

## Mild heat shocks to extend the shelf life of minimally processed lettuce

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### Abstract

Changes in ascorbic acid contents, microbial population and sensory attributes of cut Romaine lettuce subjected to thermal shocks were investigated. Immersion of cut lettuce in the thermal baths produced reduction in the ascorbic acid contents between 190 and 300 g kg<sup>-1</sup>, with the greater losses corresponding to the higher bath temperatures. However, the rate of ascorbic acid degradation during refrigerated storage was independent of the thermal treatment and all samples presented a sharp decrease during the first day of storage and a gradual decrease thereafter. Thermal shocks did not reduce the initial microbial population. During storage, an increment in microbial counts was observed, being more notorious in samples that had been exposed to the highest shock temperature (50 °C). The thermal treatment at 50 °C was the only one to delay the onset of midrib and edge browning up to four days of refrigerated storage. This midrib and edge browning was considered to be the most relevant to the overall visual quality of the product.

**Key words:** Cut-lettuce, heat shock treatments, ascorbic acid, total microbial counts, sensory attributes.

### Introduction

Minimal or fresh-cut processing of vegetables provides convenience to food services and retail customers, but may result in limited post cutting shelf life because of undesirable physiological changes (Cantwell *et al.*, 2001). Tissue disruption caused by cutting results in elevated respiration and transpiration, which can lead to rapid deterioration. In addition, cut tissues trigger chemical reactions and release nutrients that support the growth of the microflora present on raw produce. Browning of fresh fruits and vegetables reduce quality and is often the factor limiting shelf life and marketability (Saltveit, 1998). Enzymatic and non-enzymatic reactions with phenolic compounds produce brown pigments in plant tissue. Preventing browning in these tissues requires deactivation of the enzymes (*e.g.* polyphenoloxidase) responsible for browning, exclusion of oxygen, or application of chemical antioxidants.

Heat treatments have been demonstrated to be effective as a non-chemical means of improving postharvest quality for a variety of horticultural products. Moreover, the applications of mild heat shocks constitute an alternative for the preservation of organically cultivated crops for which the use of synthetic chemicals is objectionable. Heat treatments may affect ripening and protect against physiological disorders and have been used as an effective alternative treatment for decay control (Cantwell and Nie, 1996). Heat shock treatments prevented browning of minimally processed lettuce (Loaiza-Velarde *et al.*, 1997). Wounding lettuce leaves induces the synthesis of specific enzymes and the accumulation of specific phenolic compounds associated with tissue browning (Ke and Saltveit, 1989; Brech, 1995; Tomás-Barberán and Espin, 2001). Non-stressed Iceberg and Romaine lettuce leaves contain low levels of phenolic compounds. When wounded, phenylalanine ammonia-lyase (PAL), the first

committed step in the synthesis of phenylpropanoid compounds, is synthesized *de novo*. Later, phenolic compounds are synthesized and accumulated, and tissue browning occurs (Kang and Salveit, 2003). A brief heat shock (90 s at 45 °C) disrupts the wound-induced increase in PAL activity, delaying and diminishing the accumulation of phenolic compounds and tissue browning (Loaiza-Velarde *et al.*, 1997). Murata *et al.* (2004) showed that heat shock treatment is useful for prolonging the shelf life of cut lettuce, repressing the induction of PAL activity and phenolic accumulation during storage, and preventing tissue browning. Most published results on the use of thermal shocks to extend the shelf life of vegetables correspond to the use of chlorinated water. However, in processing organic vegetables, the use of chemical additives would be objectionable.

Moreira *et al.* (2005) found that immersion of whole Romaine lettuce leaves in unchlorinated water at 50 °C for 120 s reduced browning. However, this beneficial effect was accompanied by undesired phenomena. This treatment produced important losses in the initial ascorbic acid contents and fastened its rate of degradation during refrigerated storage. In addition, it affected the texture of the leaves. Finally, although the treatment produced some initial reductions of the native microflora, it induced faster growth during storage and microbial counts eventually surpassed those of the controls.

Fresh cut, minimally processed lettuce, presents a shorter shelf life than whole leaves. This comes as a result of some concurring factors such as higher metabolic rates induced by physical stress and the liberation of cellular contents by membrane disruption. Our group have done work related to the development of technologies for the preservation of horticultural crops compatible with organic production methods, with low energy inputs and low environmental impact by the use of preservation factors of

natural origin. The purpose of the present work was to investigate if, during the shorter shelf life of processed lettuce, the beneficial effects of mild thermal shocks associated to reduction of browning and initial microbial populations make a contribution to the quality of the products before the adverse effects take over. Since mechanical processing also reduces ascorbic acid contents, the relevance of losses associated to thermal treatments was also analyzed.

