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Calcium for Extending the Shelf Life of Fresh Whole and Minimally Processed Fruits and Vegetables

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Effect of ozone and calcium lactate treatments on browning and texture properties of fresh-cut lettuce

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Abstract: The effects of three treatments, 1 mg L^{-1} ozone at 18–20 °C, 15 g L^{-1} calcium lactate (CLac) at 50 °C and a combination thereof, were compared on fresh-cut lettuce over 10 days of refrigerated storage. Respiration rate, browning and texture were examined as main quality indicators. The use of ozone produced a significantly ($P < 0.05$) higher oxygen decline than the use of CLac (from day 3 to day 10). At the end of storage, CLac (alone or combined with ozone) samples had higher oxygen content (~9%) than ozone samples (~6%). Enzymatic activity decreased significantly ($P < 0.05$) in ozone samples. Polyphenol oxidase activity in fresh-cut lettuce treated with ozone (alone or combined with CLac) showed lower values on day 1 (<2500 units g^{-1}) and at the end of storage (<3000 units g^{-1}) than CLac samples (4000–4800 units g^{-1}). Ozone also reduced peroxidase activity to ~300 units g^{-1} after treatment. Finally, pectin methylesterase activity was also reduced with ozone, showing a negative effect on textural properties. Data suggested that CLac maintained quality markers better than treatments with ozone and ozone/CLac combination over 10 days of storage.

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Keywords: ozone; calcium lactate; texture; browning; Iceberg lettuce

INTRODUCTION

Consumers increasingly require food products that preserve their nutritional value, retain a natural and fresh colour, flavour and texture and contain fewer additives such as preservatives.[1] Recent research has underlined the importance of fruit and vegetable consumption to health and has reported new techniques to preserve the nutritional and sensory qualities demanded by consumers.

Even though fresh-cut produce has been sold since the 1940s, the quality is unpredictable and the shelf life limited. The extension of quality retention for fresh-cut products is relevant for the industry owing to its economic impact. It is important that the washing treatments applied to fresh vegetables help maintain their quality, since consumers demand a fresh product as well as convenience and long shelf life.[2]

Tissue browning is one of the major causes of loss of quality of fresh-cut vegetables. Browning is influenced by the concentration of phenolic compounds and by other factors such as the activity of polyphenol oxidase and peroxidase enzymes. Wound-induced loss of cellular compartmentalisation of phenolic compounds (mainly in the vacuole) and polyphenol oxidase (in the cytoplasm) results in tissue browning at a rate that increases with temperature and water loss.[3] The other cause of quality loss is decrease in firmness. Tissue softening and associated loss of integrity and leakage of juice from some fresh-cut products can be the primary cause of poor quality and unmarketability.

Chlorine solutions have been widely used to sanitise fruit and vegetables in the fresh-cut industry. However, the association of chlorine with the possible formation of carcinogenic chlorinated compounds in water has called into question the use of chlorine in food processing.[4,5]

For that reason, there is a real need to find alternatives for preservation of fresh-cut vegetables in order to improve the efficacy of washing treatments. This will lead to increased microbial safety and extend the quality retention of products. Alternatives or modified methods have been proposed, e.g. antioxidants, irradiation, ozone, organic acids, modified atmosphere packaging, whey permeate, etc.,[6–11] but none have yet gained widespread acceptance by the industry.

Calcium lactate (5–30 g L^{-1}) has been used as a firming agent for fruits such as cantaloupe, strawberry, lettuce and others.[10–14] It has been reported to be a good alternative to calcium chloride because it avoids the bitterness or off-flavours associated with that salt[15] and the antibacterial properties.[10,16] Heat shock combined with calcium lactate has been used to prevent browning reactions in vegetables and fruits.[17–21] Firming effects obtained from heat

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treatments alone or combined with calcium treatments have been attributed to the action of heat-activated pectin methylesterase and/or to increased calcium diffusion into tissues at higher temperatures.[19,22]

The main objective of this work was to determine the effect of calcium lactate, an effective and safe biopreservative, combined with heat shock on fresh-cut lettuce and compare it with that of ozone, a novel preservative washing solution. Ozone is a strong antimicrobial agent with high reactivity and penetrability and spontaneous decomposition to a non-toxic product.[23,24] Several researchers have shown that treatment with ozone appears to have a beneficial effect in extending the storage life of broccoli and seedless cucumber.[23,25] Although the antimicrobial capacity of ozone has been widely reported, few studies on quality have been carried out,[26,27] and none have evaluated the potential for synergistic effects of ozone combined with other washing treatments.

This study is focused on the respiration rate, appearance of brown discolouration and loss of firmness thorough monitoring of suitable quality markers.

