

Respiratory activity and phenolic compounds in pre-cut celery

Sonia Z. Viña, Alicia R. Chaves *

CIDCA (Centro de Investigación y Desarrollo en Criotecología de Alimentos), Facultad de Ciencias Exactas, UNLP – CONICET, Calles 47 y 116 S/No., La Plata (B1900AJJ), Buenos Aires, Argentina

Received 13 June 2005; received in revised form 15 December 2005; accepted 15 December 2005

Abstract

The effect of temperature on respiratory activity and total phenol and flavonoid contents in pre-cut celery was assessed within the 24 h following this minimal processing. To this end, celery petioles were cut as strips, conditioned in crystal polyethylene terephthalate trays covered with PVC film and stored at 0, 10 and 20 °C. Samples were removed at 0, 2, 4, 6 and 24 h after processing to analyse respiratory activity and concentrations of total phenols and flavones. For the samples kept at 0 °C for 24 h, chlorogenic acid was also determined, along with total flavonoids and antioxidant power. At this temperature, the total phenol contents remained basically constant, though at 10 °C it increased considerably two hours after applying the cutting stress. At 20 °C the increase observed was less important. The flavones, identified by HPLC in pre-cut celery were apigenin and luteolin, whose concentrations also increased between two to six hours after processing. However, exposure for 24 h at 0 °C produced a considerable decrease in total flavonoids.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: Fresh-cut vegetables; Respiratory increase; Total phenols; Flavones; Wounding response

1. Introduction

Among the so-called secondary metabolites, phenylpropanoids and related phenolic substances are represented by about 8,000 compounds whose common origin is the shikimic acid and malonate-acetate metabolic pathway (Croteau, Kutchan, & Lewis, 2000). Although some phenolic compounds play structural roles in the cell wall (for instance, the lignin), others are non-structural constituents with defensive functions in plants, being induced by various biotic and abiotic stress conditions, such as high light intensity, UV radiation, pathogen attack, nutritional deficiencies, low temperatures, and mechanical damage (Dixon & Paiva, 1995). Flavonoids are included among such compounds.

According to the modifications of the central C-ring, flavonoids can be divided into different structural classes, e.g. flavanones, isoflavones, flavones, flavonols, flavanols

and anthocyanins (Schijlen, Ric de Vos, van Tunen, & Bovy, 2004). Fruits, vegetables, and beverages, such as tea and red wine, are major sources of flavonoids in the human diet (Aisling Aherne & O'Brien, 2002). There is evidence that the geographic distribution of many chronic diseases affecting humans is irregular, and worldwide epidemiological studies relate the prevalence of some of these diseases to food intake patterns. In fact, occurrence shows a reciprocal relationship with fruit and vegetable consumption, especially in cardiovascular diseases and cancer (Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999).

From a nutritional standpoint, flavones and flavonols are of particular importance as there is evidence that they act as antioxidants (Shahidi & Wanasundara, 1992; Vinson, Dabbag, Serry, & Jang, 1995). The chemistry of flavones and flavonols is predictive of their free radical scavenging activity because the reduction potentials of flavonoid radicals are lower than those of alkyl peroxy radicals and the superoxide radical, which means that flavonoids may inactivate these oxidized-species and thus prevent deleterious consequences of their reactions (Rice-Evans, Miller, & Paganga, 1996). Therefore, investigation

* Corresponding author. Tel.: +54 221 424 9287/489 0741; fax: +54 221 425 4853/424 9287.

E-mail address: arch@quimica.unlp.edu.ar (A.R. Chaves).

of the effect of processing on the various flavonoids present in plant tissues becomes important.

On the other hand, respiratory rate variations, caused by mechanical damage in tissues and the effect of applied temperature, are closely related to metabolic activity of plant tissue. Respiratory rate noticeably affects quality variations during post-harvest storage and the magnitude of the response to damage produced during processing of fruits and vegetables.

Among horticultural products, celery easily adapts to minimal processing and constitutes an important regional crop in Argentina. According to the information provided by the *Horticultural Census of the Great Buenos Aires Green Belt*, its 1998 production under cover reached 4400 t. Previous work has been devoted to analysing overall appearance, CO₂ and O₂ concentrations in packages, surface colour, total chlorophyll, free sugars, lignin content and textural changes in fresh-cut celery (cultivar Golden Boy) stored at 0 or 10 °C during 4 weeks (Viña & Chaves, 2003). Likewise, antioxidant responses in this product kept at 0, 4 and 10 °C during extended storage (28 days) were addressed, analysing browning potential, total phenol content, chlorogenic acid concentration, ascorbic acid content and antioxidant power (Viña & Chaves, 2006). Nevertheless, changes in antioxidant compounds of celery cv Golden Boy - especially those related to phenolic metabolism and browning development - within the few hours following processing have not been addressed. There is evidence that this short period of time could be critical for maintaining quality of plant tissues subjected to wounding stress. For example, Choi, Tomás-Barberán, and Saltveit (2005) found that excision (i.e. wounding) of lettuce mid-rib tissue segments induced a rise in phenylalanine ammonia lyase (PAL) activity within 4–8 h, depending on segment thickness. Authors pointed out that PAL activity peaked at 18 or 36 h for 5 or 20 mm-thick segments, respectively, and that phenolic concentration increased in concert with the rise in PAL activity.

