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Original article

Effect of heat treatment and refrigerated storage on antioxidant properties of pre-cut celery (*Apium graveolens* L.)

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Summary This work studies the effect of two types of heat treatment, dry air at 48 °C for 1 h and water immersion at 50 °C for 90 s, and of storage time at 0 °C on a number of quality parameters for pre-cut celery: browning potential, soluble phenols content, total flavonoids, chlorogenic acid, ascorbic acid and antioxidant capacity. Pre-cut celery was placed in crystal polyethylene terephthalate trays covered with polyvinyl chloride film. Samples were taken after 0, 1, 7, 14 and 21 storage days. Treatments reduced browning potential and chlorogenic acid content and, in addition, allowed ascorbic acid concentration to be retained for a longer time. For this reason, the application of heat treatments in minimally processed celery would be beneficial.

Keywords Antioxidant activity, *Apium graveolens* L., controlled stress, fresh cut vegetables, phenolics.

Introduction

The objectives of heat treatments, developed primarily for fruits, are to achieve insect disinfestation, to control diseases, to modify tissue response to other types of stress and to maintain product quality during storage (Paull & Jung Chen, 2000). These aims are the same as those set for the post-harvest of vegetables. To put them into practice, several time–temperature combinations must be tested in advance, taking into account that the botanical origin of edible parts (fruits, stems, petioles, leaves, buds, inflorescences and the like) is, in vegetables, considerably broader. Moreover, the optimum time and temperature combination chosen to extend fresh product quality during storage depends on cultivars, maturity stage, size and growing conditions (Fallik, 2004). In addition, selection of treatment type among heating in dry air, steam or water, may depend on product characteristics.

All biological systems are known to respond to heat treatment, inducing transcription and selective translation of a determined gene group. The induced synthesis of heat shock proteins (HSP) correlates with an improved tolerance to a number of different abiotic stress factors (Loaiza-Velarde *et al*., 1997). HSP synthesis is noticeably favoured when compared with the production of most other proteins (Vierling, 1991), so the application of a controlled thermal stress would condition vegetable tissue to withstand other stress types, amongst them those caused by cutting or senescence processes during subsequent storage.

Application of heat treatments in minimally processed products would comprise additional objectives, namely to reduce physiological alterations in the plant, induced by mechanical and oxidative damage, as well as to lessen the responses linked to cicatrisation or wounding protection (Saltveit, 2000). Modifications to phenolic metabolism and tissue antioxidant capacity constitute very common defence mechanisms in plants. Diverse phenolic compounds can be induced by biotic or abiotic stress factors, such as high light intensity, UV radiation, pathogen attack, nutritional deficiency, low temperature and mechanical damage (Dixon & Paiva, 1995). Derivatives of cinnamic acid such as caffeic, *p*-coumaric and ferulic acids, referred to collectively as hydroxycinnamic acids, and chlorogenic acid are mostly synthesised by phenylalanine ammonia lyase catalysed conversion of L-phenylalanine to *trans*-cinnamic acid, which is stimulated in response to wounding or physiological stress (Wen *et al*., 2003). Several studies have shown that chlorogenic and *iso*-chlorogenic acids, derived from phenylpropanoid metabolism, accumulate in cut iceberg lettuce tissue (Ke & Saltveit, 1989; Tomás-Barberán *et al*., 1997; Fukumoto *et al*., 2002). Moreover, phytochemicals such as flavonoids and other phenolics have

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antioxidant activity and may help to protect cells against the oxidative damage caused by free radicals (Wada & Ou, 2002).

Celery adapts easily to minimal processing but the main detrimental factors for its quality are vascular browning at the ends of cut petioles, flaring of the cut ends and development of pithiness (i.e. the formation of aerenchyma in the pith) (Saltveit & Mangrich, 1996; Loaiza-Velarde *et al.*, 2003). It has been shown that a heat-shock treatment can diminish wound-induced physiological changes leading to reduced quality (i.e. tissue browning) and shortened shelf life (Loaiza-Velarde *et al.*, 2003).

