Influence of postharvest UV-C treatment on refrigerated storage of minimally processed broccoli (*Brassica oleracea* var. *Italica*)

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Abstract: Minimally processed broccoli was treated with UV-C light (8 kJ m^{-2}) and subsequently stored for 21 days at 4 °C. The UV-C treatment delayed yellowing and chlorophyll degradation during storage. Treated broccoli florets displayed lower electrolyte leakage and respiratory activity, indicating higher tissue integrity. Treated samples showed higher phenolic and ascorbic acid contents as well as higher antioxidant activity than controls. Treated samples also had a higher content of soluble sugars, but no differences in the content of soluble proteins between control and treated samples were detected. The UV-C treatment also affected bacterial and mould populations. After 21 days at 4 °C the number of colony-forming units of both populations was lower in treated than in control broccoli florets. The results suggest that UV-C treatment reduces tissue damage of minimally processed broccoli during storage at 4 °C, thus maintaining nutritional quality and reducing microbial growth.

Keywords: broccoli; UV-C treatment; cold storage; minimally processed vegetables

INTRODUCTION

Vegetables are beneficial for consumers since they represent an important source of vitamins and antioxidant compounds. Minimally processed products have the additional advantage of a shorter time of processing.[1] This fact promotes increased consumption of vegetables by consumers. Harvesting of fruits and vegetables induces severe stress due to a reduction in sources of energy, nutrients, hormones and water, leading to rapid initiation of senescence.[2] If vegetables are also processed after harvest, the products become more perishable, since they have been subjected to additional severe physical stress due to peeling, cutting, slicing, shredding, trimming, etc.[3] Moreover, processing of vegetables promotes faster microbial degradation of the products in comparison with the raw commodities.[4]

Broccoli has been described as a vegetable with a high nutritional value owing to its important content of vitamins, antioxidants and anticarcinogenic compounds.[5] Immature broccoli florets are highly perishable.[2] The inflorescences are harvested while they are totally immature, which implies severe changes in nutrient, water and hormonal status. Harvesting and subsequent processing cause severe stress, determining the appearance of accelerated senescence symptoms. Several techniques have been used to extend the postharvest life of broccoli, including refrigeration,[6] modified atmospheres[7,8] and various types of packaging.[7,9]

Recently, several new physical technologies to extend the postharvest life of fruits and vegetables have become of interest to consumers, since they do not employ chemical compounds. Among them, UV-C radiation (254 nm) has been used on various commodities.[10–12] It has been widely demonstrated that high doses of UV radiation are harmful to plants. However, if UV radiation is applied in low doses, the tissue may generate a defence mechanism, modify its metabolism and react positively to this new type of stress. This concept was termed 'hormesis' by Luckey.[13] In this sense, application of low doses of UV-C can reduce postharvest pathogen incidence in fruits such as apple, peach and grapefuit[14] and vegetables such as cabbage[15] and sweet potato.[10] Also, UV-C treatments reduce microbial populations in fresh processed vegetables.[16] These treatments can also induce profitable responses in tissues. For example, UV-C treatments can reduce the incidence of chilling injury in peppers[12] and delay senescence in tomatoes.[11] Recently, it was demonstrated that short UV-C pulses can delay postharvest senescence of intact broccoli heads at 20 °C.[17]

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In this study the effect of a low UV-C dose (8 kJ m^{-2}) on several senescence and quality parameters of minimally processed broccoli florets during storage at 4 °C was analysed.

MATERIALS AND METHODS

Plant material

Broccoli (*Brassica oleracea* var. *Italica*, cv. Cicco) heads were obtained from a local producer in La Plata, Argentina and immediately transported to the laboratory for processing. The heads were separated into florets with stems and utilised for treatments.

Combination of UV-C treatment and cold storage

Broccoli florets were immersed in chlorinated water (150 mg sodium hypochlorite L^{-1}) for 15 min. Then approximately 100–120 g of broccoli florets were placed vertically in plastic trays in order to ensure homogeneous irradiation and exposed to light from four UV-C lamps (TUV G30T8, 30 W, Philips, Amsterdam, The Netherlands). Sixteen trays containing broccoli florets were irradiated at a distance of 30 cm with a dose of 8 kJ m^{-2}. The flux intensity of the lamps was measured with a digital radiometer (Cole-Parmer Instrument Company, Vernon Hills, IL, USA). After treatment, florets were loosely covered with PVC (15 μm thick) film to minimise water loss and stored at 4 °C for 21 days. Sixteen trays containing broccoli florets without UV-C treatment were also loosely covered with the same PVC film, stored under the same conditions and used as controls. Broccoli florets were sampled immediately after treatment and after 7, 14 and 21 days of storage. Four trays of each treatment and storage time were sampled. Samples were immediately processed or frozen in liquid nitrogen and stored at −20 °C until analysis. The entire experiment was repeated three times. Since the same trends were found, results from only one experiment are shown.

