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Calcium salts and heat treatment for quality retention of fresh-cut 'Galia' melon

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ABSTRACT

'Galia' melon is one of the most common cv produced in Spain destined for fresh consumption and/or for the fresh-cut processing industry. Nevertheless, fresh-cut melon is very susceptible to softening during storage, even under chilling and modified atmosphere packaging. This softening process is related to Ca levels in fruit tissue. After preparing trapezoidal shaped sections of 'Galia' melons, the pieces were dipped for 1 min a 60 °C in Ca chloride, citrate, lactate, ascorbate, tartrate, silicate, propionate or acetate using a Ca concentration equivalent to 0.4% (0.15 g g^{-1}) pure Ca chloride, combined with 50 mg L^{-1} H_2O_2 for controlling microbial growth. Dipping in sterile distilled water (without Ca salt) at 60 °C for 1 min was used as a control treatment. Firmness, pectin methylesterase and polygalacturonase activity, Ca content, microbial growth, respiration rate, and sensory evaluation, were evaluated throughout 10 days of storage at 5 °C under a passive modified atmosphere reaching 4.5 kPa O_2 and 14.7 kPa CO_2. At the end of shelf life, Ca ascorbate, chloride and lactate provided melon pieces with a lower respiration rate, increased tissue total Ca content, and maintained a good firmness. In addition, those Ca salts reduced microbial growth. Sensory parameters, such as flavor perception, were kept above the upper limits for marketability. A considerable loss of flavor was found in all treatments except with Ca chlorine, lactate and ascorbate, the only treatments found acceptable from the consumer point of view.

1. Introduction

'Galia' melon is one of the most common melon cultivars produced in Spain (MARM, 2009). Due to its highly appreciated sensory attributes, it currently has a potential as raw material for the fresh-cut or minimally fresh processed industry (Aguayo et al., 2004). Nevertheless, fresh-cut melon is very susceptible to softening during storage, even under chilling; this process is related to the enzymatic degradation of the middle lamella of the cell wall and loss of cell adhesion. Enzymes such as pectin methylesterase (PME) and polygalacturonase (PG) play an important role in 'Galia' melon softening (Chisari et al., 2009). In addition, the softening rate is related to Ca levels in the fruit tissue (Fallahi et al., 1997). For this reason, Ca dips have been used as firming agents to extend postharvest life of several products.

Firmness and resistance to softening, resulting from addition of Ca, have been attributed to the stabilization of membrane systems and formation of Ca pectate, which increases rigidity of the middle lamella and cell walls, leading to increased resistance to PG activity and to improved turgor pressure (Mignani et al., 1995). Ca ions form intermolecular bridges by interaction with free carboxyl groups of pectic acid polymers to form insoluble salts with ionic linkages between pectin molecules (McFeeters and Fleming, 1991). Ca application often results in reduced respiration rates and ethylene production, increased firmness, and reduced incidence of physiological disorders and decay (García et al., 1996).

Studies on temperature effects of Ca solutions have been limited to application in conjunction with heat treatments. A combination of heat treatments followed by Ca dips has also been applied for the primary purpose of controlling postharvest pests and/or diseases and has been found to have very good results in maintaining or improving the texture of several products. In this sense, Ca application, combined or not with heat treatments, maintained firmness in a wide variety of fruit and vegetables including whole honeydew melon (Lester and Grusak, 1999), sliced carrot (Rico et al., 2007), fresh-cut lettuce (Martin-Diana et al., 2006), fresh-cut cantaloupe (Luna-Guzmán et al., 1999; Luna-Guzmán and Barret, 2000); fresh-cut honeydew melon (Saftner et al., 2003), fresh-cut 'Piel de Sapo' melons (Oms-Oliu et al., 2007) and fresh-cut *saccharinus* melon (Aguayo et al., 2008). Heat treatment allows demethylation of PME to form anionic carboxyl groups with which calcium ions can form salt bridge cross-links (Alonso et al., 1997). During low temperature thermal treatments, loss of selective permeability of the membrane also occurs, giving rise to diffusion of cations, such as Ca, into the cell wall. It has been showed that warm temperatures (40–60 °C) increase the beneficial effects of the Ca treatment due

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to higher washing solution retention inside the product (Rico et al., 2007).

Ca carbonate and Ca citrate are the main Ca salts added to foods in order to enhance nutritional value (Brant, 2002). Others Ca salts used in the food industry are lactate, chloride ($CaCl_2$), phosphate, propionate, ascorbate and gluconate, when the objective is the preservation and/or the enhancement of the product firmness (Luna-Guzmán and Barret, 2000; Alzamora et al., 2005; Manganaris et al., 2007; Quiles et al., 2007; Aguayo et al., 2008).

The aim of this work was to determine the combined effect of the application of different Ca salts and heat treatments, on the keeping quality of fresh-cut 'Galia' melon.

