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Effect of combined treatment with hot air and UV-C on senescence and quality parameters of minimally processed broccoli (*Brassica oleracea* L. var. *Italica*)

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Abstract

Combined treatments with hot air and UV-C were applied to minimally processed broccoli (*Brassica oleracea* L.) florets to investigate their effects on several quality and senescence parameters. To select the optimum treatment, florets were treated with three temperatures (42, 45 and 48 °C) for 3 h and three UV-C doses (5, 8 and 10 kJ m^{-2}) in all combinations and then placed in darkness at 20 °C. In general, results suggest that the effect of heat was more important than that of UV-C to prolong postharvest life of broccoli florets. Treatment at 48 °C combined with a UV-C dose of 8 kJ m^{-2} caused the higher retention of green color and the higher maintenance of organoleptical quality and was chosen to analyze its effect on other parameters during storage. The selected combined treatment delayed both yellowing and chlorophyll degradation during storage. Moreover, treated broccoli florets showed a higher retention of protein content in relation to controls. The treatment did not affect the total content of sugars but greatly reduced the level of reducing sugars. Results indicate that a combined treatment of UV-C and heat could be a useful method to delay postharvest senescence of minimally processed broccoli during storage at 20 °C.

Keywords: Broccoli; UV-C treatment; Heat treatment; Minimally processed vegetables

1. Introduction

Fresh fruits and vegetables have both important nutritional and economic value. Recently, the market demand for minimally processed fruits and vegetables has undergone an important rise because of busy lifestyles, increasing purchasing power and increasing health-conscious consumers (Baldwin et al., 1995).

Harvesting of fruits and vegetables induces a severe stress due to a reduction in the sources of energy, nutrients, hormones and water; and leads to a rapid initiation of senescence (King and Morris, 1994). Besides, if vegetables are also processed after harvest, then the product becomes higher perishable than intact ones since they have been subjected to severe physical stress, such as peeling, cutting, slicing, shredding, trimming, etc. (Watada et al., 1996).

Broccoli has been described as a vegetable with a high nutritional value due to its important content of vitamins, antioxidants and anti-carcinogenic compounds (Nestle, 1998). Broccoli inflorescences are harvested while they are totally immature, which implies severe changes in nutrient, water and hormonal status. Harvesting and the following processing cause a severe stress determining the appearance of accelerated senescence symptoms.

The use of chemical compounds to extend postharvest life of fruit and vegetables has become lesser accepted by consumers since these compounds may be contaminant of the environment or harmful to human health. Recently, new physical technologies to extend postharvest life of fruit and vegetables have become of interest. Among them, heat treatments and UV-C radiation have been used in different products. Heat has been used successfully in minimally processed vegetables like onion (Hong et al., 2000), celery (Viña and Chaves, 2007), leek (Tsouvaltzis et al., 2006), cantaloupe (Luna-Guzman et al., 1999) and garlic (Cantwell et al., 2003). Treatments with UV-C have been utilized to extend

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postharvest life of products like fresh processed lettuce (Allende and Artés, 2003) and pomegranate (López-Rubira et al., 2005). Moreover, combination of these two treatments has been applied in products like strawberry (Pan et al., 2004).

Both heat (Costa et al., 2005), and UV-C (Costa et al., 2006; Lemoine et al., 2007) treatments have been used separately to extend the postharvest life of broccoli. In this work, we analyzed the effect of a combined treatment of heat and UV-C on several quality and postharvest parameters of minimally processed broccoli florets during storage at 20 °C.

2. Materials and methods

2.1. 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 and processed. Heads were separated into florets and stems and utilized for treatments.

