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Comparison of calcium lactate with chlorine as a washing treatment for fresh-cut lettuce and carrots: quality and nutritional parameters

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Abstract: The efficacies of calcium lactate and chlorine washing treatments of fresh-cut lettuce and carrots were compared during storage at 4 °C over 10 days. The gas composition of packages, colour, enzyme activity, texture, sensory attributes, microflora and levels of ascorbic acid and carotenoids were evaluated at 1, 3, 7 and 10 days. Calcium lactate treatment was not significantly different to chlorine treatment ($p < 0.05$) in terms of maintaining colour, texture and acceptability of fresh-cut lettuce and carrots during the entire storage period. The washing treatments did not affect levels of ascorbic acid of fresh-cut lettuce or carrots. Carotenoid levels were higher in calcium lactate-treated carrots than chlorine-treated samples at the end of storage. Mesophilic, psychrotrophic and lactic acid bacteria counts were not significantly different between treatments for both vegetables.

Keywords: calcium lactate; chlorine; quality parameters; fresh-cut lettuce; sliced carrots

INTRODUCTION

The fresh-cut vegetable industry represented $10–12 billion in 2000 in the USA.[1] In Ireland, current fruit and vegetable production amounts to ~€300 million per annum.[2] A significant growth in the ready-to-use (RTU) vegetable industry (~10% p.a.) has been largely driven by increasing demand for convenient, fresh, healthy foods. A problem of commercial significance is the shelf-life of fresh-cut products. It is limited owing to quality and microbial deterioration during storage, compared with intact, unprocessed vegetables.

Chlorine solutions (50–200 mg l^{-1}) are widely used to wash fruits and vegetables and also fresh-cut products in order to reduce microbial growth[3] and maintain quality. The controversy about the formation of carcinogenic chlorinated compounds in water (chloramines and trihalomethanes) has called into question the use of chlorine.[4,5] Therefore, there is great interest in the development of alternative washing treatments for fresh-cut vegetables that guarantee the quality and safety of the products and reduce the use of chlorine.

There have been many attempts to find alternative washing treatments to chlorine.[6] These include the use of organic acids, organic acid salts and ozone, among others.[7] Treatments that can be nutritionally beneficial and enhance quality retention are particularly interesting.

Pre- and postharvest calcium solution applications have been used to extend the postharvest shelflife of fruits and vegetables. The used of calcium treatment has been reported to be effective in reducing chlorophyll and protein loss and inhibiting plant tissue senescence.[8,9] In apples it has been reported to reduce respiration[10] and increase firmness retention[11] and also to reduce in general the incidence of physiological disorder and decay.[11,12]

Of the many calcium salts, calcium lactate has the advantage that it avoids the bitterness or off-flavours associated with other salts.[13] Calcium lactate is widely used in delicate fruits and those with a high senescence index such as strawberries.[14] Lactate has been reported to have potent antibacterial properties owing to its ability to uncouple microbial transport processes.[15]

Calcium lactate has not previously been evaluated as a treatment for fresh-cut lettuce or sliced carrots, and for that reason the main objective was to evaluate comprehensively the use of calcium lactate as a washing treatment for fresh-cut vegetables and compare it with the widely used chlorine treatment.

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Quality, nutritional and microbiological parameters were used to compare both treatments. Carrots and iceberg lettuce were selected, as they are the most common vegetables used in this industry in Ireland.

MATERIALS AND METHODS

Materials

Iceberg lettuce (*Lactuca sativa* sp) was grown in Ireland and carrots (*Daucus carota* sp) were grown in France. Both vegetables were grown under commercial conditions and were purchased from a local supermarket and stored for 18 h at 4 °C before use.

Methods

Vegetable preparation

Experiments were conducted from June to September 2003. All procedures were performed in a special food processing room at 18–20 °C. For each vegetable, lettuce or carrot, two treatments were conducted in parallel and prepared from the same batch of product. The first treatment consisted of samples washed with chlorinated water and the second of samples treated with calcium lactate. For lettuce, the two outer leaves were removed and the core was excised with a stainless-steel knife. The lettuce was cut in half and each half was further cut into four pieces. Carrots were rinsed briefly prior to peeling (1 min at 18–20 °C) in order to avoid soil contamination during the peeling. They were hand peeled using a manual peeler in one direction, removing a minimal amount of surface tissue. The carrots were then sliced manually with a sharp knife into discs ~5 mm in thickness. All experiments were repeated three times in duplicate.

Washing treatment

Washing treatments of lettuce and carrots were performed by immersion of the fresh-cut lettuce and sliced carrots in each treatment solution. Chlorinated water was prepared by adding sodium hypochlorite solution (120 g l^{-1} available chlorine) to distilled water to obtain 120 mg l^{-1} free chlorine (pH ~8). Calcium lactate (Sigma-Aldrich, St. Louis, MO, USA) was diluted to 30 g l^{-1} (pH ~6.5). For all treatments solutions were prepared using distilled water stored at room temperature (18–20 °C). Each vegetable was placed in a different basket and immersed in both chlorine and calcium lactate solutions for 5 min with agitation and subsequently dried for 5 min using a salad spinner.