2. Materials and methods

2.1. Minimally fresh processed melon

'Galia' melons (*Cucumis melon* var. *cantalupensis* Naud.) of the commercial cv Solarking, were grown under Mediterranean climate conditions of the Campo de Cartagena (Murcia, Spain), were hand-harvested at the commercial ripening stage, using the scale color of Difrusa Export, SA for 'Galia' melon (scale 1–9) and according to soluble solids content (SST), expressed as °Brix. The maturity stage corresponded to 6 on the color scale and 11 °Brix. This soluble solids content level is considered to correspond to the optimal commercial ripening stage for allowing the usual time lag for distribution and retail sale (Artés et al., 1993). Fruit were selected according to their size and external skin color, in a commercial packinghouse with damaged fruit discarded. Selected sound melons were transported about 30 km to the Pilot Plant and stored at 10 °C until the next morning. Minimal processing started by washing the fruit with tap water, draining, and blotting dry with paper towels. Melons were hand-cut into eight slices, parallel to the longitudinal axis, and blossom and stem-ends discarded. Seeds, placenta and peel were also discarded and the pulp was hand-cut in trapezoidal-shaped sections (3.4 ± 0.4 cm wide, 4.4 ± 0.5 cm length). All operations were carried out in a disinfected cold room at 10 °C. In order to reduce the stress produced during processing, sharp knives were used. Knives were disinfected with chlorinated (1.4 mM) water for 30 min. After cutting, melon pieces were dipped in one of different Ca solutions.

2.2. Calcium dipping and packaging

Melon pieces were dipped in one of the following Ca solutions for 1 min at 60 °C: Ca chloride ($CaCl_2$, anhydrous purum), Ca citrate ($C_{12}H_{10}Ca_3O_{14}{\cdot}4H_2O$, ≥99% purity), Ca lactate ($Ca(CH_3CHOHCOO)_2{\cdot}5H_2O$, 98% purity), Ca ascorbate ($C_{12}H_{14}CaO_{12}{\cdot}2H_2O$, ≥99% purity), Ca tartrate ($C_4H_4O_6Ca$), Ca silicate ($CaSiO_3$, purum), Ca propionate ($C_6H_{10}CaO_4$, 97% purity) and Ca acetate ($C_4H_6CaO_4$, ≥99% purity). All chemicals were from Panreac Chemistry, Coorp, Spain. Different concentrations of Ca salts were added ensuring that they always provide the same amount of Ca corresponding to 0.4% ($0.15\,g\,g^{-1}$) than the pure $CaCl_2$. Ca levels were chosen from the results of previous validation work (data not published) and taken into account the results of Aguayo et al. (2008). To maintain the relevant temperature, a water-bath (Selecta, J.P. Barcelona, Spain) with continuous cold or hot water recirculation plus stirring was used. After the Ca dips, melon sections were chilled and disinfected by immersing for 5 min in water at 0 °C containing H_2O_2 ($50\,mg\,L^{-1}$). The final internal temperature 22 °C was monitored using a thermometer inserted in the centre of a piece of melon. As controls, melon pieces were dipped in distilled water at 60 °C for 1 min and then dipped in an H_2O_2 solution as Ca treatments.

Melon sections (160–180 g), previously drained, were then packed in polypropylene (PP) trays and heat-sealed with oriented polypropylene (OPP) of 35 μm thickness with a permeability of $5.5\,L\,m^{-2}\,d^{-1}\,atm^{-1}$ to O_2 and $10\,L\,m^{-2}\,d^{-1}\,atm^{-1}$ to CO_2, at 23 °C and 75% RH, according to the manufacturers' information (Plásticos del Segura, Murcia, Spain).

The prepared trays were stored at 5 °C and 90% RH. Three replicates for each treatment were evaluated at each time of analysis. The final gas concentrations found inside the trays were 4.5 kPa O_2 and 14.7 kPa CO_2.

2.3. Flesh firmness

A puncture test was used to evaluate melon piece firmness, based on the resistance of each piece to a pressure applied by a Lloyd instrument (LR10K, Fareham, Hants, U.K.). During the puncture test, a 4.5 mm diameter flat-head stainless steel cylindrical probe penetrated the middle of the longitudinal axis of the pieces (5 mm depth) at a speed of 50 mm s^{-1} (Aguayo et al., 2004). At each sampling day (0, 7 and 10 days at 5 °C), the firmness of 10 pieces from each treatment was monitored.

2.4. Calcium content

To determine Ca content, 15 mm diameter cylinders were extracted from the melon piece with a cork borer. Each cylinder was cut to a 5 mm depth, obtaining a disc with one side which had been in contact with the dipping solution. Sample size for each replicate comprised 18 discs from six pieces of melon, 15 g total weight. Three replicate samples were prepared for each treatment. Free and bound Ca content were determined using a modification of the methodology of Carvajal et al. (1999) reported by Aguayo et al. (2008).

2.5. Pectinmethylesterase (PME) and polygalacturonase (PG) activities

Enzymatic activities were determined in extracts prepared using a modified version of the method of Stevens et al. (2004). PME activity was determined by measuring the release of acid per time at pH 7.0 and 22 °C (Fachin et al., 2002). The reaction mixture consisted of 1000 μL of sample and 30 mL of a 1% apple pectin solution (90% esterification, Sigma, Spain) containing 200 mM of NaCl (≥99.5% purity, Panreac, Spain). Consumption of 0.01 N of NaOH (98% purity, Panreac, Spain) was recorded each minute during a 20 min reaction period. The PME activity is proportional to the rate of consumption of NaOH. PME activity was expressed in units (U), defined as micromoles of acid produced per minute at pH 7 and 22 °C.

PG activity was measured using a spectrophotometric method (Gross, 1982). One hundred microlitres of the extracted enzyme solution was incubated with 0.3 mL of 0.5% (v/v) polygalacturonic acid at 35 °C for 30 min. To stop the reaction, 2 mL of 0.1 M borate buffer, pH 9.0 and 0.4 mL of 1% (v/v) cyanoacetamide (99% purity, Sigma, Spain) were used. After cooling, the absorbance was measured at 295 nm and 25 °C (Hewlett Packard, 8453). Blank samples were determined in the same way without addition of enzyme. Enzyme activity was expressed as micromoles of galacturonic acid reducing equivalent per gram of fresh tissue per minute. D-galacturonic acid (≥98% purity, Sigma, Spain) was used as a standard.