MATERIALS AND METHODS

Raw material

Iceberg lettuce (Lactuca sativa sp.) was grown in Ireland. The product was purchased from a local supermarket and stored at 4 ◦ C for 2–4 h until used.

Experimental design

Experiments were conducted from December 2003 to May 2004. All procedures were performed in a special food-processing room at 18–20 ◦ C. The treatments were conducted in parallel and prepared from the same batch of product. Each batch was ~20 kg (~50 heads of lettuce of ~400 g each). The first washing treatment consisted of 1 mg L^{-1} ozone in distilled water, the second treatment was a solution of 15 g L^{-1} calcium lactate (CLac) at 50 ◦ C and the third treatment consisted of first washing the samples in 1 mg L^{-1} ozone and then treating them with 15 g L^{-1} CLac at 50 ◦ C. Three independent trials (165 bags per batch, 55 bags per treatment) were conducted (Fig. 1). For each treatment: three bags were used for headspace monitoring throughout the entire storage period; sensory analysis required two bags per panellist (14), i.e. 28 in total; also, on each of days 1, 3, 7 and 10, two bags for texture, two bags for colour and two bags for enzymes were used after being opened and pooled. For texture and colour, more than 25 samples were analysed per treatment and day. For enzymes, measurements were duplicated.

Selection of washing treatments

Previous work showed that CLac was a promising alternative to the industrial standard of 120 mg L^{-1} free chlorine in washing treatments for fresh-cut lettuce.[10] CLac treatment proved to be as effective as chlorine treatment and enhanced the nutritional value of the final product. A later extensive study of the effect of temperature and CLac concentration

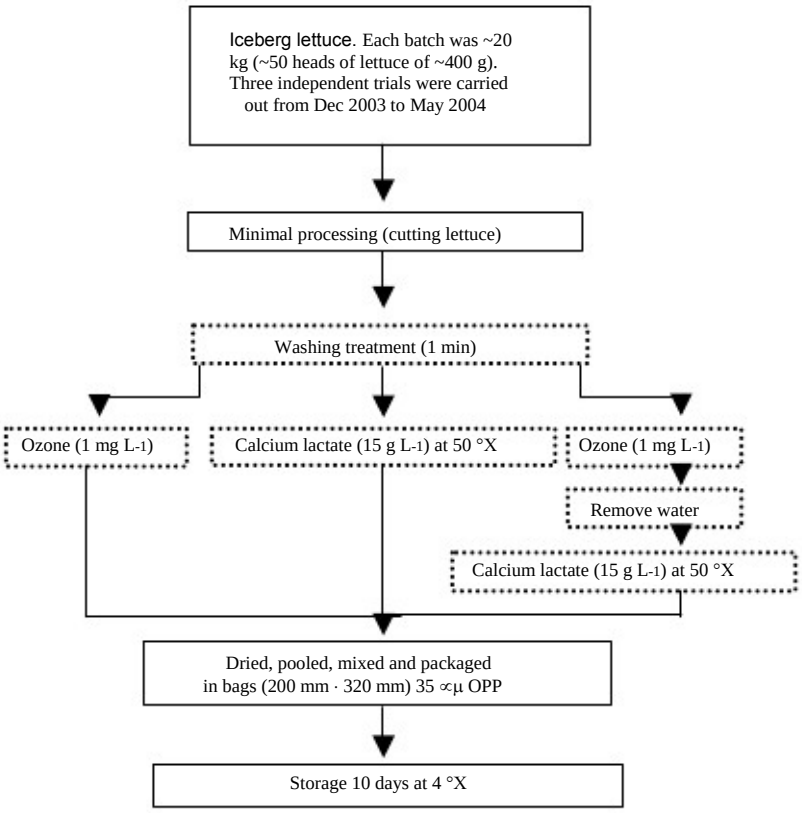

Figure 1. Flow diagram showing the procedure for the production of minimally processed fresh-cut lettuce.

on fresh-cut lettuce was investigated.[11] The results showed that 15 g L^{-1} CLac at 50 °C was the optimal condition to reduce browning and maintain firmness (crispness).[11,21,28] The second treatment selected was ozone as a novel treatment to preserve fresh-cut vegetables, since ozone has been reported to be an efficient reducer of browning[23,29] owing to its high reactivity and penetrability and its spontaneous decomposition to a non-toxic product. Following guidelines and levels reported in the literature, 1 mg L^{-1} ozone treatment was used as optimal for maintaining the shelf life in optimal and safe conditions.[30]

Processing (washing and packaging procedure)

Lettuce samples were treated according to minimal processing procedures. Each lettuce was prepared according to accepted practices. 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. Different sanitised knifes were used during the process in order to avoid cross-contamination.