The objective of the present work was to analyse the influence of two types of heat treatment and of refrigerated storage on several chemical components contributing to the antioxidant power of pre-cut celery.

Materials and methods

Plant material and processing

Celery plants (*Apium graveolens* L.) cv Golden Boy, grown in greenhouse, were received from a La Plata grower (Province of Buenos Aires, Argentina). This is a white or self-whitening variety, widely cultivated in the zone. Once the plants reached the commercial size (after about 2 months of being transplanted), they were harvested early in the morning, brought to the laboratory and processed immediately. Leaves and basal segments of the rosettes were eliminated to obtain unbranched petioles. They were washed in running drinking water to remove any soil residues, and subsequently cut with a sharpened knife in 4-cm long sticks. These were disinfected by immersion in chlorinated water (100 ppm active chlorine, pH 6–6.5, 8 °C) for 3 min and blotted dry.

Selection of treatments

For immersion heat treatments, the following preliminary temperature–time combinations were tested: 45 °C–120 s, 50 °C–90 s and 55 °C–60 s, the storage period being of 6 days at 20 °C to speed up the manifestation of damage. Based on these results, a second stage of testing comprised immersion at 50 °C–90 s and 55 °C–30 s, with a storage time at 0 °C of 28 days. Immersion treatments were carried in heated distilled water using a thermostatic bath with permanent stirring. Celery cuts were placed in a plastic basket, and dipped during the selected times. Samples were subsequently immersed in chlorinated water with ice (100 ppm of active chlorine, pH 6–6.5) for 3 min for cooling and disinfestation. The product was packaged after eliminating the excess water by draining on absorbent paper.

With regard to air heat treatments, they were carried out after applying chlorinated water to samples as mentioned earlier. Once disinfested, the product was treated in a heated air oven for combinations of 48 °C–60 min and 50 °C–20 min, followed by storage at 0 °C for 28 days. Once air treatments were finished, samples were allowed to cool at room temperature before packaging.

To evaluate and select the diverse treatments, their effect on sensorial attributes and damage development was considered (specially rot, yellowing and softening).

Selected treatments and storage conditions

The selected treatments to be studied in this work are: (i) control sample (C), i.e. not exposed to heat treatment; (ii) thermally treated product by immersion in water at 50 °C for 90 s (I, immersion); (iii) thermally treated product in dry-heated air (oven) at 48 °C for 1 h (HA, hot air).

In all tests, trays 17-cm long, 13-cm wide and 5-cm deep were used, which were made of crystal polyethylene terephthalate (PET) covered with self-adhering polyvinyl chloride (PVC) film (thickness, 10 μm; O_2 permeability, 11 232 $cm^3\ m^{-2}\ atm^{-1}\ day^{-1}$; CO_2 permeability, 48 552 $cm^3\ m^{-2}\ atm^{-1}\ day^{-1}$; water vapour permeability, 40 $g\ m^{-2}\ day^{-1}$). The trays contained 175 g of product and were kept for 3 weeks in a cold store at 0 °C with a relative humidity of 85%. Samples were taken for analysis at 0, 1, 7, 14, and 21 days. The whole experiment was repeated twice.

Chemical analysis

For each sampling point, the material coming from three trays was combined and homogenised. Immediately before the analysis, part of the pool was frozen in liquid N_2 and crushed in a laboratory mill (Janke & Kunkel Ika Labortechnik A10, Staufen, Germany). From this material, subsamples of exact weight were taken to carry out the corresponding determinations.

Browning potential

Extraction was performed with ethanol 96° and absorbance (320 nm) of the solutions was measured (Loaiza-Velarde *et al.*, 1997). Extractions and determinations were carried out in duplicate and final results were expressed as absorbance units (AU) per gram of fresh tissue.

