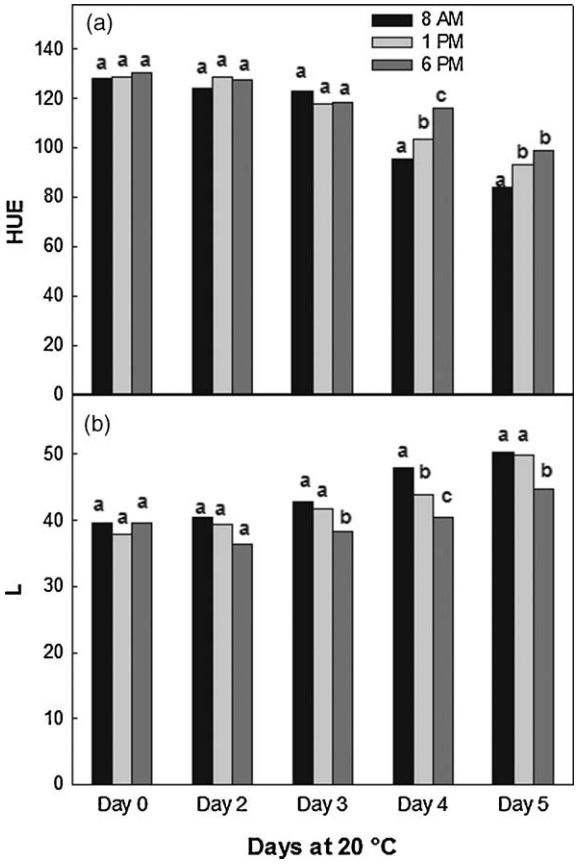


**Fig. 1.** Changes in Hue angle (a) and *L* parameter (b) of broccoli heads harvested at different hours of the day (8 AM, 1 PM and 6 PM) and stored at 20 °C. Different letters indicate significant differences at the same storage time.

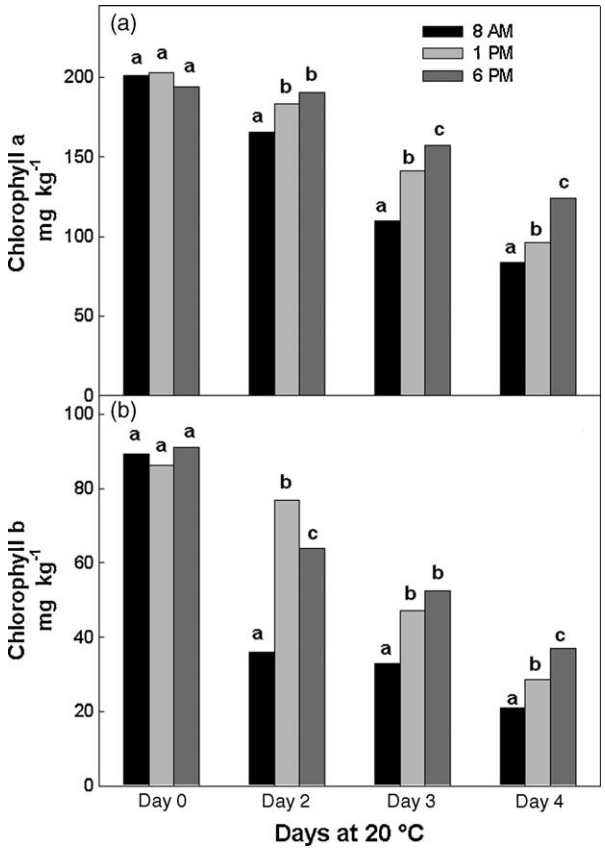


**Fig. 2.** Changes in chlorophyll a (a) and chlorophyll b (b) content during storage at 20 °C of broccoli heads harvested at different hours of day. Different letters indicate significant differences at the same storage time.

## 3. Results and discussion

### 3.1. Harvesting at different times of the day

Broccoli is a highly perishable vegetable with an accelerated senescence which leads to tissue deterioration and loss of nutritional and commercial quality (Page et al., 2001). Methods utilized to maintain the greenness of broccoli heads usually imply postharvest treatments (Toivonen, 1997; Jacobsson et al., 2004; Ku and Wills, 1999; Costa et al., 2006). However, scarce attention has been given to preharvest factors which could affect postharvest life. In this work, we analyzed the effect of harvesting at different times of the day on the postharvest senescence of broccoli. As a first approach to evaluate the progress of senescence we harvested heads and measured superficial colour during postharvest storage (Fig. 1). At harvest, all samples had a dark green colour and similar Hue and *L* values, indicating no differences in superficial colour. Samples turned from dark green to light green colour and Hue values presented a minor decrease for the first 3 d, but no differences were detected among samples. After that, samples harvested at 8 AM became yellow and showed a greater decrease of Hue compared to those harvested at sunset, which remained green. Heads harvested at 1 PM showed an intermediate behaviour (Fig. 1a). Similar results were detected for *L* parameter. In this case, as samples yellowed, *L* values increased. Samples harvested at 6 PM showed the lowest *L* increment while those obtained at 8 AM presented the highest *L* values (Fig. 1b). According to superficial colour values, samples harvested at the end of the day showed an addition of approximately 1 d in their shelf life in comparison with those harvested at 8 AM.

