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Effectiveness of antibrowning agents applied by vacuum impregnation on minimally processed pear

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ABSTRACT

Different isotonic solutions containing antibrowning compounds (EDTA, 4-hexylresorcinol, citrate and ascorbate), combined or not with calcium lactate, were applied to minimally processed (MP) pear samples (cv. Blanquilla) by using the vacuum impregnation (VI) technique in order to reduce enzymatic browning. Vacuum impregnated samples were packaged and colour, mechanical properties, development of respiration gases and volatiles in the package headspace and microbial counts were monitored throughout storage at 4 °C. VI treatments with ascorbate solutions and calcium lactate were the most effective to extending the shelf life of MP pear. These treatments caused fewer changes in colour, mechanical properties and volatile composition and slowed microbial growth. Calcium lactate led to a better preservation in terms of mechanical parameters but had minor effects on colour development during cold storage.

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1. Introduction

In the last few years, a great increase in the consumption of minimally processed (MP) fruits and vegetables has been observed (Ragaert, Verbeke, Devlieghere, & Debevere, 2004). This market is limited by the short shelf life and rapid deterioration of these products. These MP products are usually washed, peeled, cut into small pieces, treated with preservative compounds and adequately packaged. The key to these treatments is to preserve all the quality attributes while maintaining the overall fresh-like appearance. Minimal processing operations damage the product integrity resulting in cellular decompartmentalisation of enzymes and substrates and leading to various forms of biochemical deterioration such as browning, off-flavours and texture breakdown (Varela, Salvador, & Fiszman, 2007).

One of the main factors related to quality loss in MP fruits is the development of enzymatic browning due to oxidative reactions of phenolic compounds by polyphenoloxidase (PPO). This undesirable colour change is especially remarkable in the processing of some cultivars of pears, such as Blanquilla (Siddiq, Cash, Sinkha, & Akhter, 1994) and so the use of different antibrowning agents is essential to preserve quality.

Citric and ascorbic acids have been widely used to control the enzymatic browning in minimally processed fruits and vegetables. EDTA compound has been used, with or without other antibrowning agents, to preserve colour, flavour and texture in different fruits such as melon (Lamikanra & Watson, 2001) and avocado (Soliva-Fortuny, Elez-Martinez, Sebastian-Caldero, & Martin-Belloso, 2002). Excellent results using 4-hexylresorcinol (4-HR), alone or combined with other compounds, as an antibrowning agents (Arias, Gonzalez, Peiro, Oria, & Lopez-Buesa, 2007; Oms-Oliu, Aguilo-Aguayo, & Martin-Belloso, 2006; Shah & Nath, 2008), antimicrobial (BIAM, 1992) and textural preservative (Buta & Abbott, 2000; Oms-Oliu et al., 2006) have been described in minimally processed apple and pear slices. Nevertheless, other studies showed a limited antibrowning effect of this compound which in some cases promote browning phenomena (Dong, Wrolstad, & Sugar, 2000; Rojas-Grau, Sobrino-Lopez, Soledad-Tapia, & Martin-Belloso, 2006).

The effectiveness of antibrowning agents can be enhanced by the combination with different compounds (Shah & Nath, 2008) and other factors such as the selection of processing conditions like temperature and pH. Although antibrowning solutions are usually acidic, an increase of their effectiveness when the pHs of solutions are nearly neutral has been described in minimally processed pear (Gorny, Hess-Pierce, Cifuentes, & Kader, 2002).

Another problem associated with the processing of MP vegetables is the loss of the integrity of the cellular structure, and consequently the reduction of the firmness of the plant tissue. The

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maintenance of this integrity depends partially on the bonds between calcium and pectic compounds of the middle lamella. It has been demonstrated that the addition of soluble calcium leads to firmer tissue by binding carboxyl groups of polygalacturonate chains and stabilising pectin–protein complexes, for example in pear and strawberry slices (Rosen & Kader, 1989) and in MP apple (Varela et al., 2007). Moreover, soluble calcium used as calcium lactate seems not to modify or change the taste and flavour of pears or other fruits (Swailam, Hammad, Serag, Mansour, & El-Nour, 2007). Additionally, calcium can play a remarkable role in the enzymatic browning reduction because the structural strengthening can reduce the amount of PPO and/or other reactive substrates released, such as has been reported by Rosen and Kader (1989). Luna-Guzman and Barrett (2000) also reported possible antimicrobial effects of calcium ions.

