Effect of immersion solutions on shelf-life of minimally processed lettuce

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Abstract

Prior to detailed quality and physiology evaluations, different immersion solutions (SIs) were analysed by means of a nontrained sensory panel. Each SI (calcium chloride, pectin, potassium sorbate, garlic extract and citric acid) was analysed at three concentrations. Based on at least 70% acceptance, the best mixed SI ($P < 0.05$) was determined to be 2 g/L citric acid, 1 g/L calcium chloride and 250 g/L garlic extract (SI). The control treatment was washed only with 0.05 g/L active chlorine. Treated lettuce was stored at 5°C in darkness in sealed polypropylene bags, for 9 days. Some senescence indicators (weight loss, colour), certain enzymes related to colour (chlorophyllase and polyphenoloxidase), and a nutrient (ascorbic acid), were measured during storage. SI showed a positive effect on shelf-life of minimally processed lettuce, controlling enzymatic browning, chlorophyllase activity and weight loss. The microorganisms growth was not significantly controlled, but the fact that the organoleptic results of SI-treated lettuce showed about 80% acceptability during 9 days of storage, suggests that some slight modifications on SI could be the basis of a promising formula for minimally processed lettuce.

Keywords: Lettuce; Minimal processing; Calcium chloride; Citric acid; Garlic extract; Storage

1. Introduction

The consumer preference of commercially grown head lettuce *Lactuca sativa* L. var. *crispa* cv. Lorka (Bianco, 1990) is due to crispiness and yellow–green colour, but is very susceptible to enzymatic browning when cut. Therefore, to be introduced as a minimally processed product, there is a need to improve the shelf-life.

After cutting, the surface of perishable product is exposed to air and to possible damage, being vulnerable to dehydration or change in colouration. Colour changes may be principally due to enzymatic browning or loss of chlorophyll. The general condition of quality, including firmness and vitamin content, is affected mainly by temperature, because respiration rate is altered. Some of the damage can be avoided using sharp tools, inhibitors of enzymatic browning, modified atmospheres and active packaging (Ahvenainen, 1996; Abbott, 1999; Agar, Massantini, Hess-Pierce, & Kader, 1999; Watada & Qi, 1999). During cutting, the surface of product is also exposed to possible contamination with bacteria, yeasts and moulds. In the case of minimally processed lettuce, which falls into the low-acid category of horticultural products (pH 5.8–6.0), high humidity and the large number of cut surfaces can provide ideal conditions for the growth of microorganisms (Ahvenainen, 1996; Behrsing, Winkler, Franz, & Premier, 2000).

Using gamma irradiation for textural studies of minimally processed apple slices (3–4 mm thick), CaCl$_2$ prevented softening and maintained the cell wall structure through cross-linking the pectic acid in the cell wall (Gunes, Hotchkiss, & Watkins, 2001). In addition, the anhydrous hygroscopic CaCl$_2$ salt, in atmosphere of high relative humidity, has the ability of absorbing water, as has been demonstrated in cut carrots (Cisneros-Zevallos, Saltveit, & Krochta, 1997). However, without the external protective tissue, the cut products lose weight easily. In contrast, when storing horticultural products inside bags or protected with edible coatings, the relative humidity remains high to keep products from the weight loss (Baker, Baldwin, & Nisperos-Carriedo, 1994). Furthermore, there can be

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problems if the packaging film is not sufficiently permeable to O₂, CO₂ and H₂O (Watada & Qi, 1999). Moisture can condense on the inner surface of the film, which added to the acidity of leafy vegetables (pH 5.8–6.0), can end up as the cause of deterioration and growth of microorganisms (King, Magnuson, Török, & Goodman, 1991; Ahvenainen, 1996; Brackett, 1999). Behrsing et al. (2000) tested the efficacy of chlorine dipping for inactivation of Escherichia coli on recent inoculated lettuce and found a significant reduction of E. coli counts.