The chlorophyll content during postharvest storage was also evaluated (Fig. 2). Immediately after harvest, samples had similar levels of chlorophylls a and b. Content of both chlorophylls decreased during storage in all samples. However, after 2 d, this decrement was higher in samples obtained at 8 AM. After 3 and 4 d samples obtained at 6 PM had highest content of both chlorophylls, heads harvested at 8 AM had the lowest levels and samples harvested at 1 PM showed an intermediate behaviour (Fig. 2a and b). It is noteworthy that after 2 d of storage an important decrease in chlorophyll b content was detected in samples harvested at 8 AM (Fig. 2b).

Flower development is associated with several metabolic changes which require the energy provided by sugars. Harvesting interrupts the input of nutrients from the plant and triggers senescence, which in turn interrupts photosynthesis in sepals (Page et al., 2001). Both events greatly modify the supply of sugars necessary to maintain tissue integrity. We analyzed the content of total and reducing soluble sugars as well as the content of starch during postharvest senescence. As well as other parameters, immediately after harvest, the level of total and reducing soluble sugars was similar in all samples (Fig. 3a and b). During storage, samples harvested at 8 AM showed a decrease in total sugar content after 1 d, while heads obtained at midday maintained the same content for 4 d before declining. On the other hand, samples harvested at 6 PM presented an increment in total sugars after 3 and 4 d and a subsequent reduction (Fig. 3a). As a consequence, throughout storage, samples obtained at sunset had a higher content of total sugars than those obtained at 8 AM, while heads harvested at 1 PM showed intermediate values.

A similar pattern was detected in the level of reducing sugars. Samples obtained at 8 AM showed an important drop off during

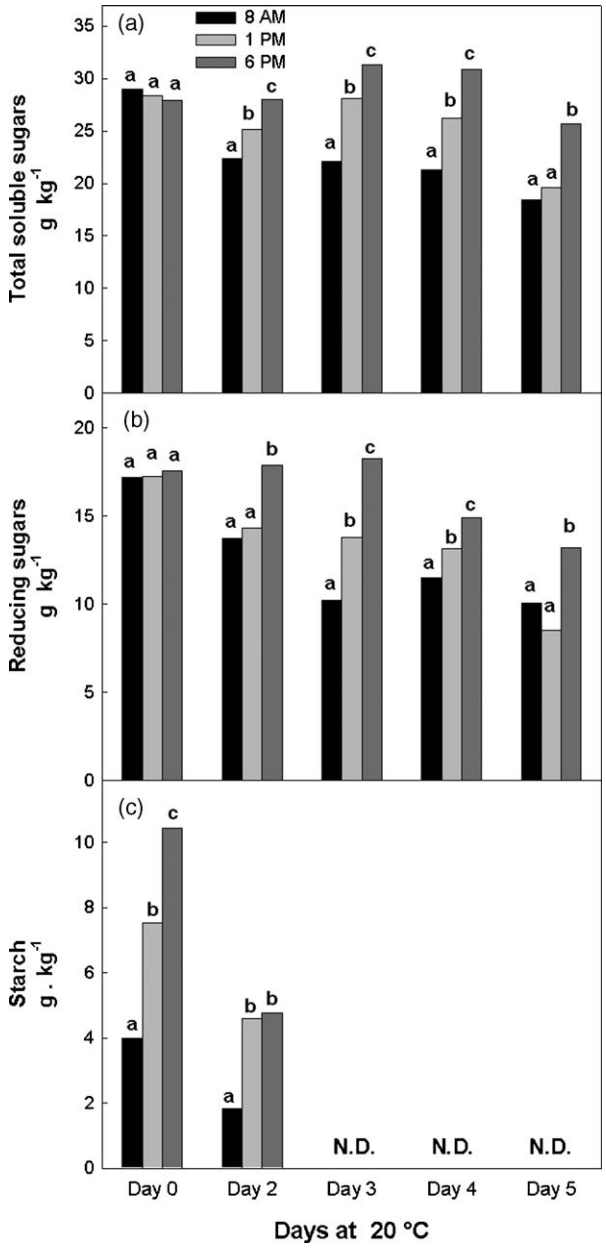


**Fig. 3.** Changes in total sugars (a), reducing sugars (b) and starch (c) content during storage at 20 °C of broccoli heads harvested at different hours of day. Different letters indicate significant differences at the same storage time.

the first 3 d and then remained constant, while the 6 PM samples decreased their reducing sugars levels at days 4 and 5 (Fig. 3b).