The free chlorine concentration in the washing water was determined using a DREl/2000 spectrometer with appropriate AccuVac pillows (Hach, Loveland, CO, USA), using the DPD method.

To minimise product heterogeneity, processed vegetables were pooled, mixed and subsequently packaged in bags (200 × 320 mm) of 35-μm oriented polypropylene (OPP) (Amcor Flexibles Europe, Brighouse, UK). Each package contained ~250 g of product. The packages were chilled in a blast freezer at 0 °C for 2 min before heat-sealing under atmospheric conditions and storage at 4 °C for 10 days.

Quality parameters

Headspace analysis. A Gaspace analyser (Systech Instruments, Oxfordshire, UK) was used to monitor levels of CO_2 and O_2 during storage. Gas extractions were performed with a hypodermic needle, inserted through an adhesive septum previously fixed to the bags, at a flow rate of 150 ml min^{-1} for 10 s. Three bags per treatment were monitored for each experiment and the bags for other analyses were measured separately.

pH measurement. A 10-g sample of vegetable tissue was blended for 2 min in 20 ml of deionised water. The pH of the slurry was measured at room temperature using an Orion research pH-meter (Model 420A; New York, NY, USA) between 18 and 20 °C.

Exudates. Exudate was quantified by the method of Carlin and Nguyen-the[16] A weighed piece of green lettuce tissue (the rib was discarded) or a slice of carrot (a disc ~5 mm thick) was placed between two Rundfilter No.615 filter-papers (Machery-Nagel, Düren, Germany) and a force of 11 kg applied for 10 s. Exudate was calculated by measuring the weight before and after applying the force to the sample and expressed as: weight after (mg)/weight before (g).

Dry matter. A weighed piece of lettuce or a slice of carrot was heated at 100 °C for 2 h in a Universal Oven (Memmert, Schwabach, Germany). Dry matter was calculated by measuring the weight before and after heating and expressed as: weight after (mg)/weight before (g).

Colour measurement. Colour was quantified using a colorimeter (ColorQuest® XE; HunterLab, Reston, VA, USA). A piece of lettuce or carrot slice was place directly on the colorimeter sensor (3.5 cm diameter) and measured; 20–30 measurements were taken per treatment. The instrument was calibrated with a white tile standard ($L^* = 93.97$, $a^* = -0.88$ and $b^* = 1.21$) and a green tile standard ($L^* = 56.23$, $a^* = -21.85$, $b^* = 8.31$) under identical luminosity conditions. The L^* parameter (lightness index scale) ranges from 0 (black) to 100 (white). The a^* parameter measures the degree of red ($+a$) or green ($-a^*$) colour and the b^* parameter measures the degree of yellow ($+b$) or blue ($-b^*$) colour. The CIE $L^*a^*b^*$ parameters were converted to Hue (arctan b^*/a^*), Chroma $(a^2 + b^2)^{1/2}$ and total colour difference $\{\Delta E = [(L_f - L_i)^2 + (a_f - a_i)^2 + (b_f - b_i)^2]^{1/2}\}$, where L_i = initial luminosity, L_f = final luminosity, $a_f = a^*$ value at final time, $a_i = a^*$ value at initial time, $b_f = b^*$ value at final time and $b_i = b^*$ value at initial time.

Texture analysis. Texture properties of lettuce and carrots were assessed using an Instron texture analyser (Model 4302 Universal Testing Machine, Instron, Canton, MA, USA). A 500 N load cell was attached. A puncture test was performed using a 1 cm diameter aluminium probe with star-shaped section. The speed setting for the experiment was 1000 mm min^{-1} and the maximum load was reported (kN). Each test was performed on a single piece of lettuce (~2.5 g of green tissue) or slice of carrot (one slice). Data were analysed with the Instron Series IX software for Windows. Initially, several whole lettuce heads were tested, to examine variability within the lettuce head. The results showed that it was necessary to obtain a minimum of 20–25 samples to approximate results to a log-normal curve.

Sensory analysis. Sensory analysis was performed for lettuce and carrot over a storage time of 10 days by a panel of untrained members with an age range of 25–40 years. Appearance, aroma, mouth-feel texture, taste and acceptability of samples were scored on a hedonic scale of 1–10. The sensory panel was selected from among the faculty members of the department and the evaluation was carried out in the sensory evaluation laboratory. Data analysis was carried out with Compusense Five software (Release 4.4) (Compusense Inc, Guelph, ON, Canada).

Browning-related enzymes [peroxidase (POD), EC 1.11.1.7, and polyphenol oxidase (PPO), EC 1.10.3.1]. Both enzymes were assayed in homogenates that were prepared as follows: 10 g of vegetable were placed in a homogeniser (Model PT 3000, Polytron, Markham, ON, Canada) in a 1:2 (w/v) ratio with 0.5 M phosphate buffer, pH 6.5, containing 50 g l^{-1} polyvinylpyrrolidone. Homogenisation was carried out twice at 4 °C and 5500 rpm, for 1 min each time, with a break of 3 min between homogenisations in order to avoid excess heating of the sample. The homogenate was then centrifuged at 12 720 g for 30 min at 4 °C. It was filtered through one layer of crepe bandage. The resulting crude extract was used without further purification. All the extracts were maintained at 4 °C and kept in the dark during use.