Different methods can be used for applying antibrowning compounds in processed fruits and vegetables. The use of vacuum impregnation (VI) technique has been shown to be highly effective for transferring the compounds from an external solution to the tissue (Chiralt, Fito, Andres, Barat, Martinez-Monzo, & Martínez-Navarrete, 1999). During the vacuum step, the expansion and outflow of occluded gas in the product pores take place. Afterwards, the atmospheric pressure is restored and the subsequent compression of the residual gas and the penetration of the external liquid occur. Several works have shown different advantages of incorporating preservative compounds in MP fruits by means of this VI method, thereby improving the final product quality (Fito & Chiralt, 2000; Fito, Chiralt, Barat, Spiess, & Behsnilian, 2001), as has also been described in recent studies carried out in MP litchi fruits (Shah & Nath, 2008).

In this study, the antibrowning effect of different compounds, combined or not with calcium lactate and applied by vacuum impregnation methods, was analysed in MP pear samples (cv. Blanquilla) throughout their cold storage (4 °C). Colour and mechanical parameters, development of gases and volatiles in the sample headspace and microbial counts were determined throughout storage.

2. Materials and methods

2.1. Raw materials

Pear (*Pirus comunis*, *cv.* Blanquilla), selected on the basis of a similar ripening degree and size, were purchased in a local market. The moisture and soluble solid contents of pears used in the study were 0.85 ± 0.02 g water/g of sample and 0.13 ± 0.05° Brix (g soluble solids/g liquid phase of sample), respectively, with pH and a_w values of 4.79 ± 0.09 and 0.986 ± 0.002, respectively.

Cylinders (2 cm height × 2 cm diameter) from the central section of the fruit piece were extracted using a sharp tubular cork borer. The impregnation solutions consisted of an isotonic sucrose solution (14°Brix) containing the different antibrowning agents: trisodium citrate 2-hydrate (citrate), sodium L-ascorbate (ascorbate), ethylenediamine tetraacetic acid 2-hydrate disodium salt (EDTA) and calcium lactate 5-hydrate (Ca), provided by Panreac (Barcelona, Spain) and 4-hexylresorcinol (4-HR) and sucrose provided by Sigma–Aldrich Química (Madrid, Spain) and Azucarera Ebro (Madrid, Spain), respectively.

2.2. Treatments and storage conditions

Pears were immersed in a 14 °Brix sucrose solutions (isotonics with the pear liquid phase) containing different antibrowning agents (ascorbate, 4-HR, EDTA, citrate), combined or not with 1 g of calcium lactate/100 g solution. Table 1 shows the concentrations of the antibrowning agents used and the physicochemical characterisation of these solutions. Previously to the VI treatment, impregnation solutions were sterilised at 121 °C for 15 min.

Vacuum impregnation was carried out in a vacuum laboratory equipment (Chafer, González-Martínez, Chiralt, & Fito, 2003) in cylindrical samples immersed in beakers containing the different sterilised solutions at 4 °C, using a solution:fruit ratio of 1:20. During the vacuum step, 5000 Pa of pressure for 5 min were applied and afterwards, the atmospheric pressure was restored for 10 min more. The impregnated samples were drained and liquid excess was removed from the surface with absorbent tissue.

Pear samples (55–60 g/container) were packaged at 4 °C for further measurements in polypropylene containers (6.5 cm height, 6 cm diameter) and heat-sealed in their perimeter with a sterile polyethylene film of 76 μm thickness (CO_2 and O_2 permeabilities were 6000–7000 and 2000–3000 $cm^3\ m^{-2}\ day^{-1}$, respectively) provided by Seward Medical Ltd. (West Sussex, UK). The film was chosen to prevent moisture losses and to obtain an equilibrium atmosphere in the headspace of the package throughout the studied storage time.

Fresh samples non-vacuum treated (control) were also analysed as reference samples.