2.6. Respiration rate

Three samples of 150 g each of melon pieces taken at random from each treatment were placed into 1 L glass jars and connected

to a gas flow panel with an air flow of 0.1–0.2 L h^{-1}, humidified to 95% RH. The jars were closed for 2 h, then the increase in CO_2 inside the jars was measured by taking a 0.5 mL gas sample from the headspace. This sample was injected into a gas chromatograph (Thermo Finningan Trace, ThermoQuest, Milan, Italy) equipped with a thermal conductivity detector at 200 °C. Oven and injector temperature were 50 and 100 °C respectively. Helium was used as a carrier gas (flux: 30 mL min^{-1}) and Chromosorb 102 column (2 m × 1/8″SS Supelco, Inc., Bellefonte, Penn, EE UU). The measurements were done every 2 days during 10 days at 5 °C. In between the measurements, and in order to avoid an excess of CO_2 accumulation (>0.3 kPa), the jars were flushed with humidified air flow.

2.7. Microbial growth

From each replicate, three random samples of 30 g of fresh-cut melon were collected from trays and homogenized for 2 min in 270 mL of sterile peptone buffered water (Scharlau, Barcelona, Spain) in a sterile stomacher bag with a Colorworth Stomacher 400 (Steward Laboratory, London, UK). Serial dilutions were prepared in the same peptone solution. Mesophilic, psychrotrophic aerobic bacteria, *Enterobacteriaceae* and yeast were quantified on days 0, 3, 7 and 10. Plate count agar was used for enumeration of mesophilic and psychrotrophic aerobic bacteria, incubated for 48 h at 30 °C or 7 days at 7 °C, respectively. Violet-red bile dextrose agar, overlaid with the same medium and incubated at 37 °C for 24 h was used for *Enterobacteriaceae*. Potato dextrose agar with oxytetracycline (0.1 g L^{-1}) was used for yeast and moulds, incubated at 22 °C for 3 and 7 days, respectively. Microbial counts were expressed as $\log_{10}$ cfu g^{-1}. Microbial quality was evaluated following the Spanish microbial legislation for minimally fresh processed vegetables (R.D. 3484/2000, 2001). According to this, the maximum microbial loads tolerated are 7 log cfu g^{-1} for aerobic bacteria, 5 log cfu g^{-1} for yeast, and 3 log cfu g^{-1} for moulds.

2.8. Sensory evaluation

A panel of five people carried out the sensory evaluations in a room at 15 °C. The members of the panel (3 men and 2 women; aged 25–60) were trained to recognize and score the quality attributes of processed melon using fresh and stored samples. Appearance was assessed using a 9-point scale to record visual appearance where, 1 = inedible, 3 = poor, 5 = fair, 7 = good, and 9 = excellent. Taste and texture were scored on a similar scale, where 1 = completely lacking or soft, 5 = moderate, and 9 = full characteristic or fresh, respectively. In both scales, the limit of marketability was 5. The term acceptability refers to the overall appreciation of a sample measured on the same scale.

2.9. Statistical analysis

The experiment followed a completely randomized design of three replicates per treatment ($n = 3$). The mean standard error was calculated using Statgraphic Plus version 2.1 (Manugistic, Inc., Rockville, MD., U.S.A.) and analysis of variance (ANOVA) and least significant difference test ($P \leq 0.05$) used to compare means within each sampling date.

3. Results and discussion

3.1. Flesh firmness

Results showed that regardless of Ca treatment, melon firmness decreased throughout the storage period (Fig. 1). Nevertheless, some treatments were more effective than others in reducing the loss of flesh firmness. The greatest differences were found at 7 days at 5 °C. At the end of the storage period, the control, and Ca citrate and silicate showed the greater firmness loss, with 31, 23 and 24%, respectively (expressed as percentage of initial firmness values). No significant differences were found among the remaining treatments, with values between 17 and 20%. The differences between Ca treatments and, in particular, between these treatments and the control, show the synergistic effects of combining a heat treatment with Ca salts.

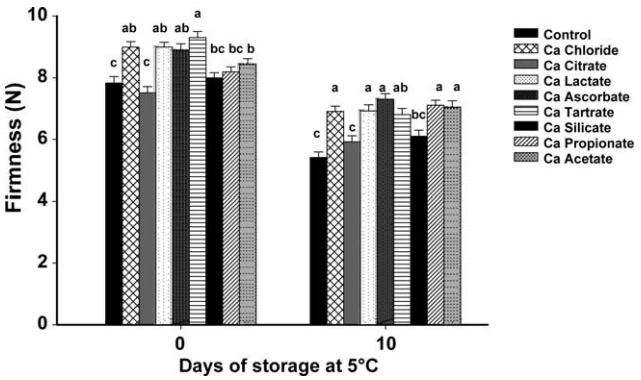

Fig. 1. Flesh firmness of fresh cut 'Galia' melon stored under modified atmosphere packaging at 5 °C during 10 days. Vertical bars indicate the standard error of the means ($n = 3$).