## Materials and methods

**Sample preparation:** Heads of Romaine lettuce (*Lactuca sativa*, type Cos, variety Logifolia) were harvested at optimal maturity. They were transported to the laboratory within 1 h of harvesting (in container at ca. 5 °C), and were immediately subjected to preliminary operations and conditioning. Outer leaves were discarded and only photosynthetic leaves (green leaves) were included in the samples. Lettuce leaves were cut before the immersion in different bath treatments. Cuts, 1 cm wide stripes, were made perpendicular to the midrib with sharp stainless-steel knives. To apply the heat-shock, the following steps were carried out: lettuce leaves were dipped in water baths with gentle agitation (10 L volume capacity) for 2 min at four temperatures (20, 30, 40 and 50 °C), at a ratio 1: 10 w/v. The introduction of cold material in the warm water baths resulted in a temperature drop. The initial water temperature was correspondingly higher to account for this and reach the desired final temperature in the system. Bath temperatures were monitored with a Data Logger (Testo GMBH & Co. Testo- Str. 1, D-79853 Lenzkirch, Germany). It was assumed that the leaves reached the bath temperature almost immediately because they were placed loose in the agitated baths, they are thin and have a thermal diffusivity similar to that of water.

Afterwards, lettuce leaves were dipped in a water bath at 5 °C for 30 s and then centrifuged for 30 s at 500 rpm to eliminate surface water. Samples treated in water at 20 °C were taken as the control samples. In each separate experimental run, for each temperature (20, 30, 40 and 50 °C) and for each storage time (0, 2, 4, 6, 16, 20, 24, 28, 48, 72 and 96 h), three lettuce lots were prepared. Each lot consisted of cut lettuce leaves (100 g), placed in polyethylene bags (25 cm x 20 cm, useful volume: 1.8 L) with an O<sub>2</sub> permeability of ca. 1000 cm<sup>3</sup> m<sup>-2</sup> day<sup>-1</sup>, CO<sub>2</sub> permeability of ca. 5000 cm<sup>3</sup> m<sup>-2</sup> day<sup>-1</sup> and water vapor of ca. 6 g m<sup>-2</sup> day<sup>-1</sup>. During sample packing environmental temperature was 15 °C. Samples were placed in boxes with overall dimensions of (0.4 x 0.3 x 0.3 m), made of heavy-duty, 0.60 cm thick, transparent acrylic, with 97-99% relative humidity, and stored at 5-7 °C. The experimental design consisted in three independent runs. In each run, two or three lots were analyzed by duplicate or triplicate.

**Determination of ascorbic acid:** Ascorbic acid contents were determined by the titrimetric assay described by Roura *et al.* (2003). Ground lettuce leaves (20 g) were extracted with 100 mL of metaphosphoric acid solution (60 g kg<sup>-1</sup>) for 3 min using a tissue homogenizer (Multiquick, MR 5550 CA, Braun) with a speed of 3500 to 7000 rpm. The homogenate was made up to 250 mL with 30 g kg<sup>-1</sup> metaphosphoric acid, and filtered through Whatman # 42 filter paper. Temperature during ascorbic acid extraction was maintained at 0 °C. Aliquots (5 mL each) of the filtrate were titrated with 2,6-dichloroindophenol. Ascorbic acid contents (mg 100 g<sup>-1</sup>) were reported on a wet basis and were performed

by triplicate on three lots from three separate experimental runs (Moreira *et al.*, 2003).

**Microbiological studies:** Lettuce leaves (25 g) were macerated in 90 mL PO<sub>4</sub>K<sub>3</sub> buffer solution (0.1 mol L<sup>-1</sup>), pH = 7.2, with a homogenizer (Stomacher 400 Circulator Homogenizer). Macerated lettuce was accomplished by spread plating. The enumeration and differentiation of mesophilic aerobic bacteria were performed on PCA (Plate Count Agar) and incubated at 35 °C for 48 h (Moreira *et al.*, 2003). Microbial counts were performed in duplicate on two lots from three separate experimental runs.