Analysis of starch content showed a different pattern. Samples presented important differences at harvest: heads which received a full day's sun light before harvest (6 PM) had the highest starch levels, while those harvested at sunrise (8 AM) had levels almost 3-fold lowest (Fig. 3c). In all samples the content of starch decreased during storage but differences between samples obtained at 8 AM and 6 PM remained after 2 d. Degradation of starch continued and reached undetectable levels after 3 d of storage in all samples.

According to the data obtained immediately after harvest, there were no changes in soluble sugars during the day but starch content increased substantially from 8 AM to 6 PM. In *Arabidopsis* leaves, reducing sugars remain almost constant during the diurnal cycle, while sucrose slightly increases and starch greatly increases (Bläsing et al., 2005). Similar results were observed in portulaca leaves, an herbaceous ornamental plant (Rapaka et al., 2007). However, in stems of the same plant, while soluble sugars do not change during the diurnal cycle, starch content presents such variations. These previous results, as well as our own, indicate that the soluble sugar levels in plant tissues change slightly (in the case of leaves) or do not change (in the case of stems or inflorescences), while starch accumulates during the diurnal cycle. Probably, the excess of carbohydrate generated by photosynthesis are incorporated into the starch pool during the day and then remobilized during the night.

Differences in the starch content among samples probably caused the different soluble sugars levels detected during storage. As presented in our results and reported by Tian et al. (1997), starch degrades quickly during broccoli postharvest and reaches very low or undetectable levels after 24–48 h. Products of starch degradation enter into the soluble sugar fraction and by this way contribute to maintain soluble sugar levels. Therefore, those samples that had higher starch content (6 PM harvest) maintained their soluble sugar levels for a longer period of time.

An increment in the levels of reactive oxygen species is a common feature of the senescence process (Navabpour et al., 2003). The presence of substances or enzymes which contribute to the antioxidant system can prevent or delay senescence symptoms of the tissue (Hodges et al., 2001). We analyzed the level of antioxidant capacity immediately after harvest and no significant differences among samples harvested at different moments of the day were found (Fig. 4a). After 2 d of storage, the antioxidant capacity decreased in all samples. On the third day, samples harvested at 8 AM remained unchanged, whereas samples harvested at 1 PM and 6 PM increased in antioxidant capacity followed by a decrease. As a consequence, significant differences between samples were only detected on day 3. The higher levels of antioxidants detected in samples harvested at 6 PM could also contribute to a delay in the senescence process.

Levels of soluble phenolic compounds correlate with antioxidant activity (Leja et al., 2001). In our experiments, no differences were detected immediately after harvest among samples harvested at different moments during the day (Fig. 4b). The content of phenolic compounds remained almost constant during storage of samples harvested at 8 AM. However, after 2 d, samples harvested at 1 PM and 6 PM had an increase in their phenolic compound levels and then reduced to levels similar to those taken initially. The increment of antioxidants detected after 3 d in samples harvested at 6 PM could probably, be tightly related to the increment of phenols detected in the same day.

Considering all the results, heads harvested at the end of the day clearly showed a slower rate of senescence in comparison to those obtained in the morning. These samples presented higher retention of green colour, chlorophyll and soluble sugar content, as well as antioxidant capacity. Analysis performed immediately after harvest indicated that there were no differences among samples in superficial colour, chlorophylls, soluble sugars and antioxidant status. The only parameter assayed which presented differences immediately after harvest was the content of starch. This suggest that carbohydrates accumulated as starch during the day, as a product of photosynthesis, allowing the broccoli to maintain a higher soluble sugar contents during senescence, which in turn contributes to a slower rate of senescence.

An extension in postharvest shelf life was also detected in baby salad leaves of roquette (*Eruca vesicaria* ssp. *sativa*), lollo rosso lettuce (*Lactuca sativa* L. 'Ravita') and red chard (*Beta vulgaris* L. var. *flavescens)* when harvested at end of the day (Clarkson et al., 2005). The extension in shelf life was associated with higher levels of sucrose in roquette and higher levels of starch in lollo rosso and red chard due to the products of photosynthesis following prolonged exposure to daylight. Differently, cabbage harvested at different times of the day did not show differences in postharvest life (Klieber et al., 2002). In this case, samples harvested at different times had

the same content of soluble sugars, although starch levels were not measured. Authors indicated that the protective function of the wrapper leaves which cover the head could lessen the stress induced by field temperatures. In this sense, the fact that external leaves cover internal ones could also block photosynthesis and thus limit diurnal fluctuations of non-structural sugar contents.