The beneficial effect of Ca treatments on firmness combined with high temperature on fresh-cut products have been reported by others researchers. Luna-Guzmán et al. (1999) treated melon cylinders using 2.5% $CaCl_2$ and Ca lactate at 60°C, and Rico et al. (2007) treated shredded carrot with 1% $CaCl_2$ at 50°C, obtaining between 6 and 16% more firmness than the water control. Saftner et al. (2003) using 40 mM Ca propionate, $CaCl_2$ and chelate on 'Honeydew' pieces increased tissue Ca content by more than double, inhibiting firmness loss. However, Ca sources have different behaviors and different beneficial effects on firmness maintenance. A better response with Ca lactate (2.5%, w/w) compared with $CaCl_2$ (2.5%, w/w) was found by Luna-Guzmán and Barret (2000). In 'Amarillo' melon, despite using equal Ca concentrations (0.18 g, 100 mL L^{-1}) supplied by different Ca salts (lactate, carbonate and propionate), the Ca tissue contents and their effect on retarding softening delays was different (Aguayo et al., 2008). Propionate showed the least firmness loss of 1.3%, followed by chlorine and lactate with 3.2 and 7.7%, respectively. These authors suggested that several factors such as solubility, the diffusive capacity of the tissues and the ability to form bridges with cell wall pectates vary depending on the source used and must be taken into account in their choice. These same reasons could explain why, in this experiments, citrate, silicate and acetate treatments resulted in significantly lower firmness compared to the other salts evaluated.

3.2. Calcium content

Initially, fresh-cut melon dipped in water (control) followed by Ca citrate, tartrate, silicate and acetate treatments resulted in the lowest free Ca content, with the maximum values corresponding to $CaCl_2$ dipped melon. At the end of the experiment, control melon pieces continued showing the lowest free Ca values while the highest content was found in melon tissue dipped in $CaCl_2$, lactate and ascorbate Ca salts (Fig. 2).

Tissue bound Ca level increased in melon pieces dipped in different Ca salts. Initially, slight differences among treatments were found with melon dipped in Ca acetate and ascorbate showing the highest levels (Fig. 3). After 10 days of storage, the highest content corresponded to Ca ascorbate followed by Ca chloride, Ca propi-

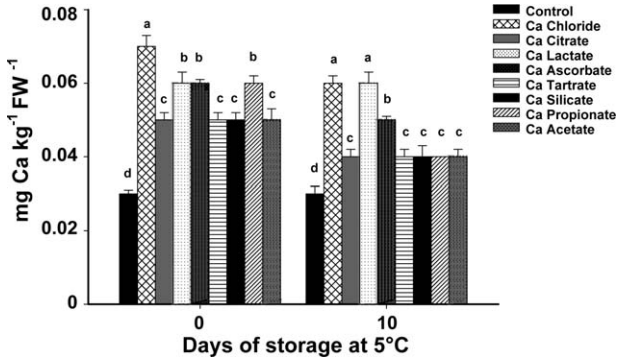

Fig. 2. Free calcium (mg Ca kg^{-1}) of fresh cut 'Galia' melon stored under modified atmosphere packaging at 5 °C during 10 days. Vertical bars indicate the standard error of the means ($n = 3$).

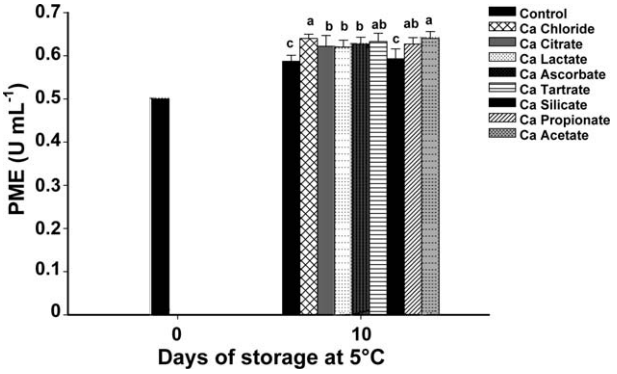

Fig. 4. Pectin methylesterase (U mL^{-1}) activity of fresh cut 'Galia' melon stored under modified atmosphere packaging at 5 °C during 10 days. Vertical bars indicate the standard error of the means ($n = 3$).

onate and Ca acetate. Bound Ca values had a good correlation with firmness retention, while after 10 days of storage, melon pieces dipped in Ca ascorbate, propionate and acetate were firmer than the control and Ca citrate.

According to these results, Ca dipping increased free Ca, that represented between 14 and 20% of total Ca content, and bound Ca that representing between 80 and 86%. The results confirming previous findings of Siddiqui and Bangerth (1996) who reported that $CaCl_2$ infiltration increased both free and bound Ca levels in apple tissues. Also Aguayo et al. (2008) reported an increase in Ca levels, mainly of bound Ca, in 'Amarillo' melon pieces treated with $CaCl_2$, propionate, lactate and carbonate for 1 min combined with heat treatment (60 °C). In this sense, the findings also support the hypothesis that an increase in Ca levels had a direct influence on softening reduction as observed on ascorbate, propionate and acetate compared to the control, Ca citrate and silicate.

The differential effect of Ca salts on flesh firmness maintenance might be due to the differences in solubility and diffusion capacity of Ca salts earlier reported by Aguayo et al. (2008). In addition, high temperatures (50–60 °C) combined with Ca dipping have a positive effect on tissue Ca fixation that determines the reduction in softening. The beneficial effects reached with high temperatures have generally been explained in terms of pectin methylesterase (PME) activation allowing Ca incorporation. Another related effect of higher temperature is the increase of Ca diffusion, especially throughout apoplast pores, which also increases the solubility of Ca salts in vegetable tissue (Harker et al., 1989).