Total phenols content

Aliquots (20 mL) of the alcoholic extracts were concentrated at reduced pressure (30 mm Hg, 40 °C) in a rotary evaporator R-124 (Büchi Labortechnik AG, Flawil, Switzerland), until dryness. Residues were resuspended in doubly distilled water. Total phenols were quantified employing the Folin-Ciocalteu reagent (Swain & Hillis,

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1959), whereas absorbance readings were taken at 760 nm. Catechin was used as standard in the 3.75–12.75 μg mL^{-1} concentration range. Duplicate extractions and determinations were conducted and final results were expressed as μmol g^{-1} of fresh tissue.

Chlorogenic acid concentration

This determination was carried out as reported in a previous work (Viña & Chaves, 2006). Samples were extracted and concentrated as mentioned earlier. Here, residues were resuspended in 1 mL high-performance liquid chromatograph (HPLC) grade methanol, and analysed in a Waters Model 6000A (Milford, MA, USA) HPLC, fitted with UV–VIS detector. A C_{18} column was employed (particle diameter 5 μm; internal diameter 4.6 mm; length 25 cm), using an 85:10:5 mixture of water:methanol:formic acid as running solvent. A flow rate of 1 mL min^{-1} was used. Detection was conducted at 320 nm. A standard solution of chlorogenic acid with a concentration of 0.87 μg mL^{-1} was used both to identify and quantify this compound. The UV–VIS spectrum of the fraction resulting from chromatographic runs was compared with the standard solution to confirm identification. Extractions and determinations were conducted in duplicate and results were expressed as nmol g^{-1} of fresh tissue.

Total flavonoids content

It was determined by the technique described by Kim *et al.* (2003), with modifications. Samples were extracted, concentrated and resuspended in doubly distilled water as described earlier. To prepare reaction mixtures, a test tube was added with 1500 μL of doubly distilled water and 500 μL of the concentrated samples. Other compounds were added sequentially: initially (zero time) a volume of 150 μL of 5% $NaNO_2$; after 5 min, 150 μL of 10% $AlCl_3$ and finally, after additional 6 min, 500 μL of 1 M NaOH. Solutions were mixed by stirring in a vortex and then absorbance at 510 nm was measured. A standard curve was constructed based on catechin concentrations in the range of 7.5–36.6 μg mL^{-1}. Extractions and determinations were conducted in duplicate. Total flavonoid levels in the samples were expressed as nmol g^{-1} of fresh tissue.

Ascorbic acid content

A modified version of the method proposed by Wimalasiri & Wills (1983) was used. Samples were taken from the homogenised-frozen-crushed material, each weighed accurately to 3 g and extracted with 5 mL of aqueous solution of 3% citric acid. After 10 min, they were centrifuged at 11 500 ***g*** for 5 min at 5 °C. Aliquots of 1 mL from each extract were centrifuged again in an Eppendorf 5415C equipment for 2 min at 14 000 r.p.m. The same HPLC equipment described in 'Chlorogenic acid concentration' section was used, with the same column. In this determination, however, the mobile phase was a 70:30 mixture of acetonitrile:water with 0.01 M $NH_4H_2PO_4$ and pH adjusted to 4.3 with orthophosphoric acid. Flow rate was 2 mL min^{-1}, detection being carried out at 254 nm. For identification and quantification, a standard ascorbic acid solution of 35 μg mL^{-1} was employed. Extractions and determinations were carried out in duplicate and final results were expressed as milligram of ascorbic acid per 100 g of fresh tissue.

