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Optimisation of Calcium Lactate Washing Treatment on Salad-cut Lettuce: Quality Aspects

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Keywords: Salad-cut lettuce, enzymatic browning, calcium lactate, temperature, quality, safety

Abstract

Markers of quality retention: colour, texture, browning, texture related enzymes and sensory properties, were analysed during storage. The use of high temperatures (50°C) showed a positive effect on enzymes related to quality maintenance. It reduced the activity of the browning-related enzymes polyphenol oxidase and peroxidase but it increased the activity of pectin methyl esterase, an enzyme involved in the maintenance of texture. High calcium lactate concentrations (3 %) produced a reduction in the respiration rate of the salad-cut lettuce during storage, but also a loss of luminosity and greenness (a*). The use of high temperatures and intermediate calcium lactate concentrations (1.5 %) proved to be the best washing treatment to maintain the quality of salad-cut lettuce over 10 days storage. These conditions (50 °C and 1.5 % calcium lactate) gave higher freshness scores and lower browning scores than conventional chlorine treatment when evaluated by a sensory panel.

INTRODUCTION

The minimally processed fruit and vegetable market has grown rapidly in recent years due to the health benefits associated with these foods. Its growth has heightened awareness about the microbiological and physiological parameters associated with quality in fresh ready-to-use vegetables. The negative effects of postharvest stress in terms of quality loss is well known (Wiley, 1994), but the beneficial effects of such treatments are not always appreciated. The extension of quality retention for fresh-cut products is relevant for industry due to its economic impact. It is important that the washing treatments applied to fresh vegetables and fruit help maintain their quality (colour, absence of excessive exudate in the package, crisp texture, etc.) since consumers demand a fresh product as well as convenience and long shelf life. Fruit and vegetables treated with calcium are generally firmer than controls during storage. It has been reported to be a good alternative to calcium chloride because it avoids the bitterness or off-flavours associated with this salt (Luna-Guzman and Barret, 2000). The antibacterial properties of calcium lactate washing solutions have been described for treatment of honeydew melon and minimally processed vegetables. Heat shock treatments, alone or combined with other agents, have also been used to prevent browning reactions in various vegetables and fruits (Hisaminato et al., 2001). Firming effects obtained from heat treatments alone or combined with calcium treatments has been attributed to the action of heat-activated pectin methyl esterase (PME) and/or to increased calcium diffusion into tissues at higher temperatures (Garcia et al., 1996).

To the best of our knowledge no studies have been carried out using calcium lactate washing treatment of lettuce. The objective of this research was to evaluate the effect of calcium lactate washing treatments at different concentrations (0.5, 1.5 and 3 %) and different temperatures (4, 25 and 50 °C) on selected quality parameters for Iceberg lettuce.

MATERIALS AND METHODS

Iceberg lettuce (*Lactuca sativa* sp.) grown in Ireland, was purchased from a local supermarket (Dunnes Stores, Ireland) and stored at 4°C until use. In the calcium lactate

test series, a full factorial screening design containing 9 tests was used (Table 1). The salad-cut lettuce was dipped in water solutions of different calcium lactate (Sigma-Aldrich, St. Louise, USA) concentrations (0.5, 1.5 and 3 %) and different temperatures (4, 25 and 50 °C) for 1 min with constant agitation.

The individual analyses, and a short summary of the methods, are listed below:

Headspace gases: A Gaspacer analyser (Systech Instruments, UK) was used to monitor levels of CO₂ and O₂ in the package during storage. Headspace gas samples were obtained using a hypodermic needle inserted through an adhesive septum fixed to the bags. The gas content was expressed in percentage (%).

pH measurement: A 10 g sample of lettuce 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.

Exudate: Exudate was quantified by the method of Carlin and Nguyen-the (1999). The sample was weighed and the percentage loss in weight recorded as the exudate. The average of triplicate values was expressed as a percentage: g exudate/ g of vegetable.

Dry matter: A weighed piece of salad-cut lettuce was heated at 100 °C for 2 hours in a Universal Oven (Mettler, Schwabach, Germany). Dry matter was calculated using the weight after heating as a percentage of the initial weight.