2.3. Colour measurements

The optical properties of pear samples were measured in triplicate by a spectrocolorimeter CM-3600d (Minolta Co, Tokyo, Japan). Colour coordinates CIE L*a*b* were obtained by using D65 illuminant and 10° observer and the psychometric coordinates, chroma ($C_{ab}^* = (a^{*2} + b^{*2})^{1/2}$), hue angle ($h_{ab}^* = \tan^{-1} b^*/a^*$) and colour differences ($\Delta E = ((L_t^* - L_0^*)^2 + (b_t^* - b_0^*)^2 + (a_t^* - a_0^*)^2)^{0.5}$)

Table 1
Characterization of the impregnation solutions.

Treatment[a]	Antibrowning/calcium lactate concentration (g/100 g solution)	a_w (20 °C)	pH (20 °C)	° Brix (g soluble solids/100 g of liquid phase)
Citrate	3/0	0.986 ± 0.002	6.97 ± 0.19	17.6 ± 1.7
Ascorbate	2/0	0.989 ± 0.002	6.7 ± 0.4	17.2 ± 1.4
EDTA[b]	0.4/0	0.991 ± 0.002	4.6 ± 0.2	14.9 ± 0.2
4-HR[c]	0.02/0	0.992 ± 0.002	6.03 ± 0.38	14.5 ± 1.4
Isotonic	0/0	0.991 ± 0.002	5.9 ± 0.5	14.2 ± 1.1
Citrate-Ca	3/1	0.984 ± 0.001	6.7 ± 0.3	18.4 ± 1.7
Ascorbate-Ca	2/1	0.985 ± 0.001	6.4 ± 0.3	18.8 ± 1.6
EDTA-Ca	0.4/1	0.990 ± 0.002	3.92 ± 0.07	16.5 ± 1.4
4-HR-Ca	0.02/1	0.991 ± 0.002	6.5 ± 0.2	15.2 ± 0.3
Isotonic-Ca	0/1	0.989 ± 0.002	6.62 ± 0.13	16.5 ± 0.3

[a] All treatments contain a sucrose solution of 14°Brix.
[b] Ethylenediamine tetraacetic acid 2-hydrate disodium salt.
[c] 4-Hexylresorcinol.

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were calculated and compared to the newly treated sample (impregnated at time $t = 0$).

2.4. Measurement of mechanical properties

Mechanical properties were evaluated by means of a compression test (1 mm min^{-1} and 80% strain) using a Texture Analyser TA-XT-plus (Stable Micro Systems, Surrey, UK) and a flat plate of 75 mm diameter. Prior to testing, sample dimensions were measured with callipers and the Hencky strain and true stress were estimated from force-deformation data, by assuming constancy of sample volume during compression (Dobraszczyk & Vincent, 1999; Peleg, 1984). Measurements were carried out by quadruplicate.

2.5. Analysis of respiration gases and volatiles

Concentration of O_2, CO_2, ethanol and acetaldehyde in the headspace of the packages were determined every 5 days until the 25th day, using a gas micro-chromatograph (mGC) (Hewlett Packard, Model 200, Agilent, Barcelona, Spain) equipped with a thermal conductivity detector and a OPM-PU08 Pora Plot U column at 65 °C. Calibration was performed using a gas mixture of 21% O_2/4% CO_2/75% N_2, and 57 ppm acetaldehyde-100 ppm ethanol (Abello Linde, Barcelona, Spain). Measurements were carried out by triplicate.

2.6. Microbial analysis

Standard methods were used to enumerate microorganisms present in each sample (Mossel, Moreno, & Struijk, 2003). The following media and incubation conditions were used: total aerobic psycotrophic count was pour plated on Plate count agar (01-161, Scharlau, Barcelona, Spain) and incubated at 20 °C for 48–72 h; mesophilic aerobic count was incubated at 30 °C for 48 h. Violet red bile dextrose agar (01-295, Scharlau, Barcelona, Spain) pour plates for an Enterobacteriaceae count at 37 °C for 24–36 h; a spread-plate with potate dextrose agar (01-483 Scharlau, Barcelona, Spain) supplemented with 0.05 g L^{-1} chloramphenicol incubated at 30 °C for 48 h for yeast and 72 h for moulds. Microbial counts were expressed as 10 log cfu g^{-1}. Analyses were carried out by triplicate on refrigerated (4 °C) samples, aseptically packaged into sterile bags, at 0, 5, 10, 15, 20 and 25 storage days.