POD activity was assayed spectrophotometrically. The reaction mixture comprised 0.2 ml of extract, 2.7 ml of 0.05 M phosphate buffer, pH 6.5, 100 µl of hydrogen peroxide solution (11 ml l^{-1}) as oxidant and 200 µl of *p*-phenylendiamine solution (0.01 g ml^{-1}) as hydrogen donor. The oxidation of *p*-phenylendiamine was monitored at 485 nm and 25 °C. An enzyme activity unit was defined as an increment of 0.1 in absorbance per minute.

PPO activity was assayed spectrophotometrically by a modified method based on Galeazzi *et al*.[17] and Tan and Harris.[18] The reaction mixture contained 0.1 ml of crude extract and 2.9 ml of substrate solution (0.020 mol l^{-1} catechol as substrate in 0.05 mol l^{-1} phosphate buffer, pH 6.5). The rate of catechol oxidation was followed at 400 nm for 2 min at 25 °C. An enzyme activity unit was defined as an increase of 0.1 in absorbance per minute.

Nutritional parameters

Total carotenoids content. Total carotenoids were extracted in the dark by homogenizing carrot tissue (5 g) with 30 ml of an acetone–ethanol (1:1 v/v) solution, containing 200 mg l^{-1} of butylated hydroxytoluene (BHT). The homogenate was filtered under suction in a Buchner funnel and washed with acetone–ethanol solvent until colourless. The filtrate was adjusted to 100 ml with acetone–ethanol. An aliquot was placed in a 1-cm cuvette and its absorbance was measured at 470 nm. Total carotenoids (mg g^{-1} sample) were calculated as described by Gross.[19]

Ascorbic acid content. Ascorbic acid determination was carried out using the 2,6-dichloroindophenol method recommended by the AOAC[20] for the analysis of vitamin C.

Microbiological analysis

A 10-g amount of vegetable was homogenised in 90 ml of peptone saline with a Stomacher homogeniser (Model Stomacher 400; Seward, London, UK). Enumeration and differentiation of microorganisms were carried out as follows: mesophilic bacteria were quantified at 30 °C in plate count agar (PCA) over 72 h. Psychrotrophic bacteria were quantified in PCA at 21 °C over 25 h. Enumeration of lactobacilli was carried out using DeMan rogosa sharpe agar (MRS) at 35 °C over 48 h.

Statistical analysis

Differences among the treatment and storage samples were tested by analysis of variance (ANOVA). Differences are reported as significant to 95% LSD interval using Statgraphics software (version 2.1) (Statistical Graphics, Rockville, MD, USA).

RESULTS AND DISCUSSION

Quality parameters

Headspace analysis

Figure 1i shows the changes in the gas concentration in fresh-cut lettuce stored at 4 °C over 10 days. The oxygen content decreased significantly during storage and carbon dioxide increased for both treatments ($p < 0.05$). Values measured at day 1 (24 h after package sealing) showed a high variation compared with the initial conditions (210 ml l^{-1} oxygen, 0.3 ml l^{-1} carbon dioxide). From day 3 to day 7 a drop in gas concentration was observed. By day 10, oxygen concentrations had reached an almost hypoxic level (12.2 ml l^{-1} for chlorine treatment and 06.2 ml l^{-1} for

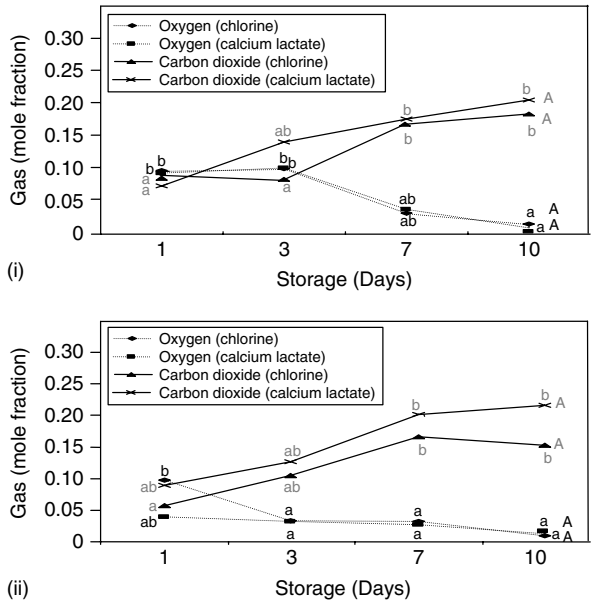

Figure 1. Effect of washing treatments (120 mg l^{-1} and 30 g l^{-1} calcium lactate) on the headspace oxygen and carbon dioxide gas content (partial pressure) during 10 days of storage at 4 °C for fresh-cut lettuce (i) and carrots (ii). Points designated on any curve by the same letter are not significantly different ($p < 0.05$). Lower-case letters are used for comparisons during storage and upper-case letters for comparisons between treatments. Black letters refer to oxygen content and grey letters refer to carbon dioxide content.

calcium lactate treatment). No significant differences ($p < 0.05$) between treatments were observed.