Enzymatic browning of shredded lettuce has been slowed down using low O₂ modified atmosphere or some vacuum level inside packaging (Heimdal, Kühn, Poll, & Larsen, 1995). In apple pieces, the enzymatic browning decreases with the use of erythorbate antioxidant and CaCl₂, depending on the cultivar and the storage time. The selection of the cultivar, to control enzymatic browning, is also important in lettuce. The phenols of the reaction catalysed by the phenylalanine ammonia-lyase are used as a possible browning index and their concentrations were seen as sufficient to inhibit browning; therefore 1, 2 and 3g/L of citric acid were tested. According to published data, the solutions chosen were: 1, 3 and 5g/L pectin (Toft, Grasdalen, & Smidsrød, 1986), 1, 2 and 3g/L calcium chloride (Cisneros-Zevallos et al., 1997; Kukura, Beelman, Peiffer, & Walsh, 1998), 3, 4 and 5g/L potassium sorbate, and were mixed as follows: citric acid+calcium chloride+garlic extract, citric acid+calcium chloride+potassium sorbate, and were mixed as follows: citric acid+calcium chloride+garlic extract, citric acid+calcium chloride+potassium sorbate, and were mixed as follows: citric acid+calcium chloride+garlic extract, citric acid+calcium chloride+potassium sorbate, and were mixed as follows: citric acid+calcium chloride+garlic extract, citric acid+calcium chloride+potassium sorbate, and were mixed as follows: citric acid+calcium chloride+garlic extract, citric acid+calcium chloride+potassium sorbate.

A loss of green pigmentation during storage can be observed in shredded Iceberg lettuce (Bolin & Huxsoll, 1991) and also in spinach leaves (Abe & Watada, 1991). The loss of chlorophyllous pigments during senescence of green vegetable tissues is due to several factors, among these, including chlorophyllase (Schoch & Ihl, 1998; Jacob-Wilk, Holland, Goldschmidt, Riov, & Eyal, 1999), magnesium dechelatase (Langmeier, Ginsburg, & Matile, 1993; Shioi, Tomita, Tsuchiya, & Takamiya, 1996), and low pH, depending on the tissue (Heaton & Marangoni, 1996).

The purpose of the present work is to improve the shelf-life of cut lettuce, after treatments with immersion solutions (SIs), by monitoring: (1) certain senescence parameters such as weight loss and colour, (2) certain enzymes related to colour changes such as chlorophyllase and polyphenoloxidase, (3) ascorbic acid as a nutrient, and (4) aerobic plate count as microbiological quality.

2. Materials and methods

2.1. Plant material

Head lettuce (Lactuca sativa L. var. crispa) cv. Lorka (Bianco, 1990), commercially grown in La Araucanía Region, Chile (39°39’15”/S), was used in the testes. Lettuce heads, harvested at optimal maturity, were processed immediately. The lettuce leaves were selected, cut with a sharp knife in transversal long narrow strips of 2 cm, dipped for 40 s in tap water containing 0.05 g/L active chlorine (Hong & Gross, 1998), solution prepared using sodium hypochlorite (50 g/kg active chlorine in filtered water, Clorox™, Clorox Chile, Santiago, Chile) and manually centrifuged in a kitchen centrifuge of 24 cm diameter (Meliconi Spa. 40057 Emilia BO, Italy). After treatments, the cut lettuce (50 g) was placed in 15 cm × 15 cm sealed polypropylene bags of low density (0.04 mm thickness), permeable to water vapor of 0.33–0.42 g/m²h (Dantas Cabral, 1986) and stored for 9 days at 5°C ± 0.2°C in darkness.