2.7. Sensory evaluation

Sensory evaluation was carried out in different sessions performed in three consecutive days by means of comparison tests, using a semi-trained panel of 16 judges. Coded samples impregnated with acetic and ascorbate solutions with and without calcium (at $t = 0$ storage time) were compared against a reference (impregnated sample with isotonic solution) in terms of the intensity of each of the following sensory parameters: aroma, flavour, sweetness, acidity, transparency and browning degree, firmness, juiciness and overall preference, by means of a multiple comparison test with a seven points scale.

2.8. Statistical analysis

Analyses of variance (ANOVA) was performed on the colour and mechanical parameters, gases and volatiles concentrations and microbial counts using storage time, type of antibrowning agent, presence of calcium and their interactions as variation factors, by using Statgraphics Plus (Statistical Graphics Corp. version 5.1) The least significant difference (LSD) test was employed to determine differences between means at a 5% significance level.

3. Results and discussion

3.1. Colour development

Impregnated samples (in comparison with the non-treated samples) underwent a decrease in luminosity and chroma values (less pure colour) with few changes in the hue, as can be observed in Fig. 1.

Colour attributes have been observed to behave in the same way by other authors when applying VI treatments (Chiralt & Talens, 2005; Talens, Martinez-Navarrete, Fito, & Chiralt, 2002). These changes are due to the air-solution substitution in the sample pores, thus leading to a homogenisation of the refractive index in the tissue with the subsequent increase in the absorption/reflection ratio of the incident light on the sample surface (Chiralt & Talens, 2005). This effect implies an increase in the transparency of the samples which has an impact on the colour perception. Nevertheless, the lack of changes in the hue supposes a non- negative impact in consumer perception, since this is the attribute that represents the most drastic colour changes.

The effect of the different factors (kind of antibrowning agent, calcium addition and storage time) on the L*, h_{ab}* and C_{ab}* parameters was analysed by means of a multifactor ANOVA. For the L* parameter, the antibrowning agent, the addition of calcium and its interaction were the statistically significant effects, thus indicating that the addition of calcium depended on the antibrowning compound used.

There were few observed changes in the L* values of treated samples (Fig. 1.a) throughout time (maximum of three units of variation). No significant differences were detected between the luminosity of samples treated with citrate-ascorbate and those treated with EDTA-isotonic solutions. Treatments with 4-HR solutions gave rise to the darker samples (lower luminosity).

In general, the addition of calcium (Fig. 1.b) significantly increased the L* values of samples, except for those treated with 4-HR that showed a decrease in this parameter. The structural effect of calcium enhances the cellular compartmentalisation and consequently makes the release of polyphenoloxidase or its substrates difficult thus improving the colour preservation of the samples. Several authors have observed similar effects in treatments with ascorbate-Ca (Dong et al., 2000; Gorny, Gil, & Kader, 1998; Gorny et al., 2002). On the other hand, the increase in L* values observed in most of the treatments could be due to the precipitation of calcium in the wall and middle lamellae, thus increasing the sample opacity.

The different behaviour observed for 4-HR–Ca treatment can be related with the change in the selective permeability of the cellular membranes when interacting with this compound, as has been pointed out by BIAM (1992).

Luminosity values slightly decrease throughout cold storage in every treatment: this effect was more marked in non-treated samples and in those carried out with EDTA and 4-HR (with and without calcium). This decrease could be due to the structural changes in the tissue which result in a development of browning phenomena. The slight increase observed in some samples throughout storage could be related with sample surface drying and/or calcium precipitation.

Chroma was the parameter that showed the fewest changes in the treated samples, oscillating in a very small range (5–8). Chroma of non-treated samples increased till reach a maximum value due to the development of brown colour in the initially white surface.

Colour changes in non-treated samples throughout storage were more marked due to the greater light reflection on their surface (more opaque samples) than in the VI samples, where the transparency increased and light absorption occurred to greater extent.