In sliced carrot (Fig 1ii), a rapid loss of oxygen was observed at day 1 compared with the initial conditions. This behaviour was more obvious in calcium lactate-treated carrots; however, differences between treatments were not found. From day 7 to day 10 the levels of carbon dioxide and oxygen were in agreement with recommended gas levels for fresh sliced carrot conservation.[21]

pH, dry matter and exudates analysis

In fresh-cut lettuce the pH values (Table 1) increased during storage until day 7 but decreased by day 10 for both treatments. At day 10 the pH values of chlorine-treated samples were significantly lower (pH 5.05) than those of calcium lactate-treated samples (pH 5.96). The pH range in these studies (5–6.5) is considered adequate for quality retention.[22,23]

Ke *et al*.[24] suggested that high levels of carbon dioxide are related to the lower pH of stored packaged lettuce. Elevated CO_2 suppresses succinate dehydrogenase (SDH) and activates glutamate decarboxylase, causing a decrease in pH. In agreement with this finding, chlorine-treated lettuce had a significantly ($p < 0.05$) lower CO_2 concentration than lettuce treated with calcium lactate.

In sliced carrots the pH values at day 1 were significantly ($p < 0.05$) lower in samples treated with calcium lactate than in samples treated with chlorine. These differences were statistically significant at days 1 and 3. At the end of the storage the values were similar to the initial values in both treatments.

Exudate values increased significantly during storage (Table 1) for both vegetables with no significant differences between treatments in fresh-cut lettuce. In sliced carrots the exudate values were higher in

Table 1. Physicochemical and nutritional parameters in fresh-cut lettuce and carrots treated with 120 mg l^{-1} of chlorine or 30 g l^{-1} of calcium lactate stored for 10 days at 4 °C

Vegetable	Physiological parameter	Treatment	Storage (days)[a] 1	3	7	10
Lettuce	pH	Chlorine	5.89 ± 0.03^{b}	6.00 ± 0.04^{c}	6.23 ± 0.04^{d}	5.05 ± 0.0^{a}
		Calcium lactate	5.54 ± 0.16^{b}	6.00 ± 0.00^{c}	6.12 ± 0.03^{cd}	5.96 ± 0.08^{c}
	Exudate (mg g^{-1})	Chlorine	7.5 ± 1.0^{ab}	32.4 ± 17.9^{ab}	27.1 ± 10.5^{ab}	23.1 ± 14.6^{ab}
		Calcium lactate	9.9 ± 2.0^{a}	19.3 ± 2.3^{a}	36.0 ± 11.5^{b}	38.7 ± 8.2^{b}
	Dry matter (mg g^{-1})	Chlorine	21.2 ± 1.8^{a}	25.3 ± 1.9^{a}	33.7 ± 3.3^{b}	33.0 ± 3.8^{b}
		Calcium lactate	22.5 ± 2.4^{a}	23.3 ± 4.2^{a}	34.9 ± 2.8^{b}	36.3 ± 5.2^{b}
	Ascorbic acid (mg g^{-1})	Chlorine	0.052 ± 0.00^{d}	0.020 ± 0.00^{b}	0.006 ± 0.00^{a}	0.020 ± 0.00^{b}
		Calcium lactate	0.040 ± 0.00^{c}	0.026 ± 0.00^{b}	0.004 ± 0.00^{a}	0.026 ± 0.00^{b}
	Carotenoids (mg g^{-1})	Chlorine	N/A	N/A	N/A	N/A
		Calcium lactate	N/A	N/A	N/A	N/A
Carrot	pH	Chlorine	6.00 ± 0.03^{c}	6.00 ± 0.28^{c}	6.21 ± 0.07^{cd}	6.12 ± 0.17^{c}
		Calcium lactate	5.72 ± 0.08^{b}	5.00 ± 0.14^{a}	6.39 ± 0.04^{d}	5.78 ± 0.11^{bc}
	Exudate (mg g^{-1})	Chlorine	16.4 ± 5.8^{c}	1.9 ± 1.7^{ab}	9.8 ± 7.4^{bc}	9.0 ± 11.5^{bc}
		Calcium lactate	7.0 ± 2.1^{b}	1.3 ± 1.1^{a}	6.8 ± 6.8^{ab}	3.8 ± 0.4^{ab}
	Dry matter (mg g^{-1})	Chlorine	78.8 ± 12.1^{a}	124.7 ± 13.7^{b}	71.5 ± 13.3^{a}	60.8 ± 2.6^{a}
		Calcium lactate	86.9 ± 10.6^{ab}	98.5 ± 2.5^{ab}	83.2 ± 20.0^{a}	113.9 ± 5.6^{b}
	Ascorbic acid (mg g^{-1})	Chlorine	0.045 ± 0.00^{c}	0.032 ± 0.00^{b}	0.012 ± 0.00^{a}	0.032 ± 0.00^{b}
		Calcium lactate	0.052 ± 0.00^{c}	0.030 ± 0.00^{b}	0.010 ± 0.00^{a}	0.032 ± 0.00^{b}
	Carotenoids (mg g^{-1})	Chlorine	11.61 ± 0.35^{d}	10.66 ± 0.17^{c}	9.75 ± 0.14^{b}	7.76 ± 0.03^{a}
		Calcium lactate	13.36 ± 0.58^{d}	10.13 ± 0.67^{bc}	9.72 ± 0.44^{b}	9.70 ± 0.30^{b}

[a] Values followed by different letters in the same row indicate significant differences during storage. Significant ($p < 0.05$) differences between treatments were found for lettuce (pH) and for carrots (pH, exudate, dry matter and carotenoids). N/A, not analysed.

samples treated with chlorine than calcium lactate ($p < 0.05$).