Antioxidant power

Samples previously frozen in N_2 and crushed were treated with 5 mL of methanol. The antioxidant power (AP) of the extracts was determined by reaction with the stable radical 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) in a methanol solution, using a modified version of the method proposed by Brand-Williams *et al.* (1995). Concentration of the extracts was varied in the reaction mixtures adding 0, 200, 400, 600, 800 or 1000 μL of each of them to a 3.9 mL methanol solution of $DPPH^{\bullet}$ (25 ppm), completing a final volume of 4.9 mL with methanol. The reaction was allowed to progress and absorbance was measured at 515 nm after a constant value was reached. Then, $DPPH^{\bullet}$ was calculated through a calibration straight line obtained in a range of concentrations of this substance. Finally, the remaining $DPPH^{\bullet}$ concentration was plotted as a function of the extract volume in the reaction mixture, to calculate EC_{50} (effective mean concentration) for each sampling point. EC_{50} was defined as the mass (grams) of tissue required to reduce $DPPH^{\bullet}$ concentration to half its initial value. Extractions and determinations were carried out in duplicate. Final results were expressed as AP, defined as the reciprocal of EC_{50} ($AP = 1/EC_{50}$).

Statistical analysis

All data were treated by analysis of variance (ANOVA). Sources of variation were time (five levels) and treatment (three levels). Means were compared using Fisher's least significant difference (LSD) test. Differences at $P < 0.05$ were considered significant.

Results and discussion

Selection of treatments

Concerning immersion treatments, the time–temperature combination 50 °C–90 s led to lower damage by pathogens and a good retention of surface colour in samples stored at 20 °C (data not shown). By using 55 °C–60 s, pieces retained their green colour, but rot and softening were noticeable. Therefore, to proceed further with conservation tests at 0 °C, the treatment at

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Table 1 Incidence of damage in pre-cut celery treated by water immersion or hot air after 28 storage days at 0 °C

Treatment	Temperature and time combination	Soft rot incidence (%)	Yellowing	Softening
Control	–	3	++	–
Immersion	50 °C–90 s	4	–	–
	55 °C–30 s	8	–	+
Hot air	48 °C–1 h	4	+	–
	50 °C–20 min	7	++	+

–, unaffected product; +, moderately affected product; ++, seriously affected product. Percentage of soft rot incidence was calculated by the number of pieces affected related to the total number of inspected pieces in each sampling point.

50 °C for 90 s was chosen; besides the exposure time of the 55 °C treatment was reduced from 60 to 30 s. Again, the treatment with the best results was 50 °C–90 s (Table 1). With respect to heated air treatments, the 48 °C–1 h combination caused lower yellowing and minimised firmness losses (Table 1).

For these reasons, the treatments selected to study their effect on the antioxidant properties of pre-cut celery were immersion in water at 50 °C for 90 s and heated air at 48 °C for 1 h.

Browning potential

Figure 1 shows the results in this topic. No significant differences of browning were observed between the controls and the thermally treated pieces immediately after heat stress by immersion or dry air application.

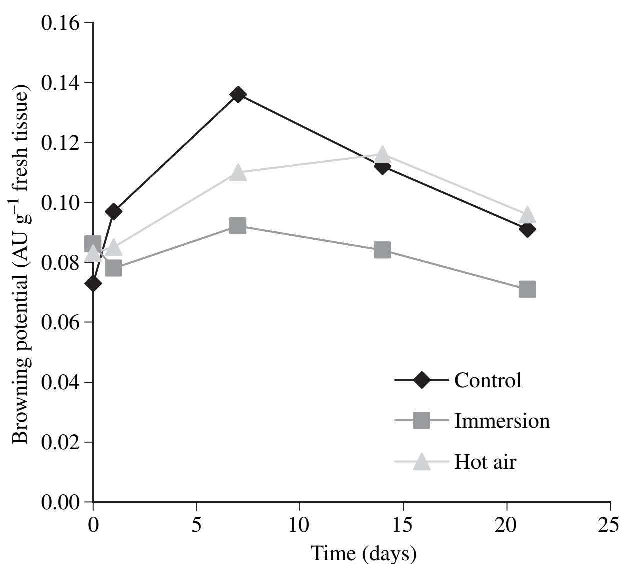

Figure 1 Browning potential of pre-cut celery exposed to heat treatments and stored for 21 days at 0 °C ($LSD_{0.05} = 6 \times 10^{-3}$).