On this basis, the objective of the present work was to analyse variations induced by wounding in the respiratory activity and content of phenolic compounds in pre-cut celery, over the first 24 h after mechanical damage. The effect of temperature was also evaluated.

2. Materials and methods

2.1. Plant material, processing and storage conditions

Celery plants (*Apium graveolens* L.) cv Golden Boy, grown in greenhouse, were provided by a La Plata green belt farm (Province of Buenos Aires, Argentina).

Plants were harvested once achieving commercial size (about 2 months after transplanting), early in the morning, and soon brought to the laboratory, where they were processed immediately. Leaves and the lower 4 cm of basal rosettes were eliminated to obtain unbranched petioles. These were washed in drinking water to completely remove

soil residues and then cut with a sharp knife in pieces 4 cm long. Segments thus obtained were disinfected in chlorinated water (100 ppm active chlorine, pH 6–6.5, 8 °C) for 3 min and dewatered in a manual kitchen centrifuge. Then, pieces were packaged in crystal polyethylene terephthalate (PET) trays (17 × 13 × 5 cm³) covered by self-adhering polyvinyl chloride (PVC) film (thickness, 10 μm; O₂ permeability, 11,232 cm³ m⁻² atm⁻¹ day⁻¹; CO₂ permeability, 48,552 cm³ m⁻² atm⁻¹ day⁻¹; water vapour permeability, 40 g m⁻² day⁻¹).

Trays containing about 175 g of product were kept at 0, 10 or 20 °C for 24 h. Samples were taken for analysis at 0, 2, 4, 6 and 24 h after processing. Experiments were carried out in duplicate and, in view of results obtained in both experiences depicting similar trends, only the data of one of them are presented here.

2.2. Determinations

2.2.1. Respiratory activity

The respiratory activity of celery pieces was determined in a static system (Fonseca, Oliveira, & Brecht, 2002). Samples (200 g of recently processed product) were placed in hermetically sealed glass containers of known volume, and kept at 0, 10 or 20 °C for 4 h, to take the corresponding samples at the end of this period.

To measure the levels of CO₂, a Shimadzu (Kyoto, Japan) gas chromatograph (Model GC-6APTF) fitted with a thermal conductivity detector was used. An Alltech (Deerfield, IL, USA) CTR1 column was employed with helium as carrier gas, at a flow rate of 30 ml min⁻¹. The injector and detector temperatures were both 120 °C, while the column was held at 30 °C. A calibration curve was constructed, based on several CO₂ concentrations. Final results were expressed as ml CO₂ kg⁻¹ h⁻¹. Determinations were carried out in triplicate.

To calculate the apparent activation energy for the respiratory activity of cut celery, the classic Arrhenius model was applied (Exama, Arul, Lencki, Lee, & Toupin, 1993):

$$R_{\text{CO}_2} = R_{\text{CO}_2}^* \exp(-E_{\text{CO}_2}^R/RT), \quad (1)$$

where T is the temperature (K), R_{CO_2} the rate of CO₂ production (ml kg⁻¹ h⁻¹) at temperature T , $R_{\text{CO}_2}^*$ the respiration pre-exponential factor for CO₂ production (ml kg⁻¹ h⁻¹), $E_{\text{CO}_2}^R$ the activation energy of CO₂ respiration (J mol⁻¹) and R the gas constant (8.314 J mol⁻¹ K⁻¹). The activation energy was obtained from the slope of the plot of $\ln R_{\text{CO}_2}$ vs. $1/T$.

2.2.2. Total phenolics content

A pool of material from three trays was used in this analysis for each time-temperature combination. A fraction of it was frozen in liquid N₂ and crushed in a mill (Janke and Kunkel Ika Labortechnik A10, Staufen, Germany). From two accurately weighed (10 g) samples of frozen and ground tissue, the extraction was done in 96% v/v ethanol. Extracts

were centrifuged (11,500g, 30 min, 10 °C) to retain the supernatants. A final volume of 25 ml was completed, and 20 ml aliquots of the extracts were concentrated in a rotary evaporator R-124 (Büchi Labortechnik AG, Flawil, Switzerland) at 30 mmHg and 40 °C, to dryness. The residues were resuspended in doubly distilled water.

