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FULL ARTICLE

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The time of the day to harvest affects the degreening, antioxidant compounds, and protein content during postharvest storage of broccoli

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Abstract

Harvesting of broccoli at several moments of the day affects the rate of senescence during storage. In this work, broccoli heads were harvested at several moments and then kept at 20°C in order to analyze protein metabolism and antioxidant compounds. Almost no differences were detected in the contents of total and soluble proteins, and free amino acids. Only an increment in free amino acids was detected by day 3 in samples obtained at 8:00 hr. With reference to antioxidants, the contents of ascorbic acid, carotenoids and xanthophylls, phenols, and flavonoids were similar in samples harvested at different moments. However, an increment was detected in carotenoids, phenols, and flavonoids during storage of samples collected at 18:00 hr on day 3 and samples collected at 13:00 hr on day 5. The combination of delay of senescence and increment in antioxidants suggest harvesting at 12:00 or 18:00 hr.

Practical application

Broccoli is a vegetable with an important level of nutrients. However, it is also highly perishable and suffers a high rate of senescence and loss of quality during postharvest. In this work, it is demonstrated that the simple practice of harvests in different moments of the day can affect the postharvest behavior of broccoli, and it is suggested to carry out the harvest toward the end of the day.

KEYWORDS

ascorbic acid, *Brassica oleracea*, carotenoids, phenolics, senescence

1 | INTRODUCTION

Broccoli is a product with high levels of ascorbic acid, glucosinolates, flavonoids, carotenoids, vitamins, and dietary fibers (Vallejo, Tomás-Barberán, & García-Viguera, 2003). However, it is also a very perishable vegetable with a high senescence velocity. In the course of its postharvest life, broccoli exhibits a severe degreening due to chlorophyll catabolism that leads to a loss of organoleptic quality. In addition, a significant loss of nutritional compounds can also take place during this period, including important bioactive chemicals such as ascorbic acid, glucosinolates, and folic acid (Jones, Faragher, & Winkler, 2006). Moreover, senescence is also connected to fast degradation of proteins. Proteins are catabolized early conducting to the decrease photosynthetic activity (Costa, Millan Montano, Carrión, Rolny, & Guiamet, 2013). In broccoli, it has been observed that the expressions of several protease genes are augmented in the course of senescence (Wang, Yang, Chen, Lin, & Shaw, 2004).

Broccoli heads possess a high concentration of ascorbic acid, although rapid degradation of this compound has been observed in heads stored at ambient temperatures after harvest (Nishikawa et al., 2003). The process of senescence involves the accumulation of active oxygen species and consequently a reduction in the content

of antioxidants, particularly ascorbic acid (Ivanov, 2014). Broccoli has a high content of another group of antioxidants, the phenolic compounds (Ramos dos Reis et al., 2015). Among them are flavonoids, which display a radical scavenging activity (Van Acker et al., 1996), and could have protective roles against the development of vascular dysfunction and the advancement of atherosclerosis (Schroeter et al., 2006). In general, the contents of flavonoids in broccoli depend on several factors as well as: cultivar (Vallejo et al., 2003), environmental conditions (Dixon & Paiva, 1995), and postharvest transportation and handling (Vallejo et al., 2003).

Procedures to keep commercial and nutritional quality of broccoli have studied technologies such as refrigeration (Serrano, Martinez-Romero, Guillén, Castillo, & Valero, 2006), heat treatments (Costa, Civello, Chaves, & Martínez, 2006), modified atmospheres (Barth, Kerbel, Broussard, & Schmidt, 1993), UV (Aiamla-or, Yamauchi, Takino, & Shigyo, 2009), and 1-MCP (Gómez-Lobato, Hasperué, Civello, Chaves, & Martínez, 2012) among others. Nevertheless, postharvest shelf life of vegetables can also be influenced by a group of preharvest conditions such as soils, climate, plant stress, and crop and plant management. Other possible and relevant factor is the moment of the day at which the heads are obtained. The diurnal cycle can greatly influence many physiological processes and plant metabolisms (Bläsing et al., 2005). In that sense, production of reactive oxygen species and their scavenging can be regulated by diurnal cycles (Lai et al., 2012).