3.3. Pectinmethylesterase activity

PME activity increased significantly during storage for all treatments (Fig. 4). After 4 days, Ca ascorbate and $CaCl_2$ showed an increase of 32 and 31% of the initial PME activity, respectively. On day 7, Ca tartrate induced and increased PME activity reaching 49% of the initial value. After 10 days, the PME activity was lowest in control and Ca silicate treatments. PME is an enzyme known to participate in the vegetable softening processes, catalyzing the hydrolysis of methylester groups of cell wall pectin. This process is not always negative since in the presence of divalent cations such as Ca^{2+}, free carboxyl groups produced can form bridges causing cell wall fortification (Van-Buren, 1974). Temperature and Ca are necessary to delayed flesh softening, since at high temperatures, PME activation occurss, generating free pectic acids which contain newly available carboxyl groups. Endogenous and exogenous ion Ca^{2+} bind to these carboxyl groups resulting in cellular wall stabilization and maintenance of flesh firmness (Ni et al., 2005). In relation to this point, there are several opinions in the literature. One is that high temperature, Ca content and high PME activity are highly correlated (Ni et al., 2005; Manganaris et al., 2007). On the other hand, some researchers report that temperature is the main factor involved in firmness maintenance while Ca salts have a marginal effect (Martin-Diana et al., 2006). Beirão et al. (2008) reported that the firming effect found on kiwifruit slices dipped in $CaCl_2$ solution (1, 2 and 3%, w/v), combined with mild heating (25 min, 45 °C), is due to the activation of PME by high temperature while the presence of Ca reduces or inhibits enzyme activation.

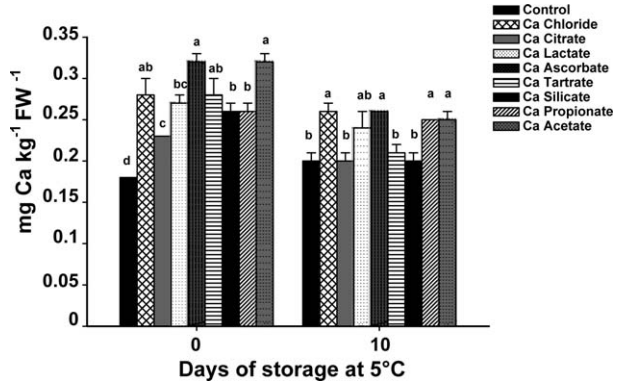

Fig. 3. Bound calcium (mg Ca kg^{-1}) of fresh cut 'Galia' melon stored under modified atmosphere packaging at 5 °C during 10 days. Vertical bars indicate the standard error of the means ($n = 3$).

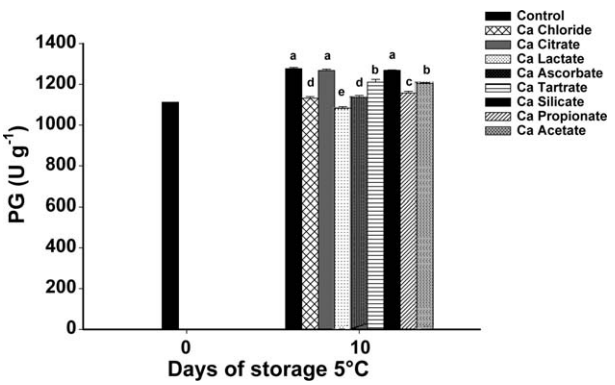

Fig. 5. Polygalacturonase activity (U g^{-1}) of fresh cut 'Galia' melon stored under modified atmosphere packaging at 5 °C during 10 days. Vertical bars indicate the standard error of the means ($n = 3$).

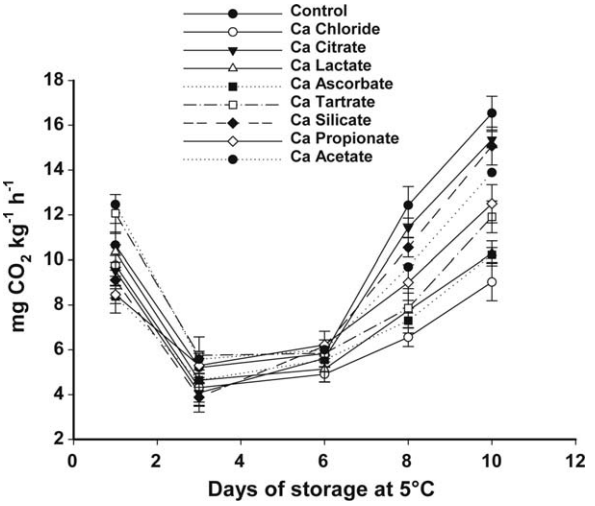

Fig. 6. Respiration rate of fresh cut 'Galia' melon stored under modified atmosphere packaging at 5 °C during 10 days. Vertical bars indicate the standard error of the means ($n = 3$).

The results of this work show that increased PME activity induced by high temperature, but along with lower rates of tissue softening was found in treatments where there was increased Ca incorporation, demonstrating that both factors are needed.

3.4. Polygalacturonase activity

The high initial PG activity observed could be explained by stress induced by the minimal processing operations (Fig. 5). After 4 days of storage, the PG activity was slightly reduced in all treatments, being higher on Ca ascorbate, propionate and chloride, with 21, 14.6 and 13.5% of the initial activity, respectively. At 7 and 10 days of storage, PG activity was highest in control, Ca silicate and citrate treatments.