Dry matter showed a significant increase during storage for fresh-cut lettuce samples ($p < 0.05$), but no significant differences between washing treatments (Table 1). Sliced carrots showed a significant increase in dry matter during storage, reaching significantly higher values in samples treated with calcium lactate (Table 1).

Colour analysis

CIE $L^*a^*b^*$ parameters were evaluated during storage for fresh-cut lettuce and sliced carrots (data not shown). Lightness index values (L^*) were significantly higher in chlorine-treated fresh-cut lettuce (81.6) than in calcium lactate-treated samples (79.8) ($p < 0.05$). The high luminosity in lettuce treated with chlorine could be due to the bleaching effect of chlorine on the tissue. The a^* parameter increased significantly ($p < 0.05$) during storage, with no significant effect due to the washing treatment. The increase in a^* (greenness to redness) is related to the appearance of browning. Hue diminished during storage, with a significant decrease from day 7 to day 10. A change in the intensity of colour (Chroma) was not found for either treatment during storage. Total colour difference (ΔE) increased during storage with no difference between chlorine- and calcium lactate-treated samples (Fig 2).

For sliced carrots, the L^* index showed significant differences between treatments ($p < 0.05$). Throughout the storage period luminosity increased in chlorine-treated carrots whereas it decreased in calcium lactate-treated carrots. The increase in L^* could be due to white surface development in chlorine-treated samples;[25] this process did not affect calcium lactate-treated samples, perhaps owing to the inhibitory effect of calcium lactate on lignification.[26]

The greenness–redness parameter (a^*) decreased significantly ($p < 0.05$) from day 1 to day 7 in sliced carrots treated with either chlorine or calcium lactate. At day 10, the samples treated with calcium lactate maintained their a^* values whereas a^* decreased in the samples treated with chlorine. This a^* decrease could be associated with a carotenoid loss in sliced carrots (Table 1). The b^* parameter and Chroma (intensity) decreased during storage of samples processed with either treatment. ΔE showed significant differences ($p < 0.05$) during the 10 days of storage; differences between treatments were not found (Fig 2).

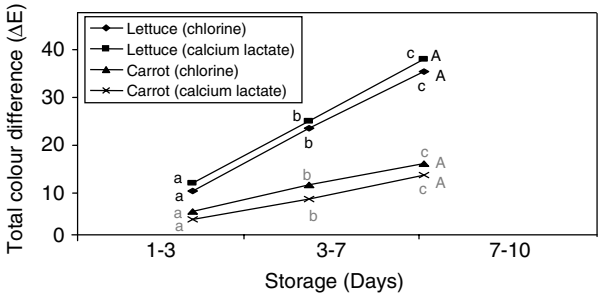

Figure 2. Total colour (CIE $L^*a^*b^*$) variation during storage at 4 °C for fresh-cut lettuce and carrots treated with 120 mg l^{-1} of chlorine and 30 g l^{-1} of calcium lactate as washing treatments. Points designated on any curve by the same letter are not significantly different ($p < 0.05$). Lower-case letters are used for comparisons during storage and upper-case letters for comparisons between treatments. Black letters refer to lettuce and grey letters refer to carrots.

Texture measurement

The maximum load (kN) was measured as a crispness parameter in fresh-cut lettuce. Significant differences between treatments were not observed ($p < 0.05$) although calcium treatments have been reported to increase the firmness in fruits. The reason for this may the low test sensitivity or perhaps longer storage times are required to observe this difference. From a safety point of view, longer storage times are not of interest for this type of fresh-cut product.

A significant change in the maximum load was observed from day 1 (0.035/0.036 kN) to day 10 (0.041/0.042 kN) for chlorine and calcium lactate, respectively. This increase in load is related to an increase in flexibility (loss of crispness). The highest values for water loss (Table 1), on day 10, correspond to maximum crispness readings. The loss of turgor of the cell (loss of moisture) can produce dehydration of the tissues and an increase in elasticity. In sliced carrots, significant differences in maximum fracture load could not be observed between treatments or throughout storage. The maximum load values during the storage for both treatments remained constant at ~0.05 kN.

Sensory evaluation

Sensory analysis was used to assess the quality of fresh-cut iceberg lettuce and sliced carrots over 10 days (Table 2). The general appearance is the main attribute that consumers use to evaluate the quality of vegetables and fruits, since people 'buy with their eyes'.[27,28] Fresh-cut lettuce showed lower scores for appearance at the end of the storage period than at days 1–7. Acceptability was significantly dependent on storage time but not on treatment. As with acceptability, appearance had the lowest values at the end of storage.

Crispy and crunchy textures are deliverable qualities and they are particularly important in fruits and vegetables where consumers associate them with freshness. The integrity of fresh processed vegetables is usually altered during processing. In this study the perception of texture as acceptable by the panel (crispness), was high until day 7. The panel could not discern differences between treatments, but at the end of the storage period (day 10) scores were significantly lower than on previous days.