Total phenols were quantified by the colorimetric method, based on the Folin–Ciocalteu reagent (Swain & Hillis, 1959). A standard curve was constructed with several catechin concentrations, in the range of 3.75–12.8 $\mu\text{g ml}^{-1}$. Absorbance readings were obtained at 760 nm and final results were expressed as $\mu\text{mol g}^{-1}$ fresh tissue.

2.2.3. Chlorogenic acid concentration

This determination was carried out as described in a previous work (Viña & Chaves, 2006). The extraction and concentration procedures were conducted as mentioned in Section 2.2.2. Residues were resuspended in 1 ml HPLC-grade methanol, and analysed in an HPLC Waters Model 6000A (Milford, MA, USA), fitted with a UV–vis detector. A C_{18} column Ultrasphere ODS (Beckman Instruments Inc., San Ramón, CA, USA) 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 was utilized, both to identify and quantify chlorogenic acid. The UV–vis spectrum of the fraction corresponding to the chlorogenic acid peak, resulting from chromatographic runs, was compared with that from the standard solution. Determinations were carried out in duplicate, using samples that remained at 0 °C for 24 h. Results were expressed as $\mu\text{mol g}^{-1}$ fresh tissue.

2.2.4. Total flavonoid levels

These determinations were done using the technique described by Kim, Jeong, and Lee (2003), with modifications. Samples were extracted, concentrated and resuspended in doubly distilled water, as described in Section 2.2.2. To prepare reaction mixtures, 1500 μl of doubly distilled water and 500 μl of concentrated samples were added to a test tube. The following reagents were added sequentially: at time 0, 150 μl of 5% NaNO_2 , after 5 min, 150 μl of 10% AlCl_3 and finally, after 6 min more, 500 μl of 1 M NaOH. The resulting mixture was homogenized in a vortex and absorbance of the solutions was measured at 510 nm. A standard curve was developed, based on several catechin concentrations in the range of 7.5–36.6 $\mu\text{g ml}^{-1}$. Determinations were carried out in duplicate, for those samples remaining at 0 °C for 24 h. Total flavonoid levels were expressed as $\mu\text{mol g}^{-1}$ fresh tissue.

2.2.5. HPLC identification and quantification of flavonoids

2.2.5.1. *Extraction and hydrolysis conditions.* Samples were obtained and homogenized as described in Section 2.2.2. Procedures described by Crozier, Lean, Mc Donald, and

Black (1997), with slight modifications, were followed for flavonoid extraction and hydrolysis. From frozen, crushed and homogenized material, two accurately weighed samples (5 g each) were taken. A volume of 15 ml of an 80% v/v solution of methanol was added, along with 1 ml of 0.5 M sodium diethyldithiocarbamate as antioxidant and 5 ml of 10 M HCl. The resulting combination was heated to boiling under reflux for 4 h. Extracts were then allowed to cool, centrifuged (11,500g, 30 min, 10 °C) to retain the supernatant and measure its final volume.

2.2.5.2. *Identification and quantification.* Samples of the supernatant thus obtained were analysed in an HPLC Waters Model 6000A (Milford, MA, USA) fitted with an UV–vis detector. Reverse-phase separations were carried out in a C_{18} column (as described in Section 2.2.3). The mobile phase was an acetonitrile:water mixture in 27:73 proportions, the pH being adjusted to 2.5 with trifluoroacetic acid. Flow rate was 1.5 ml min^{-1} and the fractions were detected at 365 nm. Standard solutions of apigenin and luteolin were applied, which were prepared following the same procedure as for sample hydrolysis. Final results were expressed as nmol g^{-1} fresh tissue.

2.2.6. Antioxidant power

Three grammes samples of frozen and crushed tissue 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 \cdot) in a methanol solution, using a modified version of the Brand-Williams, Cuvelier, and Berset (1995) method. Concentration of the extracts was varied in the reaction mixtures by adding 0, 200, 400, 600, 800 or 1000 μl of the extract to a 3.9 ml methanol solution of DPPH \cdot (25 ppm). A final volume of 4.9 ml was completed with methanol. The reaction was allowed to progress to measure absorbance at 515 nm after reaching a constant value. Then, DPPH \cdot remaining concentration was calculated through a calibration straight line obtained in a range of concentrations of this compound. Finally, the DPPH \cdot remaining 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 DPPH \cdot concentration to half its initial value. Extractions and determinations were carried out in duplicate, in samples that remained at 0 °C for 24 h. Final results were expressed as antioxidant power (AP) in g^{-1} , defined as the reciprocal of EC_{50} ($\text{AP} = 1/\text{EC}_{50}$).