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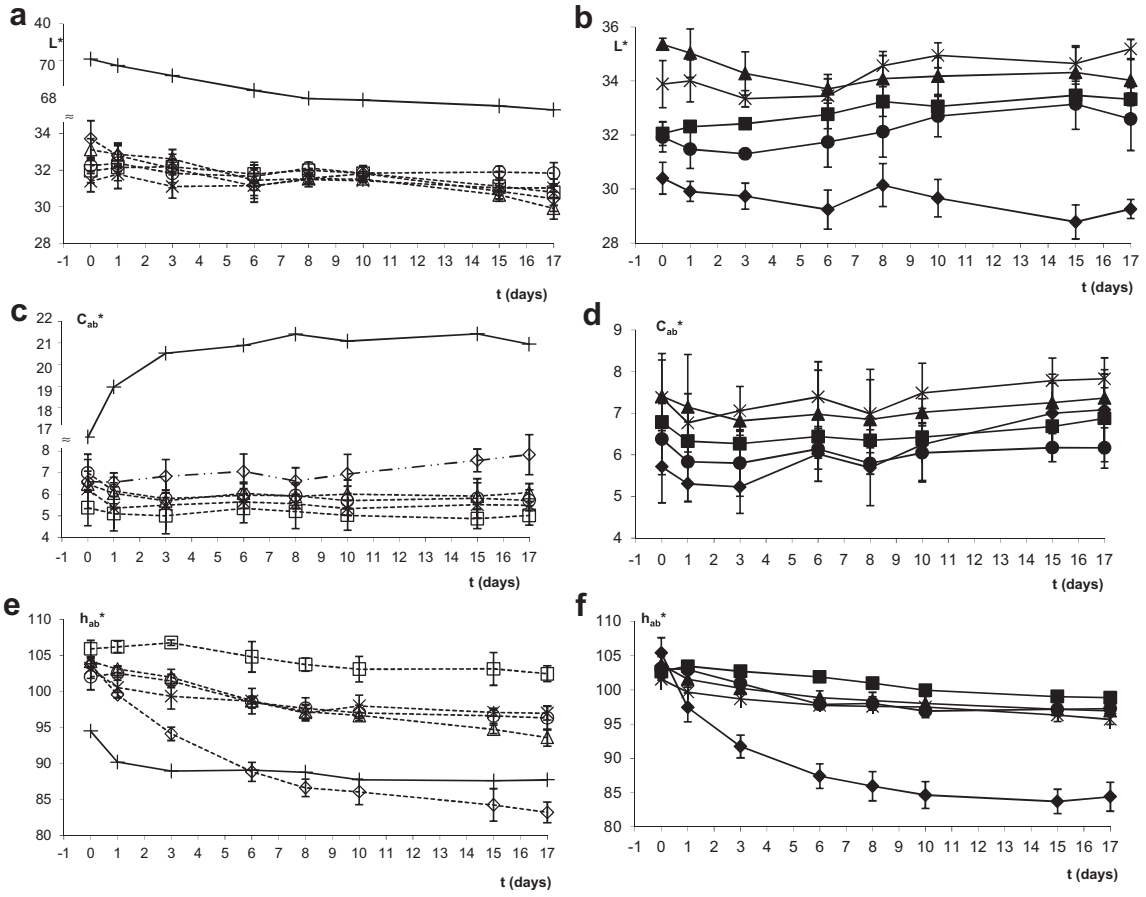

Fig. 1. Average of Luminosity (L*, $LSD^{(*)} = 0.30$), chroma (C_{ab}*, LSD = 0.19) and hue (h_{ab}*, LSD = 0.49) in pear samples with different antibrowning solutions during storage at 4 °C: without calcium (empty symbols) a, c and e) or combined with calcium lactate (full symbols) (b, d and f). (o citrate; ascorbate; x EDTA; ◆ 4–HR; Δ isotonic, + non-treated) (*) Least significant difference.

The addition of calcium, the antibrowning agent and its interaction were the most statistically significant factors. The presence of calcium had a different effect depending on the kind of treatment, as can be observed in Fig. 1.d.