Weight loss

Trays were weighed after UV-C treatment and after 7, 14 and 21 days of refrigerated storage. Results were expressed as % weight loss relative to the initial weight.

Respiration rate

A sample of fresh broccoli florets weighing approximately 150 g was allowed to reach 20 °C, then placed in a 1000 mL jar, sealed and incubated at 20 °C for 1 h. Gas samples were withdrawn with a 1 mL syringe through a septum fitted in the lid of the jar. The CO_2 content in the jar was determined using a gas chromatograph (CX 3400, Varian, Palo Alto, CA, USA) equipped with an Alltech CTRI column (Deerfield, IL, USA) and a thermal conductivity detector. The temperatures of the injector, column and detector were set at 120, 30 and 120 °C respectively. Helium was used as carrier gas at a flow rate of 25 mL min^{-1}. Calibration was made with a mixture of 10% CO_2 and 15% O_2 and performed at five points by increasing the amounts of the mixture. Results were expressed as mg CO_2 g^{-1} h^{-1}. Two jars were prepared per condition and each jar was measured twice.

Colour measurement

The superficial colour of fresh broccoli florets was determined by measuring the parameters L^*, a^* and b^* with a chromameter (CR300, Minolta, Osaka, Japan). The hue angle was calculated as $h° = \tan^{-1}(b/a)$ when $a > 0$ and $b > 0$ or as $h° = 180° + \tan^{-1}(b/a)$ when $a < 0$ and $b > 0$. Three positions on each of 25 broccoli florets were measured per treatment and storage time.

Electrolyte leakage

Fresh broccoli florets were incubated in 150 mL of 0.6 mol L^{-1} mannitol. The conductivity of the solution was measured at the beginning of incubation (a) and after 1.25 h (b). To evaluate the residual amount of electrolytes remaining in the tissues, samples were ground in an Omnimixer (Sorvall Inc., Norwalk, CT, USA), filtered through paper and centrifuged at 16 000 × g for 10 min. The conductivity of the resultant solution was measured (c). Results were expressed as the % of electrolytes that had leaked out from the tissues after 1.25 h and calculated as $[(b - a)/(b + c - a)] \times 100$. Three replicates were analysed per treatment and storage time.

Soluble protein content

Frozen broccoli florets were ground and 0.5 g of the resultant powder was homogenised in 10 mL of buffer (50 mmol L^{-1} Tris-HCl, 2 mmol L^{-1} ethylene diamine tetraacetic acid (EDTA), 0.4 mL L^{-1} mercaptoethanol, pH 7.5). The mixture was centrifuged at 12 100 × g for 20 min at 4 °C. The soluble protein content in the supernatant was determined according to the method of Bradford,[18] using bovine serum albumin as standard. Results were expressed as mg soluble protein g^{-1} fresh tissue. Four replicates were analysed per treatment and storage time.

Chlorophyll content

Frozen broccoli florets were ground and 0.4 g of the resultant powder was stirred into 5 mL of acetone/water (80:20 v/v) and centrifuged at 5000 × g for 15 min. The chlorophyll content in the supernatant was determined according to Lichtenthaler.[19] Results were expressed as μg chlorophyll g^{-1} fresh tissue. Four replicates were analysed per treatment and storage time.

Reducing and total sugar contents

Approximately 60 g of frozen broccoli florets were ground in a refrigerated mill and 2 g of the resultant powder was homogenised in 12 mL of ethanol. The mixture was centrifuged at 12 000 × g for 15 min at

4 °C. The reducing sugar content in the supernatant was determined by the Somogyi–Nelson method.[20] For total sugar determination an aliquot of the supernatant was first hydrolysed with 100 g L^{-1} HCl for 10 min and then analysed as described above. For quantification a standard glucose solution was employed. Results were expressed as mg glucose g^{-1} fresh tissue. Four replicates were analysed per treatment and storage time.