Washing treatments were performed by immersion of the fresh-cut lettuce in the ozone and CLac treatment solutions. Ozonated water was generated by bubbling gaseous ozone into distilled water. Ozone gas mixture was produced using an active oxygen generator machine (model HV-103, Analytical Technology Inc., Hampshire, UK) connected to a dissolved ozone monitor (model C15-64, Analytical Technology Inc.). The gas was then pumped into the system using an aquatic air pump at a flow rate of 2.5 L min^{-1}. Ozonated water was held in a container until it reached a concentration of 1 mg L^{-1} at 4 °C. The ozone concentration was measured in the range 0–10 mg L^{-1} with a sensitivity of 0.001 mg L^{-1} above 0.005 mg L^{-1}. Repeatability was ±0.01 mg L^{-1} and linearity was 0.5%. Calcium lactate (Sigma, St Louis, MO, USA) was diluted to 15 g L^{-1} (pH 6.5) in distilled water at 50 °C.

Each treatment was carried out in a different basket (~200 g vegetable product L^{-1}) by immersion in the corresponding washing solution for 1 min with agitation and subsequently drying for 5 min in an automatic salad spinner. To minimise product heterogeneity, processed vegetables were pooled, mixed and subsequently packaged in bags (200 mm × 320 mm) of 35 ∝m oriented polypropylene (OPP, Amcor Flexibles Europe, Bristol, UK). The permeability of the film was defined by oxygen (O_2) and carbon dioxide (CO_2) transmission rates of ~12 000 and ~13 000 mL m^{-2} day^{-1} atm^{-1} respectively at 5 °C. Each package contained ~100 g of product. The packages were chilled in a blast freezer at 0 °C for 2 min before being heat sealed under atmospheric conditions.

Quality markers

Various quality markers were analysed as indicators of respiration, browning and texture: headspace, colour measurement, browning-related enzymes, texture-related enzymes, Instron and sensory analysis.

Headspace analysis

A Gaspace analyser (Systech Instruments, Oxon, 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 30 mL min^{-1} for 10 s, monitoring with a sensitivity of 0.001 (O_2) and 0.1 (CO_2). Accuracy of readings was: ±0.5% below 10% and ±0.1% above 10% for O_2; ±0.1% below 1%, ±0.2% between 1 and 10% and ±2% above 10% for CO_2. Three bags per treatment were monitored for each experiment, and the bags for other analyses were measured separately. The trials were also triplicated.

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. Lettuce (10 g) was placed in a homogeniser (Polytron model PT 3000, Ontario, Canada) in a 1:2 (w/v) ratio with 0.5 mol L^{-1} 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 and filtered through one layer of crepe bandage. The resulting crude extract was used without further purification. All extracts were stored at 4 °C in the dark and used immediately.

POD activity was assayed spectrophotometrically. The reaction mixture consisted of 0.2 mL of extract and 2.7 mL of 0.05 mol L^{-1} phosphate buffer, pH 6.5, containing 100 ∝L of hydrogen peroxide (1 mL L^{-1}) as oxidant and 200 ∝L of p-phenylendiamine as hydrogen donor. The oxidation of p-phenylendiamine was monitored at 485 nm and 25 °C. One unit of enzyme activity was defined as an increment of 0.1 in absorbance per minute.

PPO activity was assayed spectrophotometrically by a modified method based on Refs 31 and 32. 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. One unit of enzyme activity was defined as an increment of 0.1 in absorbance per minute. Three independent trials were carried out. All enzymatic measurements were made in duplicate.

Pectin methylesterase (PME, EC 3.1.1.11)

PME activity was measured using the method described by Kimball.[33] Briefly, 10 g of sample was

diluted in an extraction solution (0.2 mol L^{-1} sodium phosphate buffer, pH 7.5, containing 1 mol L^{-1} sodium chloride and 10 mmol L^{-1} dithiothreitol) and homogenised at 4 °C for 2 min at 5500 rpm. The macerate was incubated at 4 °C for 30 min with agitation and then centrifuged at 12 500 × g for 30 min at 4 °C. A 1 mL aliquot of this extract was mixed with 40 mL of substrate solution (1 g L^{-1} pectin). The solution was adjusted to pH 7 with 1 mol L^{-1} NaOH and then readjusted to pH 7.5 with 0.05 mol L^{-1} NaOH. After the pH had reached 7.5, 0.2 mL of 25 mmol L^{-1} NaOH was added. The time required to return to pH 7.5 was recorded. Activity was quantified as carboxyl groups formed by the hydrolysis of methyl esters of pectin and was measured by titration using a pH electrode to monitor the production of H^+. PME activity units were calculated using the formula[33]

$$\begin{aligned} \text{PME} = {} & (25\ \text{mmol L}^{-1}\ \text{NaOH}) \times (\text{X mL extracted}) \\ & \times (0.2\ \text{mL NaOH}) \times 10^6/(1\ \text{mL sample}) \\ & \times (10\ \text{g sample}) \times \text{time (min)} \end{aligned} \quad (1)$$

Three independent trials were carried out. All enzymatic measurements were made in duplicate.