2.3. Statistical analysis

Data were treated by analysis of variance (ANOVA). Sources of variation were time elapsed after processing (five levels) and temperature (three levels). Comparison of means was conducted with Fisher's least significant difference (LSD) test at a significance level $p = 0.05$.

3. Results and discussion

3.1. Respiratory activity

Fresh-cuts are generally much more perishable than intact products because they have been subjected to severe physical stress. Respiration rates of fresh-cuts are generally higher than those of intact products, this increase going from only a few percentage points for green beans, grape and zucchini to over 100% for kiwifruit and lettuce (Cantwell, 1995; Watada, Ko, & Minott, 1996). On the other hand, the O₂ consumption rate of shredded endive was only 1.2 times that of the intact counterpart (Chambroy, 1989). This ratio increased to 1.4 for broccoli (Ballantyne, 1987) and to two for shredded lettuce (Ballantyne, 1986). Respiration rate averaged three to seven times that for intact tissue in grated carrots (Carlin, 1989; MacLachlan & Stark, 1985).

For whole celery plants (petioles + leaves), respiratory rates were reported by Ryall and Lipton (1979) to be 3.7 ml CO₂ kg⁻¹ h⁻¹ at 0 °C and 14.2 ml CO₂ kg⁻¹ h⁻¹ at 10 °C. The results obtained in our experiments in pre-cut celery petioles are comparable to those published, since, at 0 °C, a value of 3.16 ml CO₂ kg⁻¹ h⁻¹ was found. In contrast, cut petioles at 10 °C showed a respiratory rate of 7.44 ml CO₂ kg⁻¹ h⁻¹, i.e. about half the value reported for the whole plant at this temperature. Fonseca et al. (2002) have pointed out that vegetables comprise a great range of plant organs with diverse metabolic activity and so different respiration rates. Thereby, comparing our data to those by Ryall and Lipton (1979), the contribution of leaves to the total respiratory activity in whole celery plants was considerably greater than that for petioles.

Fast temperature reduction is an essential tool to minimize the effect of mechanical damage on the increase of respiration rate and other detrimental processes in minimally processed products, thus reducing quality losses. As temperature increases from 0 to 10 °C, the respiratory rate increases substantially, causing a parallel speeding up of damage processes (Watada & Qi, 1999). Thus, in pre-cut zucchinis and kiwis a Q₁₀ (temperature or van't Hoff quotient) of about 3.5 was observed, while, in tomatoes, this was close to seven (Watada et al., 1996).

In our determinations on just processed celery, the respiratory activity experienced a significant increase. The amount of CO₂ produced at 10 °C per kg of product and hour was 2.4 times higher than that at 0 °C (Q₁₀ 0 → 10 °C). Exama et al. (1993), have reported for celery, a Q₁₀ 0 → 10 °C of 2.29, this being comparable to the value found in pre-cut celery petioles. Concerning the results at 20 °C, CO₂ production reached 17.1 ml kg⁻¹ h⁻¹, being 5.4 times higher than at 0 °C and 2.3 times higher than at 10 °C. Therefore, the Q₁₀ in the range of 10 → 20 °C was comparable to that for 0 → 10 °C. Such respiratory increase is below those reported for other pre-cut products, such as sliced zucchinis, cucumber, squash, peppers and tomatoes, in the same temperature range (Watada et al., 1996).

Previous work, conducted on pre-cut celery, has showed that these respiration rates established an equilibrium atmosphere inside the packages composed of 11.1 ml CO₂ l⁻¹ and 170.3 ml O₂ l⁻¹, after 14 days at 0 °C (Viña & Chaves, 2003). When the product was stored at 10 °C, the internal atmosphere was composed of 23.3 ml CO₂ l⁻¹ and 146 ml O₂ l⁻¹, after 14 days (Viña & Chaves, 2003).

Calculation of the apparent activation energy for CO₂ production rate ($E_{\text{CO}_2}^R$) in pre-cut celery yielded a value of 56.1 kJ mol⁻¹ and a pre-exponential factor ($R_{\text{CO}_2}^*$) equal to 1.72×10^{11} ml kg⁻¹ h⁻¹. Exama et al. (1993) have calculated values for $R_{\text{CO}_2}^*$ y $E_{\text{CO}_2}^R$ for diverse fruit and vegetables: for white celery, they found that $E_{\text{CO}_2}^R$ was 53.1 kJ mol⁻¹, and $R_{\text{CO}_2}^*$ was equal to 4.72×10^{10} ml kg⁻¹ h⁻¹. Therefore, the value obtained here for $E_{\text{CO}_2}^R$ was very similar to that found in the literature, being also in the same order as those for products, such as beets, cabbage, lettuce, broccoli, Brussels sprouts and cauliflower. On the other hand, $E_{\text{CO}_2}^R$ obtained for pre-cut celery was higher than the value reported for the O₂ consumption rate of mushrooms (43.4 kJ mol⁻¹) by Varoquaux, Gouble, Barron, and Yildiz (1999). Concerning the pre-exponential factor, the value found for pre-cut celery was 3.7 times higher than that obtained by Exama et al. (1993) for white celery.