Polyphenol oxidase (PPO) and Peroxidase (POD) activity measurement: Both enzymes were assayed in homogenates that were prepared as follows: 10 g of tissue was placed in a polytron homogeniser (Polytron model PT-MR-3000, Kinematica, Switzerland) in a 1:2 (w:v) ratio with 0.5 M phosphate buffer pH 6.5 containing 50 g/l polyvinylpyrrolidone. Homogenisation was carried out two times 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 centrifuged at 12,720 x g for 30 min at 4 °C. It was then filtered through one layer of crepe bandage. The resulting crude extract was used without further purification. All the extracts were kept at 4 °C in the dark and used immediately (within one hour). PPO activity was assayed spectrophotometrically by a modified method based on Tan and Harris (1995). The reaction mixture contained 0.1 mL crude extract and 2.9 mL substrate solution (0.02 mol/l catechol as substrate in 0.05 mol/l phosphate buffer, pH 6.5). The rate of catechol oxidation was followed at 400 nm for 2 min at 25 °C. A unit of enzyme activity was defined as an increase of 0.1 absorbance units per minute. POD activity was assayed spectrophotometrically, the reaction mixture contained 0.2 mL of extract and 2.7 mL of 0.05 M phosphate buffer pH 6.5 containing 100 µl of hydrogen peroxide (1.5 % v/v) as oxidant and 200 µl of p-phenyldiamine as hydrogen donor. The oxidation of p-phenyldiamine was monitored at 485 nm and 25 °C. A unit of enzyme activity was defined as an increase of 0.1 absorbance units per minute.

Pectin methyl esterase (PME) activity: This was measured using the method described by Kimball (1991). Ten grams of tissue was diluted in an extraction solution (0.2 M sodium phosphate buffer, pH 7.5 containing 1M sodium chloride and 10 mM 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 centrifuged at 12,500 g for 30 min at 4 °C. 1 mL of this extract was mixed with 40 mL of substrate solution (0.1% pectin). The solution was adjusted to pH 7.0 with 1.0 M NaOH, and the pH of the solution was re-adjusted to pH 7.5 with 0.05 M NaOH. After the pH reached 7.5; 0.2 mL of 0.05N 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 titrimetrically using a pH electrode to monitor the production of H⁺.

Colour measurement: The first method used a colorimeter (HunterLab, UK). CIELAB* colour values (L*, a* and b*) were measured. The instrument was calibrated with a white standard tile and with a green standard tile under illumination conditions. The CIELAB* parameters were converted to Hue ($\arctan b^*/a^*$) and Chroma ($(a^{*2}+b^{*2})^{1/2}$). Hue values indicate product colour, Chroma values indicate colour saturation and variation of colour during the storage was calculated by using the following formula: ($\Delta E = [(L_f - L_i)^2 + (a_f - a_i)^2 + (b_f - b_i)^2]^{1/2}$)

Images of salad-cut lettuce were captured during the storage period (10 days) using a computer vision system (Canon Power Shot, CCD 3.1 MPixels, Japan). A lighting unit with two fluorescent lamps provided the only illumination in a dark room in order to obtain images with the same brightness and contrast (Kaiser Fototechnik, RB 5000 DL. Copy Lighting Unit 5556, Buchen, Germany). Images were captured and colour was analysed qualitatively and quantitatively using Photoshop® image analysis software (Adobe System, 2002). CIELAB parameters were obtained using this software.

Texture analysis: Texture properties of Iceberg lettuce were assessed using an Instron texture analyser (Instron 4302 Universal Testing Machine, Canton MA, USA) fitted with a puncture cell. The speed setting for the experiment was 1000 mm/min and maximum load for the puncture test was expressed in kN.

Sensory analysis: Fresh appearance, browning, off-flavours, texture, and overall acceptability of samples were scored on a hedonic scale of 1 to 5. 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, Ontario, Canada).

Statistical analysis: Statgraphics software (version 2.1; Statistical Graphics Co., Rockville, USA).

RESULTS AND DISCUSSION

Previous work showed calcium lactate washing treatments were a good alternative to chlorine washing (~120 ppm) for salad cut lettuce. Calcium lactate treatment proved to be as effective as chlorine treatment and enhanced the nutritional value of the final product. In the present work a study of the effect of temperature and calcium lactate concentration on lettuce has been investigated in order to find the optimum combination of calcium lactate concentration and temperature for salad-cut lettuce washing treatment. Response surface methodology has been adopted for the optimisation process.