It is likely the role of Ca probably lies in reducing the expression or activity of PG, as observed in tomato pericap discs (Mignani et al., 1995). On the other hand, Ca binding to the carboxyl groups of the pectic homogalacturonan backbone and more specifically in the middle lamella (Damarty et al., 1984), may protect the pectic backbone from PG-mediated depolymerization (Wehr et al., 2004), which would explain why treatments that retained firmness were those with highest Ca contents.

Temperature also plays an important role in reducing PG activity, as observed by Vicente et al. (2005) on heat-treated strawberries (45 °C, 3 h). The results of this work also show a synergistic effect of temperature and Ca on PG activity, at the beginning there was less activity in all treatments, which could be due to the heat treatment, and towards the end of shelf life, there was increased PG activity in treatments that showed greater softening and lower Ca contents and greater softening. Thus it is plausible to establish a relationship between enzyme activity and firmness given that firmer treatments showed also a higher initial PME and lower PG activity at the end of the storage period, as occurred in the control, Ca silicate and Ca acetate treatments. The effect of temperature on PG activity is reversible since the enzyme activity increased from the fourth day. This effect has been found previously in tomato fruit, where PG activity returned after a 6 days lag when fruit were exposed to 25 °C (Yoshida et al., 1984). A similar reversibility was found in apple fruit softening at 38 °C for 4 days (Lurie and Klein, 1990).

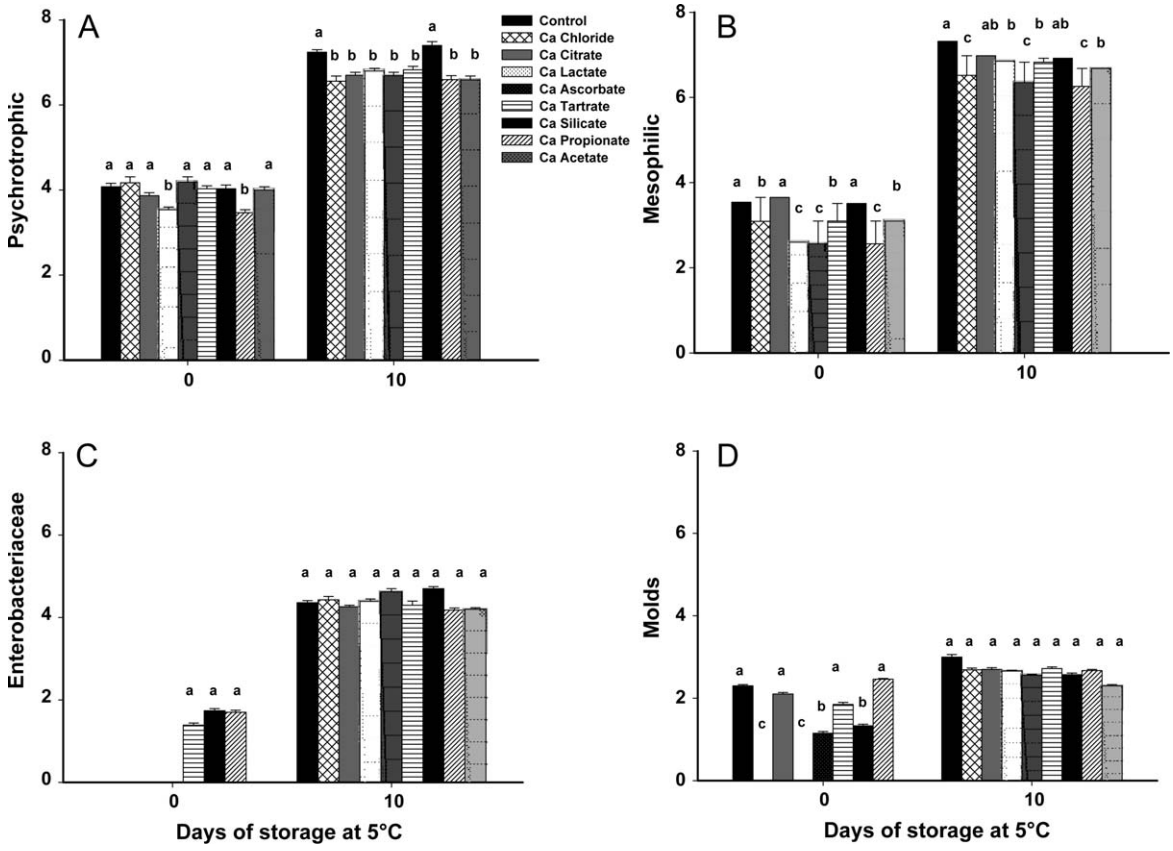

Fig. 7. Microbial growth ($\log_{10}$ cfu g^{-1}) from fresh-cut 'Galia' melon stored under modified atmosphere packaging at 5 °C during 10 days. (A) Psychrotrophic, (B) mesophilic, (C) Enterobacteriaceae and (D) moulds growth. Vertical bars represent standard error of the means ($n = 3$).