Higher levels of sugars in crops harvested at the end of the day can protect against not only the stress produced by harvest, as observed in broccoli, but also against chilling injury as detected in tomato seedlings (King et al., 1988). In this work, the authors suggest that changes in chilling sensitivity over the diurnal period are regulated by the light cycle and that increased sensitivity at the end of the dark period could be due to carbohydrate depletion.

Another beneficial effect of accumulation of sugars during the diurnal cycle was observed in *Portulaca*, an ornamental plant. This plant is commonly dispersed after shoot-tip cutting and a significant number of leaves usually abscise after cutting, which is related to the ethylene produced in the process (Rapaka et al., 2007). The authors detected that the increase in preharvest endogenous carbohydrate levels as the photoperiod progresses reduces the subsequent postharvest ethylene responsiveness and thus diminishes the number of abscised leaves.

### 3.2. Postharvest treatment with sugars

In order to check the effect of the level of single sugars in postharvest senescence of broccoli heads, we performed treatments by placing florets in sugar solutions, assuring a continuous supply. Several solutions were assayed and their effects on superficial colour changes were evaluated during postharvest at 20 °C. The results shown in Table 1 indicate that treatments with 10% sucrose and glucose caused the highest green colour retention. Lower and higher concentrations were not as effective probably due to insufficient sugar supply or a hyperosmotic toxic effect, respectively. Based on these results we chose 10% sucrose and 10% glucose to evaluate their effects on senescence during storage. A continuous supply of sucrose or glucose delayed both chlorophyll a and chlorophyll b degradation (Fig. 5a and b), mainly in the case of 10% sucrose after 2 and 3 d of storage. When total sugar content was measured, a reduction was found in all samples after 2 or 3 d of storage (Fig. 6a). Such reduction continued for the controls, but it was not detected

**Table 1**
Evolution of superficial color (HUE and L) of broccoli heads stored at 20 °C with their stems placed in contact with solutions having different sugar concentrations.

| | HUE | | | | L | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Day 0 | Day 2 | Day 3 | Day 4 | Day 0 | Day 2 | Day 3 | Day 4 |
| Control | 120.2 | 116.2 | 110.9 | 98.8 | 34.43 | 38.18 | 40.82 | 43.95 |
| Glucose 2% | 120.2 | 117.2 | 111.0 | 100.0 | 34.43 | 38.20 | 40.80 | 43.66 |
| Glucose 10% | 120.2 | 119.1* | 114.9* | 105.1* | 34.43 | 37.82 | 40.05 | 43.09 |
| Sucrose 2% | 120.2 | 117.9 | 112.8 | 102.9* | 34.43 | 37.98 | 40.79 | 43.77 |
| Sucrose 10% | 120.2 | 118.6 | 115.5* | 106.2* | 34.43 | 38.08 | 40.03 | 42.99* |
| Sucrose 15% | 120.2 | 117.3 | 111.1 | 100.2* | 34.43 | 37.85 | 40.55 | 43.69 |

* Indicate significative differences when compared to control ($P = 0.05$).