2.2. Selection of UV-C treatments and heat treatments combination

The broccoli florets were immersed in chlorinated water (150 ppm as sodium hypochlorite) during 15 min at 15 °C. After that, approximately 100–120 g of broccoli florets were placed vertically in plastic trays in order to assure a homogeneous irradiation on florets and exposed to light from four UV-C lamps (TUV G30T8, 30 W, Philips). Trays containing broccoli florets were irradiated at a distance of 30 cm with doses of 5, 8 and 10 kJ m^{-2}. The flux intensity of lamps was measured with a digital radiometer (Cole-Parmer Instrument Company, Vernon Hills, IL, USA). Immediately after UV-C treatments, florets were loosely covered with a PVC film (15 μm thick) to decrease water loss, placed in a heater and treated with hot air at 42, 45 and 48 °C for 3 h. After treatments trays were stored at 20 °C for 5 d. Sixteen trays were utilized for each UV-C and heat treatment combination. The same number of trays containing broccoli florets without treatments were stored in the same conditions and used as controls. Broccoli florets of four trays of each combination treatment were sampled immediately after the treatments and during storage. For treatment selection, weight loss, color measurement and organoleptical quality were determined. The entire experiment was repeated three times and, since the same trends were found, results from only one experiment are shown.

2.3. Optimal UV-C and heat combination treatments

A group of 16 trays containing broccoli florets were treated as described in the previous paragraph in order to obtain a dose of irradiation of 8 kJ m^{-2} and a heat treatment of 48 °C for 3 h. Another group of 16 trays containing broccoli florets were not treated and utilized as controls. The two groups of trays were stored in the same conditions at 20 °C during 4 d. Broccoli florets were sampled immediately after the treatments and after 2, 3 and 4 d of storage. Samples were immediately processed or frozen in N_2(l) and stored at −20 °C until analysis. The entire experiment was repeated three times and, since the same trends were found, results from only one experiment are shown.

2.4. Weight loss

The trays were weighed after the treatments and during storage at 2, 3, and 4 d. Results were expressed as percentage of weight loss relative to the initial weight.

2.5. Loss of organoleptic quality

Organoleptic quality (OQ) of samples was determined through the evaluation of seven parameters. Each parameter was scored on a scale with the following reference points: condensation inside the packaging (1 without, 2 with); dehydration (0 without, 1 incipient, 2 regular, 3 severe); visual moulds development (0 absence, 1 slightly visible presence, 2 from 10% to 40% of surface with moulds, 3 more than 40% of surface with moulds); exuded presence (1 without, 2 with); unpleasant smell (1 no off-odors, 2 presence of off-odors); browning (0 without, 1 slightly visible, 2 from 10% to 40% of surface with browning, 3 more than 40% of surface with browning); and yellowing (0 absence or 0% of surface with yellowing, 1 from 0% to 25% of surface with yellowing; 2 from 25% to 75% of surface with yellowing, 3 more than 75% surface with yellowing). Each floret was evaluated individually for each of seven described parameter by a semi-trained panel of six persons. These values were averaged to obtain the sensory score of an individual floret. The sensory scores of florets from a tray were averaged to obtain the sensory score of an individual tray. Finally, the sensory scores of individual trays were averaged to obtain the sensory score corresponding to samples of a particular treatment and day of storage. A lesser score (OQ) indicates a better organoleptic quality.

2.6. Superficial color

Superficial color of fresh broccoli florets was determined by measuring parameters L^*, a^*, and b^* with a chromameter which covered an area of 8 mm^2 (Minolta CR300, Osaka, Japan). The hue angle (h°) was calculated as $h^\circ = \tan^{-1}(b/a)$, when $a>0$ and $b>0$, or as $h^\circ = 180^\circ + \tan^{-1}(b/a)$ when $a<0$ and $b>0$. Three positions on each of 25 broccoli florets were measured for each treatment and storage time.

2.7. Chlorophyll content

Frozen broccoli florets were crushed by a mill and 0.4 g of the obtained powder was poured into 5 mL of acetone:water (80:20), stirred and then centrifuged at 5000 × *g* for 15 min. The supernatant was used to determine the content of chlorophyll according to Lichtenthaler (1987). Results were expressed as chlorophyll mass on a fresh weight basis (mg kg^{-1}). Four replicates were done for each treatment and storage time.