Initial values averaged 0.08 AU per gram of fresh tissue. A significant increase in browning potential was observed for control samples over the first week of storage ($P < 0.05$), reaching a maximum at day 7, of almost twice the initial value. From then on, there was a tendency for browning to decrease, though nevertheless the browning potential in the untreated product stored at 0 °C for 21 days was significantly higher ($P < 0.05$) than its initial level. In immersion-treated samples, browning potential was mostly constant ($P > 0.05$) over the first two weeks in cold store, to reduce towards day 21, to values slightly below the initial value. Samples treated in hot air experienced a significant increase ($P < 0.05$) of browning potential up to 14 days of storage. At this time, a maximum of 1.4 times of the initial value was reached.

In our experiments, the initial browning potential values were 2.5 times as low as those found by Loaiza-Velarde *et al.* (2003). These authors published browning potentials of 0.2 AU per gram of fresh tissue for petiole pieces 5 mm in length, increasing up to 0.6 AU per gram fresh tissue after 5 weeks at 0 °C. Besides, for immersion-treated samples at 50 °C for 90 s, the increase in browning potential was delayed by 3 weeks compared with the controls (Loaiza-Velarde *et al.*, 2003), showing a similar trend as that observed in our experiments, where the immersion treatment hindered browning. Our results showed, in turn, that the hot air treatment was less effective. In spite of the preceding facts, enzymatic browning in the variety under study here (Golden Boy) did not produce severe damage because, although there was some development in the stored product (control and treated), manifestations were highly localised (brown-orange spots coincident with exposed vascular strands).

Total phenols

Figure 2 shows total phenol concentration in cut celery as a function of time. Total phenol content in samples, measured immediately after applying immersion and hot air treatments, was slightly higher than that for the control, though not different enough to be statistically significant ($P > 0.05$). Such content averaged 0.20 μmol g^{-1} of fresh tissue at the beginning of storage. Instead, no significant differences were found between measured values during storage, nor with respect to their initial values. The same behaviour was found in treated samples. In the three cases, a tendency of total phenol content to increase was observed up to day 14. Loaiza-Velarde *et al.* (1997) have analysed results from immersion heat treatments applied to lettuce, and found combinations of 50 °C–90 s and of 55 °C–60 s to slow down the increase of phenolic compounds concentration with respect to the control, within 72 h of storage at 10 °C.

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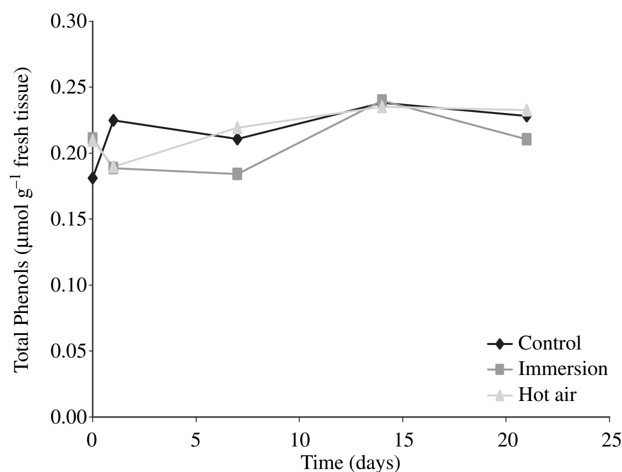

Figure 2 Total phenols in pre-cut celery experiencing heat treatment and stored for 21 days at 0 °C ($LSD_{0.05} = 0.06$).