3.2. Total phenols content

The results obtained are listed in Table 1. Total phenols content of minimally processed celery, observed for 0 °C, remained substantially constant over the whole period tested. At 10 °C, in turn, an increase of free phenols was observed within the two first hours (26% above the starting value), though, from then on, the value remained constant. At 20 °C, the trend was towards an increase after 2 h of storage, although the value reached (9% above the initial) was not significantly different from the initial value. From this point onwards, free phenols concentration at 20 °C remained constant. Therefore, there seemed to be an increase of free phenols over the first 2 h after cutting. Product conservation at 0 °C would possibly act by inhibiting the response to damage, hence the relative constancy of the values measured at such temperature.

Babic, Amiot, Nguyen-The, and Aubert (1993) have found an increase in phenolic content between 0 and 3 days in shredded carrot tissues stored at 4 °C after mechanical

Table 1
Total phenols content (μmol g⁻¹ fresh tissue) of minimally processed celery stored at 0, 10 or 20 °C for 24 h (LSD_{0.05} = 0.03)

Time (hours)	Temperature		
	0 °C	10 °C	20 °C
0	0.21	0.21	0.21
2	0.21	0.27	0.23
4	0.20	0.22	0.22
6	0.22	0.27	0.24
24	0.20	0.24	0.24

damage caused by the process. Hanotel, Fleuriet, and Boisseau (1995) have investigated several biochemical parameters of browning in minimally processed witloof chicory and found that phenolic concentrations were immediately increased by wounding stress and tended to decrease toward 3 days of storage at 10 °C.

3.3. Chlorogenic acid concentration

Chlorogenic and hydroxycinnamic acids, such as caffeic, *p*-coumaric and ferulic acids, are mostly synthesized by phenylalanine ammonia lyase-catalysed conversion of L-phenylalanine to *trans*-cinnamic acid, which is enhanced in response to wounding or physiological stress (Wen, Delaquis, Stanich, & Tivonen, 2003).

The chlorogenic acid content of just processed celery (Table 2) represented almost 7% of the total phenols and increased ($p < 0.05$) after 24 h at 0 °C, until practically doubling the initial value. Babic et al. (1993) have also found increases of chlorogenic acid content in grated carrot, especially between 1 and 3 days of storage at 4 °C. Ke and Saltveit (1989), Tomás-Barberán, Loaliza-Velarde, Bonfanti, and Saltveit (1997) Fukumoto, Toivonen, and Delaquis (2002) have found build-up of chlorogenic and *iso*-chlorogenic acids in cut iceberg lettuce tissues.

3.4. Total flavonoids, antioxidant power and flavones

The initial content of total flavonoids (Table 2) in minimally processed celery represented almost 22% of total phenols and this significantly decreased after 24 h at 0 °C, reaching a concentration 38% below the initial value.

The antioxidant power (Table 2) remained at the initial values in samples stored at 0 °C for 24 h ($p > 0.05$).

On the other hand, the analysis of flavones in celery petioles allowed identification of luteolin and apigenin with initial concentrations at the beginning of tests of 0.88 $\mu\text{g g}^{-1}$ and 4.61 $\mu\text{g g}^{-1}$ fresh tissue, respectively. Concentration of luteolin represented 1.5% of total phenols, while that for apigenin signified 8%. Crozier et al. (1997) have also identified luteolin and apigenin in several celery varieties: Greensleeves, Ista, Celebrity and Victoria. The authors worked with whole plants (leaves + petioles) and found, for hydrolysed celery extracts, considerable amounts of both compounds (on average, 38 μg of luteolin and 97 μg apigenin per g fresh tissue of white varieties). They pointed out, in addition, that flavonols, such as quercetin and kampferol, were not detected. The concentrations

found in our experiments with pre-cut celery were lower than those described by Crozier et al. (1997), and this may indicate that the flavonoid content of petioles is markedly lower than that in leaves.

At the beginning of our tests, luteolin and apigenin made up 44% of the total flavonoid content. Both compounds behaved similarly at the temperatures tested. At 0 °C, luteolin concentration (Fig. 1) was observed to increase during the first 2 h to twice the initial value, to remain constant over the remaining 22 h. At 10 °C, during the first 6 h after processing, the increase was 2.5 times over the initial value, to then show a slight decrease. At 20 °C, the luteolin content increased quite rapidly (2.6 times in 2 h) but then it decreased after four hours. No further variations were observed at this temperature.