**Sensory evaluation:** The ability of panelists (members of our laboratory with experience in sensory evaluation of leafy vegetables) to discriminate and reproduce results was tested in replicate test on fresh leaves treated with heat shocks and no treated. At each sampling time (0, 1, 2, 3 and 4 d of storage) lettuce leaves were removed 20 min prior evaluation to let them reach room temperature. They were subjected to sensory evaluation in duplicate on three lots from three separate experimental runs.

The coded (3 digit) samples were presented one at a time in random order to the judges. Sensory sessions were conducted in an air-ventilated room under white light (daylight equivalent). The sensory attributes color (uniformity and intensity), midrib and edge browning, texture appearance and sensorial acceptability (overall visual quality) were scored on a five-point scale. A score of 5 points represented excellent quality; scores of 4, 3 and 2 represented, respectively, very good, good and poor qualities. Finally, a score of 1 represented very poor quality. Therefore, samples receiving scores less than 3 in any of the sensory attributes analyzed was considered to be unmarketable (Roura *et al.*, 2000).

**Phenylalanine ammonia lyase (PAL) activity measurements:** PAL activity was assayed following the methodology described by Ke and Saltveit (1989) and Pereyra *et al.* (2005). One unit of PAL activity is defined as the amount of PAL that produces 1 μmol of cinnamic acid in 1 h under the specified conditions and is expressed as 1 μmol g<sup>-1</sup> h<sup>-1</sup>. PAL activity was measured during 24 h of storage and the assays were performed in triplicate on two lots from two separate experimental runs.

**Statistical analysis:** Differences among samples were tested by least significant differences variance analysis (Box *et al.*, 1978). Differences in slopes for ascorbic acid evolution in minimally processed lettuce subject to different heat temperature shocks were tested according to Volk (1980). Wherever differences are reported as significant, a 99.5 or 99.9% confidence level was used.

## Results

**Heat shock effects on ascorbic acid degradation and microbial growth:** The initial ascorbic acid content of fresh whole lettuce leaves dipped in water baths for 2 min at 20 °C was 0.210 ± 0.029 (g kg<sup>-1</sup> of fresh weight). The time elapsed between lettuce harvest and ascorbic acid determination in lettuce samples was one hour. Because of cutting lettuce leaves into 1 cm wide stripes, the ascorbic acid contents were reduced in the range of 100 to 150 g kg<sup>-1</sup>, reaching a final ascorbic acid content value of 0.180 ± 0.026 (g kg<sup>-1</sup> of fresh weight).

The exposure of cut lettuce to mild heat shocks by immersion in heated water for 120 s produced decreases in the initial ascorbic acid contents. Compared to control samples (cut lettuce immersed in a water bath at 20 °C), samples exposed to 30, 40 and 50 °C lost 190, 200 and 300 g kg<sup>-1</sup> of ascorbic acid, respectively.

The evolution of ascorbic acid contents, represented as the ln of the ratio of ascorbic acid contents over the initial contents against storage time, is presented in Fig. 1. Apparently, there would be two periods for ascorbic acid degradation. There was a rapid decrease in the first 24 h of refrigerated storage. Thereafter, the ascorbic acid contents degraded at a lower rate. No significant differences were found among the slopes of the tendency lines for the different bath temperatures, neither during the first 24 h of storage nor thereafter. However, the slopes of the tendency lines before and after the first 24 h of storage were significantly different.

Fig. 2 presents the evolution of total microbial counts in fresh cut-lettuce dipped in water at 20, 30, 40 and 50 °C during 4 days of storage. Total microbial counts in control samples were  $6.90 \pm 0.39$  (log colony forming units [CFU]. g<sup>-1</sup>, n= 13). Samples subjected to mild heat treatments at 30, 40 and 50 °C did not present reductions in the microbial populations respect to control samples (20 °C).

During refrigerated storage, samples treated at different heat shock temperatures presented a same pattern for microbial populations

Table 1. Sensory attributes (midrib and edge browning, color, texture and overall visual quality) in fresh cut-lettuce as influenced by heat shock temperatures and by refrigerated storage at 5 °C for up to 4 d