2.2. Choice of solutions for mixed immersion treatment

Preliminary experiments were done to determine the concentrations of SIs. Although Castañer, Gil, Artes, and Tomas-Barberan (1996) tested 10–100 g/L citric acid and found complete inhibition of browning of Iceberg lettuce stems only with 100 g/L citric acid, in the present work, with crispy lettuce cv. Lorka, one-tenth of those concentrations were seen as sufficient to inhibit browning; therefore 1, 2 and 3 g/L of citric acid were tested. According to published data, the solutions chosen were: 1, 3 and 5 g/L pectin (Toft, Grasdalen, & Smidsrød, 1986), 1, 2 and 3 g/L calcium chloride (cisneros-Zevallos et al., 1997; Kukura, Beelman, Peiffer, & Walsh, 1998), 3, 4 and 5 g/L potassium sorbate (Dziezak, 1986), and 160, 250 and 330 g/L previously filtered garlic suspension in water (Yu & Wu, 1989; Kyung, Park, & Kim, 1996). The cut lettuce was dipped in these solutions for 40 s, then the excess liquid was eliminated with the manual centrifuge of 24 cm diameter (Meliconi Spa) and the lettuce packaged for storage as described in Section 2.1.

The concentration used for each SI was selected through a nontrained consumers panel, based on the method described in Vankerschaver, Willocx, Smout, Hendrickx, and Tobbak (1996). The number of panellists for each session fluctuated between 20 and 30 and treatments were evaluated on day 0, 1, 4, 6 and 9 of storage. The consumer panel was asked to answer yes or no to the question “Would you consume the sample?” For each evaluation date, the products presented to each panelist were randomised, and for each sample, the percentage of the panel that would consume the product was calculated.

Using the method of Vankerschaver et al. (1996), the solutions chosen were 2 g/L citric acid, 1 g/L calcium chloride, 1 g/L pectin, 250 g/L garlic extract and 5 g/L potassium sorbate, and were mixed as follows: citric acid + calcium chloride + garlic extract, citric acid + calcium chloride + potassium sorbate, citric acid + pectin + garlic extract, citric acid + pectin + potassium sorbate. The same method used for the determination of the SI concentration was employed to choose which of these four treatments of mixed SIs best maintained the visual quality of lettuce.
2.3. Sampling and treatment

Sampling procedure was carried out between March and June 1999 and January and March 2000, with 3–6 replicates, depending on the analysis. Sampling for each quantitative chemical analysis was done taking a portion of salad-cut lettuce (leaf to stem of 1:1). Repetitions were performed on different dates to avoid sampling errors resulting from heterogeneity of plant material. The nondestructive analysis (colour with five sealed bags and weight loss with six sealed bags, per treatment), were made on 0, 3, 6, 9 days. The destructive analysis (microbiological evaluation, ascorbic acid, polyphenoloxidase (PPO) activity, chlorophyllase activity) were done always on sealed bags and were also carried out every 3 days for up to 9 days per treatment, using three bags for each analysis-day (three separate replicates).

The treatments used were the best mixture of SI as described in Section 2.2, which was called SI in all further experiments, and the control (cut-lettuce, dipped for 40 s in water containing 0.05 g/L active chlorine and manually centrifuged); SI and control were packaged as described in Section 2.1.