Colour measurement

Colour was quantified using a colorimeter (Hunter-Lab, Reston, UK). A lettuce piece was placed directly on the colorimeter sensor (3.5 cm diameter) and measured; 20–30 measurements were taken per treatment. The instrument was calibrated with white tile ($L_* = 93.97$, $a_* = -0.88$, $b_* = 1.21$) and green tile ($L_* = 56.23$, $a_* = -21.85$, $b_* = 8.31$) standards under D_{65} luminosity conditions. Total colour change $E = [(L_f - L_i)^2 + (a_f - a_i)^2 + (b_f - b_i)^2]^{1/2}$ was analysed, 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. Three independent trials were carried out. More than 25 samples were analysed per treatment and day.

Texture analysis

Textural properties of lettuce were assessed using a texture analyser (Instron 4302 universal testing machine, Canton, MA, USA) with a 500 N load cell attached. A Kramer shear cell with an eight-blade probe attached to the instrument was used. The speed setting for the experiment was 100 mm min^{-1}. Tissue segregation and orientation were done for the analysis. All tests were performed with photosynthetic tissue. Rectangular pieces (3 cm × 6.5 cm) were cut and placed in the Kramer cell. Data were analysed with the Instron series IX software for Windows. The instrumental measurement of lettuce texture is difficult, mainly owing to the high variability of the product. Lettuce contains two different types of tissue (vascular and photosynthetic) that are not always easy to differentiate and have an irregular distribution, and the relative position of vascular packages (parallel, oblique or perpendicular orientation) with respect to the shear cell blades directly affects the measurement.[34] For this reason, photosynthetic tissue with perpendicular orientation was selected for the experiments. Values were expressed as (maximum load - minimum load)/maximum load, which was defined as the crispiness coefficient (CC).[21] Three independent trials were carried out. More than 25 samples were analysed per treatment and day.

Sensory analysis

Sensory analysis of lettuce was carried out over 10 days of storage by a panel of 14 untrained members with an age range of 25–40 years. Fresh appearance of samples was scored on a hedonic scale from 0 (poor fresh appearance) to 5 (excellent fresh appearance). The sensory panel was selected from members of the department and the analysis was carried out in the sensory evaluation laboratory. Data analysis was performed using Compusense® Five software (release 4.4, Ontario, Canada). Three independent trials were carried out.

Statistical analysis

Analysis of variance (multifactor and one-way ANOVA) was used to find differences between treatments, storage and their interaction for each of the variables studied. Means were compared by the least significant difference (LSD) test at a significance level of P = 0.05 using Statgraphics software (version 2.1, Statistical Graphics Co., Rockville, MD, USA). Three independent trials were carried out.

RESULTS AND DISCUSSION

Headspace analysis

Changes in headspace gas composition (oxygen and carbon dioxide) of fresh-cut lettuce were measured over 10 days. The oxygen content in the packages decreased during storage, while the carbon dioxide content increased, as expected (Fig. 2). Changes in oxygen and carbon dioxide concentration were more dramatic from day 1 to day 3 than from day 3 to day 10 for all treatments.

Oxygen decreased from its initial atmospheric concentration (21%) to values around 15% after 1 day of storage (Fig. 2(a)). This rapid decrease reflected a high respiration rate, presumably caused by the stress produced by minimal processing.[35] On the other hand, carbon dioxide increased significantly ($P < 0.05$) in all samples over the entire storage period (Fig. 2(b)), with the highest increases being observed from day 0 to day 2.

Washing treatments significantly affected ($P < 0.05$) the oxygen and carbon dioxide levels (Fig. 2). The use of ozone in the washing treatment produced a more rapid oxygen decline than the use of CLac in the storage period from day 3 to day 10 (Fig. 2(a)). This was evident at the end of storage, when samples treated with CLac had higher oxygen contents than

ozone-treated samples. The fresh-cut lettuce washed with the combined treatment had intermediate values, which indicates that the stress produced by the use of ozone was partially reduced by the use of CLac.