Sensory Attributes*	Heat shock temperature (°C)	Storage time (days)				
		0	1	2	3	4
Midrib Browning	20	5.0±0.0a x *	4.7±0.4b x	4.2±0.4c x	3.5±0.7d x	3.4±0.3d x
	30	5.0±0.0a x	4.8±0.2b x	4.0±0.3b x	3.7±0.6d x	3.6±1.2d x
	40	5.0±0.0a x	4.9±0.2a x	4.7±0.1b y	4.0±0.5c y	4.0±0.8c y
	50	5.0±0.0a x	5.0±0.0a y	5.0±0.0a z	4.9±0.1a z	4.9±0.2a z
Edge Browning	20	5.0±0.0a x	4.5±0.2b x	4.0±0.5c x	3.1±0.7d x	2.5±0.6d x
	30	5.0±0.0a x	4.9±0.1b y	4.2±0.3c x	3.2±0.4d x	3.0±0.2d y
	40	5.0±0.0a x	5.0±0.0a y	4.5±0.2b y	3.5±0.5c y	2.9±0.3d y
	50	5.0±0.0a x	5.0±0.0a y	5.0±0.0a z	4.9±0.1a z	4.8±0.1a z
Texture	20	4.9±0.3 <sup>a</sup> x	4.8±0.2a x	4.4±0.4b x	4.3±0.3b x	3.4±0.5c x
	30	4.8±0.2a x	4.7±0.2 <sup>a</sup> x	4.6±0.2a x	4.3±0.6a x	3.5±0.7b x
	40	4.6±0.1 <sup>a</sup> y	4.5±0.2 <sup>a</sup> x	4.3±0.3a x	3.9±0.4b x	3.6±0.4b x
	50	4.4±0.1a z	4.4±0.1a y	4.1±0.4a x	3.8±0.2b x	3.1±0.5c x
Colour	20	5.0±0.0a x	4.4±0.2b x	4.1±0.2c x	3.7±0.4d x	3.1±0.5e x
	30	5.0±0.0a x	4.6±0.4b x	4.0±0.1c x	3.8±0.5c x	2.9±0.2d x
	40	5.0±0.0a x	4.4±0.2b x	4.3±0.2b x	3.9±0.4c x	3.0±0.1d x
	50	4.8±0.4a x	4.3±0.2b x	3.9±0.3c x	3.6±0.6c x	3.1±0.3d x
OVQ	20	5.0±0.0a x	4.5±0.3b x	3.8±0.5b x	3.2±0.4c x	2.2±0.5d x
	30	4.9±0.2a x	4.6±0.3b x	4.1±0.2c x	3.3±0.5d x	2.7±0.2e x
	40	4.9±0.1a x	4.6±0.2b x	4.4±0.2c y	3.7±0.3d y	2.9±0.1e y
	50	4.8±0.3a x	4.3±0.8b x	3.9±0.3c x	3.7±0.2c y	3.3±0.7d y

OVQ= overall visual quality

\*Mean scores of three independent lots. Samples in each lot were run by duplicate.

Five point scale: 5 = very good and 1 = poor

a,b,c,d,e : Values within a same row with a different letter are significantly different ( $P<0.05$ ).

x,y,z : Values within a same column with a different letter are significantly different ( $P<0.05$ ).

evaluation (Fig. 2). There was a sharp increase in the number of CFU g<sup>-1</sup> during the first day of storage. During day 2 and 3, the microbial populations stabilized around 8.2 log CFU g<sup>-1</sup>. Finally, between days 3 and 4 of storage, another increase in microbial

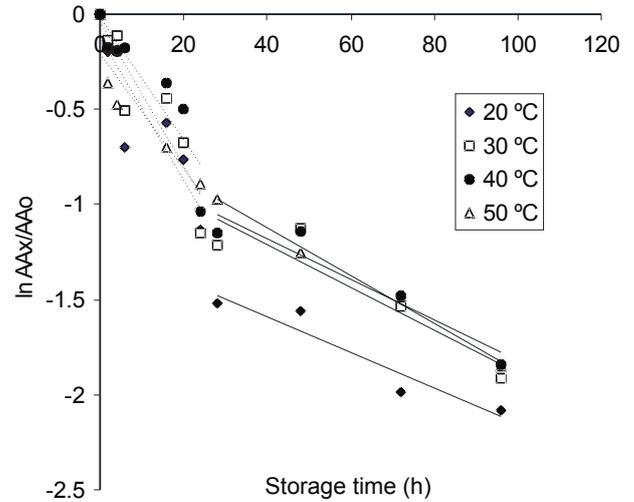


Fig. 1. Ascorbic acid degradation (ln AAx/AAs) in fresh cut-lettuce dipped in water at 20, 30, 40 and 50 °C. Dotted lines correspond to tendency lines obtained during the first 24 h of storage with a slope average of  $-0.03475$ . Full lines correspond to tendency lines obtained during the period 24-96 h of storage, with a slope average of  $-0.0109$ . Each assay was performed by triplicate of 3 lots on 3 separate experimental runs.