Total phenolic compounds

Approximately 60 g of frozen broccoli florets were ground in a refrigerated mill and 2 g of the resultant powder was homogenised in 12 mL of ethanol. The mixture was centrifuged at 12 000 × *g* for 15 min at 4 °C. A sample of the crude extract (100 μL) was added to 1110 μL of water and 200 μL of a 1:1 dilution of Folin–Ciocalteu commercial reagent. After 3 min at 25 °C, 1.5 mL of a solution of 20 g L^{-1} Na_2CO_3 and 0.1 mol L^{-1} NaOH was added. The reaction mixture was incubated for 1 h at the same temperature. The absorbance was measured at 760 nm and the total phenolic content was calculated using phenol as standard. Results were expressed as mg phenol g^{-1} tissue. Four replicates were analysed per treatment and storage time.

Ascorbic acid content

Frozen broccoli florets were ground in a refrigerated mill and a 1 g sample of the resultant powder was extracted in 5 mL of an aqueous solution of citric acid (30 g L^{-1}). The mixture was centrifuged at 9000 × *g* for 10 min at 4 °C. The extract was then centrifuged at 23 600 × *g* for 5 min at 5 °C to clarify it. A high-performance liquid chromatograph (Model 6000A, Waters, Milford, MA, USA) fitted with a UV–visible detector and a C_{18} column (particle diameter 5 μm, internal diameter 4.6 mm, length 25 cm) was used to determine the ascorbic acid concentration. The mobile phase was 0.2 mol L^{-1} KH_2PO_4/H_3PO_4, pH 2.4, at a flow rate of 1 mL min^{-1}. Detection was at 254 nm. For identification and quantification a standard ascorbic acid solution (prepared fresh daily) was employed. Results were expressed as g ascorbic acid kg^{-1} fresh tissue. Four replicates were analysed per treatment and storage time.

Antioxidant capacity

The free radical-scavenging capacity of broccoli florets was determined by the procedure of Brand-Williams *et al.*[21] Frozen broccoli florets were ground in a refrigerated mill and 2 g of the resultant powder was homogenised in 12 mL of ethanol. The mixture was centrifuged at 12 000 × *g* for 15 min at 4 °C. Aliquots of the extract were added to test tubes containing 3.9 mL of 0.025 g L^{-1} 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol (prepared fresh daily). The absorbance at 515 nm was measured at different times until the reaction reached a plateau. The % of DPPH remaining was then plotted against the volume of extract to obtain the amount of extract necessary to decrease the initial DPPH concentration by 50%, defined as $EC_{50}{}^{-1}$. The antioxidant capacity was expressed as $EC_{50}{}^{-1}$. Measurements were performed in duplicate for each treatment and storage time.

Microbiological assays

Approximately 25 g of fresh broccoli florets were homogenised with 225 mL of sterile peptone saline solution (1 g peptone L^{-1}) for 15 min. From the resulting suspension, two series of dilutions from 10^{-1} to 10^{-6} were prepared and 1 mL of each dilution was seeded in the appropriate medium. For total aerobic bacteria, sterile plate count agar (Biokar Diagnostic, Allone, France) was used. Plates were incubated at 37 °C for 36 h. For yeasts and moulds, sterile peptic digest of United States Pharmacopeia (USP) (Biokar Diagnostic) meat was used. Plates were incubated at 20 °C for 120 h. Results were expressed as log colony-forming units (CFU) g^{-1} fresh tissue. Plates for total aerobic bacteria and for yeasts and moulds were analysed in triplicate for each treatment and storage time.

Statistical analysis

Experiments were performed according to a factorial design. Data were subjected to analysis of variance (ANOVA) and means were compared by the least significant difference (LSD) test at a significance level of 0.05 using the SYSTAT software package (SYSTAT Inc, Evanston, IL, USA).