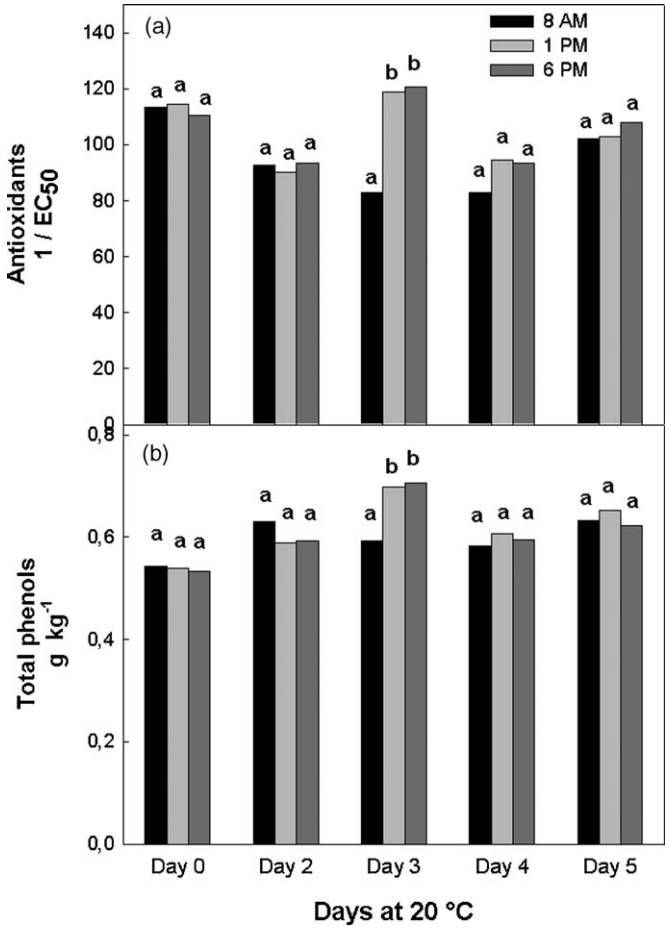


**Fig. 4.** Changes in antioxidant power ($EC_{50}^{-1}$) and total phenols during storage at 20 °C of broccoli heads harvested at different hours of day. Different letters indicate significant differences at the same storage time.

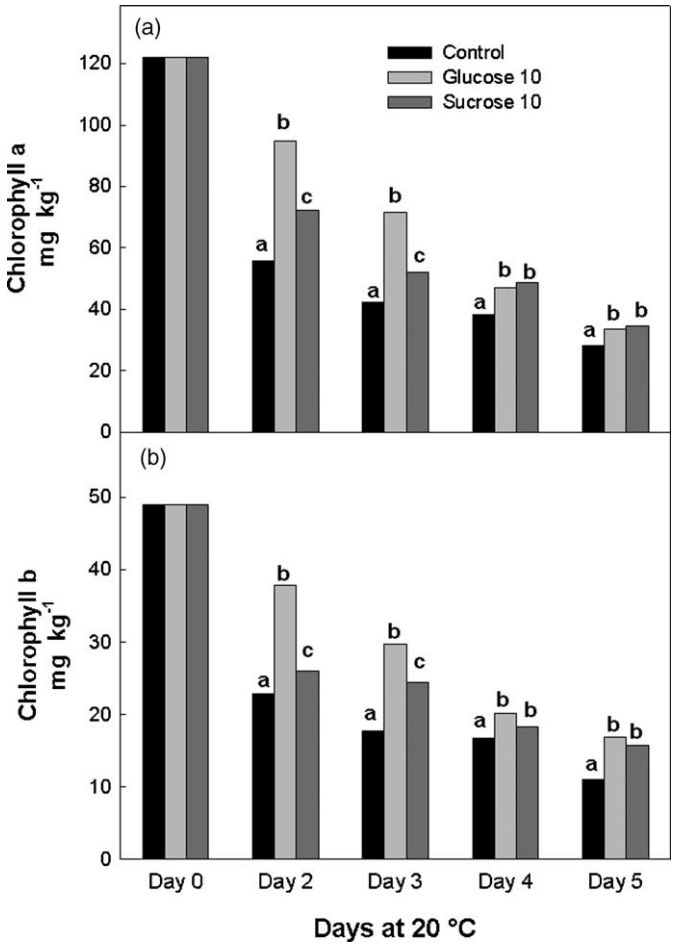


**Fig. 5.** Changes in chlorophyll a (a) and chlorophyll b (b) content of broccoli heads during storage at 20 °C. Heads were maintained in glucose or sucrose solutions during storage time. Different letters indicate significant differences at the same storage time.