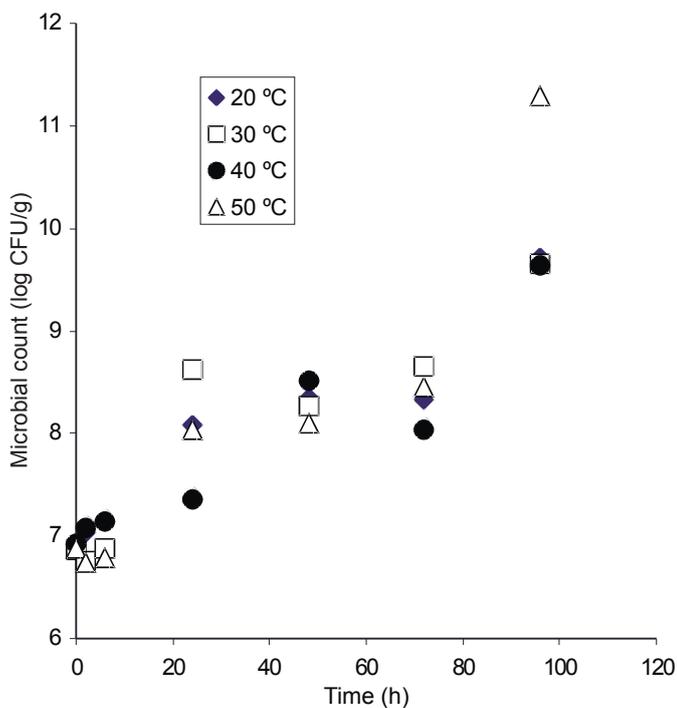


Fig. 2. Evolution of total microbial counts (CFU. g<sup>-1</sup>) in fresh cut-lettuce dipped in water at 20, 30, 40 and 50 °C during 4 days of storage. Each assay was performed in duplicate of 2 lots on 3 separate experimental runs.

counts was found. The largest increases in microbial populations corresponded to samples that had been exposed to the highest bath temperatures, that is 50 °C.

**Sensory acceptability and PAL activity:** Table 1 presents the sensory attributes (midrib and edge browning, color, texture appearance and overall visual quality) in fresh cut-lettuce as influenced by heat shock temperatures and by refrigerated storage at 5 °C for up to 4 d. The application of the thermal shocks did not cause any noticeable changes in the sensory evaluations at the beginning of the storage period. Although the texture appearance of samples treated at 40 and 50 °C received lower scores, associated to a little softening of the tissues, the differences were not significant. The scores attributed to cut-lettuce color were similar in all the samples, independent of the temperature at which they had been exposed. Samples treated at 20 and 30 °C consistently obtained higher scores in texture appearance than those treated at 40 and 50 °C. Nevertheless, after 4 d of storage, this particular index did not reach rejection levels.

On the other hand, samples treated at 50 °C received significantly higher scores in midrib and edge browning throughout the 4 d of storage. The scores assigned to these samples for midrib and edge browning corresponded to excellent quality even after 4 d of storage. Since browning has an important impact on the overall visual quality (OVQ) of this product, samples treated at 50 °C were the only ones to receive scores of OVQ above 3 at day 4.

Fig. 3 represents the phenylalanine ammonia lyase (PAL) activity in midrib tissue of fresh cut-lettuce treated at 50 °C compared with control cut-lettuce (20 °C) during the first hours of storage. No significant increase in PAL activity was observed in the first 12 h of storage in both samples, so only PAL values obtained at storage times higher than 13 h were presented. Heat shock treatments at 50 °C significantly decreased the rate of PAL activity