The oxygen and carbon dioxide content of the packages containing the salad-cut lettuce were analysed following treatment with different concentrations of (0.5-3 %) calcium lactate and different wash temperatures (0-50 °C) (Fig. 1). Changes in package gas composition during storage as a function of wash treatment temperature were observed. The oxygen content and carbon dioxide content changed significantly ($p < 0.05$) during storage. At day 10 the lowest values of oxygen (Fig. 1A) and the highest values of carbon dioxide (Fig. 1C) were observed.

Calcium lactate treatment significantly affected ($p > 0.05$) the O₂ concentration but not the CO₂ concentration ($p > 0.05$) throughout the storage period (Fig. 1 B and C). High calcium lactate concentrations produced a decrease in the oxygen content at all temperatures. These results are in agreement with other authors who suggest that calcium lactate can reduce the respiration of minimally processed fresh vegetables (Hewett and Watkins, 1991).

Both gases were not affected by temperature until the end of the storage period for all the treatments. Samples treated at the high temperature showed the lowest oxygen content at the end of the storage period. A significant interaction between calcium lactate treatment and temperature was observed for both gasses (data not shown). Temperatures between 30-45 °C showed the lowest values of oxygen and carbon dioxide at high concentrations of calcium lactate. At the end of the storage period oxygen levels had decreased to ca. 7 % (Fig. 1A, 1B) while carbon dioxide levels had risen to ca. 10 % for all calcium lactate concentrations and temperatures.

The pH of salad-cut Iceberg lettuce increased significantly with storage time but not with temperature or calcium lactate concentration. The interaction between temperature and storage time had a significant effect ($p < 0.05$) on pH values. Samples treated at low temperatures (4 °C) showed the highest pH values, and samples washed at 50 °C the lowest. Thus, treatment at the higher temperature exhibited a slower increase in pH over the storage period and reached lower final pH values. This effect could be due to the inhibitory effect of temperature on bacterial growth. Delaquis et al. (1999) have

described a reduction in bacterial counts in lettuce treated with warm water. In this study low pH wash solutions were not considered, however, calcium lactate itself has an inhibitory effect on microbial populations. The antimicrobial properties of calcium lactate depend on its forming the undissociated acid in solution. The undissociated form acts to uncouple microbial substrate transport and oxidative phosphorylation during electron transport. Previous studies have shown that calcium lactate (3 %) was as effective as chlorine (~120 ppm) in microbial population control (mesophiles, psychrophiles and lactic acid bacteria).

Exudate values decreased significantly during storage ($p < 0.05$). The exudate was not affected by calcium lactate concentration but temperature had a significant effect on exudate. Samples treated at 50 °C had the lowest exudate values. This finding was in agreement with the dry matter data (see below). Dry matter values did not vary with the calcium lactate concentration, but treatment temperature and storage time affected this parameter significantly ($p < 0.05$). In the samples treated at high temperatures (50 °C) the loss of water was lower than in the samples treated at 4 °C and 25 °C. Samples treated at 50 °C showed lower loss of water than samples washed at lower temperatures over all the.

Polyphenol oxidase (PPO) is an enzyme implicated in the enzymatic browning process. There were significant differences between treatments in PPO activity with respect to storage time, calcium lactate concentration and temperature (Fig. 2). The activity of this enzyme significantly increased during storage. Calcium lactate had a significant ($p < 0.05$) inhibitory effect on PPO activity, resulting in decrease of the activity with increasing calcium lactate concentration. The 50 °C treated samples had a lower PPO activity than samples treated at 25 °C and 4 °C. This may be due to the effect of heat-shock on the enzyme producing a decrease in its activity (Saltveit, 2000).

A decrease in POD activity during storage was observed (Fig. 3). Activity did not change significantly with respect to calcium lactate concentration until the end of storage (Fig. 3). POD activity was significantly affected by temperature. It was found to be lower at 50 °C than at 25 °C and 4 °C (Fig. 3). Several authors support the idea of a synergistic activity between POD and PPO enzymes; the products of POD are used by the PPO so a decrease in POD activity can produce a decrease in the substrate available for PPO (Castañer et al., 1999).