2.8. Total protein content

Frozen broccoli florets were crushed in a mill and 0.4 g of the powder obtained was homogenized with 10 mL of 0.1 mol L^{-1} NaOH containing 10 g L^{-1} sodium dodecyl sulfate (SDS) and heated in a boiling water bath for 10 min. Samples were centrifuged at 10,000 × *g* for 20 min at 4 °C and protein were then precipitated from the supernatant with 100 g L^{-1} trichloroacetic acid. Samples were left in ice water for 1 h and then centrifuged at 12,000 × *g* for 10 min at 4 °C. The pellet was suspended in 0.2 mL of 0.1 mol L^{-1} NaOH containing 10 g L^{-1} (SDS), and protein content was measured by the method of Lowry et al. (1951). Bovine serum albumin was used as standard. Results were expressed as protein mass on a fresh weight basis (g kg^{-1}). Four replicates were analyzed for each treatment and storage time.

2.9. Soluble protein content

Frozen broccoli florets were crushed in a mill and 0.5 g of the resultant powder were suspended and homogenized with 10 mL of buffer (50 mmol L^{-1} Tris–HCl, 2 mmol L^{-1} EDTA, 0.04% (v/v) mercaptoethanol, pH 7.5). The mixture was centrifuged at 12,100 × *g* for 20 min at 4 °C. The supernatant was used to determine the content of soluble protein according to Bradford (1976), using bovine serum albumin as standard. Results were expressed as soluble protein mass on a fresh weight basis (g kg^{-1}). Four replicates were analyzed for each treatment and storage time.

2.10. Reducing and total sugars content

Approximately 60 g of 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 × *g* for 15 min at 4 °C. The supernatant was utilized to determine the content of reducing sugars by using the Somogyi–Nelson method (Nelson, 1944). For total sugar determination, an aliquot of the supernatant was mixed with 2 mL of 0.5 g L^{-1} anthrone in 660 g L^{-1} H_2SO_4. The mixture was heated for 10 min at 100 °C. Samples were then cooled in ice water and absorbance at 620 nm was measured. For quantification, a standard glucose solution was employed. Results were expressed as glucose mass on a fresh weight basis (g kg^{-1}). Four replicates were analyzed for each treatment and storage time.

2.11. 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.

3. Results

3.1. Selection of UV-C treatments and heat treatments combination

Florets obtained from minimally processed broccoli heads were treated with UV-C radiation and hot air and then stored during 5 d at 20 °C. Three doses of UV-C radiation (5, 8 and 10 kJ m^{-2}) were utilized in all possible combinations with three temperatures (42, 45 and 48 °C). Immediately after treatments, no visible symptoms of dehydration were observed in each group of samples. During storage at 20 °C, weight loss varied from 1% to 1.5% per day in all samples. No significant differences were detected between control and 48 °C heat-treated samples, but the weight loss was significantly higher in samples treated at 42 and 45 °C. No differences were found among 5, 8, and 10 kJ m^{-2} UV-C doses in each heat treatment (data not shown).

Initial hue values ranged from 126 to 128 and decreased during storage while initial *L** values were approximately 40.5 and increased during storage (Table 1). Samples treated with 42 °C showed the same values of hue and *L** after 5 d of storage in relation to controls, regardless of the UV-C doses utilized. Treatment at 45 °C led to different results according to the dose of UV-C. After 5 d of storage, samples treated with 5 kJ m^{-2} did not show differences in relation to controls, whereas those treated with 8 and 10 kJ m^{-2} presented a higher hue and a lower *L** with respect to control. Samples treated at 48 °C showed an important retention of hue and *L** values in relation to controls after 5 d of storage. Among them, samples treated with a dose of 8 kJ m^{-2} presented a complete inhibition of the hue decrement

Table 1
Changes of hue, lightness (*L**) and organoleptic quality (OQ) in control and UV-C/heat-treated broccoli florets at 0 d (initial) and after 5 d of storage at 20 °C (LSD, $P < 0.05$; $LSD_{hue} = 3.58$; $LSD_{L^*} = 1.74$; $LSD_{OQ} = 0.97$)