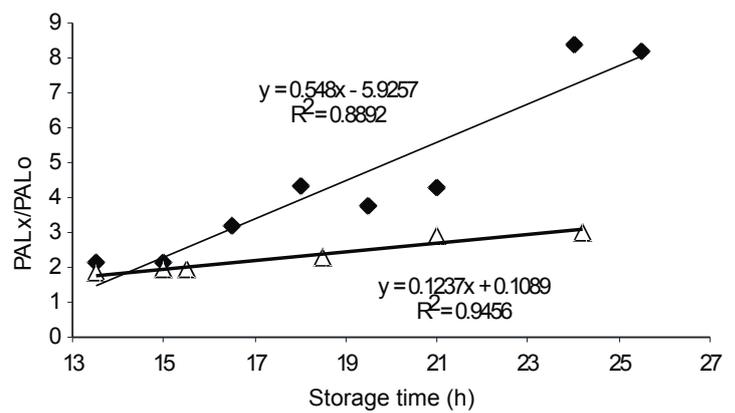


Fig. 3. PAL activity in midrib tissue of fresh cut-lettuce during the first 24 h of storage.  $\Delta$ - Cut-lettuce subjected to heat-shock treatment at 50 °C,  $\blacklozenge$ - Control cut-lettuce. Each assay was performed by triplicate of 2 lots on 2 separate experimental runs.

in the first day of storage (Fig. 3), being 4 times lower than in control samples.

## Discussion

The initial contents of ascorbic acid in fresh vegetables depend on various pre and post harvest factors such as climatic conditions, maturity at harvest, harvesting methods or exposure to sunlight (Lee and Kader, 2000). Since ascorbic acid is degraded during storage of these commodities, the time elapsed between harvest and analysis, that is the freshness of the sample, plays an important role in its initial levels. The narrow period (1 h) between lettuce harvest and ascorbic acid determination resulted in higher initial ascorbic acid content in the lettuce samples assayed. The values obtained were significantly higher than those previously obtained with the same lettuce variety (Moreira *et al.*, 2005; Roura *et al.*, 2003).

Ascorbic acid is lost during the different processing operations: some is lost in the cutting operation and some due to application of heat shocks. Cutting resulted in loss of ascorbic acid content in the range 100 to 150 g kg<sup>-1</sup>. Kader (1992) reported that fresh vegetables lose ascorbic acid when they are severely cut or shredded. Cut-lettuce samples subjected to a heat shock at 50 °C presented additional losses in ascorbic acid around 300 g kg<sup>-1</sup>. Similarly, Moreira *et al.* (2005) reported that the immersion of whole lettuce leaves in hot water produced losses in ascorbic acid content.

During storage, ascorbic acid degradation in fresh cut-lettuce subjected to mild heat shocks presented two periods with different degradation rates: a rapid decrease in the first 24 h of refrigerated storage and thereafter, a period of lower rate (Fig. 1). This would indicate different mechanisms for ascorbic acid degradation during the first stages of refrigerated storage that could be attributed to residual stress factors and to leaching and oxidation of the ascorbic acid exposed by the cuts. The physical damage caused by preparation increases respiration and ethylene production within minutes, and associated increases occur in the rates of other biochemical reactions responsible for changes in color, flavor, texture and nutritional quality (Kader, 1992). In whole lettuce leaves, subjected to the same thermal treatments, the increase in ascorbic acid degradation rate during the first day of storage was not observed (Moreira *et al.*, 2005), indicating

that in cut-lettuce samples the first period of degradation would be attributed at stress factors accumulation due to cutting operation.

Although different ascorbic acid degradation rates before and after the first 24 h of storage were observed, no significant differences were found among the slopes of the tendency lines for the different bath temperatures. Murata *et al.* (2004) reported that exposure of cut lettuce to 50 °C for 90 s did not affect the ascorbic acid contents.

Microorganisms play an important role in the shelf life of fresh vegetables. Both, cutting and thermal treatments may affect the microbial populations that will proliferate in minimally processed lettuce. The initial microbial counts found in control cut-lettuce (20 °C) were similar at those previously reported for whole lettuce leaves of the same variety (Moreira *et al.*, 2005; Roura *et al.*, 2003). Thermal treatment applications did not produce any initial microbial counts reductions in fresh cut-lettuce (Fig. 2). In contrast, initially microorganisms reductions were observed when whole lettuce leaves were subjected to mild heat shocks (Moreira *et al.*, 2005). Therefore, this different behavior would be due to the cutting operation. Fresh, whole vegetables usually have a hard, protective layer and waxy material on the outer surface. In minimally processed vegetables, the continuity of this protective layer is affected and some cells are ruptured. This would expose cellular material high in water contents, sugars, organic acids and other organic substances (King and Bolin, 1989). Damaged tissue would not only provide a portal for the entry of microorganisms but would also facilitate their access to nutrients for their metabolism. Furthermore, microorganism can establish itself inside broken cells or cells adjacent to broken tissue and although fresh-cuts are washed with sanitizing agents, microorganisms can survive when they are located within cells or areas not penetrated by the washing treatment (Watada *et al.*, 1996). Delaquis *et al.* (2004) reported reduction in the microbial populations of about 1 log cycle on fresh-cut iceberg lettuce treated for 1 min at 50 °C in chlorinated water. Li *et al.* (2001) also reported reductions in the populations of mesophilic aerobic bacteria in the range of 1.71 to 1.96 log cycles on lettuce treated for 90 s at 50 °C in chlorinated water. The presence of chlorine would be responsible for the differences with our results.