The panel did not find differences in taste during storage but off-odour increased significantly by day 10. The development of off-odours may be due to the high concentrations of carbon dioxide and low levels

Table 2. Sensory analysis of fresh-cut lettuce and carrots treated with 120 mg l^{-1} of chlorine or 30 g l^{-1} of calcium lactate and stored for 10 days at 4 °C

Vegetable	Sensory parameter	Treatment	Storage (days)[a] 1	3	7	10
Lettuce	Appearance[b]	Calcium lactate	4.42 ± 3.10^{ab}	5.40 ± 1.41^{b}	5.10 ± 2.09^{ab}	2.80 ± 2.79^{a}
		Chlorine	5.26 ± 3.20^{ab}	5.78 ± 1.99^{b}	4.64 ± 2.26^{a}	3.40 ± 2.90^{a}
	Browning[c]	Calcium lactate	5.62 ± 2.41	5.28 ± 1.43	5.62 ± 2.41	4.38 ± 2.64
		Chlorine	4.12 ± 2.43	5.67 ± 1.97	4.12 ± 2.42	3.60 ± 2.42
	Texture[d]	Calcium lactate	7.97 ± 2.33^{b}	7.66 ± 1.96^{b}	8.00 ± 0.40^{b}	4.40 ± 1.66^{a}
		Chlorine	7.61 ± 1.42^{b}	7.34 ± 1.62^{b}	7.60 ± 1.74^{b}	5.20 ± 1.78^{a}
	Taste[e]	Calcium lactate	5.88 ± 1.02	5.88 ± 1.03	4.38 ± 1.70	3.74 ± 1.92
		Chlorine	4.76 ± 1.07	4.76 ± 1.07	5.22 ± 1.79	3.76 ± 2.06
	Odour[f]	Calcium lactate	7.10 ± 1.88^{b}	5.02 ± 2.70^{b}	6.50 ± 1.07^{b}	2.38 ± 2.22^{a}
		Chlorine	6.25 ± 1.50^{b}	5.93 ± 2.20^{b}	5.08 ± 0.30^{b}	2.44 ± 1.97^{a}
	Acceptability[g]	Calcium lactate	5.25 ± 1.04^{b}	5.00 ± 1.08^{b}	6.00 ± 1.14^{ab}	2.80 ± 1.20^{a}
		Chlorine	5.52 ± 1.05^{ab}	5.66 ± 1.50^{b}	5.60 ± 0.88^{b}	3.40 ± 0.94^{a}
Carrot	Appearance[b]	Calcium lactate	4.50 ± 1.87	5.14 ± 2.36	5.10 ± 2.03	3.04 ± 1.42
		Chlorine	4.30 ± 2.02	3.40 ± 0.49	4.67 ± 2.77	3.20 ± 1.48
	Bleaching[c]	Calcium lactate	7.33 ± 2.70^{b}	7.22 ± 1.70^{b}	7.02 ± 2.70^{b}	3.54 ± 3.32^{a}
		Chlorine	7.11 ± 3.07^{b}	4.68 ± 0.87^{ab}	5.97 ± 3.29^{ab}	3.86 ± 3.07^{a}
	Texture[d]	Calcium lactate	8.00 ± 0.89^{b}	8.80 ± 0.55^{b}	7.66 ± 0.98^{b}	6.00 ± 1.00^{a}
		Chlorine	8.34 ± 0.75b	8.00 ± 0.71b	7.34 ± 1.03^{b}	5.60 ± 1.09^{a}
	Taste[e]	Calcium lactate	5.80 ± 1.45^{ab}	6.16 ± 1.02^{b}	5.62 ± 1.69^{b}	3.89 ± 1.23^{a}
		Chlorine	5.48 ± 1.64^{ab}	6.26 ± 1.34^{b}	5.33 ± 1.56^{b}	3.86 ± 1.46^{a}
	Odour[f]	Calcium lactate	7.12 ± 1.88^{b}	7.22 ± 1.70^{b}	6.43 ± 2.42^{b}	3.32 ± 1.36^{a}
		Chlorine	6.25 ± 1.30^{b}	4.68 ± 0.88^{b}	5.62 ± 1.78^{b}	3.56 ± 1.73^{a}
	Acceptability[g]	Calcium lactate	8.00 ± 2.18^{b}	8.80 ± 1.88^{b}	7.66 ± 1.50^{b}	4.00 ± 2.08^{a}
		Chlorine	8.00 ± 1.88^{b}	8.40 ± 1.66^{b}	7.34 ± 1.12^{b}	4.80 ± 2.28^{a}

[a] Values followed by different letters in the same row indicate significant differences during storage. There were no significant differences between the two treatments at $p < 0.05$ in respect of any of the parameters investigated.
[b] Appearance (10 = good, 0 = bad).
[c] Browning/bleaching (10 = no browning/bleaching, 0 = a lot of browning/bleaching).
[d] Texture (10 = very crispy, 0 = soft).
[e] Taste (10 = good, 0 = bad).
[f] Odour (10 = fresh, 0 = rotten).
[g] Acceptability (10 = good, 0 = bad).

of oxygen in the package at the end of the storage time (Fig 1).