RESULTS AND DISCUSSION

The usual method to retard senescence, deterioration and pathogen growth in broccoli is refrigeration,[22] even in the case of minimally processed heads.[23] Recently, it was shown that short UV-C treatment can delay senescence of intact broccoli heads at 20 °C.[17] In this study we combined short UV-C treatment with refrigerated storage of minimally processed broccoli heads. Florets were treated with a UV-C dose of 8 kJ m^{-2} and subsequently stored at 4 °C. Samples showed an average weight loss of 4% after 21 days of storage, and no significant differences were detected between control and treated samples (Table 1). The dose of UV-C used in this experiment can be considered as low and probably did not affect tissue integrity. Similarly, fruits such as tomato and pepper did not show increased water loss after UV-C treatment.[11,12]

Senescence is accompanied by tissue deterioration and damage. As the product becomes more senescent, plasma membranes lose their integrity, and solutes may be released from the cytosol. In addition, cutting can enhance damage and solute release from cells. In this experiment, after 7 days of storage there was no significant increase in electrolyte leakage in either control or UV-C-treated samples. However, at day 14 a significant increase in electrolyte leakage was

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Table 1. Weight loss, electrolyte leakage and respiration rate in control (C) and UV-C (8 kJ m^{-2})-treated (T) broccoli florets during storage for 21 days at 4 °C

Parameter		Days at 4 °C			
		0	7	14	21
Weight loss (%)	C		1.80 ± 0.21	2.98 ± 0.24	4.20 ± 0.22
($LSD_{0.05}$ = 0.25)	T		1.60 ± 0.20	2.75 ± 0.23	4.05 ± 0.17
Electrolyte leakage (%)	C	8.26 ± 3.31	6.12 ± 0.80	43.13 ± 3.06	67.23 ± 18.30
($LSD_{0.05}$ = 18.95)	T	4.40 ± 3.90	8.09 ± 0.19	8.93 ± 0.11	46.73 ± 11.40
CO_2 production (mg g^{-1} h^{-1})	C	0.35 ± 0.20	0.35 ± 0.05	0.62 ± 0.04	1.57 ± 0.25
($LSD_{0.05}$ = 0.26)	T	0.55 ± 0.22	0.35 ± 0.06	0.59 ± 0.05	1.19 ± 0.19

detected in control florets, while no change was found in treated samples. Similarly, treated florets showed lower electrolyte leakage than controls after 21 days (Table 1).

When vegetal tissues are naturally or artificially damaged, respiratory activity increases. In this study a slight increase in respiration rate was detected immediately after UV-C treatment, which may be attributed to the stressing effect of applied radiation. CO_2 production remained constant until 14 days and then increased. However, treated florets showed a respiratory activity almost 20% lower than that of control samples after 21 days (Table 1). These results suggest that UV-C radiation reduces cell damage that occurs during storage, thus maintaining better tissue integrity in treated samples in comparison with controls. A reduction in CO_2 production was also detected in intact broccoli heads irradiated with UV-C during senescence at 20 °C.[17] Moreover, short postharvest heat treatments that are able to delay senescence in broccoli heads also provoked a reduction in respiratory activity.[12,24] In the case of tomatoes, UV-C-irradiated fruits showed a delay in respiratory activity that was associated with the climacteric peak rather than with tissue deterioration.[11]

Loss of green colour is the main factor that determines quality during postharvest storage of broccoli, and most treatments have the objective of delaying this process. Harvested broccoli heads show an intense degreening and yellowing caused by degradation of chlorophylls.[25] In the present experiment, minimally processed broccoli also showed degreening and yellowing during storage, which can be evaluated through the decrement in hue and the increment in L^*. Samples treated with UV-C showed lower visible yellowing, with higher values of hue (Fig. 1(b)) and lower values of L^* (Fig. 1(a)) in comparison with control samples. These results correlate with the higher chlorophyll retention measured in treated broccoli florets (Fig. 1(c)). In a related work, UV-C-treated broccoli heads showed lower activities of chlorophyllase and Mg-dechelatase, two of the enzymes involved in chlorophyll catabolism.[17] Previous studies have demonstrated that chlorophyllase activity is stimulated by ethylene[26,27] and that UV-C light treatments reduce ethylene production in many vegetables.[11,14] The possible reduction in ethylene production could partially explain the delay in degreening and yellowing in UV-C-treated samples.

An interesting parameter from the nutritional point of view is the level of antioxidants. These compounds may play a critical role in human health maintenance by preventing oxidative damage to molecules and ultrastructural components of cells. In this study, antioxidant activity remained almost constant in both control and UV-C-treated broccoli florets until day 14. However, at day 21 a slight increase was detected in treated samples and a slight decrease in control samples (Fig. 2(a)), resulting in a significant difference in the level of antioxidant capacity between control and treated samples. Similarly, Costa *et al.*[28] found that UV-B radiation induces an antioxidant system defence in sunflower cotyledons, while Boveris *et al.*[29] suggested that UV-C light can increase the antioxidant capacity in soybean cotyledons. Storage of broccoli florets for 21 days at 5 °C produced almost no change in antioxidant capacity,[30] while this parameter decreased after 5 days of storage at 20 °C.[17] It is probable that storage for 5 days at 20 °C induced a more intense senescence than storage for 21 days at 4 °C, leading to a higher reduction in antioxidant capacity.