Treatment	Hue		*L**		OQ	
	0 d	5 d	0 d	5 d	0 d	5 d
Control	127.63	105.31	40.66	55.93	0	3.85
42 °C–5 kJ m^{-2}	127.66	104.46	40.75	55.52	0	3.64
42 °C–8 kJ m^{-2}	126.98	108.46	40.67	54.51	0	2.94
42 °C–10 kJ m^{-2}	127.05	105.04	40.68	52.83	0	2.75
45 °C–5 kJ m^{-2}	126.97	106.45	40.58	54.17	0	2.68
45 °C–8 kJ m^{-2}	127.00	117.62	40.61	49.15	0	1.06
45 °C–10 kJ m^{-2}	127.09	120.22	40.64	47.12	0	1.43
48 °C–5 kJ m^{-2}	126.63	123.38	40.59	44.99	0	0.92
48 °C–8 kJ m^{-2}	127.72	127.70	40.66	44.80	0	0.46
48 °C–10 kJ m^{-2}	126.47	121.86	40.66	44.90	0	0.94

and showed higher values than samples treated with doses of 5 and 10 kJ m^{-2}. In the case of L^*, no differences were detected among samples treated with different UV-C doses.

The organoleptic quality of samples decreased during storage, which can be followed by the increment in the OQ score (Table 1). Treatment with 42 °C and UV-C doses of 5 and 8 kJ m^{-2} did not avoid the loss of quality but the treatment at the same temperature with a UV-C dose of 10 kJ m^{-2} reduced the increment in the OQ score. Treatment with 45 °C with the three UV-C doses significantly reduced the loss of organoleptic quality. However, the better performance was detected with the higher doses. Finally, the treatment with 48 °C showed an important retention in the OQ score regardless the UV-C utilized.

Based on weight loss, hue and L^* values and organoleptic quality, the combination of 48 °C/3 h and 8 kJ m^{-2} was selected to analyze the effect of this combination on several postharvest quality parameters of minimally processed broccoli during 4 d of storage at 20 °C.

3.2. Effect of the selected UV-C dose and heat treatment combination

3.2.1. Loss of organoleptic quality

All parameters utilized to evaluate the loss of organoleptic quality of minimally processed broccoli florets showed an increment during storage at 20 °C, which reflects a lower quality of the product. Among these parameters, the degreening and yellowing is the main cause of loss of organoleptic quality (data not shown). Control samples showed an important increment in the loss of organoleptic quality score through storage time reaching values near to 6 after 4 d of storage. Differently, treated samples showed a slight increment in the loss of organoleptic quality score during storage (Fig. 1).

3.2.2. Superficial color and chlorophyll content

Superficial color was evaluated through the hue value and L^* parameter (Fig. 2). Just after heat treatment, no difference in hue

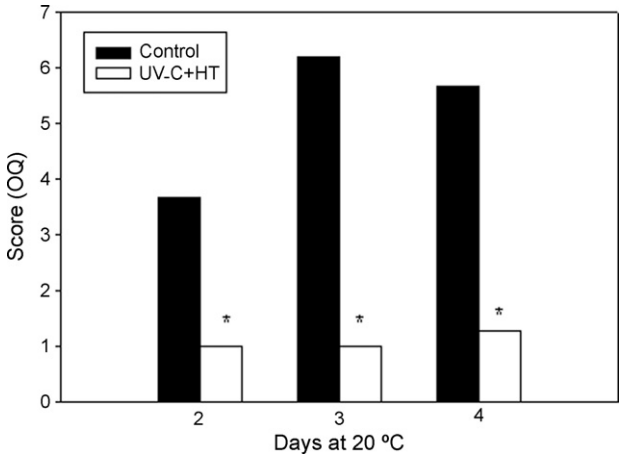

Fig. 1. Effect of combined treatment: first irradiation with UV-C light (8 kJ m^{-2}) and then heating (45 °C, 3 h in air oven) on organoleptic quality in broccoli florets stored during 4 d at 20 °C. *Value significantly different from the corresponding control (LSD, $P < 0.05$). ($LSD_{OQ} = 2.28$).