The browning scores showed no differences during storage or between treatments. The low oxygen atmospheres developed inside the storage bags could have reduced enzymatic discoloration because oxygen is a necessary substrate for the browning reaction.[29,30]

For sliced carrots, the panel did not find significant differences in appearance between treatments during storage. At day 10, significant changes in browning, texture, acceptability, taste and odour were observed.

Browning-related enzymes (peroxidase and polyphenol oxidase)

In fresh-cut lettuce the chlorine-treated samples showed similar peroxidase activity profiles to samples treated with calcium lactate. Activity peaked at both day 3 and day 10 (Fig 3i). Calcium lactate-treated fresh-cut lettuce had lower activity values throughout storage. The data indicate that calcium lactate treatment of fresh-cut lettuce may have a higher antioxidant capability than chlorine treatment.

PPO activity in fresh-cut lettuce decreased significantly ($p < 0.05$) for both treatments. This behaviour can be associated with low oxygen concentrations during storage. Although significant differences were found at day 1, both treatments showed similar activity values during storage (Fig 3iii).

In carrots, the POD activity was lower than in lettuce (Fig 3ii). This was expected since peeling removes the bulk of POD enzyme.[31] In sliced carrots no difference in activity was observed between treatments during storage. Hence it seems that both treatments had the same antioxidant capability.

Initial PPO activity values (Fig 3iv) were higher in chlorine- than in calcium lactate-treated samples, but the differences were not significant ($p < 0.05$). However, at the end of storage the amount of PPO in sliced carrots was higher in samples treated with calcium lactate than in samples treated with chlorine.

Although PPO is an enzyme directly related to browning, its activity is limited by the oxygen availability and is also influenced by peroxidase activity. Therefore, although the PPO activity value was higher at day 1 in samples treated with calcium

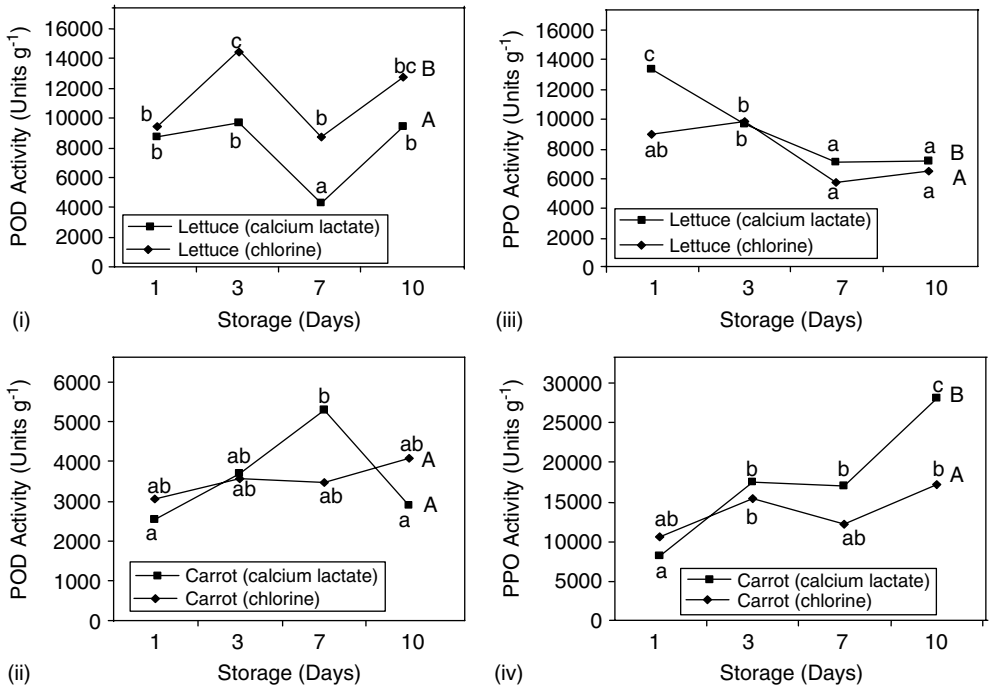

Figure 3. Peroxidase (POD) and polyphenol oxidase (PPO) activity in fresh-cut lettuce (i and iii) and carrots (ii and iv) treated with chlorine 120 mg l^{-1} and calcium lactate 30 g l^{-1} and stored for 10 days at 4 °C. The activity is expressed as units of enzymatic activity per gram. Points designated on any curve by the same letter are not significantly different ($p < 0.05$). Lower-case letters are used for comparisons during storage and upper-case letters for comparisons between treatments.

lactate in fresh-cut lettuce or at day 10 in sliced carrots, there were no differences in apparent browning (colorimeter and sensory analysis).

Nutritional parameters

Total carotenoids

Total carotenoid (Table 1) content decreased during storage in chlorine- and calcium lactate-treated samples and was significantly reduced by chlorine treatment ($p < 0.05$) compared with calcium lactate treatment. This is consistent with colour measurements, which showed significantly higher a^* values for calcium lactate-treated samples than chlorine-treated samples.

Ascorbic acid

Ascorbic acid (Table 1) in both treatments decreased during storage ($p < 0.05$) in fresh-cut lettuce and sliced carrots. In both vegetables, differences in ascorbic acid content between treatments were not found. This agrees with Ihl *et al*[32] who found a decrease in ascorbic acid content during storage but could not find differences between washing treatments.