Ascorbic acid (AA) and phenolics are compounds involved in the maintenance of antioxidant status. The content of phenolic compounds decreased after 14 and 21 days of storage in control samples, while it remained constant until day 14 and increased at day 21 in UV-C-treated florets (Fig. 2(b)). Stevens *et al.*[31] and Brown *et al.*[15] reported an increment in the activity of phenylalanine ammonia lyase (PAL) in peaches and cabbage seeds respectively after treatment with UV-C. Since PAL is one of the key enzymes in phenolic synthesis, the increment in the content of phenolic compounds in treated samples could be related to enhanced PAL activity. In contrast, the content of AA diminished in both control and treated florets during storage, reaching levels about 50% lower than the initial values (Fig. 2(c)). However, treatment with UV-C induced a lower rate of decrement in AA. Therefore treated samples showed significantly higher levels than control florets at the end of the experiment (21 days). The content of AA was found to decrease in broccoli heads during storage at 15 °C[32] and 20 °C.[33] On the other hand, no change was

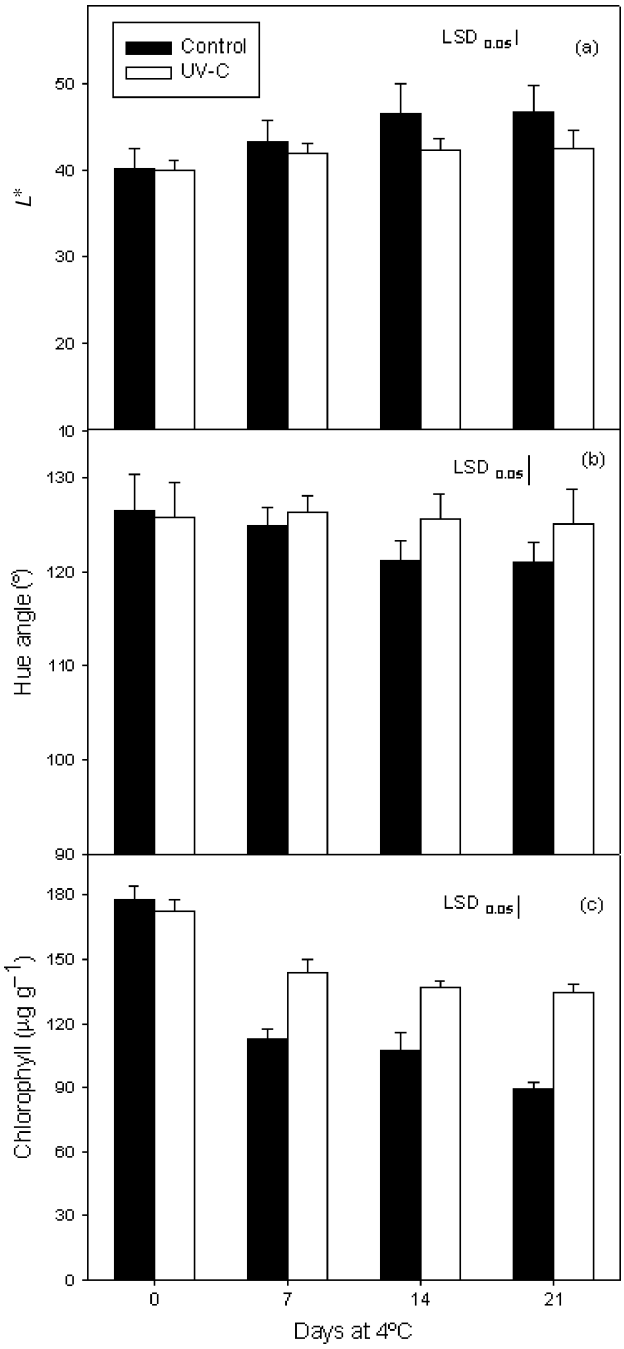

Figure 1. Changes in (a) lightness (L^*), (b) hue angle and (c) chlorophyll content in control and UV-C (8 kJ m^{-2})-treated broccoli florets during storage for 21 days at 4 °C ($LSD_{L^*} = 1.25$, $LSD_{hue\ angle} = 2.12$, $LSD_{chlorophylls} = 11.37$).