The results indicate that CLac caused a reduction in the respiration of lettuce (Fig. 2(a)), which is in agreement with the findings of other authors who suggested that CLac reduces the respiration of minimally processed fresh vegetables.[36,37] Since the rate of respiration of a vegetable is related to its senescence state,[38] a lower consumption of oxygen might be due to a lower stress response in lettuce. The lower respiration rate in CLac-treated samples could be explained by a possible post-wounding protective effect of CLac at 50 °C.[21,28] Calcium seems to maintain cell wall structure by interacting with the pectic acid present in cell walls to form calcium pectate, thereby maintaining water activity and thus reducing the stress usually associated with higher respiration rates.[38] The higher respiration rate in samples treated with ozone could be associated with tissue and photosynthetic apparatus being damaged, which might alter the metabolic state.

Browning-related enzymes (polyphenol oxidase and peroxidase)

After tissue wounding, many enzymes are released at the wound site. Two of them, PPO and POD, are involved in tissue discolouration (e.g. russet spotting, rusty brown discolouration, etc.), contributing to a reduction in quality. Previous results showed significant differences in PPO and POD activity in fresh-cut lettuce depending on the type of tissue examined (photosynthetic or vascular).[28] For this reason, the present study was carried out entirely with photosynthetic tissue to minimise variability between experiments.

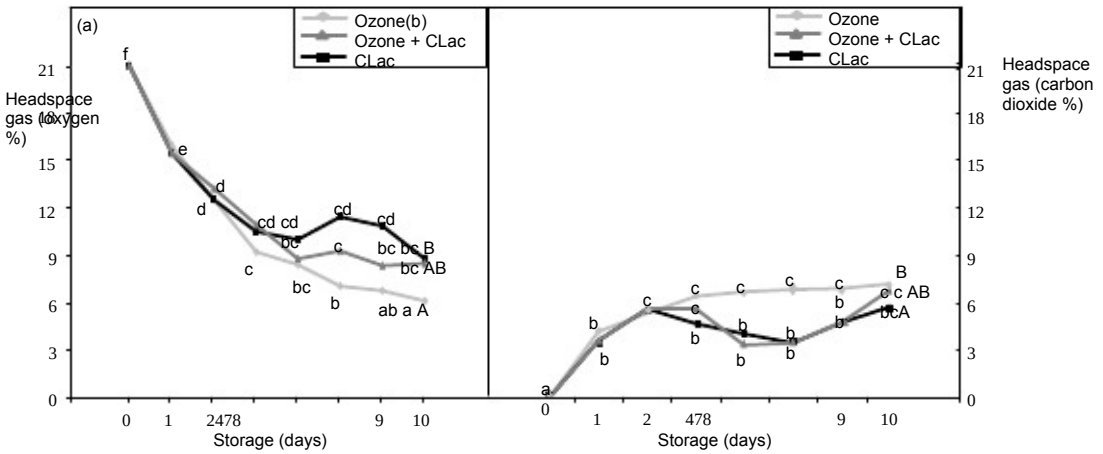

Figure 2. Changes in headspace gas composition ((a) oxygen and (b) carbon dioxide) in packages of fresh-cut lettuce stored at 4 °C for 10 days and treated with (1) ozone (1 mg L^{-1}), (2) calcium lactate (CLac, 15 g L^{-1} at 50 °C) or (3) ozone combined with calcium lactate (ozone + CLac). Points designated on any curve by the same letter are not significantly different ($P > 0.05$). Lowercase letters are used for comparisons during storage and uppercase letters for treatment comparisons. Three independent trials were carried out in triplicate.

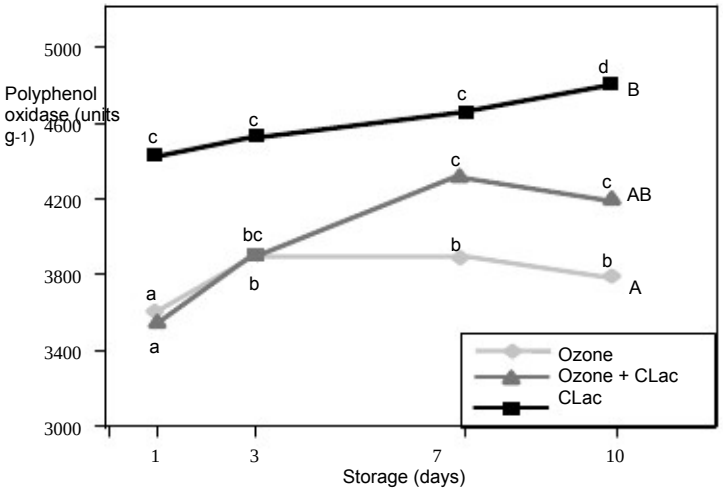

Figure 3. Changes in polyphenol oxidase activity in fresh-cut lettuce stored at 4 °C for 10 days and treated with (1) ozone (1 mg L^{-1}), (2) calcium lactate (CLac, 15 g L^{-1} at 50 °C) or (3) ozone combined with calcium lactate (ozone + CLac). Points designated on any curve by the same letter are not significantly different ($P > 0.05$). Lowercase letters are used for comparisons during storage and uppercase letters for treatment comparisons. Three independent trials were carried out in duplicate.