Chlorogenic acid

Figure 3 shows that chlorogenic acid levels in cut celery immediately after both thermal treatments was somewhat higher than in controls though the variations were not significant ($P > 0.05$). Initial values were within 14.1 and 21.2 nmol g^{-1} of fresh tissue and the controls experienced a rapid increase in chlorogenic acid, reaching twice the initial concentration after 24 h at 0 °C. Maximum concentration was observed at day 7, being 2.2 times as high as the initial value. Then, concentration decreased reaching values slightly below the initial at day 21. In immersion-treated samples, a significant increase was also observed for chlorogenic acid concentration after 24 h in cold store, but this elevation was comparatively smaller, as the maximum level attained was 1.5 times as high as the initial value. Then it decreased, reaching values alike the initial. Concerning the samples treated in heated air, a significant concentration decrease was observed for this compound, considering the first 14 days of storage at 0 °C. From then and until day 21, levels remained mostly constant. Our results agree with those found for lettuce by Loaiza-Velarde *et al.* (1997), who have observed a chlorogenic acid build-up in control samples after 3 days of storage at 10 °C. They also found that exposure at 50 °C for 90 s retained initial levels of chlorogenic acid over the 72 h of the experiment.

If a hierarchy of tissue response to different types of abiotic stress would exist (Saltveit, 2000), then it would be possible to redirect protein synthesis in minimally processed products from enzyme production related to damage response towards HSP (Saltveit, 2000). Thus, the enzyme synthesis participating in phenylpropanoid metabolism, which would be induced by mechanical injury, may be partially or totally repressed to favour HSP generation (Saltveit, 2000). A consequence of this would be the lower increase of chlorogenic acid observed in our experiments with minimally processed celery, for immersion- and hot air-treated samples. In this matter, efficiency of hot air treatment seems to be higher compared with immersion, i.e. to regulate the increase of chlorogenic acid that is observed in the untreated control.

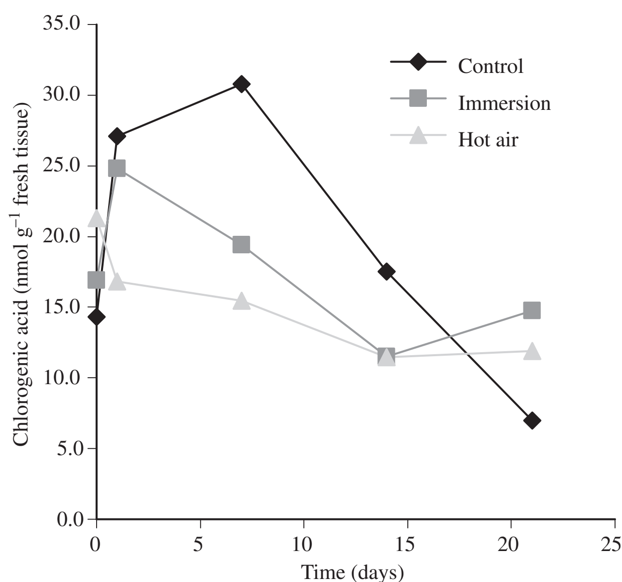

Figure 3 Chlorogenic acid content in heat-treated pre-cut celery, stored for 21 days at 0 °C ($LSD_{0.05} = 7.3$).

Total flavonoids

Studying the main group of phenolic compounds, the total flavonoid content was analysed. Results are exhibited in Fig. 4, and they indicate that the application of heat treatments, both by immersion and dry air, induced a sudden decrease in total flavonoids compared with the controls. In fact, initial flavonoid concentration in untreated samples was 1.3 and 2.5 times as high as those resulting from immersion and hot air treatments, respectively. This would indicate that thermal stress promoted a decrease of total flavonoids that was more marked after hot air treatment. Control samples experienced a decrease of 38% in total flavonoids, with respect to the initial value, after 24 h. Values kept decreasing but more slowly up to day 14, and remained almost constant up to day 21, where the concentration was of 53% of the starting level. In immersion-treated samples, flavonoid evolution was similar to that for controls though, in contrast, flavonoids increased slightly in those samples receiving hot air treatment ($P < 0.05$) over the first week in cold store and then remained nearly constant