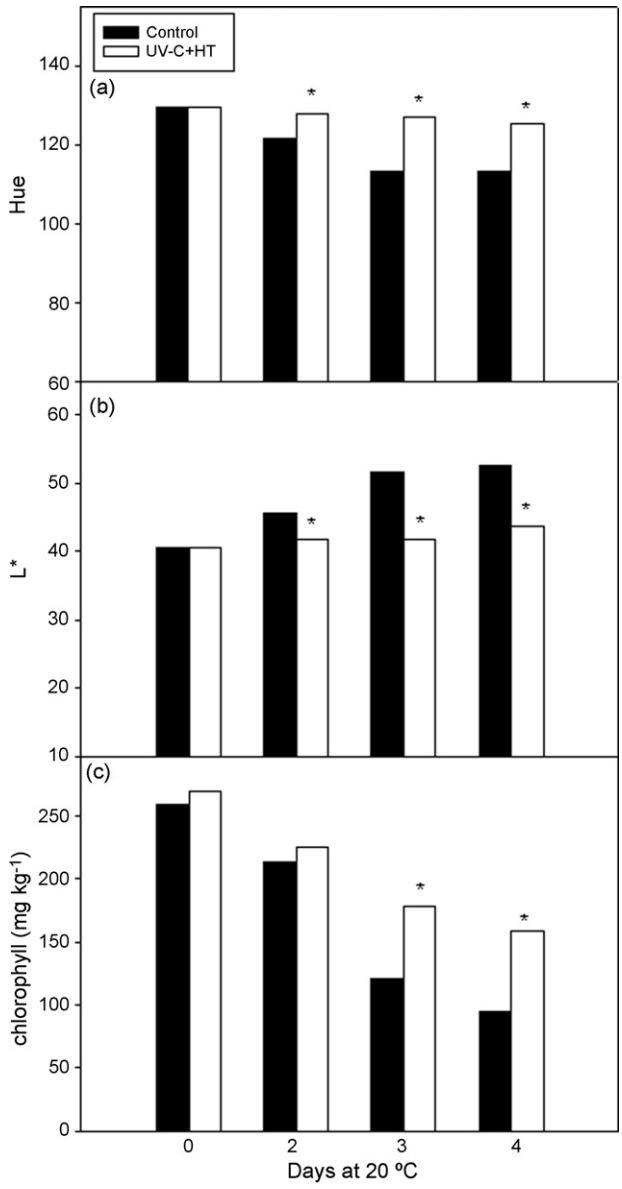

Fig. 2. Effect of combined treatment: first irradiation with UV-C light (8 kJ m^{-2}) and then heating (45 °C, 3 h in air oven) on hue angle (a), lightness (L^*) (b), and chlorophyll content (c) of broccoli florets stored during 4 d at 20 °C. Chlorophyll content was expressed as chlorophyll mass on a fresh weight basis (mg kg^{-1}). *Value significantly different from the corresponding control (LSD, $P < 0.05$). ($LSD_L = 1.47$), ($LSD_{hue} = 1.73$), ($LSD_{chlorophylls} = 23.47$).

value was observed between control and treated samples. Control samples showed an important decrease of hue during storage while treated samples showed almost no changes of this parameter. Consequently, treated samples presented significantly higher hue values than controls after 2, 3 and 4 d of storage at 20 °C. Lightness (L^*) increased significantly during storage in control samples, whereas treated samples presented only a slight increase in L^*. Again, this different behavior rendered significant differences between control and treated samples after 3 and 4 d of storage.