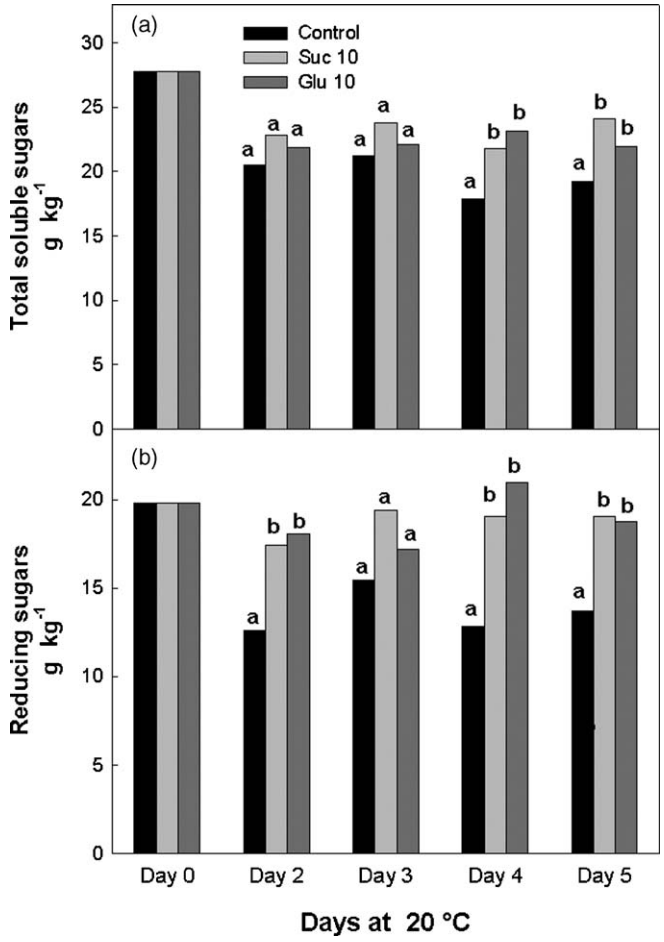


**Fig. 6.** Changes in total sugars (a) and reducing sugars (b) content during storage at 20 °C of broccoli heads. Heads were held in glucose or sucrose solutions during storage time. Different letters indicate significant differences at the same storage time.

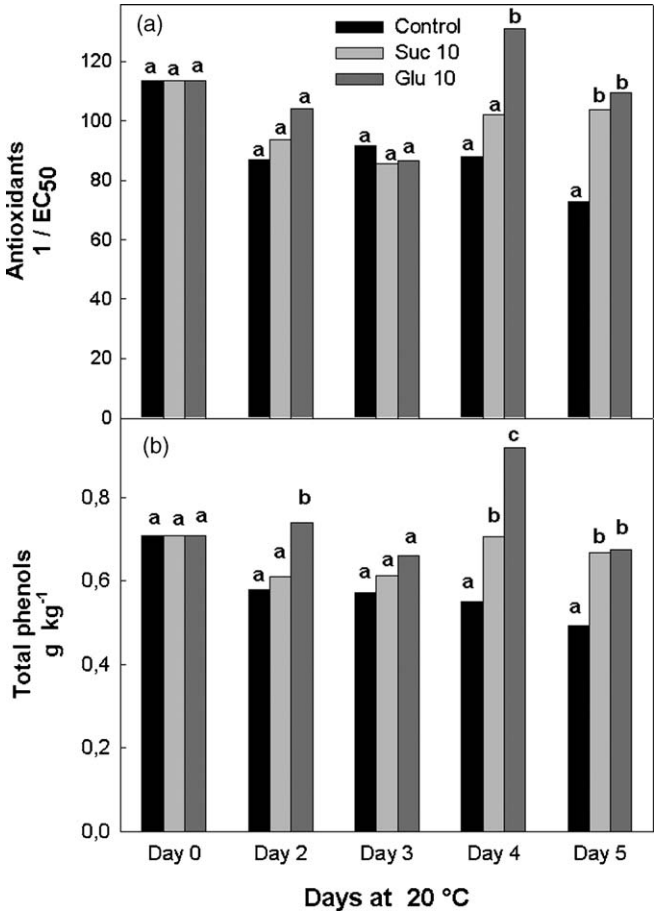


**Fig. 7.** Changes in total antioxidant power ($EC_{50}^{-1}$) and total phenols of broccoli heads during storage at 20 °C. Heads were held in glucose or sucrose solutions during storage time. Different letters indicate significant differences at the same storage time.

in treated heads. As a consequence, samples supplied with sucrose or glucose had higher levels of total sugars at the end of incubation. In the case of reducing sugar content, a reduction was detected in control samples after 2 d. Then, reducing sugars remained almost constant until the end of storage. Differently, the treated samples did not show a reduction and maintained concentration levels similar to those initially taken for 5 d (Fig. 6b).

Antioxidant power was also evaluated and control samples behaved similar behaviour to samples harvested at 8 AM and their antioxidant capacity reduced during storage. Sugar-treated heads showed levels of antioxidant compounds similar to controls until day 3, but then their values were significantly higher (Fig. 7a). A similar pattern was detected in the case of phenolic compounds (Fig. 7b), indicating a high correlation between these compounds and the antioxidant capacity of broccoli.

These results corroborate the delaying effect of sugars on postharvest senescence of broccoli as it was previously shown (Irving and Joyce, 1995). Supplementing with either glucose or sucrose maintained higher levels of total or reducing sugars during storage, which in turn delayed colour change and chlorophyll degradation as well as maintained higher levels of antioxidants.

Senescence of broccoli or ornamental flowers is accelerated by postharvest ethylene production. Studies performed on broccoli florets and carnation flowers have shown that increasing the endogenous carbohydrate levels by external sucrose resulted in decreased ethylene responsiveness (Verlinden and Garcia, 2004; Nishikawa et al., 2005).