Independently of the bath temperature assayed, microbial counts in cut-lettuce increased gradually during storage presenting samples treated at 50 °C the highest microbial counts (Fig. 2). On one side, this could be due to the availability of nutrients for microbial growth resulting from the physical disruption of tissue structures associated to the cutting operation. On the other side, during the first hours of storage there is a transient temperature until thermal equilibrium between the samples and the storage chamber is reached, that could favor bacterial growth. This would correspond to a period when the low storage temperature would hinder the proliferation of microorganisms. Moreira *et al.* (2005), found that high heat shocks temperatures also promoted faster microbial growth in whole lettuce. Li *et al.* (2001) also reported enhanced microbial growth during the storage of iceberg lettuce that had been treated at 50 °C.

Fresh leafy vegetables are very fragile foodstuffs that deteriorate rapidly. The sensory attributes that make deterioration most evident are changes in texture appearance (wilting) and color

(enzymatic discoloration). Thermal treatment application on cut-lettuce did not produce changes in the sensorial acceptability determined by color, texture appearance and overall visual quality (Table 1). In a previous work, Moreira *et al.* (2005) reported similar results for whole lettuce leaves subjected to mild heat shocks.

Thermal treatment at 50 °C was the only effective treatment in delaying the onset of midrib and edge browning up to four days of refrigerated storage. This attribute was considered by the panelist as the most relevant in the overall visual quality of the product. Minimal processing generally increases the rates of metabolic processes that cause deterioration of fresh produce. Control of the wound response is the key to provide processed products of good quality (Kader, 1992). Apparently, the impact of wounding was reduced by the application of heat shock treatments at 50 °C, reducing wound-induced metabolic activity. Loaiza-Velarde *et al.* (1997) reported that treatments at 45 °C for 90 s disrupt the wound-induced increase in PAL activity, delays the accumulation of phenolic compounds and diminishes tissue browning. Salveit (2000) reported that treatment at 45 °C for 90 s was so persistent in fresh-cut lettuce that it did not show any browning, even after 15 d in air at 5 °C.

A significant decrease in PAL activity in cut-lettuce as a consequence of thermal treatment at 50 °C was obtained (Fig. 3). The ability of heat-shock treatments to reduce wound-induced browning may be the result of the decreased synthesis of wound-induced PAL activity (Saltveit, 2000). Ke and Saltveit (1989) indicated that wound-induced PAL activity is evident in the first hours of storage and reaches a maximum after 1 day. Pereyra *et al.* (2005) working with the same lettuce variety (Romaine lettuce) found a high regression coefficient when the slopes of the tendency lines of PAL activity during the first day of storage were plotted against the slopes of the tendency lines of OVQ scores. They reported that changes in OVQ scores and in PAL activity during the first day of storage could be concurrent phenomena and therefore the initial rate of change in PAL activity could be used to predict the shelf life of minimally processed lettuce.

Cut lettuce treated at 50 °C received significantly higher scores in midrib and edge browning throughout the 4 d of storage. Since browning has an important impact on the overall visual quality (OVQ) of this product, samples treated at 50 °C were the only ones to receive scores of OVQ above 3 at day 4. Since fresh-cut lettuce presents a shorter shelf life than whole lettuce leaves and midrib and edge browning are enhanced by wounding, the retardation of discoloration disorders becomes the more important quality index in cut vegetables and thermal shocks at 50 °C could make a great contribution to extend the shelf life of minimally processed lettuce.

This work presented evidence for the potential application of mild heat shocks as a novel preservation technology for minimally processed vegetables compatible with organic and low input farming systems.