Chlorophyll content decreased in both control and treated samples during storage (Fig. 2). However, chlorophyll degradation rate was lower in treated samples than controls. At

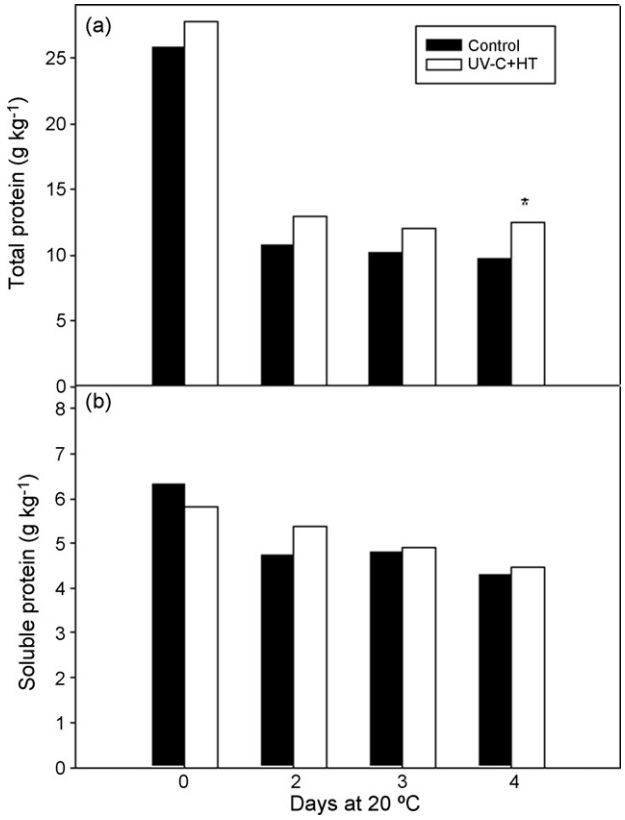

Fig. 3. Effect of combined treatment: first irradiation with UV-C light (8 kJ m^{-2}) and then heating (45 °C, 3 h in air oven) on total (a) and soluble (b) protein in broccoli florets stored during 4 d at 20 °C. Protein contents were expressed as protein mass on a fresh weight basis (g kg^{-1}). *Value significantly different from the corresponding control (LSD, $P < 0.05$). ($LSD_{total\ protein} = 2.44$), ($LSD_{soluble\ protein} = 1.04$).

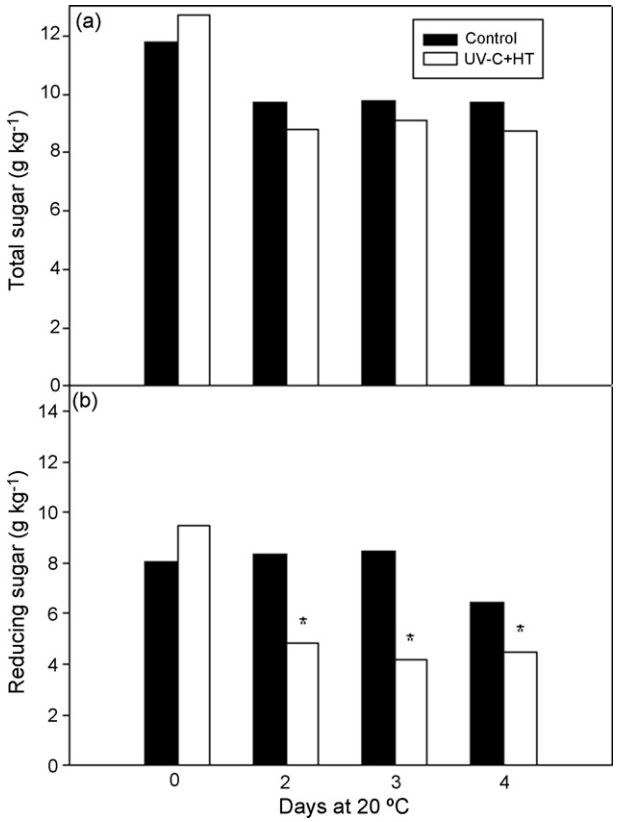

Fig. 4. Effect of combined treatment: first irradiation with UV-C light (8 kJ m^{-2}) and then heating (45 °C, 3 h in air oven) on total (a) and reducing (b) sugar content in broccoli florets stored during 4 d at 20 °C. Sugar content were expressed as glucose mass on a fresh weight basis (g kg^{-1}). *Value significantly different from the corresponding control (LSD, $P < 0.05$). ($LSD_{total\ sugar} = 1.05$), ($LSD_{reducing\ sugar} = 1.54$).

day 4, treated florets had 60% of initial chlorophyll content, while control florets presented only 38% of initial value.

