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Antioxidant responses in minimally processed celery during refrigerated storage

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Abstract

This work studies the effect of storage temperature (0, 4 and 10 °C) and time on the antioxidant capacity of cut celery packaged in polystyrene trays sealed with PVC film. Samples were taken at 0, 7, 14, 21 and 28 days of storage to determine total phenols, chlorogenic acid and ascorbic acid. The browning potential and antioxidant capacity of the product were also evaluated. The antioxidant power presented similar behaviour for the three temperatures tested, decreasing after the first 7 days and then increasing up to day 14. Such increase coincided with an elevation of the ascorbic acid content, which was stronger for higher temperatures. As a general conclusion, minimally processed celery retained its initial antioxidant capacity for a period of 21 days at 0 °C, showing the lowest levels of browning potential at this temperature.

Keywords: Fresh-cut vegetables; Ascorbic acid; Phenolics; Enzymatic browning; Antioxidant activity

1. Introduction

During minimal processing, fruits and vegetables are treated in a series of stages where their structure and tissues are generally damaged or removed. By cutting, the size of diverse organs is reduced to obtain ready-to-use products that are packaged in small portions for convenience. During handling, cutting, washing and rinsing, important mechanical damage occurs, which is accompanied by oxidative stress. Disinfection by immersion in chlorinated water is still widely used for simplicity and low cost, though it constitutes an additional damaging factor because of hypochlorous acid reactivity (Wei, Cook, & Kirk, 1985).

The synthesis of several phenylpropanoid compounds (flavonoids, isoflavonoids, psoralens, coumarins, phenolics acids, chlorogenic acid, lignin and suberin) is induced in plants by biotic and abiotic stress, factors such as wounding, low temperature and attack of pathogens (Dixon & Paiva, 1995). In vegetables, responses can be oxidation of preformed phenolic compounds, synthesis of monomeric phenols and production of polymeric phenolic compounds (Rhodes & Wooltorton, 1978). Some notable consequences of these mechanisms are enzymatic browning and lignification of growing tissues, which damage various minimally processed products. Besides, the antioxidant capacity of this food group may be affected, with important consequences on nutritional quality.

Phenolic compounds are known to constitute one of the most important groups of natural antioxidants, owing to their diversity and extensive distribution. They possess biological and chemical properties in common: reducing character, capacity of sequestering reactive oxygen species (ROS) and several electrophiles, for chelating metallic ions, tendency to self-oxidation and

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capacity for modulating the activity of some cell enzymes (Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999).

Functions of diverse phenolic antioxidant in the diet have been discussed in many works (Astley, 2003; Block, Patterson, & Subar, 1992; Gutteridge, 1993; Hertog & Hollman, 1996; Kinsella, Frankel, German, & Kanner, 1993; Trewavas & Stewart, 2003). The biological activity of phenylpropanoids and their function as antimicrobial agents are well recognised, as are their antiallergenic and anti-inflammatory properties, along with their antimutagenic action (Rice-Evans, Miller, & Paganga, 1996).

On the other hand, ascorbic acid is an antioxidant that directly or indirectly sequesters harmful free radicals, usually present in live cells. For instance, it plays an essential role in capturing hydrogen peroxide and protects thiol groups of enzymes and proteins from oxidation (Foyer, 1993). According to McCarthy and Matthews (1994), minimal processing of fruits and vegetables may reduce ascorbic acid content of tissues. By contrast, other authors have reported that ascorbate synthesis is increased under stress conditions, and that modifications in the ascorbate pool would provide a good index of the stress experienced by a vegetable tissue (Stegmann, Schuler, Ruff, Knollmüller, & Loreth, 1991).

Celery is a plant material that easily adapts to minimal processing and constitutes an important regional crop. According to information provided by the Horticultural Census of the Great Buenos Aires Green Belt, its annual production in 1998, carried out under cover, reached 4400 t in this region.

The aim of the present work on minimally processed celery was to evaluate the effect of cutting, storage temperature and time on the contents of substances with antioxidant activity, such as ascorbic acid, total phenols and chlorogenic acid.

2. Materials and methods

2.1. Plant material, processing and storage conditions

Celery plants (*Apium graveolens* L.) cv Golden Boy, grown in a greenhouse, were received from a La Plata grower (Province of Buenos Aires, Argentina). Golden Boy is a white or self-whitening variety, widely cultivated in the zone. Two months after being transplanted, and once reaching commercial size, plants were harvested early in the morning, brought to the laboratory, and processed immediately.