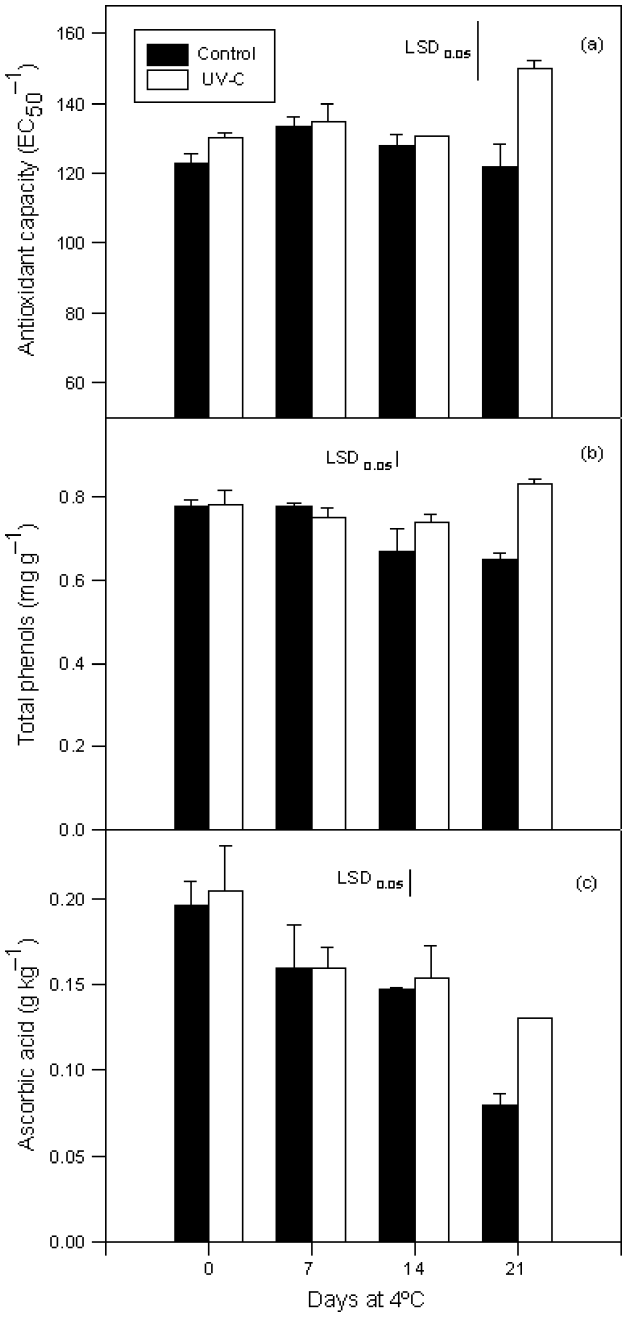

Figure 2. Changes in (a) antioxidant capacity, (b) total phenol content and (c) ascorbic acid content in control and UV-C (8 kJ m^{-2})-treated broccoli florets during storage for 21 days at 4 °C ($LSD_{antioxidant\ capacity} = 8.33$, $LSD_{total\ phenols} = 0.0396$, $LSD_{ascorbic\ acid} = 0.016$).

observed in the content of AA in broccoli stored for 21 days at 4 °C.[34] These differences could be due to the cultivars used in the experiments. Taken together, the higher antioxidant capacity of treated samples could be related to their higher levels of AA and phenolic compounds compared with controls. However, the slight increase in antioxidant capacity in treated samples after 21 days could not be attributed to the levels of phenolic compounds and AA, since the former did not change and the latter diminished during storage. It is possible that another compound (or perhaps more than one) with antioxidant capacity increased in content during storage.