In a former research, our group found out that harvesting at different moments of the day (Hasperué, Chaves, & Martínez, 2011) can influence the rate of senescence during postharvest conservation. This simple practice allows improving postharvest shelf life of broccoli without using chemicals or technologies that require high economic costs. Now, we proposed to analyze the effect of the time of day at harvest on several compounds associated with nutritional quality (antioxidants and proteins) during postharvest storage of broccoli.

2 | MATERIALS AND METHODS

2.1 | Plant material

Forty-five heads of broccoli (*Brassica oleracea* var. Italica cv. Legacy) were obtained from a farm in the surroundings of La Plata, Argentina (34° 59′ S and 58° 3′ O) at each of the moments of day: 08:00 , 13:00 , and 18:00 hr, and carried directly to the laboratory. Heads were deposited in plastic trays and wrapped with PVC films possessing four holes per tray, to restrict abundant dehydration. Broccoli heads were stored in darkness at 20°C during 5 days and 50 heads were collected periodically. Florets were separated from heads and frozen with liquid nitrogen and subsequently kept at −80°C until use.

2.2 | Determination of superficial color

Superficial color of broccoli heads was measured according to Perini et al. (2017). Five heads were used per each condition and five measurements were done on each head.

2.3 | Determination of contents of chlorophylls, carotenoids, and xanthophylls

Frozen broccoli florets were ground in liquid nitrogen, and 0.2 g of the resulting powder was incorporated to 2 ml of acetone and kept for 4 hr in darkness. The suspension was centrifuged at 10,000 × *g* for 10 min at 4°C. Chlorophylls, xanthophylls, and carotenes were measured in the supernatant according to Lichtenthaler (1987) and calculated as grams per kg of fresh tissue. Three biological replicates and three technical repetitions were performed.

2.4 | Determination of reduced and total ascorbic acid content

The ascorbic acid (AA) and dehydroascorbate (DHA) were extracted from liquid nitrogen-frozen broccoli florets. Approximately 200 mg of tissue was processed with 1 ml of 3% v/v trifluoroacetic in cold mortar and pestle. The samples were laid in an Eppendorf tube and centrifuged at 16,000 × *g* for 10 min. The pellet was discarded and supernatant was maintained in an ice bath for the measurement. Samples were eluted in C18 cartridges (Sep-Pak® Vac 3 cc, 500 mg, Waters, Ireland). The AA content was measured in a HPLC equipped with a LC-10 AT pump (Shimadzu®, Japan) at a flow rate of 0.5 ml/min of 100 mM potassium phosphate buffer adjusted at pH 3.0 and using a UV-VIS detector (Model SPD-10AV, Shimadzu®, Japan) at 265 nm. For total AA determination, 1 volume of 100 mM phosphate buffer pH 7.0 was mixed with 1 volume of the supernatant and incubated for 10 min with 5 mM DL-dithiothreitol for reducing the DHA pool and measured as previously described. The AA content was expressed in mmol gr^{-1} of fresh weight basis. L-ascorbic acid was used as a standard (Bártoli et al., 2006). Three biological replicates and three technical repetitions were performed.

2.5 | Determination of contents of phenolic compounds and flavonoids

For the determination of phenolic compound content, 0.2 g of frozen broccoli was pulverized with liquid nitrogen and homogenized with 1 ml of ethanol 100% for 5 min. The tubes were centrifuged at 9,000 × *g* for 15 min at 4°C. Then, the supernatant was collected and utilized to measure phenolic compounds (Costa, Vicente, Civello, Chaves, & Martínez, 2006) with slight changes. Fifty microliters of ethanolic extract was added to 0.45 ml of distilled water and 0.1 ml of Folin's reagent (1:1 with distilled water). After 3 min, 0.5 ml of Na_2CO_3 10% w/v was added and then the tubes were homogenized and stored for 1 hr in darkness. The absorbance was measured at 760 nm and total phenolic content was estimated using gallic acid as standard. The results were expressed as mg of gallic acid equivalent per 100 g of fresh tissue. Three biological replicates and three technical repetitions were performed.