Leaves and 4-cm long segments of the basal rosette 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 strips. These were disinfected by immersion in chlorinated water (100 ppm active chlorine, pH 6–6.5, 8 °C) for 3 min and rinsed in a manual domestic centrifuge. Finally, the material was packaged in polystyrene trays ($15 \times 10 \times 5$ cm^3), and covered with self-adhering 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 $^{-1}$).

Trays containing about 175 g of product were kept for 28 days in cold stores at 0, 4 and 10 °C with a relative humidity of 85%. Samples (three trays for each time–temperature combination) were taken for analysis at 0, 7, 14, 21 and 28 days. Storage experiments were done in triplicate. Since the results were very similar for different conditions, here we provide those corresponding to one of them.

2.2. Determinations

2.2.1. Sampling

For each combination of time and temperature, the material from three trays was combined and homogenised. Part of the pool was frozen with liquid N_2 and crushed in a mill (Janke and Kunkel Ika Labortechnik A10, Staufen, Germany). From this material, exactly weighed subsamples were taken in order to carry out the corresponding determinations.

2.2.2. Browning potential

Ten grammes of tissue frozen in liquid N_2 and crushed as described above were treated with ethanol 96° for 60 min and then centrifuged at 11,500*g*, 10 °C for 30 min, retaining the supernatants. A further amount of ethanol was added to complete a final volume of 25 ml. Absorbance at 320 nm was measured on an aliquot of this extract. Extractions and determinations were carried out in duplicate, and the final results were expressed as absorbance units (AU) g^{-1} fresh tissue.

2.2.3. Total phenols content

Extraction was conducted as described in Section 2.2.2. Aliquots (20 ml) of the extracts were concentrated at reduced pressure (30 mm Hg, 40 °C) in a rotary evaporator R-124 (Büchi Labortechnik AG, Flawil, Switzerland), to dryness. Residues were resuspended in doubly distilled water. Total phenols were quantified employing the Folin–Ciocalteu reagent (Swain & Hillis, 1959). Absorbance readings were carried out at 760 nm. Duplicated extractions and determinations were conducted. Catechin was used as standard in a 3.75–12.75 μg ml^{-1} concentration range. Final results were expressed as μmol g^{-1} fresh tissue.

2.2.4. Chlorogenic acid concentration

Extraction was conducted as described in Section 2.2.2. Aliquots of the extracts (20 ml) were concentrated

as mentioned in Section 2.2.3. Here, residues were resuspended in 1 ml of HPLC grade methanol, and analysed in a HPLC Waters Model 6000A (Milford, MA, USA), fitted with a UV–vis detector. A C_{18} column was employed (particle diameter, 5 μm; internal diameter, 4.6 mm; length, 25 cm), using a 85:10:5 mixture of water:methanol:formic acid as running solvent. A flow rate of 1 ml min^{-1} was used, and detection was conducted at 320 nm.

A standard solution with a concentration 0.87 μg ml was used both to identify and quantify chlorogenic acid. Extractions and determinations were conducted in duplicate, and the results were expressed as nmol g^{-1} fresh tissue.

2.2.5. Ascorbic acid content

A modified version of the method proposed by Wimalasiri and Wills (1983) was used. Samples were taken from the homogenised-frozen-crushed material, which were weighed accurately to 3 g each, and extracted in 5 ml of an aqueous solution of citric acid 3% (w/v) and, after 10 min, they were centrifuged (11,500*g*, 5 min, 5 °C). Aliquots of 1 ml from each extract were centrifuged again in an Eppendorf 5415C equipment for 2 min at 14,000 rpm.

The same HPLC equipment as described in Section 2.2.4 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 $NH_4H_2PO_4$, bringing the pH 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 l^{-1}, was employed. Extractions and determinations were carried out in duplicate, and the final results were expressed as μmol g^{-1} fresh tissue.

2.2.6. Antioxidant power

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

2.3. Statistical analysis

Data were treated by analysis of variance (ANOVA). Sources of variation found were time (five levels) and storage temperature (three levels). Means were compared by the least significant difference (LSD) test, at a significance level $p = 0.05$.

3. Results and discussion

3.1. Browning potential

Browning of vascular bundles in the cut surface of celery petioles has been described as one of the main quality-affecting changes (Loaiza-Velarde, Mangrich, Campos-Vargas, & Saltveit, 2003). Fig. 1 shows the evolution of browning potential of minimally processed celery as a function of storage time and storage temperature.

In our experiments, the initial value was 5.4 10^{-2} AU (absorbance units) g^{-1} fresh tissue, this level being below the values found by Loaiza-Velarde et al. (2003), who reported browning potentials in celery petioles above 0.2 AU g^{-1} fresh tissue. This would indicate low susceptibility to browning, and good adequacy for minimal processing of the cv Golden Boy used in this work.