With regard to apigenin (Fig. 2), at 0 °C its concentration increased significantly over the first four hours, reaching twice the initial value. Then, the value decreased considerably to recover the initial values, remaining constant up to the end of the period. At 10 °C a slight but significant increase of apigenin content, by 60% with respect to the initial value, was observed on reaching 6 h after wounding. Such a value was significantly lower than that observed for 0 °C ($p < 0.05$). With regard to the results at 20 °C, the starting values for apigenin almost doubled only

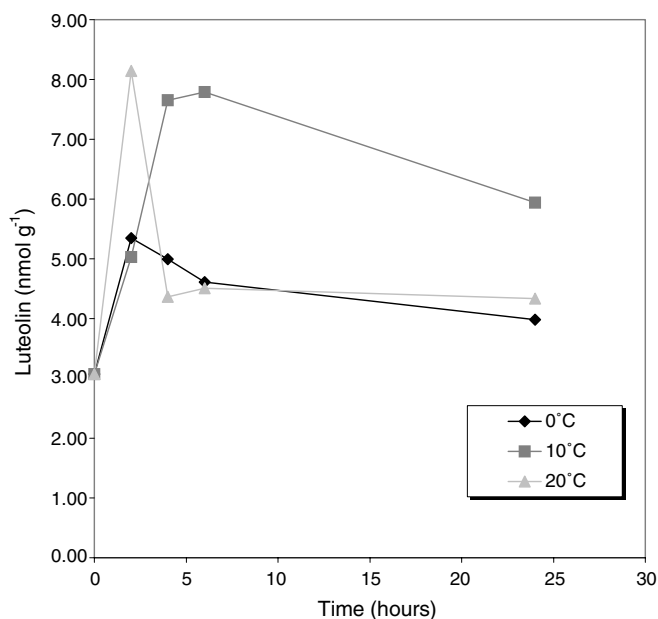


Fig. 1. Luteolin content (nmol g^{-1} fresh tissue) of minimally processed celery stored at 0, 10 or 20 °C for 24 h ($\text{LSD}_{0.05} = 2.10$).

Table 2

Chlorogenic acid, total flavonoids content and antioxidant power of minimally processed celery stored at 0 °C for 24 h

Time (hours)	Chlorogenic acid		Total flavonoids		Antioxidant power g^{-1}
	$\mu\text{mol g}^{-1}$ Fresh tissue	% Of total phenols	$\mu\text{mol g}^{-1}$ Fresh tissue	% Of total phenols	
0	1.43×10^{-2}	6.8	4.59×10^{-2}	21.9	4.6
24	2.71×10^{-2}	13.5	2.86×10^{-2}	14.3	4.3
$\text{LSD}_{0.05}$	0.73×10^{-2}		0.45×10^{-2}		0.6

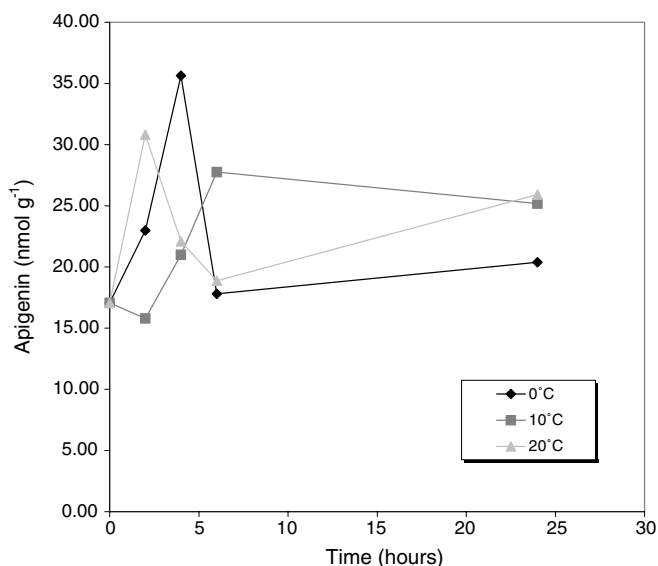


Fig. 2. Apigenin content (nmol g⁻¹ fresh tissue) of minimally processed celery stored at 0, 10 or 20 °C for 24 h (LSD_{0.05} = 5.18).

2 h after processing. However, after 4 h, a significant reduction was observed, reaching almost the initial values. An overall analysis would indicate an increase in celery flavonoid content as a response to processing-induced damage. Such increase was observed very soon (after 2 h).