Figure 3 shows the PPO activity profile during storage of fresh-cut lettuce. Samples treated with ozone and ozone combined with CLac showed significantly lower ($P < 0.05$) PPO enzymatic activities than samples treated with CLac alone. This decrease in PPO activity could be caused by the high oxidation potential of ozone compared with CLac. Although CLac has been described as an inhibitor of PPO synthesis[28] during storage, ozone showed a higher efficacy in the reduction of these enzymes. Saftner et al.[39] considered ozone to be an efficient organic matter oxidant that could reduce the microbial content, giving rise to a lower respiration rate, and decrease enzyme synthesis.[40] Intermediate PPO values in the samples treated with the combined method can be attributed to the opposing effects of the increase in stress (double washing process), which might increase enzyme activity, and the ozone, which decreases it.

The oxidative effect of ozone might also have produced lower POD activity (Fig. 4). Although significantly ($P < 0.05$) lower values were observed with the use of ozone treatments, either alone or combined, compared with CLac alone, these significant differences were only observed at the beginning of storage (days 1–3). From day 7, no effect of the treatment was observed.

Texture-related enzymes (pectin methylesterase)
PME activity increased significantly during storage for all treatments, although showing fluctuations within the storage period (Fig. 5). This irregular behaviour may be due to a wounding response and/or to changes in the solubility of the enzyme during storage.[41] Such behaviour has been observed for certain browning-related enzymes.[42] Other authors have attributed this variability to intrinsic factors and to pre- and

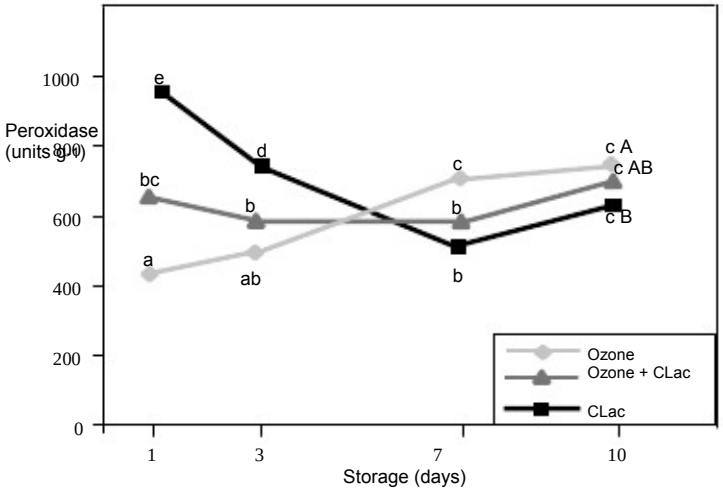

Figure 4. Changes in peroxidase activity in fresh-cut lettuce stored at 4 °C for 10 days and treated with (1) ozone (1 mg L^{-1}), (2) calcium lactate (CLac, 15 g L^{-1} at 50 °C) or (3) ozone combined with calcium lactate (ozone + CLac). Points designated on any curve by the same letter are not significantly different ($P > 0.05$). Lowercase letters are used for comparisons during storage and uppercase letters for treatment comparisons. Three independent trials were carried out in duplicate.

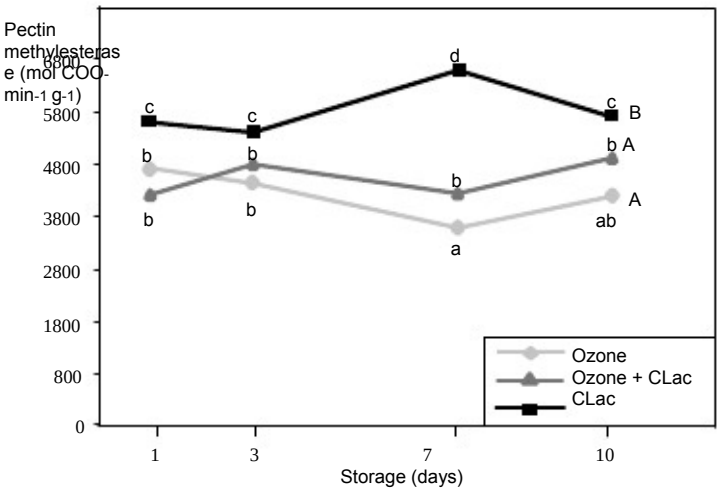

Figure 5. Changes in pectin methylesterase activity in fresh-cut lettuce stored at 4 °C for 10 days and treated with (1) ozone (1 mg L^{-1}), (2) calcium lactate (CLac, 15 g L^{-1} at 50 °C) or (3) ozone combined with calcium lactate (ozone + CLac). Points designated on any curve by the same letter are not significantly different ($P > 0.05$). Lowercase letters are used for comparisons during storage and uppercase letters for treatment comparisons. Three independent trials were carried out in duplicate.