2.4. Ethylene content inside bags

To measure the ethylene inside the sealed bags (five replicates for each treatment day), through an area of the sealed bags reinforced with silicone and adhesive tape, the air inside the bags was permitted to flow to a vacuum tube of known volume. Total volume of gas inside the bag was measured (it was always around 250 mL). A 500 µL gas samples from the vacuum tube was taken with a syringe and were injected in a Varian Star 3400 cx gaschromatograph (GC), using a 30 mL long FID temperature were maintained at 250 °C. Splitless mode injection was used. Column temperature was kept at 40 °C for 1 min, then programmed from 40 °C to 70 °C at 10 °C/min and maintained at 70 °C for 6 min. Ethylene (9 mg/L) in nitrogen (purchased at INDURA Gases Especiales, Santiago, Chile) was used for the identification and quantification, employing the method of Galeazzi, Sgarbieri, and Constantinides (1981) and Tan & Harris (1995). The reaction mixture contained 0.1 mL crude enzyme solution and 2.9 mL substrate solution (0.020 mol/L catechol as substrate added to 0.05 mol/L phosphate buffer, pH 7.0). The reference cuvette contained only substrate solution. The rate of oxidation of catechol was followed at 25 °C at 400 nm, at min 1 and 2. The enzyme activity unit was defined as the 0.001 change in absorbance, between min 1 and 2, under the condition of the assay. A preliminary reactivity of the PPO enzyme of Lorka lettuce toward different substrates (catechol, 4 methyl catechol, gallic acid, 3,4 dihydroxyphenylacetic acid) was examined, catechol being the most reactive substrate. Therefore, catechol was used for the PPO activity determinations. The enzyme extraction was done in triplicate, from experiments done in different dates, and each enzyme activity was assayed at least in triplicate.

2.6. Extraction and assay of chlorophyllase (chlorophyll-chlorophyllido hydrolase, EC 3.1.1.14)

Minimally processed lettuce (2 g) was homogenized with 3.3 mL of cold homogenizing mixture (acetone and 0.02 mol/L phosphate buffer, pH 7.5 in a ratio of 4:1) and centrifuged (Beckman GS-15) at 880 × g for 3 min. A volume of 3.0 mL of this crude extract was assayed for chlorophyllase activity, after adding 130 nmol chlorophyll. The pigments before incubation, in 1 mL of the mixture, were immediately analysed, and the rest was incubated (GFL 3032) in darkness for 1 h at 30 °C with agitation (100 oscillations min⁻¹); then an aliquot of 1 mL was analysed for the pigments after incubation. For pigments (before and after incubation) analysis, the reaction was stopped with cold acetone to a final ratio 4:1 acetone to enzyme extract, followed by a 3 min centrifugation at 880 × g. Pigments were analysed by HPLC (LaChrom Merck Hitachi L-7100 pump and a UV–vis detector Merck Hitachi L-4250) as described below. Chlorophyllase activity, measured as the appearance of chlorophyllide a and b and phophorbid a and b, was obtained after subtraction of the initial dephtylated pigments present in the assay at the start of the 1 h incubation. Results of the enzyme assay were expressed as nmol of dephtylated pigments (chlorophyllide a, b and phophorbid a, b) produced after 1 h reaction time at 30°C, in darkness, per gram of fresh lettuce tissue (FW). For each treatment, the enzyme extraction was done in triplicate, from different experiments, and each assayed for activity was replicated.
two-fold. To check the enzyme activity, a chlorophyllase activity experiment was done with the enzyme previously boiled for 5 min (Ihl, Monsalves, & Bifani, 1998; Schoch & Ihl, 1998).

The measurement of pigments by HPLC was based on the methods of Yamauchi and Watada (1991) and Fang, Bouwkamp, and Solomos (1998), with slight modifications. A Lichrospher 100 RP18 (5 μm) Merck column, an HPLC LaChrom Merck Hitachi L-7100 pump and a UV–vis detector Merck Hitachi L-4250 were used. Samples of 20 μL were injected, and at the beginning, a linear gradient was employed. The initial ratio of solvent A (80% methanol and 20% water) to B (ethyl acetate) was 100:0. The final ratio of solvent A to B was 50:50 at a flow rate of 1 mL/min. The detector was used at 420 nm for the measurements of chlorophyll a, b, chlorophyllide a, b, pheophytin a, b, pheophorbide a, b, since all these pigments absorb at 420 nm. Standard chlorophyll a and b were purchased at Sigma-Aldrich. Chlorophyllide a and b were obtained upon incubation of chlorophylls with chlorophyllase extracted from Swiss chard (Beta vulgaris L. cv. Cicla) under incubation conditions described in Schoch and Ihl (1998). Pheophorbide were prepared by adding 2–3 drops of 2 mol/L HCl to 5 mL of the chlorophyllide solution in acetone, extracted in ethyl acetate, washed until neutral pH with water and dried under nitrogen stream. Pheophitin were prepared as described above, acidifying the chlorophylls in hexane, and washing till neutrality. All standard pigments were prepared in 80% acetone solutions for the absorption spectrum (Hewlett Packard 8452 A spectrophotometer) and for the HPLC pigment analysis, where pigments were injected in triplicate, in different concentrations, to obtain the retention time and a relative area/pmol, with an error margin of 5%. The retention time was 2.8 ± 0.5 min for chlorophyllide b, 5.5 ± 0.5 min for chlorophyllide a, 9.7 ± 0.3 min for pheophorbide b, 12.5 ± 0.3 min for pheophorbide a; 21.5 ± 0.5 min for chlorophyll b, 23.7 ± 0.7 min for chlorophyll a, 26.0 ± 0.3 min for pheophytin b and 29.4 ± 0.5 min for pheophytin a.