At 0 °C, the browning potential remained stable over the first 2 weeks and then increased significantly ($p < 0.05$) by 20% with respect to the initial value

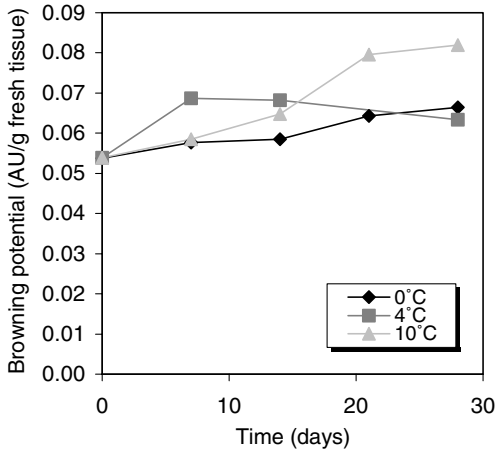

Fig. 1. Browning Potential (absorbance units, AU, g^{-1} fresh tissue) of minimally processed celery stored at 0, 4 or 10 °C for 28 days ($LSD_{0.05} = 6 \times 10^{-3}$).

(hereafter, percentages with respect to the initial values are used except when otherwise stated). At 4 °C, browning potential increased by 28% in the first 7 days, to remain constant from then on. Browning increases were higher for pieces stored at 10 °C, reaching values of 48% at day 21 of storage.

Visible manifestations of enzymatic browning were found in pieces stored at 10 °C for 21 days; these occurred on cut surfaces as orangish dots, in correspondence with exposed vascular bundles. However, enzymatic browning was not the main detrimental factor for product quality.

3.2. Total phenols content

Results from the determination of total phenols are shown in Fig. 2. At the beginning, total phenols concentration was 0.12 μmol g^{-1} fresh tissue. At 0 and 4 °C, the proportion of soluble phenols did not vary substantially with time. In the product stored at 10 °C, a slight increase ($p < 0.05$) was observed at the day 21, of some 19%.

Ke and Saltveit (1988) have observed a marked elevation in the total soluble phenols content for iceberg lettuce exposed to several kinds of stress (attack of pathogens, ethylene treatment), under conditions causing no variations in the control samples. Babic, Amiot, Nguyen-The, and Aubert (1993a) observed an increase of phenols in ready-to-use carrots, as a response to processing-induced damage. In turn, Amanatidou, Slump, Gorris, and Smid (2000) found an 8-fold increase in total phenols concentration in carrot discs stored in air for 12 days at 8 °C. The authors indicated that phenols build up as a physiological response to infections and damage.

Storage time seemed to influence quality of minimally processed celery stored for extended periods, as it led to increased total phenols concentration at 10 °C. A temperature of 0 °C would lessen such a manifestation.

3.3. Chlorogenic acid concentration

Results on the evolution of chlorogenic acid are plotted in Fig. 3. The initial level was 12.9 nmol g^{-1} fresh tissue and represented 10.5% of total phenols.

At 0 °C, a considerable decrease of chlorogenic acid content up to day 14 was observed, the value reached being 33% of the initial. At 4 °C, the decrease was even more marked; after the first week, the content was only 28% of the initial. Chlorogenic acid content, after 28 days at this temperature, dropped to 18%. In turn, at 10 °C, the decrease observed over the first 7 days was notable, to a value representing 10% of the initial. The reduction rate in chlorogenic acid content was stronger for higher storage temperatures; rate at 10 °C was twice as high as at 4 °C and four times higher than at 0 °C.

Evolution of chlorogenic acid content during storage seems to depend on the plant species, cultivar and even type of tissue. In this regard, Gil-Izquierdo, Gil, Conesa, and Ferreres (2001), working on artichokes, found the concentration to increase in product stored at temperatures between 0 and 7 °C for 14 days, this rise being more marked in internal bracts than in external ones. By contrast, as heads were stored at 10 °C, only traces of this compound were found in external bracts, and the difference was attributed to chlorogenic acid degradation as browning substrate. On the other hand, Babic, Amiot, Nguyen-The, and Aubert (1993b) observed chlorogenic acid to increase or decrease in minimally processed carrot after 1–3 storage days at 4 °C, depending on the variety tested.