For flavonoids content determination, 0.25 g of frozen broccoli was pulverized with liquid nitrogen and homogenized with 1 ml of ethanol 50% for 5 min. The tubes were centrifuged at 9,000 × *g* for 15 min at 4°C. The supernatant was reserved and utilized to measure

flavonoids content according to Costa, Vicente, et al. (2006) with slight changes. Two hundred microliters of ethanolic extract was homogenized with 0.51 ml of distilled water and 0.030 ml of $NaNO_2$ 5% w/v. After 5 min, 0.060 ml of $AlCl_3$ 10% w/v was added and tubes were homogenized. After another 5 min, 0.2 ml of 1 M NaOH was added to it. The samples were homogenized and the absorbance was read immediately at 515 nm. A calibration curve using catechin as a standard was constructed. Total flavonoids were express as microgram of catechin equivalent per gram of fresh tissue. Three biological replicates and three technical repetitions were performed.

2.6 | Determination of soluble and total proteins

Extractions of soluble and total proteins were done according to Perini et al. (2017). Soluble proteins were quantified by the method of Bradford (1976), whereas total proteins were measured according to Lowry et al. (1951). Soluble as well as total protein content were expressed as gram per kg of fresh tissue. Three biological replicates and three technical repetitions were performed.

2.7 | Determination of content of free amino acids

Quantification of total free amino acids was performed according to Lee and Takahashi (1966) with slight modifications. Frozen broccoli florets were pulverized in liquid nitrogen with pestle and mortar and 0.25 g of the resulting powder was mixed with 1 ml of 80% ethanol. The suspension was stirred, centrifuged at 9,000 × *g* for 10 min at 4°C, and 1 ml of the supernatant was recovered. The pellet was re-extracted twice, first with 500 µl of 60% ethanol and then with 500 µl of distilled water. The supernatants were pooled together. For total free amino acids determination, 100 µl of the dilution 1/5 of the ethanol extract was mixed with 375 µl of 500 mM citrate buffer pH = 5.5, 400 µl glycerol and 125 µl of 2% (v/v) ninhydrin solution. Reaction mixture was incubated at 100°C during 6 min and then put in a cold ice bath for 10 min. After that, absorbance was evaluated spectrophotometrically at 570 nm. A calibration curve was constructed using glycine as standard. Three biological replicates were performed and technical measurements were done in triplicate. Results were calculated as mg glycine/mg fresh weight.

2.8 | Experimental design and statistical analysis

The experiment was configured according to a factorial design, being the factors time of harvest and storage time. All statistical calculations were performed using the SYSTAT software package. The results were analyzed by one-way ANOVA and means were compared by using Tukey's test at significance level of 0.05.

3 | RESULTS AND DISCUSSION

The harvest of horticultural products at several moments of the day can affect their quality during postharvest storage. Clarkson, Rothwell, and Taylor (2005) detected an increase in postharvest shelf life in leaves of salad roquette, lollo rosso lettuce, and red chard, if the samples were harvested when the day ends. However, in cabbage, harvests done at different moments of the day did not influence postharvest life (Klieber, Porter, & Collins, 2002).

In previous research, our group had showed that broccoli heads collected toward the end of the day accumulate starch and that this accumulation probably contributes to delay senescence and to maintain better quality and higher soluble sugars during postharvest storage (Hasperué et al., 2011). Moreover, we also showed that samples harvested at 18:00 hr showed a lower catabolism of chlorophylls in relation with heads obtained at morning. In addition, most of genes related to chlorophyll degradation during broccoli postharvest senescence showed a lower expression or a deferment in their mRNA increments in heads harvested at 18:00 hr (Hasperué, Gómez Lobato, Chaves, Civello, & Martínez, 2013).

In this research, we studied the effect of such harvesting practice on other parameters associated with nutritional quality. To do that, heads were obtained at different moments of the day and maintained at 20°C. The progression of senescence was evaluated by measuring superficial color and chlorophyll content. At harvest, all samples showed similar Hue and L values (Table 1) and chlorophyll content (Figure 1).

During storage, broccoli heads lost their green aspect and became yellow. These variations were more pronounced in samples obtained at 08:00 hr. Heads harvested at 18:00 hr presented a lower decline in Hue and a lower enhancement in L respect to the heads collected at 8:00 hr, indicating a lessen yellowing (Figure 1).

Moreover, samples obtained at 13:00 and 18:00 hr exhibited lower chlorophyll catabolis after three days in relation to those harvested at 8:00 hr. However, after 5 days of storage, samples collected at afternoon were those with the higher total chlorophyll content. These results were in accordance to those described in a previous works (Hasperué et al., 2011; Hasperué, Lemoine, Chaves, & Martínez, 2014).