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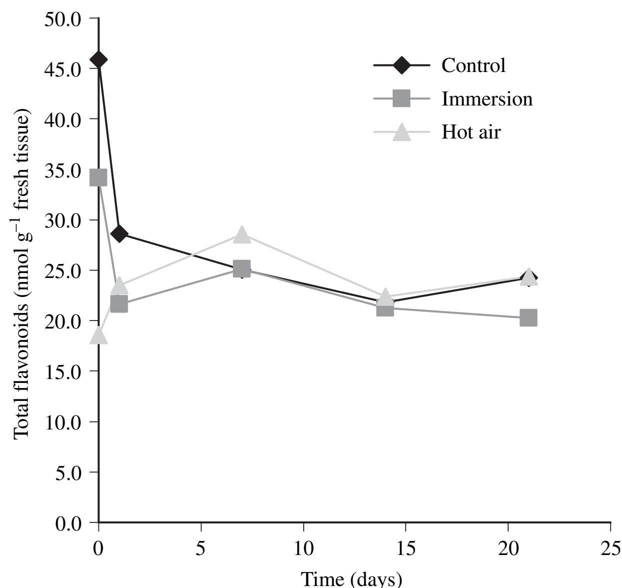

Figure 4 Total flavonoids content in heat-treated pre-cut celery, stored for 21 days at 0 °C ($LSD_{0.05}$ = 4.5).

up to the end of storage. No differences were observed from the seventh day of storage between treated and control samples ($P > 0.05$). Ewald *et al.* (1999) have analysed the effect of various thermal treatments and processing methods on the flavonoids content in onion. For steam-blanched products, losses of 39% for quercetin and of 64% in kampferol were observed. Although the effect observed for heat-treated celery petioles was also a loss of total flavonoids, the decrease produced by water immersion at 50 °C for 90 s was of 26% with respect to the initial content in the control in our experiments. Despite hot air treatments being much less drastic than conventional blanching, the use of hot air in cut celery led to a 60% decrease in total flavonoids with respect to untreated product at the beginning of the experiments.

Ascorbic acid

Table 2 shows results from ascorbic acid (AA) determinations. Just after applying immersion treatment (day 0), there was a slight though significant decrease in AA content, compared with the control. In turn, hot air did not produce changes ($P > 0.05$). With regard to the controls, a significant increase by 36% of AA content was observed over the first week of storage at 0 °C, with respect to the initial level. From then on AA content decreased significantly by 20% on day 21, compared with the initial value. In immersion-treated samples, the behaviour was very similar, though variations were less important. For instance, at day 7 the increase reached 16% of the initial value, while the subsequent reduction led to concentrations similar to the initial, at day 21. In the hot air treatment, a similar trend was presented. After 7 days of storage at 0 °C, AA concentration increased significantly, by 24%, compared with the initial value. AA levels decreased from then on, returning to practically the initial values for 21 days. Such treatment would induce higher AA retention, as measured levels were higher than in controls and samples exposed to immersion treatment.

Table 2 Ascorbic acid content (mg/100 g fresh tissue) of heat-treated pre-cut celery stored for 21 days at 0 °C ($LSD_{0.05}$ = 0.4)

	Ascorbic acid (mg/100 g fresh tissue)		
Time (days)	**C**	**I**	**HA**
0	3.6	3.1	3.4
7	4.9	3.6	4.2
14	3.0	3.0	3.5
21	2.9	3.1	3.3

C, control; I, immersion thermal treatment (50 °C, 90 s); HA, hot air thermal treatment (48 °C, 1 h).

The best known function of AA in plant cells is chloroplast protection against oxidative damage. Inactivation of O_2^- and H_2O_2 produced by such organelles is catalysed by superoxide dismutase and ascorbate peroxidase. The monodehydroascorbate formed upon the action of this enzyme can be reduced directly by ferredoxin. Moreover, a second enzymatic system known as ascorbate-gluthathion system is important to regenerate AA from dehydroascorbic acid (Horemans *et al.*, 2000). McCarthy & Matthews (1994) have indicated that minimal processing of fruits and vegetables would reduce AA content in tissues. However, ascorbate synthesis was also found to increase under stress conditions and that variation of its concentration would be a good indicator of the extent of damage experienced by the plant tissue (Stegmann *et al.*, 1991).