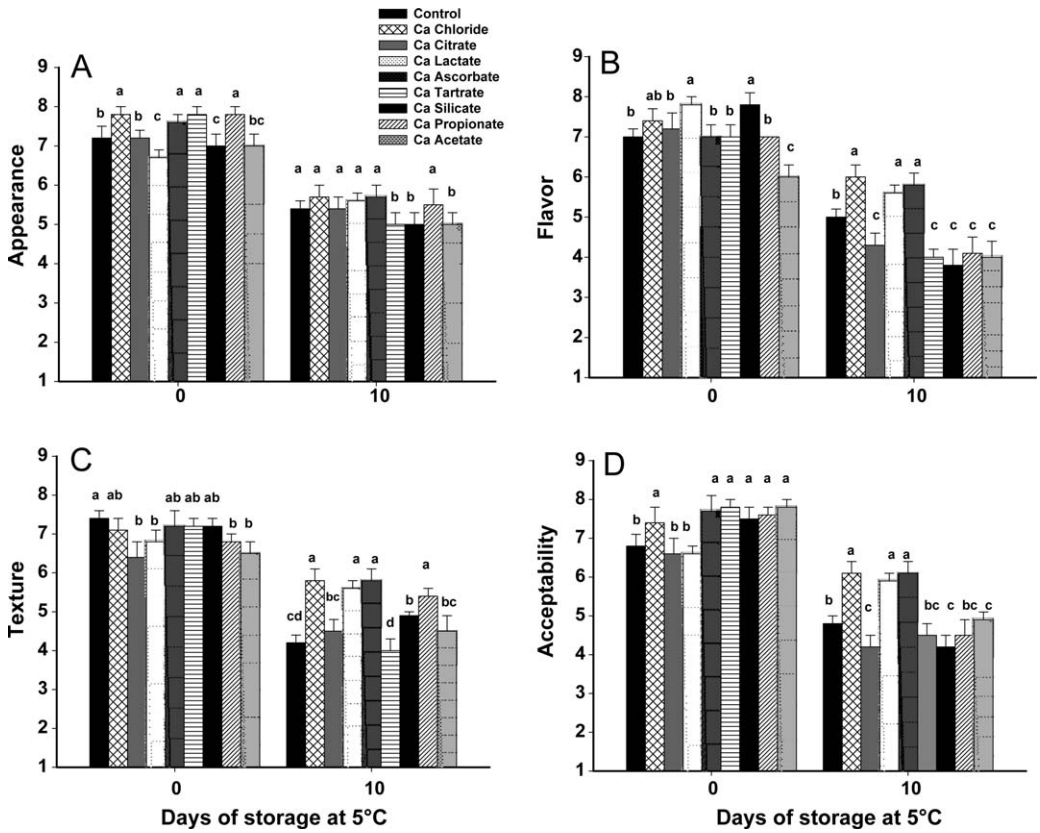

Fig. 8. Sensory evaluation (1–9) of fresh cut 'Galia' melon stored under modified atmosphere packaging at 5 °C during 10 days. (A) External appearance, (B) texture, (C) flavor and (D) acceptability. Vertical bars indicate the standard error of the means ($n = 7$).

PME has the function of deesterification of cell wall homogalacturans, which become PG substrates. Therefore, a higher initial PME activity and increased PG activity at the end of the storage, as found in this work, might be expected.

3.5. Respiration rate

A high initial respiratory activity in melon pieces, irrespective of treatment, was found on day 1 (Fig. 6). This effect confirms previous findings, attributed to wound response to cutting (Aguayo et al., 2004; Silveira et al., 2008). After that, respiration rate declined and stabilized until 6 days of storage without differences among treatments.

At the end of the shelf life, melon pieces treated with Ca propionate, tartrate, lactate, ascorbate or chlorine had lower respiration rates (9.02 and 12.50 mg CO_2 kg^{-1} h^{-1}). Similarly, Lamikanra and Watson (2004) and Luna-Guzmán and Barret (2000) found a reduction in respiratory rates of fresh-cut 'Cantaloupe' melon treated with $CaCl_2$ and lactate (2.5% at 25 and 60 °C for 1 min), an effect linked to ripening and senescence delay as a result of the combined additive effect of hot water dipping and Ca salts. This delay of senescence was discussed by Lester and Grusak (1999), who postulated that Ca applications can delay or slow down changes related to these processes having a direct effect in maintaining the functionality of the membranes. According to other authors, Ca increases membrane rigidity thereby blocking gas exchange (Saftner et al., 1998).

These explanations fit with the results obtained here; the treatments with lower respiration rates were also those which retained better flesh firmness and higher bound Ca concentrations, this also being linked to maintaining rigidity and stability of membranes. Moreover, the higher respiration rates found in the control, citrate, silicate and acetate treatments may be associated not only ripening and senescence, but also to a slight increase of microbial growth, as reported by Aguayo et al. (2004, 2008) and as discussed in the next section.

3.6. Microbial growth

With the exception of Ca silicate, the Ca salts induced a reduction in psychrotrophic load (Fig. 7A). At the beginning of the experiment, counts were about 4 log cfu g^{-1}, with no differences among treatments except for Ca propionate and lactate whose population was about 3.5 log cfu g^{-1}. At 7 and 10 days, the reductions were very low, reaching between 0.3 and 0.6 log units. At the end of the storage period, the control and silicate treatments resulted in loads exceeding the maximum legal limit (RD 3484/2000, 2001) at 7.24 and 7.4 log cfu g^{-1}, respectively. The remaining treatments did not differ statistically among themselves and resulted in counts between 6.56 and 6.83 log cfu g^{-1}.

Mesophilic counts of melon pieces dipped in Ca lactate, ascorbate and propionate showed the greatest reductions (1 log unit less than in the control), on day 0 (Fig. 7B), while the control, Ca citrate and silicate resulted in the highest counts (3.5 log cfu g^{-1}). After 10 days, $CaCl_2$, ascorbate and propionate were the most effective treatments in controlling microbial growth, showing a decrease of 0.8 to 1 log unit compared to the control. At this time, the highest counts was found in the control (7.32 log cfu g^{-1}) followed by citrate and silicate, as in the psychrotrophic counts, exceeding the legal limit of 7 log cfu g^{-1} (RD 3484/2000, 2001).