TABLE 1 Changes in Hue and L values in broccoli heads harvested at different hours and stored during 5 days at 20°C

	Hue			L		
	8 hr	13 hr	18 hr	8 hr	13 hr	18 hr
Day 0	127.3 ± 1.3 a	127.7 ± 1.7 a	128.4 ± 1.4 a	39.69 ± 2.25 a	40.06 ± 2.13 a	39.87 ± 2.11 a
Day 3	107.1 ± 2.1 a	113.3 ± 2.3 b	116.2 ± 0.8 b	49.35 ± 3.28 a	48.03 ± 2.79 a	46.26 ± 2.12 b
Day 5	84.9 ± 1.5 a	91.9 ± 2.3 b	98.6 ± 1.6 c	59.93 ± 3.64 a	58.13 ± 3.23 b	56.58 ± 3.16 c

Note: Different letters indicate significant differences ($p < 0.05$) among samples at the same time.

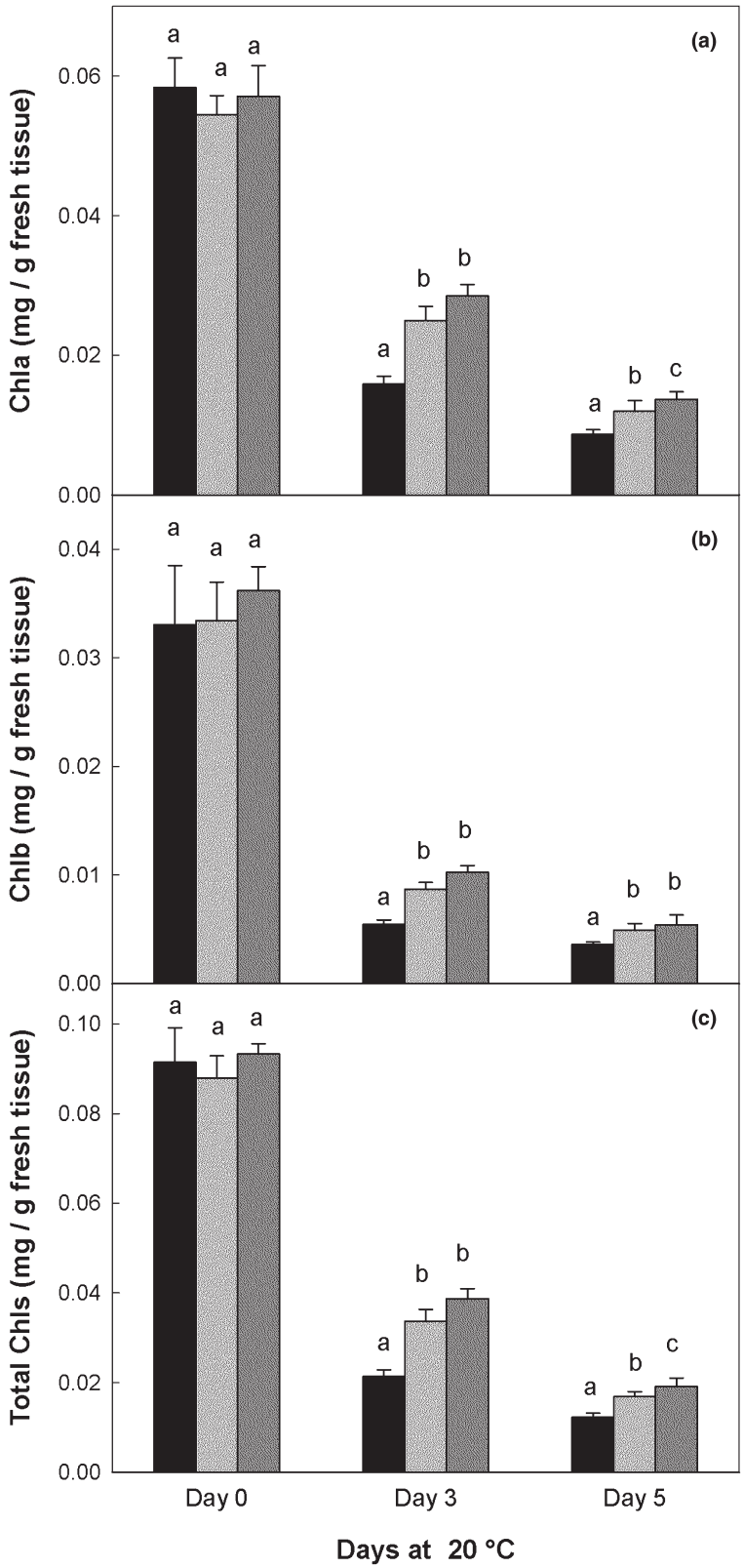

FIGURE 1 Content of chlorophyll a (panel a), chlorophyll b (panel b), and total chlorophyll (panel c) in broccoli heads harvested at different hours of the day and stored during 5 days at 20°C. Different letters indicate significant differences at the same time of storage ($p < 0.05$)

Besides the disappearance of green color and catabolism of chlorophyll, postharvest physiology of broccoli is characterized by an intense degradation of proteins (Page, Griffiths, & Buchanan-Wollaston, 2001). Numerous papers describe an increment in protease activity during broccoli senescence and a decrease in the level of total proteins (Wang et al., 2004). We also detect a decrement in the content of total