In addition to chlorophyll degradation, important compositional changes occur in broccoli during senescence. The content of total proteins, sugars and fatty acids decreases[35,36] and products of oxidation accumulate. In the present work, total sugar content diminished during storage and no significant differences were detected between control and UV-C-treated samples (Fig. 3(a)). Reducing sugar content also decreased in both control and UV-C-treated florets during storage (Fig. 3(b)), but

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the decrement was lower in treated florets. At the end of storage, treated samples had a level of reducing sugars approximately 38% higher than that in controls. The lower level of reducing sugars in control samples could be due to their higher respiratory activity, particularly at the end of storage. However, no differences in the content of total sugars were detected between control and UV-C-treated florets, indicating higher levels of non-reducing sugars in controls. Sánchez-Mata *et al.*[37] and Able *et al.*[38] found a reduction in reducing sugars and an increment in non-reducing sugars during storage of green beans and pak choy leaves respectively. They assumed that the latter increment would be due to starch degradation, as measured by Able *et al.*[38] Generally, these processes involve interconversions among different carbohydrates. In the present work, if starch is also degraded, then production of non-reducing sugars can occur during storage and senescence. In this sense, intense degradation of starch has been detected during postharvest senescence of broccoli florets;[39,40] moreover, transient accumulation of non-reducing sugars has also been described by Finger *et al.*[40] If this is the case, the lower rate of senescence observed in UV-C-treated florets could lead to lower production of non-reducing sugars, as reflected by the lower level of these compounds measured at the end of storage.

During senescence, loss of integrity and functionality of membranes may lead to solubilisation of anchored membrane proteins.[41] We found a slight increment in the content of soluble proteins during storage at 4 °C, similar to results previously reported by Masih *et al.*[42] in minimally processed broccoli stored at 12 °C and by Costa *et al.*[17] in intact broccoli heads stored at 20 °C. However, we did not detect significant differences in soluble protein content between control and UV-C-treated samples (Fig. 3(c)).

Manipulation of vegetables induces faster microbial growth in relation to non-processed products. We detected an increment in the microbial populations of total bacteria and moulds during refrigerated storage (Fig. 4). However, counts of both populations were higher in control samples than in UV-C-treated florets. Application of UV-C can reduce the incidence of several diseases, as observed in peppers,[12] or specific diseases such as brown rot in peaches[31] and black rot in cabbage.[15] UV-C light can produce a direct effect on pathogen viability or germination of conidia, as shown by Pan *et al.*,[43] because of the intense damage that radiation induces in cellular structures. However, an indirect effect of UV-C radiation could also mediate in the reduction of pathogen growth. Previous studies have found that UV-C treatments induce PAL, a key regulatory enzyme in phenolic metabolism[44,45] that is necessary for the synthesis of several antimicrobial compounds. Treatments with UV-C induce the formation of phytoalexins, compounds that participate in plant defence mechanisms, such as resveratrol in grapes[46] and hydroxyphaseollin in soybean.[47]