Evolution of AA content in cut celery, either heat-treated or not, was in agreement with our previous findings for the same cultivar (Viña & Chaves, 2006), where a slight increase of AA concentration was reported over the first week in refrigerated storage. More recently, Gómez & Artés (2005) working with a green celery cultivar have found that AA content decreased after 15 days at 4 °C, when pieces were stored in macroperforated polypropylene bags.

Referring to our results, if synthesis or 'recovery' of AA content occurs, this fact would be a response to processing damage or to gradual senescence, which comes out even during storage at low temperatures. In view that in our experiments, such elevations were observed after about 7 days in store, it is likely that they would be linked with processes occurring before the

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start of tissue senescence. Heat treatments would attenuate this response to a greater or lesser extent, affecting in some way the normal ageing processes of cut celery.

Antioxidant power

The evolution of AP in cut celery exposed to heat treatments is shown in Fig. 5. At the beginning of the tests, AP of the control and hot air-treated samples were not significantly different ($P > 0.05$), averaging 4.4 g^{-1} of fresh tissue. Immediately after application of immersion treatment, AP decreased significantly by 41% compared with the control. This observation for the initial point of storage, agrees with the results for AA, where immersion treatment also induced a concentration decrease with respect to the control. Concerning controls, AP exhibited a significant decrease which, after 7 days of storage, reached 50% of the initial value. From then on, AP showed an increase, which first recovered the initial level, and then exceeded it by 30%, remaining constant up to the end of the conservation period. This behaviour was similar to that found when studying antioxidant capacity of cut celery stored at several temperatures (Viña & Chaves, 2006). In immersion-treated samples, AP increased up to 14 days, to regain values alike those of controls. On applying hot air, AP evolved as in the controls.

In this series of experiments on the effect of thermal treatments on AP of cut celery, no differences between control and treated samples were observed after 7 days of storage.

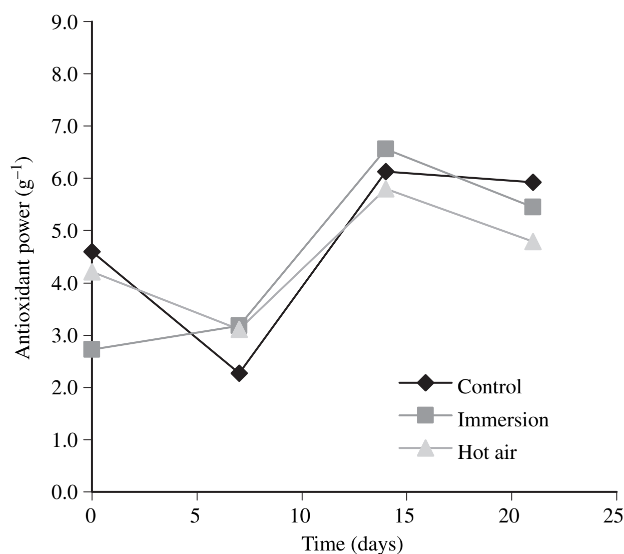

Figure 5 Antioxidant power of heat-treated pre-cut celery, stored for 21 days at 0 °C ($LSD_{0.05} = 0.6$).

Conclusions

The results of the present study indicate that heat treatments were beneficial to lessen the increases in browning potential verified in the controls over the first week at 0 °C. In the same period, the hot air treatment inhibited the concentration increase of chlorogenic acid. This last compound can act as substrate for enzymatic browning reactions. In this regard, immersion treatment was not as effective.

Immediately after application, treatments caused losses in total flavonoids. Concerning AA retention, hot air treatment was more beneficial than immersion.

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