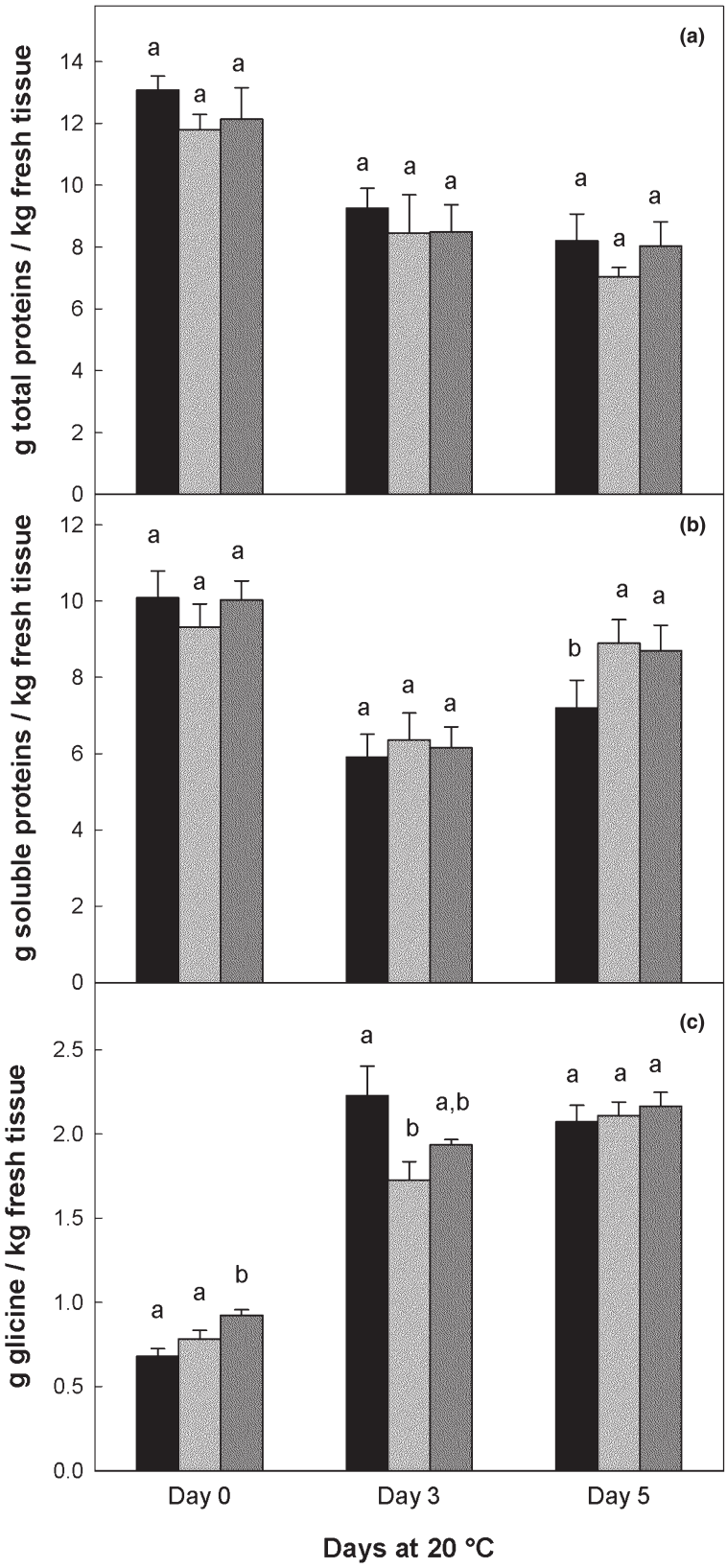

FIGURE 2 Content of total proteins (panel a), soluble proteins (panel b), and total amino (panel c) acids in broccoli heads harvested at different hours of the day and stored during 5 days at 20°C. Different letters indicate significant differences at the same time of storage ($p < 0.05$)

proteins during postharvest storage with no differences among times of harvest (Figure 2 panel a). However, we detected a slight increase in the content of soluble proteins in all samples after 5 days of storage (Figure 2 panel b). Nevertheless, this increment was higher in samples obtained at 13:00 and 18:00 hr. When senescence process is advanced, membranes lose their integrity and functionality. This fact can conduct to solubilization of bounded membrane proteins (Dangl, Dietrich, & Thomas, 2000), resulting in an increment in the content of soluble proteins. However, solubilized proteins are then degraded, and the least amount of soluble proteins in the samples harvested at 8:00 hr, would indicate a greater degradation of previously solubilized proteins.

The goal of protein degradation is the remobilization of nitrogen, so proteins are degraded to their structural components, amino acids. In our assays, we detected a continuous increase in the total amino acid content during senescence. By day 3, a greater increase of these compounds was detected in the samples obtained at 8:00 hr (Figure 2 panel c), coinciding with the fact that these samples had the highest rate of senescence according to the loss of chlorophylls.

Broccoli is a product with an elevated content of antioxidants, among which ascorbic acid, carotenoids, phenols, and flavonoids stand out (Duarte-Sierra, Forney, Michaud, Angers, & Arul, 2017; Fernández-León, Fernández-León, Lozano, Ayuso, & González-Gómez, 2013; Raseetha, Leong, Burritt, & Oey, 2013). In heads obtained at different moments of the day a slight decrease in the content of ascorbic acid was detected in the heads harvested at 18:00 hr (Figure 3 panel a). It is likely that the accumulation of active oxygen species (Foyer & Shigeoka, 2011) as a result of reactions secondary to photosynthesis toward the end of the day caused the decrease in the ascorbic content. Variations in the level of ascorbic acid during the circadian rhythm has been observed (Chang, Yang, & Riskowski, 2013) and it has been hypothesized that these variations could be due to light–dark cycles and photosynthetic activity (Ivanov, 2014). In any case, the ascorbic content decreased in the same way in all samples during storage as senescence progressed, as it is described in other researches (Ma et al., 2012, 2010).