Ke and Saltveit (1988) have observed a marked increase of flavonoid content in iceberg lettuce exposed to various stresses (e.g. russet spotting, ethylene treatment) and kept at 5 °C for 3 days. In contrast, control samples did not exhibit significant concentration variations. Our observations allowed us to understand that the effect of storage temperature on luteolin and apigenin contents would be different from those observed for total phenols in samples stored for 24 h. Both the concentrations of total phenols and flavones increased over the first 2 h after processing. However, no increase of phenols content was observed at 0 °C, while luteolin and apigenin did increase at the three temperatures tested.

4. Conclusions

The increase in respiratory activity of minimally processed celery, caused by the increase in temperature, was moderate compared with values published in the literature for other pre-cut vegetables. Although some increase in total phenol content was observed for product stored at 10 °C, this magnitude was low and hence would not indicate a deleterious response to damage. Moreover, for product stored at 20 °C, such increase was not proportionally higher.

Apigenin and luteolin concentrations increased between 2 and 6 h after processing, depending on conservation temperature. However, values measured after 24 h of processing were comparable or slightly above starting levels. Concerning the results at 0 °C, total flavonoid content, measured 24 h after processing, was half the initial value.

Acknowledgements

This work was financially supported by the Agencia Nacional de Promoción Científica y Tecnológica (AN-PCyT), PICT 1998-7088. Dr. SZV wishes to thank the CONICET for granting her a postdoctoral scholarship.

References

- Aisling Aherne, S., & O'Brien, N. M. (2002). Dietary flavonols: chemistry, food content, and metabolism. *Nutrition*, 18(1), 75–81.
- Babic, I., Amiot, M. J., Nguyen-The, C., & Aubert, S. (1993). Changes in phenolic content in fresh ready-to-use shredded carrots during storage. *Journal of Food Science*, 58(2), 351–356.
- Ballantyne, A. (1986). Modified atmosphere packaging of selected prepared vegetables. Technical memorandum No. 436. Chipping Campden, UK: Campden Food Res. Assoc. Cited by Varoquaux, P., & Wiley, R. C. (1994). Biological and biochemical changes in minimally processed refrigerated fruits and vegetables. In R. C. Wiley (Ed.), *Minimally processed refrigerated fruits and vegetables*. New York, USA: Chapman and Hall Inc.
- Ballantyne, A. (1987). Modified atmosphere packaging of selected prepared vegetables. Technical memorandum No. 464. Chipping Campden, UK: Campden Food Res. Assoc. (68 p). Cited by Varoquaux, P., & Wiley, R. C. (1994). Biological and biochemical changes in minimally processed refrigerated fruits and vegetables. In R. C. Wiley (Ed.), *Minimally processed refrigerated fruits and vegetables*. New York, USA: Chapman and Hall Inc.
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und-Technologie-Food Science and Technology*, 28, 25–30.
- Cantwell, M. (1995). Fresh-cut product biology requirements. *University of California Perishables Handling Newsletter*, 81, 4–6.
- Carlin, F. (1989). Altérations microbiologiques et désordres physiologiques de carottes râpées prêtes à l'emploi. Thèse de Docteur-Ingénieur de l'Institut National Agronomique Paris-Grignon. Cited by Varoquaux, P., & Wiley, R. C. (1994). Biological and biochemical changes in minimally processed refrigerated fruits and vegetables. In R. C. Wiley (Ed.), *Minimally processed refrigerated fruits and vegetables*. New York, USA: Chapman and Hall Inc.
- Chambroy, Y. (1989). Physiologie et température des produits frais découpés. Colloque AFF, Avignon. *Rev. Gen. Froid* (Vol. 3, pp. 78–92). Cited by Varoquaux, P., & Wiley, R. C. (1994). Biological and biochemical changes in minimally processed refrigerated fruits and vegetables. In R. C. Wiley (Ed.), *Minimally processed refrigerated fruits and vegetables*. New York, USA: Chapman and Hall Inc.
- Choi, Y.-J., Tomás-Barberán, F. A., & Saltveit, M. E. (2005). Wound-induced phenolic accumulation and browning in lettuce (*Lactuca sativa* L.) leaf tissue is reduced by exposure to *n*-alcohols. *Postharvest Biology and Technology*, 37, 47–55.
- Croteau, R., Kutchan, T. M., & Lewis, N. G. (2000). Natural products (secondary metabolites). In B. B. Buchanan, W. Gruissem, & R. L. Jones (Eds.), *Biochemistry and molecular biology of plants* (pp. 1250–1318). Rockville: American Society of Plant Physiologists.
- Crozier, A., Lean, M. E. J., Mc Donald, M. S., & Black, Ch. (1997). Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce and celery. *Journal of Agricultural and Food Chemistry*, 45, 590–595.
- Dixon, R. A., & Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. *The Plant Cell*, 7, 1085–1097.
- Exama, A., Arul, J., Lencki, R. W., Lee, L. Z., & Toupin, C. (1993). Suitability of plastic films for modified atmosphere packaging of fruits and vegetables. *Journal of Food Science*, 58(6), 1365–1370.
- Fonseca, S. C., Oliveira, F. A. R., & Brecht, J. K. (2002). Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review. *Journal of Food Engineering*, 52, 99–119.