3.2.3. Total and soluble protein content

Immediately after the combined treatment there were no differences in the total protein content. The total protein content diminished markedly in both treated and control samples after 2 d of storage (Fig. 3). No significant differences between control and treated samples were found, except after 4 d of storage, where the UV-C and hot air treated samples had approximately 20% more total proteins than controls.

There were not differences in the levels of soluble proteins between control and treated florets immediately after treatment (Fig. 3). A slight decrease of this parameter was measured through storage at 20 °C, but no significant differences were detected between treated and control samples.

3.2.4. Total and reducing sugars content

Immediately after treatment the total sugar content was slightly higher in treated florets but no significant differences were detected (Fig. 4). After 2 d of storage, the level of total sugar content decreased and then remained unchanged until 4 d of storage in both treated and control samples. No significant differences were detected through this period between control and treated samples.

No differences in the level of reducing sugars were detected immediately after treatment. However, during storage, treated samples showed an important decrease after 2 d and then remained constant, while control samples remained constant until day 3 and then decreased. As a consequence, controls samples showed a significant higher content of reducing sugars after 2, 3 and 4 d of storage.

4. Discussion

Harvesting of immature broccoli heads produces an important and sudden disruption of nutrient and hormone supplies, which induces an accelerated senescence (Page et al., 2001). Besides, processing such as cutting can enhance senescence and the consequent tissue damage and deterioration. Postharvest senescence of broccoli heads can be controlled by several physical methods. The use of heat treatments either with hot-water or with hot air has been successfully utilized to delay degreening (Forney, 1995; Tian et al., 1997; Funamoto et al., 2002; Costa et al., 2005). Also, treatment with hormic doses of UV-C can retard postharvest senescence in broccoli heads (Costa et al., 2006; Lemoine et al., 2007). In this work, we combined a

hot air treatment at different temperatures with several doses of UV-C radiation and analyzed their effects on superficial color and organoleptic quality. Temperature and UV-C doses of treatments were around those utilized in previous works (Costa et al., 2005, 2006; Lemoine et al., 2007). In general, the effect of heat was more important than that of UV-C. For example, at 42 °C it was not detected delay in the degreening at any UV-C doses utilized although a slight increment in the quality was detected at 10 kJ m^{-2}. As temperature of treatment increases the effect of the delay in the degreening becomes more noticeable. At 45 °C the effect of heat can be enhanced by UV-C doses of 8 and 10 kJ m^{-2}. However, at higher temperatures (48 °C) treatment with 8 kJ m^{-2} rendered better results than that of 10 kJ m^{-2}. In a previous work (Costa et al., 2005), it was showed that treatments higher than 48 °C are too high and the tissue suffers an irreversible stress that conduct to tissue deterioration. Treatment at 48 °C is near the limit of temperature that tissue can support. As a consequence, the additional treatment with UV-C (that causes a new stress) must be moderate (8 kJ m^{-2}) in order to obtain additional benefits.

Broccoli florets suffer a rapid deterioration after harvest that conducts to a loss of organoleptical quality. This includes, among other factors, visual symptoms, production of unpleasant odors, dehydration and growth of pathogens (Jones et al., 2006). We found a combination of heat and UV-C treatment that maintain the green color and drastically reduce the loss of organoleptic quality by almost 5 d at 20 °C.