postharvest factors, which can affect enzyme activity, vitamin content, etc.[43]

Fresh-cut lettuce treated with CLac had significantly ($P < 0.05$) higher PME activity values than samples treated with ozone or the combined method (Fig. 5). Previous studies have shown that CLac combined with heat shock produces an activation of PME.[11,19,22] The beneficial effects on texture of heat treatments and CLac solution have been explained by the activation of PME.[19] PME is responsible for cleaving the methoxyl groups from methylated pectic substances, generating free pectic acids[44] that contain newly available carboxyl groups.

This activation of PME caused by CLac and heat shock was inhibited by the treatment with ozone. Samples treated with ozone in combination with CLac showed the same levels of PME activity as samples treated with ozone alone. This suggests a stronger inhibitory effect on this enzyme (also on PPO and POD) caused by ozone treatment than the activation produced by CLac treatment.

Colour analysis

Total colour differences (E) were analysed using CIE L^* a^* b^* parameters for fresh-cut lettuce. Changes in colour during storage ($P < 0.05$) were observed (Fig. 6). Samples subjected to the double treatment showed greater changes in colour than samples subjected to individual treatments. However, no differences were found between samples treated with CLac and samples treated with ozone.

This greater change in colour with the double treatment could be associated with the damage and stress produced by the double manipulation (physical browning) rather than being caused by enzymatic browning (Fig. 3). Although colour variation can be associated with enzymatic browning by phenolic oxidation over time,[45–49] our results did not show a correlation. Fresh-cut lettuce treated with ozone and CLac combined had a similar reduction in PPO (throughout the entire storage period) and POD (during the first day of storage) activities to samples treated with ozone.

Instron analysis (crispiness coefficient)

The variation in CC was analysed during storage (10 days) for all samples (Fig. 7). During storage a significant ($P < 0.05$) reduction in CC was observed in all cases, which was associated with a loss of turgor and fresh-like textural properties. Significant differences between treatments were found. Samples treated with ozone showed the lowest values of CC, while samples treated with CLac had the highest values. This finding might be partly associated with PME activation (Fig. 6). The use of heat shock (50 °C) and the addition of CLac can make tissues firmer by binding to the pectin carboxyl groups that are generated through the action of PME.[45] Samples subjected to the double treatment had similar values to samples treated with ozone alone.

Sensory analysis

Fresh appearance is the main attribute that consumers use to evaluate the quality of vegetables and fruits, since people 'buy with their eyes'.[50,51] Appearance, browning and texture are key aspects used in sensory analysis to evaluate the general quality of a product. This is especially true for lettuce, where browning and lack of firmness are critical factors in perceived loss of quality.[52]

Sensory analysis was used to assess the quality of fresh-cut Iceberg lettuce over 10 days. Mean scores for sensory fresh appearance are given in Table 1. Fresh-cut lettuce showed significantly lower scores for appearance at the end (10 days) of storage than from day 1 to day 7. The average values on day 7

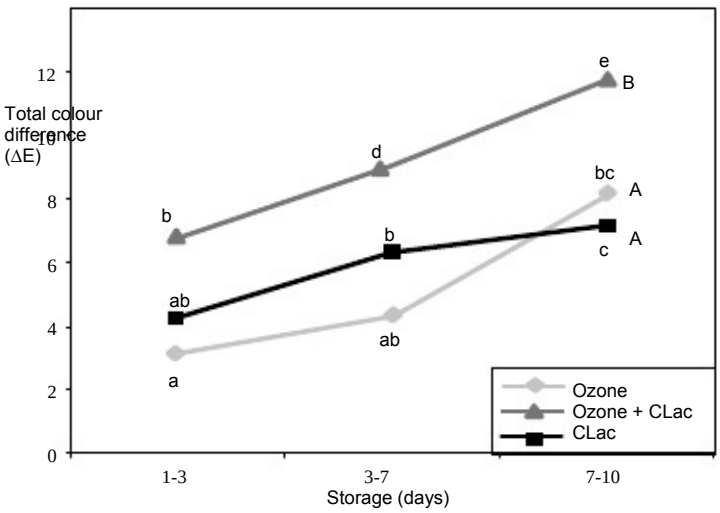

Figure 6. Total colour change (CIE L^* a^* b^* parameters) in fresh-cut lettuce stored at 4 °C for 10 days and treated with (1) ozone (1 mg L^{-1}), (2) calcium lactate (CLac, 15 g L^{-1} at 50 °C) or (3) ozone combined with calcium lactate (ozone + CLac). Points designated on any curve by the same letter are not significantly different ($P > 0.05$). Lowercase letters are used for comparisons during storage and uppercase letters for treatment comparisons. Three independent trials were carried out; >25 samples per trial were analysed.