- Fukumoto, L. R., Toivonen, P. M. A., & Delaquis, P. J. (2002). Effect of wash water temperature and chlorination on phenolic metabolism and browning of stored iceberg lettuce photosynthetic and vascular tissues. *Journal of Agricultural and Food Chemistry*, *50*, 4503–4511.
- Hanotel, L., Fleuriet, A., & Boisseau, P. (1995). Biochemical changes involved in browning of γ -irradiated cut witloof chicory. *Postharvest Biology and Technology*, *5*, 199–210.
- Horticultural Census of the Great Buenos Aires Green Belt [Online]. (1998). <http://www.sagpya.mecon.gov.ar> Accessed 12.12.03.
- Kim, D.-O., Jeong, S. W., & Lee, C. Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry*, *81*, 321–326.
- Ke, D., & Saltveit, M. E. (1988). Plant hormone interaction and phenolic metabolism in the regulation of russet spotting in iceberg lettuce. *Plant Physiology*, *88*, 1136–1140.
- Ke, D., & Saltveit, M. E. (1989). Wound-induced ethylene production, phenolic metabolism and susceptibility to russet spotting in iceberg lettuce. *Physiologia Plantarum*, *76*, 412–418.
- MacLachlan, A., & Stark, R. (1985). Modified atmosphere packaging of selected prepared vegetables. Technical memorandum No. 412. Chipping Campden, UK: Campden Food Res. Assoc. (78 pp). Cited by Varoquaux, P., & Wiley, R. C. (1994). Biological and biochemical changes in minimally processed refrigerated fruits and vegetables. In R. C. Wiley (Ed.), *Minimally processed refrigerated fruits and vegetables*. New York, USA: Chapman and Hall Inc.
- Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, *20*(7), 933–956.
- Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., & Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry*, *66*, 401–436.
- Ryall, A. L., & Lipton, W. J. (1979). Handling, transportation and storage of fruits and vegetables. *Vegetables and melons* (second ed.). Westport, CT, USA: AVI Publishing Company Inc., pp. 1–42.
- Schijlen, E. G. W. M., Ric de Vos, C. H., van Tunen, A. J., & Bovy, A. G. (2004). Modification of flavonoid biosynthesis in crop plants. *Phytochemistry*, *65*, 2631–2648.
- Shahidi, F., & Wanasundara, P. K. (1992). Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition*, *32*(1), 67–103.
- Swain, T., & Hillis, W. E. (1959). The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture*, *10*, 63–68.
- Tomás-Barberán, F. A., Loaiza-Velarde, J., Bonfanti, A., & Saltveit, M. E. (1997). Early wound- and ethylene-induced changes in phenylpropanoid metabolism in harvested lettuce. *Journal of American Society for Horticultural Science*, *122*(3), 399–404.
- Varoquaux, P., Gouble, B., Barron, C., & Yildiz, F. (1999). Respiratory parameters and sugar catabolism of mushroom (*Agaricus bisporus* Lange). *Postharvest Biology and Technology*, *16*, 51–61.
- Vinson, J. A., Dabbag, Y. A., Serry, M. M., & Jang, J. (1995). Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. *Journal of Agricultural and Food Chemistry*, *43*, 2800–2802.
- Viña, S. Z., & Chaves, A. R. (2003). Texture changes in fresh-cut celery during refrigerated storage. *Journal of the Science of Food and Agriculture*, *83*, 1308–1314.
- Viña, S. Z., & Chaves, A. R. (2006). Antioxidant responses in minimally processed celery during refrigerated storage. *Food Chemistry*, *94*, 68–74.
- Watada, A. E., & Qi, L. (1999). Quality of fresh-cut produce. *Postharvest Biology and Technology*, *15*, 201–205.
- Watada, A. E., Ko, N. P., & Minott, D. A. (1996). Factors affecting quality of fresh-cut horticultural products. *Postharvest Biology and Technology*, *9*, 115–125.
- Wen, A., Delaquis, P., Stanich, K., & Tivonen, P. (2003). Antilisterial activity of selected phenolic acids. *Food Microbiology*, *20*, 305–311.