The main symptom of senescence in broccoli florets is yellowing caused by chlorophyll catabolism (Tian et al., 1994). In previous works, it was showed that heat treatments can delay this process (Funamoto et al., 2002; Costa et al., 2005) and reduce the loss of superficial color. Application of postharvest heat treatments to fruits or vegetables can cause a transient and reversible inhibition of ripening or senescence (Paull, 1990; Klein and Lurie, 1991). UV-C treatments also can affect the normal catabolism of chlorophylls and maintain the green color (Costa et al., 2006). In these treatments, in which heat or UV-C treatments are applied separately, superficial degreening is reduced but not completely inhibited during storage. In the present work, the combined treatment provoked an almost complete maintenance of the superficial color (Fig. 2, panels a and b) indicating an additive effect of both treatments. The combined treatment also caused a delaying in chlorophyll degradation, but this effect was not as intense as that detected in the delay of superficial color. Probably, the effect of UV-C is mainly superficial and the internal tissues that did not receive UV-C radiation were only affected by heat treatment. During postharvest broccoli senescence it was detected an increment in the activities of enzymes involved in chlorophyll catabolism such as chlorophyllase and Mg-dechelatase (Costa et al., 2005). These increments may be delayed by heat (Funamoto et al., 2002; Costa et al., 2005) or UV-C (Costa et al., 2006) postharvest treatments.

Besides chlorophyll degradation, important compositional changes occur during senescence of broccoli. Harvesting interrupts the supply of sugars, which are necessary to maintain the normal respiration rate. As a consequence, the level of total sugars decreases during postharvest senescence. In the present work, total sugars content diminished during storage but no significant differences were detected between control and treated samples (Fig. 3a). In a previous work, heat treatments performed on intact broccoli heads induced a slight delay in the consumption of sugars (Costa et al., 2005). Differently, treatment with UV-C in minimally processed broccoli florets did not affect the rate of total sugars decrement (Lemoine et al., 2007). Processing of broccoli heads introduces an additional stress to tissues which may generate an intense respiration rate and consumptions of sugars. Heat treatments may reduce the loss of sugars in intact broccoli heads (Costa et al., 2005) but their effects may be not enough in minimally processed samples.

An interesting effect was observed in the content of reducing sugars. Both control and UV-C treated florets showed a decrease in the level of these compounds during storage, but the decrement was higher in treated florets. Samples treated only with UV-C (Lemoine et al., 2007) showed an opposing behavior, and treated samples maintained levels of reducing sugars higher than controls. However, the effect of heat is probably more intense than that of UV-C as it was showed previously (Table 1). If this is the case, then heat treatments may have inhibited enzymes that inter-convert different carbohydrates such as invertase. Several works have demonstrated that heat treatments may reduce the activities of enzymes that are normally enhanced during ripening, senescence or storage (Civello et al., 1997; Sozzi et al., 1996; Funamoto et al., 2002). Downs et al. (1997) described a slight increase in the content of glucose and fructose and a decrease in sucrose after harvest. Moreover, it was found an increase in the activity of invertase and an enhanced expression of two genes encoding acid invertase during broccoli postharvest senescence (Coupe et al., 2003). Therefore, the lower level of reducing sugars in treated samples could be due to a heat-induced reduction in the invertase activity.

Intense proteolysis occurs during postharvest senescence of broccoli (Pogson and Morris, 1997; Page et al., 2001), conducting to a reduction in the protein content. In advanced stage of senescence, loss of integrity and functionality of membranes may lead to solubilization of anchored membrane proteins (Dangl et al., 2000), which are then degraded. In a previous work (Costa et al., 2005) it was found that heat treatment delayed the overall proteolysis. However, UV-C treatments did not affect protein degradation in minimally processed broccoli heads stored at 4 °C (Lemoine et al., 2007). As a consequence, the higher protein amount found in treated florets after 4 d of storage could be due to heat treatment rather than UV-C radiation.

5. Conclusions

In this work, we analyzed the possibility of using a combined treatment with heat and UV-C to delay postharvest deterioration of minimally processed broccoli heads. Combined treatment contributed to the conservation of organoleptical quality, green color, and chlorophyll and protein content, but did not affect the reduction of the total sugar levels. The extent of broccoli life is mainly due to heat, but the irradiation with UV-C gives some additional and beneficial effects. The combination of UV-C and

heat treatments could be a useful method to prolong postharvest life of minimally processed broccoli heads during storage at 20 °C.

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