3.4. Ascorbic acid content

The initial ascorbic acid (AA) content in celery cuts, the initial content was 0.32 μmol g^{-1} fresh tissue and

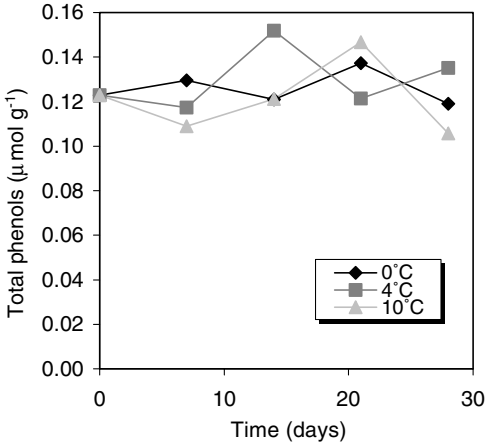

Fig. 2. Total phenols content (μmol g^{-1} fresh tissue) of minimally processed celery stored at 0, 4 or 10 °C for 28 days ($LSD_{0.05} = 0.02$).

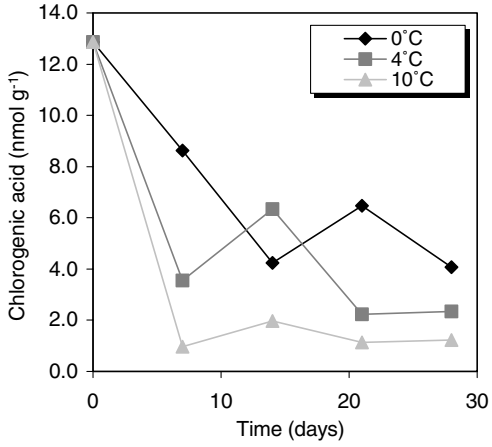

Fig. 3. Chlorogenic acid content (nmol g^{-1} fresh tissue) of minimally processed celery stored at 0, 4 or 10 °C for 28 days ($LSD_{0.05} = 3.1$).

Table 1
Ascorbic acid content (μmol g^{-1} fresh tissue) of minimally processed celery stored at 0, 4 or 10 °C for 28 days ($LSD_{0.05}$ = 0.10).

Time (days)	Temperature		
	0 °C	4 °C	10 °C
0	0.32	0.32	0.32
7	0.32	0.28	0.28
14	0.38	0.44	0.52
21	0.33	0.34	0.36
28	0.30	0.38	0.31

this value was 2.6 times lower than that reported by Kwon, Hong, and Kong (1998) for whole celery plants but eight times higher than the 0.62 mg/100 g (0.04 μmol g^{-1}) from the celery edible portion published by Salunkhe and Desai (1984). Table 1 shows the evolution of ascorbic acid content in pre-cut celery, as a function of time and storage temperature.

Non-significant variations in AA content were found at 0 °C, though there was an increasing trend at 14 days. At 4 °C, the increase observed after 2 weeks was significant ($p < 0.05$) and represented 38%. In contrast, AA concentration decreased between days 14 and 28, returning to the initial values. At 10 °C, behaviour was alike that observed at 4 °C: a significant increase was measured in the middle of the storage (day 14), representing 64%, but then the AA content decreased to initial levels. The increase of AA at 10 °C was 1.7 times as higher than that recorded at 4 °C.

Vitamin C has been considered many times as one of the more labile food constituents, so losses are frequent during processing and storage of plant products. For instance, Gil-Izquierdo et al. (2001) analysed the effect of storage temperature on the vitamin C content of artichokes, and found considerable losses of AA after 14 storage days at temperatures between 0 and 10 °C.

However, in some broccoli varieties (Eheart, 1970; Eheart & Odland, 1972) AA content was found to increase during storage at 2–3 °C. More recently, Paradis, Castaigne, Desrosiers, and Willemot (1995) studied whole and cut broccoli during 21 days storage at 4 °C, and found negligible changes in vitamin C.

On the other hand, during postharvest storage of young spinach leaves (Toledo, Ueda, Imahori, & Ayaki, 2003) and sliced strawberry (Palmer Wright & Kader, 1997), AA content first decreased and then increased. In this regard, Lee and Kader (2000) have found AA to be more stable in acidic media, which would be an advantage of fruits over vegetables.

In our experiments, with minimally processed celery, no initial decreases in AA content were observed that could be attributed to cutting-induced damage. Variable increases were observed during storage, being stronger for higher temperature, possibly because of the extent of senescence in the product.

Size and degree of reduction of the ascorbate pool would reflect tissue state in terms of antioxidant capacity, besides providing an approximation of the effects of stress on the photosynthetic apparatus (Foyer, 1993). The ascorbate function as an antioxidant is ensured by action of the ascorbate–glutathione cycle, which provides an efficient mechanism for recycling oxidised ascorbate and for keeping the AA reservoir in its reduced form (Foyer, 1993).