2.7. Colour measurement

The colour was quantified with a Minolta Chroma-meter CR-200b colourimeter on CIE L*a*b* system as described in Ihl, Shene, Scheuermann, and Bifani (1994) and Ihl et al. (1998). The instrument was calibrated with a white standard tile (Y = 93.1, x = 0.3140, y = 0.3212) and with a green standard tile (Y = 33.2, x = 0.277, y = 0.375) under illuminant condition C (6774 K). The L* variable lightness index ranges the scale from 0 for black to 100 for white. The a* scale measures the degree of red (+a*) or green (−a*) colours and the b* scale measures the degree of yellow (+b*) or blue (−b*) colours.

2.8. Ascorbic acid content determination

Lettuce tissue (5 g), homogenized in a porcelain mortar with pestle in 5 mL distilled water, was centrifuged at 880 × g for 3 min; the pellet was washed, centrifuged again, and ascorbic acid was measured in the combined supernatants. Ascorbic acid reduces yellow molybdophosphoric acid to phosphomolybdenum blue, the concentration of which is determined reflectometrically using the Merck ascorbic acid reflectometric test and the Merck RQ Flex at 570/657 ± 10 nm (Ihl et al., 1998).

2.9. Weight loss determination

Weight of cut lettuce was recorded initially and after storage and the difference was used to calculate percent weight loss. The weight was controlled in the same six sealed bags of each treatment during all the storage time.

2.10. Microbiological assay

Aerobic plate count was carried out using Plate Count Agar (Merck), with incubation time of 48 h at 35°C (Barriga, Trachy, Willemot, & Simard, 1991; King et al., 1991). The microbial population was expressed as the logarithm of colony-forming units per gram fresh weight of cut-lettuce (log cfu/g FW), which means that for 1 log cfu/g FW increase, the microbial counts increases tenfold.

2.11. Statistical analysis

The results were analysed for the influences of treatments and storage days, by using the variance method StatMost 3.0 (Statistical Analysis and Graphics) with a significance level of 5% (Steel, Torrie, & Dickey, 1997). The significance between treatments and storage days were analysed using Duncan’s multiple range test (α = 0.05).