Exogenous application of soluble sugars can also contribute to protect vegetal tissues from other stresses such as chilling sensitivity, as it was shown in tomato seedlings (King et al., 1988).

## 4. Conclusions

This work shows the potential and relevant impact that time of day at harvest can have on the postharvest life of broccoli. We found that heads harvested at the end of the day had a delayed senescence compared to those harvested at morning and that these differences could be due to the additional accumulation of starch during the diurnal cycle. Moreover, an exogenous supply of soluble sugars can mimic the endogenous contribution of starch and thus also delay senescence. These data did not demonstrate that carbohydrates accumulated during the daylight period are the only factor that delays senescence in broccoli heads harvested at end of day, but they certainly indicate that changes in starch concentrations are related to the differences detected among samples.

Finally, in relation to the possible technological application we must underline that, traditionally, farming practice implies harvesting at early morning to allow time for processing or packaging during the same day. Our results would suggest the convenience of rescheduling the time of harvest to obtain significant improvements in the postharvest conservation of broccoli.

## References


Argyroudi-Akoyunoglou, J.H., Prombona, A., 1996. Light-independent endogenous circadian rhythm in the capacity for chlorophyll formation. J. Photochem. Photobiol. B: Biol. 36, 271–277.

Able, A.J., Wong, L.S., Prasad, A., O'Hare, T.J., 2002. 1-MCP is more effective on a floral brassica (Brassica oleracea var. italica L.) than a leafy brassica (Brassica rapa var. chinensis). Postharvest Biol. Technol. 26, 147–155.

Bläsing, O.E., Gibon, Y., Günther, M., Höhne, M., Morcuende, R., Osuna, D., Thimm, O., Usadel, B., Scheible, W.-R., Stitt, M., 2005. Sugars and circadian regulation make major contributions to the global regulation of diurnal gene expression in Arabidopsis. Plant Cell 17, 3257–3281.

Chen, L.F.O., Lin, C.H., Kelkar, S.M., Chang, Y.M., Shaw, J.F., 2008. Transgenic broccoli (*Brassica oleracea* var. *italica*) with anti-sense chlorophyllase (BoCLH1) on delaying postharvest yellowing. Plant Sci. 174, 25–31.

Clarkson, G.J., Rothwell, S.D., Taylor, G., 2005. End of day harvest extends shelf life. Hortic. Sci. 40, 1431–1435.

Costa, L., Vicente, A.R., Civello, P.M., Chaves, A.R., Martínez, G.A., 2006. UV-C treatment delays postharvest senescence in broccoli florets. Postharvest Biol. Technol. 39, 204–210.

Coupe, S.A., Watson, L.M., Ryan, D.J., Pinkney, T.T., Eason, J.R., 2004. Molecular analysis of programmed cell death during senescence in *Arabidopsis thaliana* and *Brassica oleracea*: cloning broccoli LSD1, Bax inhibitor and serine palmitoyltransferase homologues. J. Exp. Bot. 55, 59–68.

Eason, J.R., Ryan, D.J., Watson, L.M., Hedderley, D., Christey, M.C., Braun, R.H., Coupe, S.A., 2005. Suppression of the cysteine protease, aleurain, delays floret senescence in *Brassica oleracea*. Plant Mol. Biol. 57, 645–657.

Ferguson, I.B., Ben-Yehoshua, S., Mitcham, E.J., McDonald, R.E., Lurie, S., 2000. Postharvest heat treatments: introduction and workshop summary. Postharvest Biol. Technol. 21, 1–6.

Hodges, D.M., Forney, C.F., Wismer, W.V., 2001. Antioxidant responses in harvested leaves of two cultivars of spinach differing in senescence rates. J. Am. Soc. Hortic. Sci. 126, 611–617.

Irving, D.D., Joyce, D.C., 1995. Sucrose supply can increase longevity of broccoli (*Brassica oleracea*) branchlets kept at 22 °C. Plant Growth Regul. 17, 251–256.

Jacobsson, A., Nielsen, T.M., Sjoholm, I., Wendin, K., 2004. Influence of packaging material and storage condition on the sensory quality of broccoli. Food Qual. Pref. 15, 301–310.

King, A.I., Joyce, D.C., Reid, M.S., 1988. Role of carbohydrates in diurnal chilling sensitivity of tomato seedlings. Plant Physiol. 86, 764–768.

King, G.A., Morris, S.C., 1994. Physiological changes of broccoli during early postharvest senescence and through the preharvest–postharvest continuum. J. Am. Soc. Hortic. Sci. 119, 270–275.