The beneficial effects on texture of heat treatments and calcium solution washes have usually been explained in terms of the activation of pectin methylesterase (PME). PME activity changed significantly during storage for all the treatments: activity initially increased to a maximum before decreasing at the end of the storage period (Fig. 4). Curiously, the concentration of calcium lactate did not show a significant effect on PME activity ($p < 0.05$).

Treatment temperature was the only parameter that had a significant effect on PME, increasing with increasing temperature (Fig. 4). PME activity was higher in samples treated at 50 °C than at 25 °C. Treatment at 4 °C showed the lowest values. These results agree with studies that show that treatment with calcium solutions at temperatures over 50 °C increases PME activity (Garcia et al., 1996).

Colour parameters were estimated with a colorimeter and digital image analysis on the surface of salad-cut lettuce during storage. The three CIELAB colour parameters (a^* , b^* and luminosity), as expected, showed significant differences during storage ($p > 0.05$). Temperature and calcium lactate concentration had a significant effect for all these colour parameters ($p < 0.05$).

Samples treated with higher calcium lactate concentration showed significantly ($p < 0.05$) lower luminosity (L values). By contrast, treatment at higher temperatures gave rise to higher luminosity. This latter effect could be related to the loss of PPO activity at higher temperature. Low levels of luminosity indicate high levels of browning since darkness is related to browning. These findings agree with King et al. (1991).

Another parameter related to browning (Castañer et al., 1999) is the a^* value since it is associated with the breakdown of chlorophyll (Bolin and Huxsoll, 1991). A significant increase in a^* was observed with increasing calcium lactate concentrations

(data not shown). The higher temperature treatments (50 °C) showed lower a^* values (greener) ($p>0.05$).

All of the above CIELAB* parameters may be combined and expressed as variation of colour (ΔE). Fig. 5 shows the effect of temperature and calcium lactate concentration on variation of colour during storage. Variation in colour increased during storage for all samples (Fig. 5). High concentrations of calcium lactate gave higher colour variation than samples treated at low or intermediates concentrations (Fig. 5). The salad-cut lettuce treated at high temperatures showed a significantly lower variation of colour ($p<0.05$) than samples treated at room temperature or 4 °C (Fig. 5).

Texture was analysed using a puncture cell attachment. The maximum puncture load (kN) was measured as a crispiness parameter in salad-cut lettuce. A significant ($p>0.05$) increment in the maximum load at day 10 was observed for all the treatments. Calcium lactate concentration did not significantly affect the maximum load measurement. High temperature treatment gave lower values of maximum load than low temperature. This increase in load is related to an increase in flexibility (loss of crispness).

In order to limit the sensory analysis to a manageable amount and since the effect of temperature was the most relevant for all the parameters, a calcium lactate concentration of 1.5 % was used for these studies. Samples treated with 1.5 % calcium lactate at 4, 25 and 50 °C and a control (chlorine ~120 ppm) were evaluated by the sensory panel. The sensory panel analysis found significant differences over the storage time ($p<0.05$) in browning, acceptability and fresh appearance parameters with different wash treatments. It is known that fresh vegetables lose their typical fresh appearance and texture following processing after a short storage time. Samples treated with calcium lactate at 50 °C had lower browning (Fig. 6A) and significantly higher scores for acceptability (Fig. 6B) and fresh appearance (Fig. 6C) than samples treated at 4 °C and 25 °C and even lower than the chlorine treatment (control). Other parameters evaluated by the sensory panel such as texture, off-odours and fresh appearance did not show significant differences between treatments (not shown). The samples treated with calcium lactate at 25 °C showed similar behaviour to the samples treated with chlorine, which was in agreement with previous studies in this laboratory. Sensorial analysis showed differences in texture as a function of storage ($p<0.05$) time but did not show a difference between treatments ($p>0.05$), although higher scores were found in samples treated at 50 °C up to day 7. All treatments gave similar values at the end of the storage time.