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## References

- Box, G.E.P., W.G. Hunter and J.S. Hunter (Eds), 1978. *Statistics for experimenters*. Wiley, New York.
- Brech, J.K. 1995. Physiology of lightly processed fruits and vegetables. *HortScience*, 30: 18-22.
- Cantwell, M.I. and X. Nie, 1996. Use of heat treatments to control postharvest pathogens on tomatoes and melons. In: *Organic 1992 Proceedings of Organic Farming Symposium*, University of California, Division of Agriculture, National Research Publication, pp. 96-101.
- Cantwell, M.I., G. Hong and T.V. Suslow, 2001. Heat treatments control extension growth and enhance microbial disinfections of minimally processed green onions. *HortScience*, 36: 732-737.
- Delaquis, P.J., L.R. Fukumoto, P.M.A. Toivonen and M.A. Cliff, 2004. Implications of wash water chlorination and temperature for the microbiological and sensory properties of fresh-cut iceberg lettuce. *Postharvest Biol Technol.*, 31(1): 81-91.
- Kader, A.A. (Ed.), 1992. *Postharvest Technology of Horticultural Crops*. University of California, Division of Agriculture and Natural Resources.
- Kang, H. and M.E. Saltveit, 2003. Wound-induced PAL activity is suppressed by heat-shock treatments that induce the synthesis of heat-shock proteins. *Physiol Plant.*, 119(3): 450-455.
- Ke, D. and M.E. Saltveit, 1989. Wound-induced ethylene production, phenolic metabolism and susceptibility to russet spotting in iceberg lettuce. *Physiol. Plant.*, 76: 412-418.
- King, A. and H. Bolin, 1989. Physiological and microbiological storage stability of minimally processed fruits and vegetables. *Food Technol.*, 43: 132-135.
- Lee, S. and A. Kader, 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biol and Technol.*, 20: 207-220.
- Li, Y., R.E. Brackett, R.L. Shewfelt and Beuchat, 2001. Changes in appearance and natural microflora on iceberg lettuce treated in warm, chlorinated water and then stored at refrigeration temperature. *Food Microbiol.*, 18: 299-308.
- Loaiza-Velarde, J.G., F.A. Tomás-Barberá and M.E. Saltveit, 1997. Effect of intensity and duration of heat shock treatments on wound-induced phenolic metabolism in iceberg lettuce. *J. Am. Soc. Hort. Sci.*, 122: 73-77.
- Moreira, M.R., S.I. Roura and C.E. del Valle, 2003. Quality of Swiss chard produced by conventional and organic methods. *Lebensm-Wiss Technol.*, 36: 135-141.
- Moreira, M., A. Ponce, C. del Valle and S.I. Roura, 2005. Ascorbic acid retention, microbial growth and sensorial acceptability in lettuce leaves subjected to mild heat shocks. *J. Food. Sci.*, 71(2): S188-192.
- Murata, M., E. Tanaka, E. Minoura and Homma, 2004. Quality of cut lettuce treated by heat shock: prevention of enzymatic browning, repression of phenylalanine ammonia-lyase activity and improvement on sensory evaluation during storage. *Biosci. Biotechnol. Biochem.*, 68: 501-507.
- Pereyra, L., S.I. Roura and C.E. del Valle, 2005. Phenylalanine ammonia lyase activity in minimally processed Romaine lettuce. *Lebens-Wiss u Technol.*, 38(1): 67-72.
- Roura, S.I., L.A. Davidovich and C.E. del Valle, 2000. Postharvest changes in fresh Swiss chard under different storage conditions. *J. Food Qual.*, 23: 143-147.
- Roura, S.I., M.R. Moreira, A.G. Ponce and C.E. del Valle, 2003. Dip treatments for fresh Romaine lettuce. *Ital. J. Food Sci.*, 3(15): 405-415.
- Saltveit, M.E. 1998. Heat shock and fresh cut lettuce. *Perishables Handling Q.*, 95: 5-6.
- Saltveit, M.E. 2000. Wound induced changes in phenolic metabolism and tissue browning are altered by heat shock. *Postharvest Biol. Technol.*, 21(1): 61-69.
- Tomás-Barberán, F.A. and J.C. Espin, 2001. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *J. Food Sci. Agriculture*, 81: 853-876.
- Volk, W. (Ed.), 1980. *Applied Statistics for Engineers, Correlation-Regression*. Mc Graw-Hill, Inc. New York.
- Watada, A.E., N.P. Ko and D.A. Minott, 1996. Factors affecting quality of fresh-cut horticultural products. *Postharvest Biol. Technol.*, 9: 115-125.