The initial *Enterobacteriaceae* growth in all treatments except Ca tartrate, propionate and silicate, was less than 1 log unit (Fig. 7C). After 10 days at 5 °C, Ca ascorbate and silicate showed a population of around 4.7 log cfu g^{-1}, although they were not statistically different from the other treatments, where counts range 4.18–4.62 log cfu g^{-1}.

A reduction of about 1 log unit in mold growth was found with $CaCl_2$, ascorbate and acetate treatments at the beginning of the experiment (Fig. 7D). At the end of the experiment the highest yeast growth were found in the control, Ca lactate and ascorbate treatments (3.47–3.62 log cfu g^{-1}), while the lowest values were found with $CaCl_2$ and citrate (data not shown). The remaining treatments did not differ statistically. In all the values were below the legal limits.

The inhibitory effect of some Ca salts on microbial growth has been related to cell wall stability by ionic Ca and polygalacturonate chains, since Ca increases the rigidity of the cell wall and middle lamella and therefore the resistance to fungal enzymes, decreasing softening and cell wall degradation (Damarty et al., 1984; Bolin and Huxsoll, 1989). Fresh-cut 'Honeydew' melon dipped for 30 s in different Ca salts (40 mM supplied as propionate, $CaCl_2$ and chelated with amino acids) reduced the microbial load by about 1 log unit (Saftner et al., 2003). These authors linked the antimicrobial capacity of propionate to the formation of undissociated propionic acid. In addition, Davidson and Juneja (1990) reported that the antimicrobial properties of Ca lactate and propionate depend on their ability to form acids in solution able to decouple microorganism transport through the membrane and oxidative phosphorylation which influencing the electron transport system. Our results confirm this hypothesis since lactate and propionate were the treatments that together with $CaCl_2$ induced the greatest bacterial reduction. Aguayo et al. (2008) also reported that $CaCl_2$ and lactate (0.18 g Ca 100 mL^{-1}) reduced microbial counts by 2 log units, while Ca propionate, at the same concentration, showed a greater reduction (4 log units).

3.7. Sensory evaluation

External appearance showed differences between Ca and control treatments on day 0 (Fig. 8A). The lowest scores corresponded to Ca citrate, lactate, silicate and acetate. The other treatments reached higher scores of about 6.8 ± 0.2. However, these initial differences were not maintained throughout shelf life with only, minor differences were found among treatments, at day 10 all of which were acceptable for consumption with scores of 5 or higher.

Flavor was the parameter most affected by Ca treatment, in particular, at the end of shelf life when greatest differences were found. At this time, citrate, tartrate, silicate, propionate and acetate were rated as unacceptable for consumption (Fig. 8B). These Ca salts did not impart an undesirable taste to the product but the low ratings given by the panel were due to a marked loss in favourable taste, maybe related to lower sugar and acid contents as a consequence of a higher respiration rate. In addition, at the end of shelf life, Ca propionate received a low rating (4.1 ± 0.3), attributed to a loss of flavor and not to an unusual taste, as Aguayo et al. (2008) reported. At the end of shelf life, $CaCl_2$ was one of the salts that allowed flavor maintenance receiving the highest scores. Bitter or salty taste was not detected, most likely due to lower $CaCl_2$ concentrations used. Saftner et al. (2003) reported that both propionate and amino acid chelated Ca (40 mM) did not affect melon taste, while $CaCl_2$ showed a slight salty taste but not enough to be rejected by the sensory panel.

Texture did not differ among treatments at the beginning of the experiment (Fig. 8C). However on day 10, control, Ca citrate, tartrate, silicate, and acetate treatments were scored as not acceptable for consumption. The lowest texture score was for Ca tartrate sample (4.2 ± 0.2). The results of texture evaluation by the sensory panel showed a good correlation with values monitored with the Lloyd instrument. The results of Aguayo et al. (2008) and Luna-Guzmán and Barret (2000), of maintaining fresh-cut melon firmness by the use of Ca salts, were confirmed by the sensory panel.

The values of the overall quality at the end of the shelf life showed that only control, Ca chloride, lactate and ascorbate resulted in values over the limit of acceptability for consumption (Fig. 8D). The other treatments were rated as not acceptable mainly due to the loss of flavor.

4. Conclusions

Ca ascorbate, chloride and lactate (0.15 g Ca g^{-1}) combined with water at 60 °C and H_2O_2 (50 mg L^{-1}) can be successfully used for overall quality retention of fresh-cut 'Galia' melon up to 10 days at 5 °C under a passive modified atmosphere reaching 4.5 kPa O_2 and 14.7 kPa CO_2. After 10 days of shelf life these three Ca salts provided 'Galia' melon pieces with a lower respiration rate, and a higher tissue total Ca content, of almost double than in the control treatment. This allowed for good maintenance of firmness with only a reduction of 17–20% compared to the initial value, while in the control was reduced 31%. These Ca salts also potentiated the antimicrobial effect of H_2O_2, allowing the melon pieces to maintain satisfactory microbial quality as well as the effect of hot water immersion allowing the maintenance of flesh firmness through an effect on PG and PME enzymes. In turn, these three salts did not affect the sensory quality of the product.

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