Regarding the hue values, the antibrowning agent and the storage time were the most statistically significant factors. In general, a very similar decrease in h_{ab}* was detected in all the samples (treated and non-treated), except in the treatments with 4-HR (with and without calcium) where a great change in hue from green (h_{ab}* = 105) to yellow (h_{ab}* = 85) was observed (Fig. 1.e and f), thus indicating the development of enzymatic browning. It is remarkable to note that the most significant changes in hue occurred during the first week. On the other hand, samples treated with ascorbate underwent the fewest changes in hue. The addition of calcium did not have an influence on hue changes, except in treatments with ascorbate, where the samples' hue decreased significantly ($p < 0.05$) when calcium was added.

Storage caused samples treated with 4-HR solutions to behave in a particular way: there was a drastic loss in luminosity and an excessive decrease in hue. Similar results have been reported applying this agent, but not using VI method, in MP pear wedges (Oms-Oliu et al., 2006) and apple slices (Luo & Barbosa-Canovas, 1995, pp. 271; Rojas-Grau et al., 2006). This can be explained by the great enzymatic activity that occurred mainly in the vascular bundles (Luo and Barbosa-Canovas, 1995) combined with the particular action mechanism of 4–-HR compound (Arias et al., 2007).

3.2. Mechanical parameters

The effect of VI on the mechanical parameters can be deduced when comparing the values of non-treated samples with those obtained for samples impregnated with the isotonic solution (Table 2). VI caused a decrease in the sample stiffness (lower E_d) and resistance to fracture (lower σ_F). Similar results have been described by Martinez-Monzo, Martinez-Navarrete, Chiralt, and Fito (1998) for other impregnated fruits, which were related with the structural deformations of samples due to the pressure changes imposed in the system during the vacuum pulse (Fito, Andres, Chiralt, & Pardo, 1996). These deformations could imply cellular damage, especially in the cell bonding zones, thus affecting the mechanical behaviour (Fito, Chiralt, Barat, & Martínez-Monzo, 2000).

Statistical analysis showed that the kind of antibrowning agent, the storage time and the interaction of antibrowning agent and presence of calcium had a significant effect ($p < 0.05$) on the analysed mechanical parameters. All the treatments caused the same mechanical changes commented on above, which can be partially attributed to the effect of the VI, but also to the interactions of the added compound and the fruit tissue. In this sense, stress at failure point ($t = 0$) in treatments with ascorbate, EDTA and 4-HR without calcium decreased around 50% with respect to the isotonically impregnated samples.

The deformation values at point of failure ranged between 9 and 15% for all samples and no great differences were observed

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Table 2
Mechanical parameters (average and deviation values) in the non-treated (fresh) and treated pear samples, at the beginning ($t = 0$) and changes occurred after 17 storage days.

	σ_F[1] (kPa)		ϵ_{HF}[2]		E_d[3] (kPa)	
Treatment	$t = 0$	Δt_{17}-t_0	$t = 0$	Δt_{17}-t_0	$t = 0$	Δt_{17}-t_0
Citrate	104 ± 19^{ab}	−88	0.17 ± 0.02^{e}	−0.05	769 ± 170^{abc}	−328
Ascorbate	53 ± 19^{a}	−41	0.09 ± 0.02^{a}	−0.001	721 ± 93^{abc}	−543
EDTA	61 ± 23^{ab}	−47	0.12 ± 0.03^{abc}	0.01	576 ± 381^{a}	−447
4−HR	42 ± 10^{a}	−35	0.102 ± 0.016^{ab}	0.06	510 ± 87^{a}	−456
Isotonic	89 ± 21^{ab}	−83	0.158 ± 0.003^{de}	−0.05	871 ± 322^{abc}	−749
Citrate-Ca	72 ± 13^{ab}	−33	0.13 ± 0.03^{bcd}	−0.03	600 ± 165^{a}	−169
Ascorbate-Ca	66 ± 16^{ab}	−26	0.13 ± 0.02^{bcd}	0.01	674 ± 212^{ab}	−282
EDTA- Ca	94 ± 29^{ab}	−42	0.14 ± 0.03^{cde}	0.09	794 ± 404^{abc}	−634
4-HR- Ca	51 ± 8^{a}	−36	0.113 ± 0.020^{abc}	0.04	621 ± 210^{a}	−490
Isotonic-Ca	111 ± 25^{bc}	−20	0.13 ± 0.03^{bcde}	0.02	1016 ± 283^{bcd}	−272
Non-treated sample	129 ± 22	−99	0.16 ± 0.02	−0.06	1122 ± 253	−493

a, b, c,…: Different superscripts within a column indicate significant differences ($p < 0.05$) among samples.
1 stress at the failure point.
2 Henky strain at the failure point.
3 deformability modulus.