Kiyota, M., Numayama, N., Goto, K., 2006. Circadian rhythms of the L-ascorbic acid level in Euglena and spinach. J. Photochem. Photobiol. B: Biol. 84, 197–203.

Klieber, A., Porter, K.L., Collins, G., 2002. Harvesting at different times of day does not influence the postharvest life of Chinese cabbage. Sci. Hort. 96, 1–9.

Ku, V.V.V., Wills, R.B.H., 1999. Effect of 1-methylcyclopropene on the storage life of broccoli. Postharvest Biol. Technol. 17, 127–132.

Leja, M., Mareczek, A., Starzynk ska, A., Rozek, S., 2001. Antioxidant ability of broccoli flower buds during short-term storage. Food Chem. 72, 219–222.

Lemoine, M.L., Civello, P.M., Martínez, G.A., Chaves, A.R., 2007. Influence of postharvest UV-C treatment on refrigerated storage of minimally processed broccoli (*Brassica oleracea* var. *italica*). J. Sci. Food Agric. 87, 1132–1139.

Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Met. Enzymol. 148, 350–382.

Ma, G., Wang, R., Wang, C.-R., Kato, M., Yamawaki, K., Qin, F., Xu, H.-L., 2009. Effect of 1-methylcyclopropene on expression of genes for ethylene biosynthesis enzymes and ethylene receptors in post-harvest broccoli. Plant Growth Regul. 57, 223–232.

Navabpour, S., Morris, K., Allen, R., Harrison, E., A.-H.-Mackerness, S., Buchanan-Wollaston, V., 2003. Expression of senescence-enhanced genes in response to oxidative stress. J. Exp. Bot. 54, 2285–2292.

Nelson, N., 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153, 375–380.

Nishikawa, F., Kato, M., Hyodo, H., Ikoma, Y., Sugiera, M., Yano, M., 2005. Effect of sucrose on ascorbate level and expression of genes involved in the ascorbate biosynthesis and recycling pathway in harvested broccoli florets. J. Exp. Bot. 56, 65–72.

Page, T., Griffiths, G., Buchanan-Wollaston, V., 2001. Molecular and biochemical characterization of postharvest senescence in broccoli. Plant Physiol. 125, 718–727.

Pan, J., Vicente, A.R., Martínez, G.A., Chaves, A.R., Civello, P.M., 2004. Combined use of UV-C irradiation and heat treatment to improve postharvest life of strawberry fruit. J. Sci. Food Agric. 84, 1831–1838.

Rapaka, V.K., Faust, J.E., Dole, J.M., Runkle, E.S., 2007. Diurnal carbohydrate dynamics affect postharvest ethylene responsiveness in portulaca (*Portulaca grandiflora* 'Yubi Deep Rose') unrooted cuttings. Postharvest Biol. Technol. 44, 293–299.

Rose, R., Rose, C., Omi, S., Forry, K., Durall, D., Bigg, W., 1991. Starch determination by perchloric acid vs enzymes: evaluating the accuracy and precision of six colorimetric methods. J. Agric. Food Chem. 39, 2–11.

Sicher, R.C., Kremer, D.F., Harris, W.G., 1984. Diurnal carbohydrate metabolism of barley primary leaves. Plant Physiol. 76, 165–169.

Tian, M., Talebul Islam, Stevenson, D., Irving, D., 1997. Color, ethylene production, respiration, and compositional changes in broccoli dipped in hot water. J. Am. Soc. Hortic. Sci. 122 (1), 112–116.

Toivonen, P.M.A., 1997. The effects of storage temperature, storage duration, hydrocooling, and micro-perforated wrap on shelf life of broccoli (*Brassica oleracea* L., *italica* group). Postharvest Biol. Technol. 10, 59–65.

Tosun, B.N., Yücecan, S., 2008. Influence of commercial freezing and storage on vitamin C content of some vegetables. Int. J. Food Sci. Technol. 43, 316–321.

Verlinden, S., Garcia, J.J., 2004. Sucrose loading decreases ethylene responsiveness in carnation (*Dianthus caryophyllus* cv. White Sim) petals. Postharvest Biol. Technol. 31, 305–312.

Vicente, A.R., Martínez, G.A., Chaves, A.R., Civello, P.M., 2003. Influence of self-produced $CO_2$ on postharvest life of heat treated strawberries. Postharvest Biol. Technol. 27, 265–275.

Viña, S.Z., Chaves, A.R., 2008. Effect of heat treatment and refrigerated storage on antioxidant properties of pre-cut celery (*Apium graveolens* L.). Int. J. Food Sci. Technol. 43, 44–51.