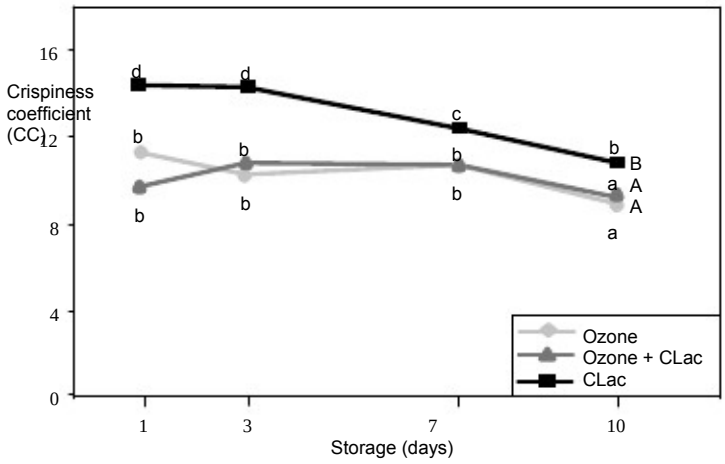

Figure 7. Effect of washing treatment on crispiness coefficient (CC). Measurement of CC of fresh-cut lettuce stored at 4 ◦ C for 10 days and treated with (1) ozone (1 mg L−1), (2) calcium lactate (CLac, 15 g L−1 at 50 ◦ C) or (3) ozone combined with calcium lactate (ozone + CLac). Points designated on any curve by the same letter are not significantly different (P > 0.05). Lowercase letters are used for comparisons during storage and uppercase letters for treatment comparisons. Three independent trials were carried out; >25 samples per trial were analysed.

Table 1. Sensory evaluation of fresh appearance rating (scale 1–5) of fresh-cut lettuce stored at 4 ◦ C for 10 days and treated with (1) ozone (1 mg L−1), (2) calcium lactate (CLac, 15 g L−1 at 50 ◦ C) or (3) ozone combined with calcium lactate (ozone + CLac)

Treatment	Day 1	Day 3	Day 7	Day 10
Ozone	3.45 ± 0.50b	3.85 ± 0.60b	3.55 ± 0.80b	2.29 ± 0.30a
Ozone + CLac	3.80 ± 0.23b	3.68 ± 0.20b	3.51 ± 0.12b	2.43 ± 0.23a
CLac	3.65 ± 0.56b	3.91 ± 0.27	3.71 ± 0.37b	2.60 ± 0.20a

Each value represents mean ± standard deviation for fresh appearance. Values followed by different letters in the same row indicate significant differences during storage. No significant differences were observed between treatments. Three independent trials were carried out; 14 panellists in each trial scored the samples. Ratings: 0 = poor fresh appearance; 3 = acceptable fresh appearance; 5 = excellent fresh appearance.

were considered acceptable (score above 3), showing that all treatments maintained good quality until the end of storage. Differences between treatments were not observed. At the end of storage, lower fresh appearance values were observed in samples washed with ozone and ozone combined with CLac. However, the differences were not significant. The results obtained in the sensory analysis did not correlate with the instrumental results, which might be due to the high variability of the product and/or the limited discriminative ability of human perception.

CONCLUSIONS

The use of an ozone washing treatment to extend the quality of fresh-cut lettuce was compared with a treatment using CLac at 50 ◦ C and a combination of both, with unequal results. A reduction in enzyme activity was produced by ozone treatments, resulting in a potentially beneficial effect of reduced enzymatic browning, although this potential reduction in browning was not revealed by colour analysis. However, this enzyme inactivation showed a negative effect, as the reduction in activity of the texture-related enzyme PME was correlated with a lower crispiness coefficient. Depending on further studies to evaluate the effect on the microbial load of these two treatments (and the combination of both), some advantages of CLac compared with ozone make the former a more suitable treatment, i.e. safety of use, no by-product production, dairy industry waste reutilisation, calcium enrichment of the product and a possibly more lasting effect, as ozone rapidly disappears. For these reasons the authors consider CLac (used at 50 ◦ C) a more suitable treatment than ozone in order to extend the quality of fresh-cut lettuce.

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