between treatments. In general, the samples broke at longer deformation values after 17 days of storage.

The addition of calcium maintained or increased the resistance to break (σ_F) and the stiffness (E_d) of samples. This coincides with many previous research papers, which have reported that calcium firms the tissue by strengthening the cell wall and middle lamella (Lee, Park, Lee, & Choi, 2003; Varela et al., 2007).

After 17 days of storage time, load mechanical parameters (stress and modulus) decreased, as expected from the tissue degradation promoted by the action of enzymes and senescence processes. This degradation was particularly intense in samples non-treated with calcium (greater differences with regard $t = 0$ were found). So, the calcium ion seems to improve the stability of the mechanical response throughout storage and the best results were obtained in both isotonic-Ca and ascorbate-Ca treatments, where the lower changes were observed.

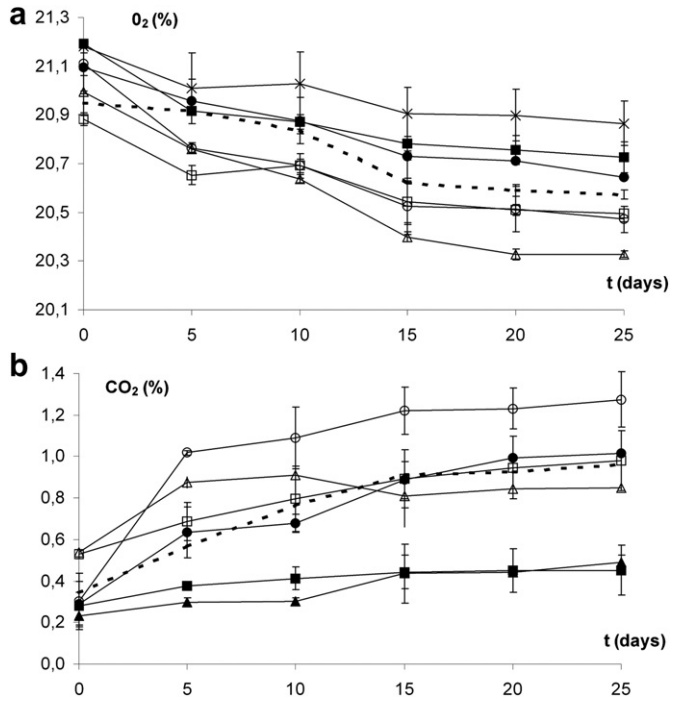

Fig. 2. O_2 (a) and CO_2 (b) concentration in the headspace of packaged pear samples during cold storage at 4 °C. (non-treated: dashed line; empty symbols: without calcium lactate; full symbols: with calcium lactate: o citrate; ascorbate; Δ isotonic).

3.3. Development of headspace gases and volatiles

Considering the preceding results, 4-HR and EDTA compounds were discarded as effective antibrowning agents in pear. Therefore, the rest of the treatments (citrate, ascorbate both with and without calcium) were submitted to the study of development of headspace gases and microbial stability and compared to control treatments (treated with isotonic solutions).

The statistical analysis (ANOVA) showed that all factors (kind of antibrowning agent, storage time and presence of calcium) and their interactions were statistically significant ($p < 0.05$).