CONCLUSIONS

Increased browning and the loss of crispy texture are two of the most important causes of losses for minimally processed lettuce in the market. From a quality point of view the use of high temperature (50 °C) treatments appeared to inhibit browning reactions and also improved texture by activation of PME. High concentration (3 %) of calcium lactate appeared to decrease respiration with respect to lower concentration (0.5 %) but increased the colour variation of the lettuce during storage. In controlling these key quality parameters the best treatment conditions found were 1.5 % calcium lactate at 50 °C.

ACKNOWLEDGEMENTS

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Figures

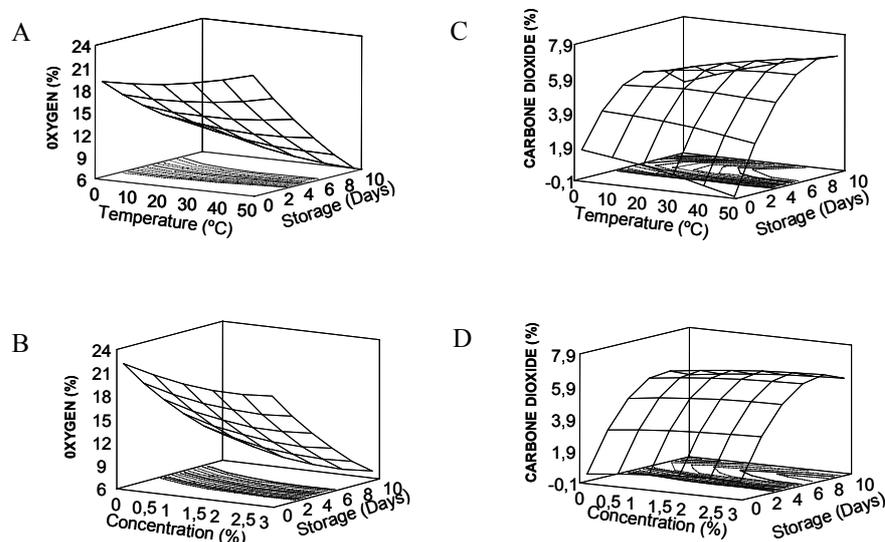


Fig. 1. Effect of calcium lactate concentration (%) and temperature (°C) on the headspace oxygen and carbon dioxide content over 10 days storage.

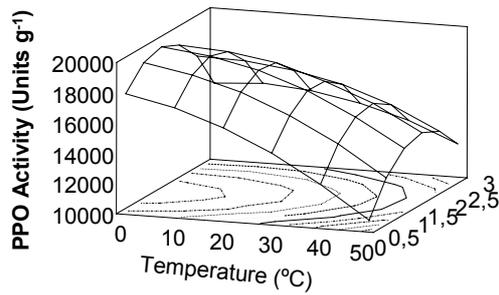


Fig. 2. Changes in polyphenol oxidase (PPO) activity as a function calcium lactate concentration (%) and storage time (days) for three different temperatures: (A), 4 °C; (B), 25 °C and (C), 50 °C.

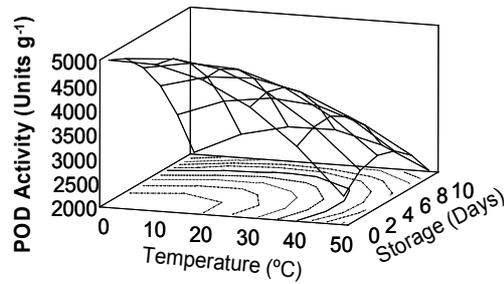


Fig. 3. Effect of temperature in the activity of peroxidase (POD) during the storage.

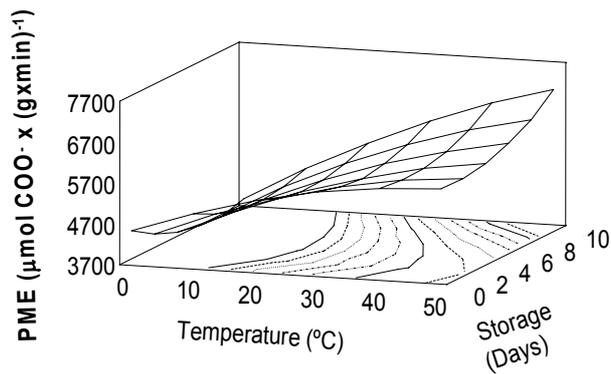


Fig. 4. Effect of temperature in the activity of pectin methyl esterase (PME) over the storage.