In circumstances where membranes are considerably damaged, as occurs during advanced tissue senescence, cells are likely to increase concentration of antioxidant substances (AA among them) to repair or compensate the effects of such damage. AA regeneration or synthesis would help, at least in the short term, to offset the growing production of ROS and other free radicals that would lead to cellular injury or death. For example, a stress factor, such as UV–B radiation increased ascorbic acid levels in cucumber cotyledons (Jain, Kataria, & Guruprasad, 2003).

If this situation occurred in minimally processed celery, the damage process under analysis would be related to the progress of storage time and, consequently, to senescence. Thus, the lower temperatures (0 °C) would attenuate but would not defer such response.

3.5. Antioxidant power

Fig. 4 shows changes in antioxidant power (AP) of minimally processed celery as related to time and

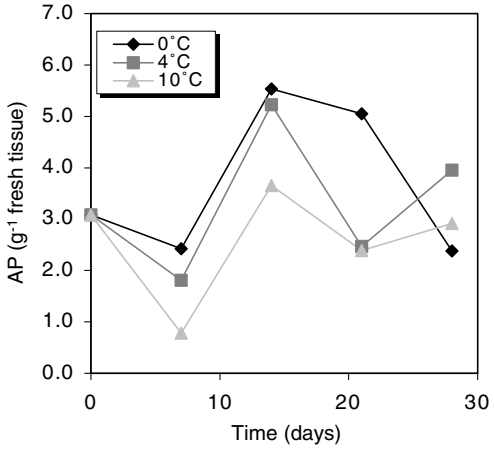

Fig. 4. Antioxidant Power (AP, g^{-1} fresh tissue = $1/EC_{50}$) of minimally processed celery stored at 0, 4 or 10 °C for 28 days ($LSD_{0.05}$ = 0.3). (EC_{50}: effective mean concentration).

storage temperature. The starting value was 3.1 g^{-1} fresh tissue. AP variations were observed to be similar over the 28 days of storage at the three temperatures tested. An initial decrease was verified, followed by an increase leading to a maximum value at 14 days of storage. The initial decrease was more considerable for higher temperatures, whereas the maximum reached was higher at 0 or 4 °C than at 10 °C.

The evolution of total phenols content (Section 3.2) would not by itself explain the decrease of AP corroborated after seven days of storage nor the increase observed at day 14. On the other hand, the chlorogenic acid content initially decreased (up to day 7) at the three temperatures tested and this was more noticeable for higher storage temperatures. This fact may have induced the initial decrease in AP.

Antioxidant capacity of vegetables is known to depend on a wide number of compounds. In this regard, Chu, Chang, and Hsu (2000) have indicated that several phytochemicals, such as flavonoids, phenolic acids, aminoacids, ascorbic acid, tocopherols and pigments, might contribute to the total antioxidant activity. Moreover, mannitol has been described as having reducing capacity (Keller & Matile, 1989) so it can also sequester hydroxyl radicals (Smirnoff & Cumbes, 1989).

In previous work (Viña & Chaves, 2003) we have followed the mannitol content in pre-cut celery after 7 days of storage; we found a decrease of 8% at 0 °C, and of 50% at 10 °C. This behaviour could have contributed to the decreased antioxidant power observed in the first week for celery petioles stored at 0 and 10 °C.

When analysing the possible relationship between the evolutions of AP and AA content during refrigerated storage of cut celery, the Pearson linear correlation coefficients were significant ($p < 0.05$), being 0.764, 0.959 and 0.652 for storage at 0, 4 and 10 °C, respectively. The correlation established is mainly caused by the increases of both AP and AA contents at 14 days in the cold store.

Miller and Rice-Evans (1997) have suggested that phenolic antioxidants of apple juice, notably chlorogenic acid, would protect vitamin C from oxidative degradation. In our experiments with cut celery, the decrease of chlorogenic acid and the constancy of ascorbic acid levels observed during the first week would be congruent with the former observation.

4. Conclusions

Chlorogenic acid content decreased during refrigerated storage of minimally processed celery, and this behaviour was more pronounced for higher storage temperatures. AA concentration increased toward day 14 in the cold store at the temperatures tested. As the elevation was stronger for higher temperatures, this phenomenon would indicate the extent of senescence in tissue. AP in cut celery initially decreased after 7 days of storage and then increased after 2 weeks, regardless of storage temperature. Its behaviour would depend on complex interactions among the different antioxidant groups present in the product. However, some direct association was found between AP and ascorbic acid contents.

As a general conclusion, minimally processed celery retained its initial antioxidant capacity for a period of 21 days at 0 °C, showing the lowest levels of browning potential at this temperature.

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