3. Results and discussion

3.1. Selection of the immersion solution

Because consumers prefer minimally processed horticultural products without defects, and with optimal freshness, appearance, firmness, and texture (Watada & Qi, 1999), some SIs were selected through a sensory panel: calcium chloride as humidity holder (Cisneros-Zevallos et al., 1997) and as a textural enhancer, which produces specific textural changes in the structure of cell wall polymers, either during ripening or processing of fruits and vegetables (Roy et al., 1994; Jackman &...
Stanley, 1995; Luna-Guzman & Barrett, 2000); pectins, to retain water, and together with calcium, to maintain the texture, due to the calcium pectate formation in the cell wall (Jackman & Stanley, 1995; Gunes et al., 2001); potassium sorbate, against yeasts and molds (Dziezak, 1986); garlic extract, as an antibacterial agent (Kyung et al., 1996); citric acid, to shift the pH from the optimum range for PPO and to chelate the copper of the active site of the enzyme (Martinez & Whitaker, 1995; Castañer et al., 1996). As described in Section 2.2, after a shelf-life study in each concentration of each SI, the SIs chosen were 2g/L citric acid, 1g/L calcium chloride, 1g/L pectin, 250g/L garlic extract and 5g/L potassium sorbate and were mixed as citric acid+calcium chloride+garlic; citric acid+calcium chloride+potassium sorbate; citric acid+pectin+garlic extract; citric acid+pectin+potassium sorbate. Fig. 1 represents the percentage acceptability of the mixed SIs to determine the treatment to best maintain the visual quality of the cut lettuce. There were significant differences ($P<0.05$) between citric acid+calcium chloride+garlic mixture (treatment A) and the control (treatment E). The solutions of citric acid+calcium chloride+garlic (A), citric acid+pectin+garlic (C) and citric acid+pectin+potassium sorbate (D) showed a similar pattern during the first 4 days of storage, with 80% acceptability on day 4 of storage. After day 4, the lettuce treated with the solution of citric acid+calcium chloride+garlic (A) was better accepted by the sensory panel, although no significant difference was shown. From the preliminary screening tool in this part of the work, the results showed that the mixture of 2g/L citric acid, 1g/L calcium chloride, 250g/L garlic extract maintained the highest acceptability and was therefore chosen as the mixed SI for all further experiments.

### 3.2. Effect of the mixed immersion solution chosen during storage

If low-acid (pH 5.8–6.0) and high-humidity environment in minimally processed vegetables, like lettuce, can provide ideal conditions for the growth of microorganisms, the use of citric acid could not only prevent browning, but also lower the pH enough to prevent some of the microorganisms growth (Ahvenainen, 1996). Within these lines, the use of garlic as an antimicrobial agent has been proved successfully by Kyung et al. (1996). As can be seen in Fig. 2 during the 9-day storage period, the SI treatment reduced, although not significantly, the microbial growth. According to the Chilean Sanitary Regulations (Chile, 1996), for fresh processed fruits and vegetables, the aerobic plate count should be less than $10^5$ cfu/g. Therefore, at day 6 of storage (Fig. 2), the SI treatment is still below the threshold value in which the product can be consumed, but the sample with control treatment must be considered spoiled.

Philosop-Hadas, Meir, and Aharoni (1991) found an ethylene emanation peak in cut tissue of spinach leaves during the first 2–3 h after cutting. In our preliminary results, the ethylene concentration inside the bags sealed after 2h was $64.5\pm 22.6$ nL/L for the SI treated lettuce, a significantly lower value than $729.5\pm 62.1$ nL/L for the control lettuce. The control treatment showed the ethylene emanation of cut-lettuce, while SI solution...
reduced the ethylene emanation corresponding to wounding stress, probably due to the CaCl₂ component of the SI solution. Ca²⁺ is suggested to inhibit stress-induced senescence by maintaining membrane integrity (Nur, Ben-Arie, Lurie, & Altman, 1986, Gekas, Oliveira, & Crapiste, 2002).

Lightness index values (L*) (Fig. 3) did not show significant difference \( (P > 0.05) \) between the control and SI treatment or between days of storage, nor did they show a correlation with PPO activity (Fig. 4). However, there is a good correlation between PPO (Fig. 4) and colour parameter a* (Fig. 3) for the control and SI treatment. Colour parameter a* (Fig. 3) on day 6 of storage was significantly lower (more green) in SI than that in the control, but in relation to storage day 0, the greenness of SI was not significant. In Fig. 4, a significant reduction of PPO activity in SI treatment was observed during all the storage time. Castañer et al. (1996) showed that colour parameter a* better represented the changes in colour of enzymatic browning in minimally processed lettuce, as did Steet and Tong (1996) with regard to greenness in pureed green peas. Colour parameter b* (Fig. 3), also on day 6 of storage, was significantly different in SI than in the control, and in relation to storage day 0. Significant differences \( (P > 0.05) \) were not found between treatments and for the other storage days. Otherwise, some enzymatic browning was visually detected at day 9. As already mentioned, the SI treatment significantly reduced PPO activity through the 9-day storage period. As far as storage time is concerned, PPO activity increased significantly \( (P < 0.05) \) between days 0 and 9, for both treatments (Fig. 4).