Microbiological analysis

Different authors have reported an increase in mesophilic counts with quality losses during storage. Fresh-cut lettuce showed a high initial content of mesophilic counts prior to the washing treatment (6.5 log). Although the initial counts in this study were higher than reported by other authors (5–6 log),[33] this may be due a number of factors that are difficult to control (ambient conditions during the postharvest period, amount of soil and irrigation conditions, amongst others).[33] Both treatments showed a reduction in mesophilic counts after the wash treatment but there were no significant differences ($p < 0.05$) between treatments during the storage period. Mesophilic counts in fresh-cut lettuce showed similar profiles for both treatments although calcium lactate-treated samples showed a transient increase at day 7 of storage. The mesophilic counts reached in fresh-cut lettuce (Fig 4) were considered acceptable to the consumer at the end of 10 days of storage. The limit for consumer consumption of fresh processed vegetables is 8 log for mesophilic bacteria.[34]

In fresh carrots, the initial mesophilic counts, prior to the washing treatment, were lower than in lettuce (4.5 log). The mesophilic counts decreased significantly after washing treatment but there was no difference between counts during storage. The final mesophilic counts at the end of the storage period were lower than 5 log in both cases.

The psychrotrophic counts profile (Fig 4) showed no significant differences with the washing treatments used. The initial count (7 log) reduction was not different for the two treatments. From day 2 the values remained similar until day 10.

In sliced carrots, initial counts (3.5 log) were reduced in both treatments, increasing similarly during the storage and showing no differences between treatments ($p < 0.05$).

The lactic acid bacteria did not grow appreciably under the conditions used; in both treatments during

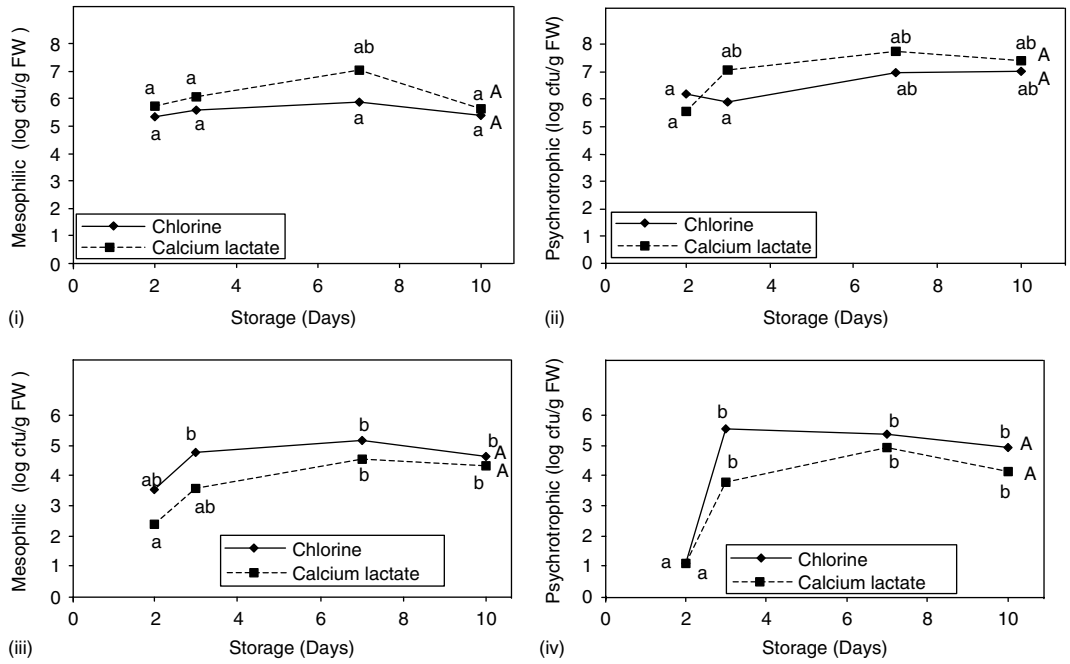

Figure 4. Microbial populations on fresh-cut lettuce (i and ii) and carrots (iii and iv) washed with chlorine 120 mg l^{-1} and calcium lactate 30 g l^{-1} and stored for 10 days. Points designated on any curve by the same letter are not significantly different ($p < 0.05$). Lower-case letters are used for comparisons during storage and upper-case letters for comparisons between treatments.

the storage the levels were lower than 1 log (data not shown).

Although, in this study, differences in mesophilic and psychrotrophic counts between treatments were not observed for the two vegetables during storage, further investigations of the effect of these treatments on pathogens may be needed.

CONCLUSION

Calcium lactate is a suitable washing treatment for fresh-cut lettuce and carrots. It has a similar antimicrobial effect to chlorine rinsing treatments and similar effects on several quality and nutritional parameters. It has advantages over chlorine in certain areas. Calcium lactate washing treatments avoid the post-treatment bleaching effect in fresh-cut lettuce and the appearance of whiteness on surfaces of sliced carrots. Calcium lactate treatment, compared with chlorine treatment, maintained similar or even better nutritional values of the samples during storage.

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