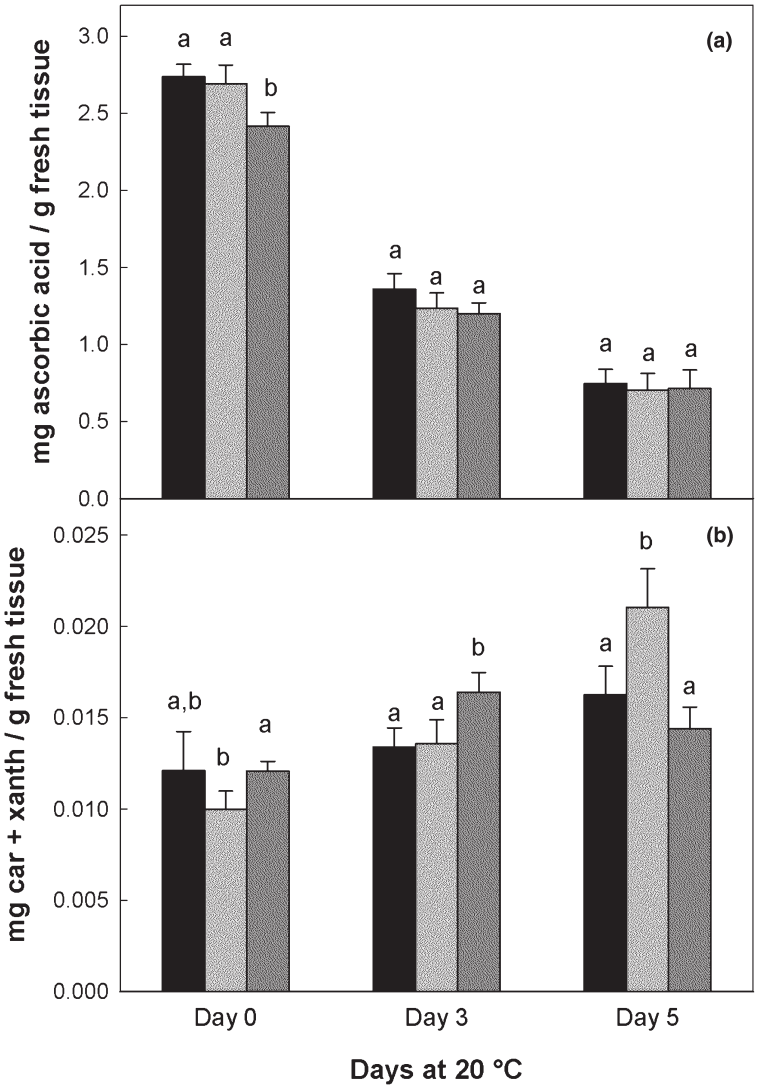

FIGURE 3 Content of ascorbic acid (panel a) and carotenoids + xanthophylls (panel b) in broccoli heads harvested at different hours of the day and stored during 5 days at 20°C. Different letters indicate significant differences at the same time of storage ($p < 0.05$)

Carotenoid pigments act as lipid-soluble antioxidant since they preserve cellular membranes by scavenging or quenching free radicals. The content of carotenoids and xanthophylls showed a slight increment in heads collected at 18:00 hr in relation to samples harvested at 13:00 hr. It was showed that genes encoding carotenoid biosynthesis are positively regulated by light (Simkin, Zhu, Kuntz, & Sandmann, 2003). In this sense, the augmentation of carotenoids toward the end of the day detected in our samples could be due to a higher exposition of broccoli heads to light. The content of carotenoids and xanthophylls increased after 3 days of storage (Figure 3 panel b) but the increment was more important in heads obtained at 18:00 hr. After 5 days, the increase was higher in heads harvested at 13:00 hr reaching levels approximately 25% higher than those detected in the other samples. The content of xanthophylls and carotenoids habitually diminishes through senescence (Biswal, 1995). However, broccoli is a singular tissue as it is an inflorescence and carotenoids and xanthophylls could concentrate in petals during growth of flower.

Regarding the content of total phenols, we detected variations in relation to the moment of day when the samples were obtained (Figure 4 panel a). The samples harvested near noon showed a lower content of total phenols and a slight increment was observed in heads collected at 18:00 hr. According to our knowledge, there are few investigations that analyze the variation in the content of phenolic compounds during the day. For example, it has been described that in broccoli and cabbage the antioxidant activity and the content of phenolics decrease toward the end of the day (Soengas, Cartea, Velasco, & Francisco, 2018). Moreover, in grapes of Tempranillo, a cultivar with black-skinned berries, the expression of genes related to phenylpropanoid biosynthesis showed a circadian variation (Carbonell-Bejerano et al., 2014).

During storage, an increment in the concentration of phenolic compounds was observed in all samples, but this increase was more important in the samples obtained at 18:00 hr after 3 days at 20°C, while heads harvested at 13:00 hr presented the greater content of phenolic compounds after 5 days of storage (Figure 4 panel a).