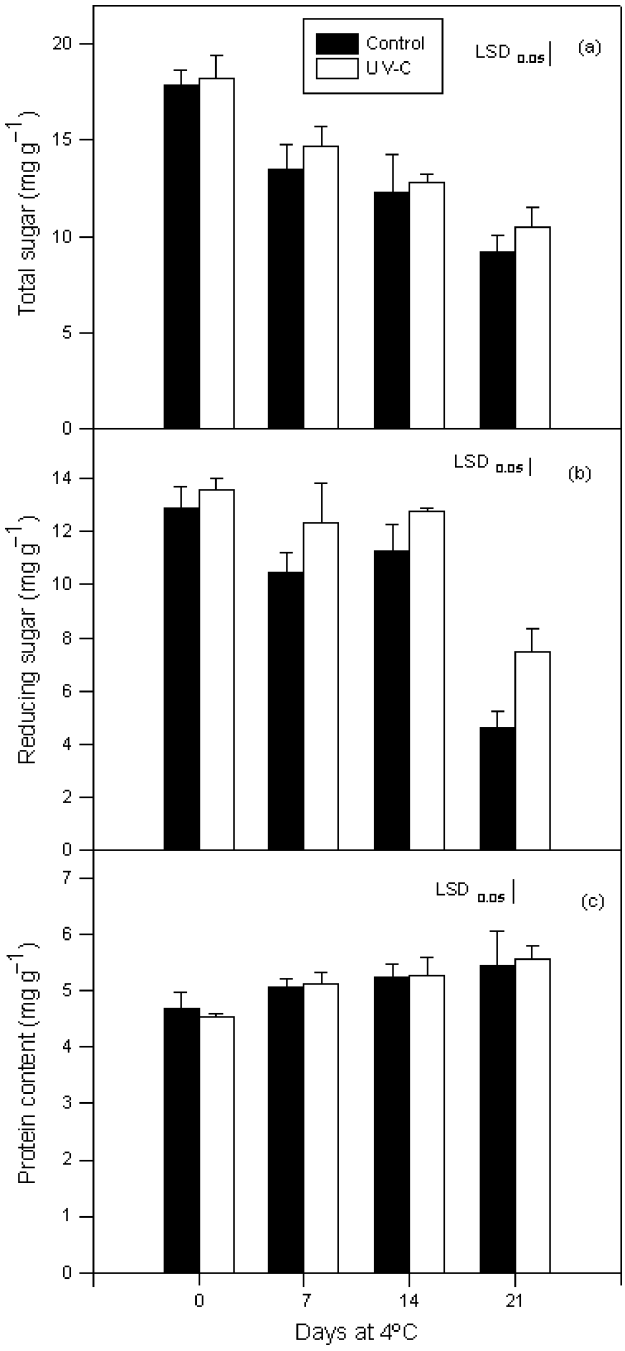

Figure 3. Changes in (a) total sugar, (b) reducing sugar and (c) soluble protein contents in control and UV-C (8 kJ m^{-2})-treated broccoli florets during storage for 21 days at 4 °C ($LSD_{total\ sugars} = 1.31$, $LSD_{reducing\ sugars} = 0.936$, $LSD_{soluble\ proteins} = 0.45$).

CONCLUSIONS

This study has shown that UV-C treatment at 8 kJ m^{-2} can delay the postharvest deterioration of minimally processed broccoli florets during storage at 4 °C. The UV-C treatment contributed to the conservation of tissue integrity, as indicated by the lower electrolyte leakage and respiratory activity in treated florets, probably by maintaining a higher antioxidant status. Moreover, degreening, chlorophyll degradation and protein solubilisation were also delayed by the UV-C treatment. Finally, the direct

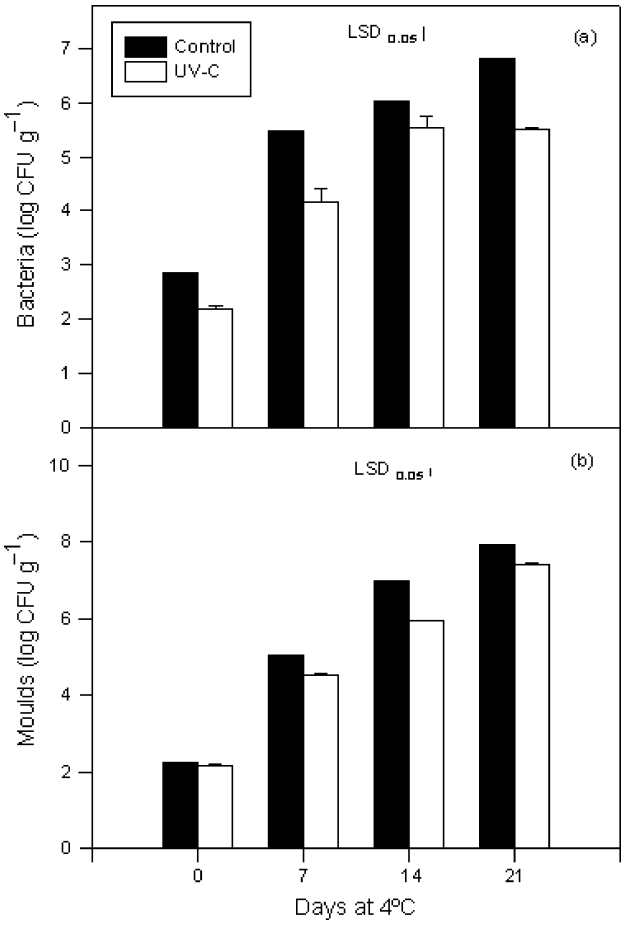

Figure 4. Changes in number of colony-forming units (log CFU g^{-1}) of (a) total bacteria and (b) moulds in control and UV-C (8 kJ m^{-2})-treated broccoli florets during storage for 21 days at 4 °C ($LSD_{bacteria} = 0.22$, $LSD_{moulds} = 0.07$).

or indirect effect on microbial growth as well as the increased antioxidant capacity showed that UV-C treatment could be beneficial to maintain the quality of minimally processed broccoli florets.

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