As described in Artes et al. (1998), ethylene produced when lettuce is cut results in the strengthening of enzymatic browning, which is attributed to the increase PPO and phenylalanine ammonia-lyase activity. It was confirmed in this experiment that, inside the package, SI treatment showed a gradual increase in ethylene (unpublished data) and also in PPO activity (Fig. 4). Artes et al. (1998) also described the decrease in enzymatic browning with NaClO treatment, and a more effective decrease when calcium was included. This is already seen at day 0, where 2 h after package sealing, the ethylene concentration was reduced in 91% with the SI mixture (control = 729.5 ± 62.1 nL/L; SI = 64.5 ± 22.6 nL/L), and the PPO activity was also reduced for the same treatment (which contained 1 g/L CaCl₂) (Fig. 4). Citric acid, one of the other components of the SI mixture, is also an effective browning inhibitor when applied on the lettuce tissue, contributing to the low PPO activity. Data suggest that the mixture of calcium chloride with citric acid was responsible for the effectiveness of the inhibition of enzymatic browning, despite the fact that Castañer et al. (1996) found that acetic acid was a better browning inhibitor for head lettuce.

Chlorophyllase was significantly reduced \( (P < 0.05) \) by SI compared to the control (Fig. 5). The SI mixture was important for maintaining a low chlorophyllase activity, likely due to a shifting of the pH from the optimal range.
for lettuce chlorophyllase in vivo. This may also explain the shift to redness found in parameter a* for the control treatment (Fig. 3), and the greenness for SI treatment. Good maintenance of chlorophyll content was also found in snowpea pods, in modified atmosphere packaging of approximately 5 kPa O₂ plus 5 kPa CO₂ (Pariasea, Miyazaki, Hisaka, Nakagawa, & Sato, 2001).

The ascorbic acid lost was independent of the treatment employed, showing no significant differences (P > 0.05) between treatments, but significantly different (P < 0.05) among times. The ascorbic acid content declined from 109 ± 25 to 65 ± 6 mg/kg in the control and 73 ± 10 mg/kg in SI, similar to the ascorbic acid content reported in the literature for capitata lettuce: 80–120 mg/kg edible portion (Nehring, 1968).

There was no significant weight loss, for SI treatment during the 9 storage days, while the control shows a continuous decrease in weight, already being significant with 6 days of storage (Fig. 6). The weight loss is a natural process of catabolism of horticultural products, catalysed by enzymes and is accelerated by cutting and slicing. This decrease in weight may be attributed to respiration and other senescence-related metabolic processes during storage (Watada & Qi, 1999).

Therefore, shelf-life of salad-cut lettuce showed a positive effect with SI (with an apparent increase from 3 to 6 days), although microorganisms development was not significantly controlled. But SI was a treatment leading to a better maintenance of the colour parameters a* and b*, significantly reduced weight loss, PPO and chlorophyllase activities, although not ascorbic acid content, compared to the control. Otherwise, the preliminary organoleptic results of SI-treated lettuce showed about 80% acceptability during 9 days of storage, which agree with the results shown of controlling enzymatic browning and weight loss during the same time and during 6 days for chlorophyllase activity. The shelf-life of salad-cut lettuce could be improved to better control the microorganisms growth, with further SI studies based on SI, increasing the garlic acid concentration inside.
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References