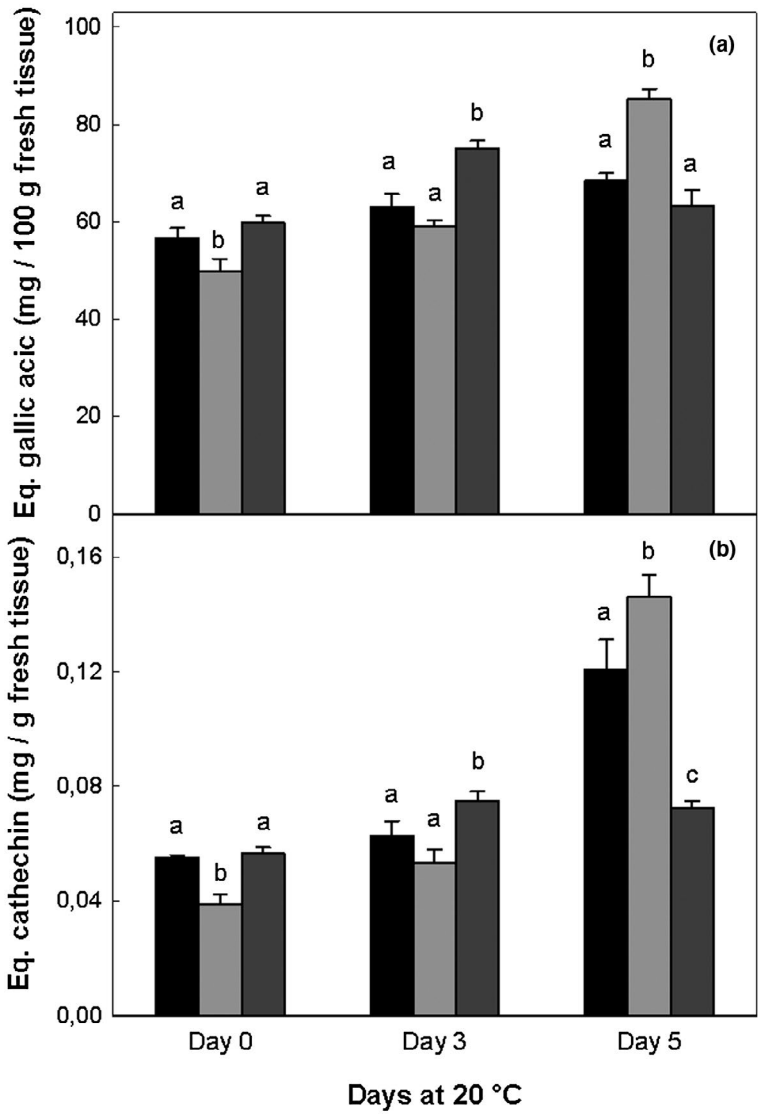

FIGURE 4 Content of total phenols (panel a) and flavonoids (panel b) in broccoli heads harvested at different hours of the day and stored during 5 days at 20°C. Different letters indicate significant differences at the same time of storage ($p < 0.05$)

The variation in the flavonoid content was similar to that observed for total phenols (Figure 4 panel b). At the time of harvest, the samples obtained at 13:00 hr had lower flavonoid content. An increase in flavonoids was also observed during storage, with the heads collected at 18:00 hr showing the highest content after 3 days. However, toward the end of the stage of storage, samples collected at 8:00 and 13:00 hr showed an increase of 2.5 and 3 times, respectively. Studies about the variation in the concentration of phenols and flavonoids through postharvest of broccoli have rendered dissimilar results. In some studies, an increase in the level of these compounds has been detected along with postharvest storage (Costa, Civello, Chaves, & Martínez, 2006; Xu et al., 2012), while in other cases a decrement in the level of these compounds was detected (Serrano et al., 2006; Villarreal-García, Nair, Cisneros-Zevallos, & Jacobo-Velázquez, 2016).

4 | CONCLUSIONS

In previous studies, we had shown the benefits of harvesting broccoli in the last hours of the day. This practice allows that postharvest senescence elapses more slowly. In this work, we confirmed that heads collected at 18:00 hr showed a lower degreening. Additionally, we focused on two parameters that affect the nutritional quality of broccoli, proteins and antioxidants. No major changes in protein metabolism were detected in heads collected in several moments of the day or during senescence, but we could confirm that samples harvested at 8:00 had an increased protein catabolism, indicating a higher senescence rate. Regarding antioxidants, the levels of ascorbic acid, carotenoids and xanthophylls, phenols, and flavonoids were similar at the moment of harvest. However, interesting increments in the content of carotenoids, phenols, and flavonoids were observed during storage of samples collected at 18:00 hr toward day 3 and in the samples obtained at 13:00 hr toward day 5. The combination of delay of senescence and increase of antioxidants reinforces the harvest recommendation around 18:00 hr, or, if samples are stored by more time, to harvest around 13:00 hr.

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CONFLICT OF INTEREST

The authors state that there is no conflict of interest.

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