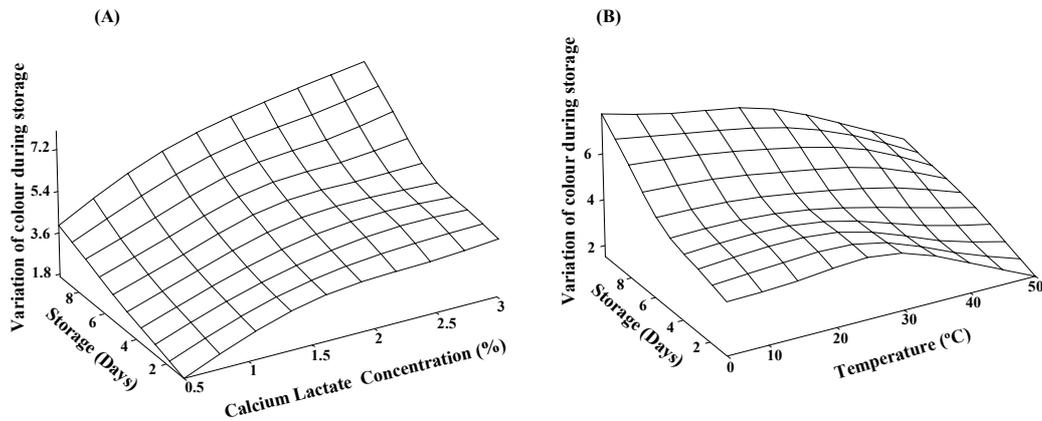
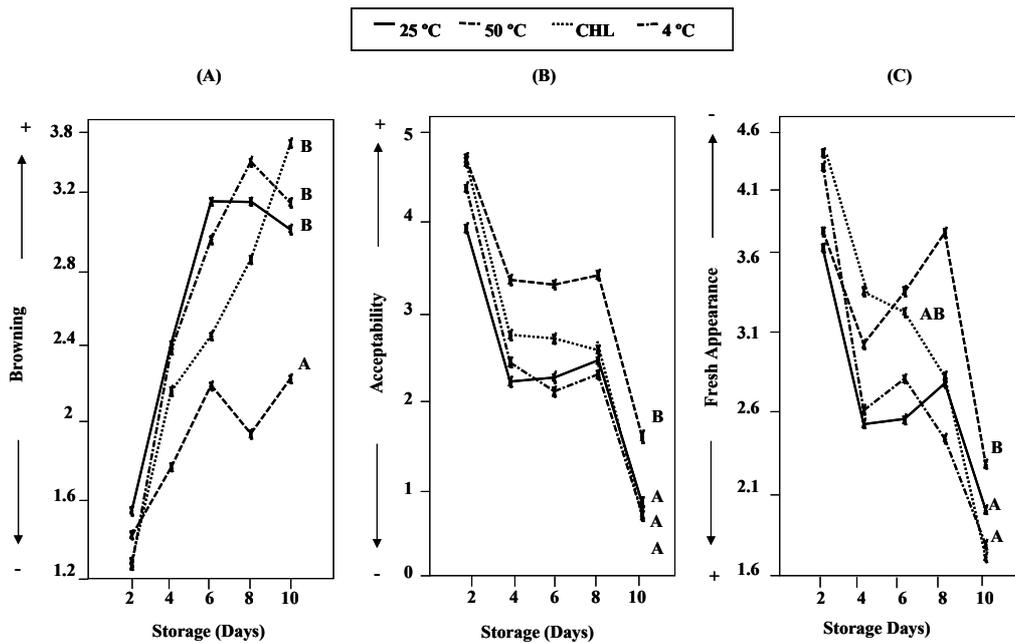


Fig. 5. Variation of colour during storage (10 days) as a function of (A) calcium lactate concentration (%) and (B) temperature (°C).



*Points designated by different letter show significant differences ($p < 0.05$)

Fig. 6. Sensory evaluation of salad-cut lettuce treated with 1.5 % calcium lactate at different temperatures during storage. Chlorine (~120 ppm, room temperature) was used as control. Parameters: browning (A), acceptability (B) and fresh appearance (C).