Fig. 2 shows changes of O_2 and CO_2 concentration in the headspace of the package in non-treated (dashed lines) and treated samples during cold storage. The tendency to reach the dynamic equilibrium, which is associated with the balance of gas fluxes of

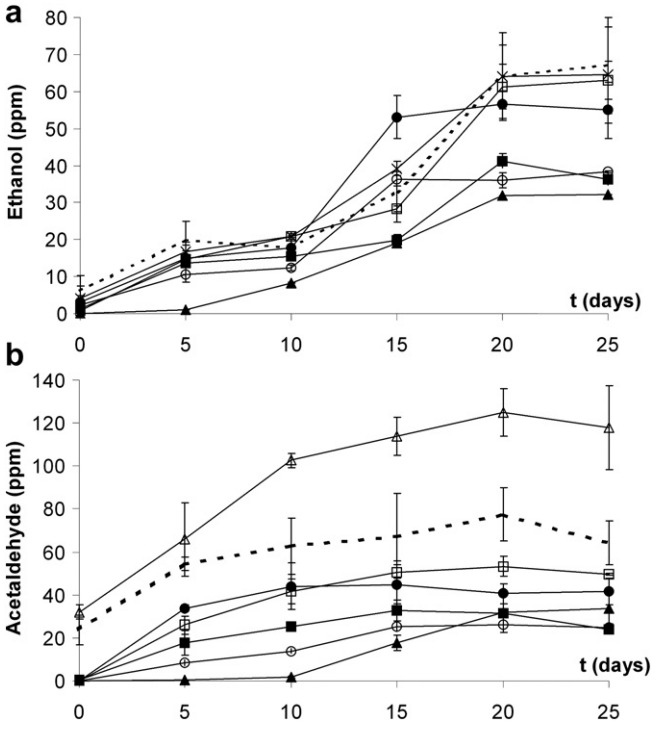

Fig. 3. Ethanol (a) and acetaldehyde (b) changes in the headspace of packaged pear samples during cold storage at 4 °C. (non-treated: dashed line; empty symbols: without calcium lactate; full symbols: with calcium lactate: o citrate; ascorbate; Δ isotonic).

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generation and permeation through the plastic bag, can be observed. The equilibrium time could be established at around 15 days of storage, at which point the CO_2 concentrations were under 1.5%, while those of O_2 were over 20% in every sample. These concentrations agree with the high permeability of the heat-sealed polyethylene in the package to both O_2 and CO_2 and with the slowing down of the respiration process at low temperatures (4 °C).

The most remarkable effect was observed in calcium treated samples. In the equilibrium state of these samples, the rate of oxygen consumption was reduced, if compared to non-treated samples, as was the rate of CO_2 generation. This effect has been observed to differing degrees in other fruits treated with calcium solutions (Torres, Castello, Escriche, & Chiralt, 2008) and has been related with the role of calcium in the physiological pattern of the fruits (Artes, Conesa, Hernandez, & Gil, 1999; Luna-Guzman, Cantwell, & Barrett, 1999; Saftner, Bai, Abbtt, & Lee, 2003).

In Fig. 3, the development of ethanol and acetaldehyde concentrations in the package headspace throughout time has been plotted. As can be observed, the concentration of these volatiles increased until reaching an equilibrium value. These volatile compounds can be generated due to the beginning of fermentative processes (Baldwin, Nisperos-Carriedo, Shaw, & Burns, 1995) and also, to the microbiological contamination and the fruit senescence (Kays, 1991). Nevertheless, the levels of volatile compounds reached were lower than those reported by other authors for similar products (Raybaudi-Massilia, Mosqueda-Melgar, Sobrino-Lopez, Soliva-Fortuny, & Martin-Belloso, 2007; Soliva-Fortuny, Ricart-Coll, Elez-Martinez, & Martıin-Belloso, 2007).

When using the antibrowning compounds, the composition of the headspace changes less when compared to the non-treated sample and the isotonic treatment, leading to less developed of undesirable volatile compounds. When studying VI carried out with isotonic solutions Castello, Fito, and Chiralt (2006) also found an increase in the respiratory quotient and therefore in the development of fermentative routes of cut strawberries, as compared with fresh-cut samples. This was attributed to the difficulties oxygen had to diffuse in the sample intercellular space occupied by the impregnated solution.

The presence of calcium contributed significantly to decreasing the concentration of ethanol and acetaldehyde, except for the samples treated with citrate solution, which can be attributed to the chelating effect of citrate on this ion. The calcium effect of

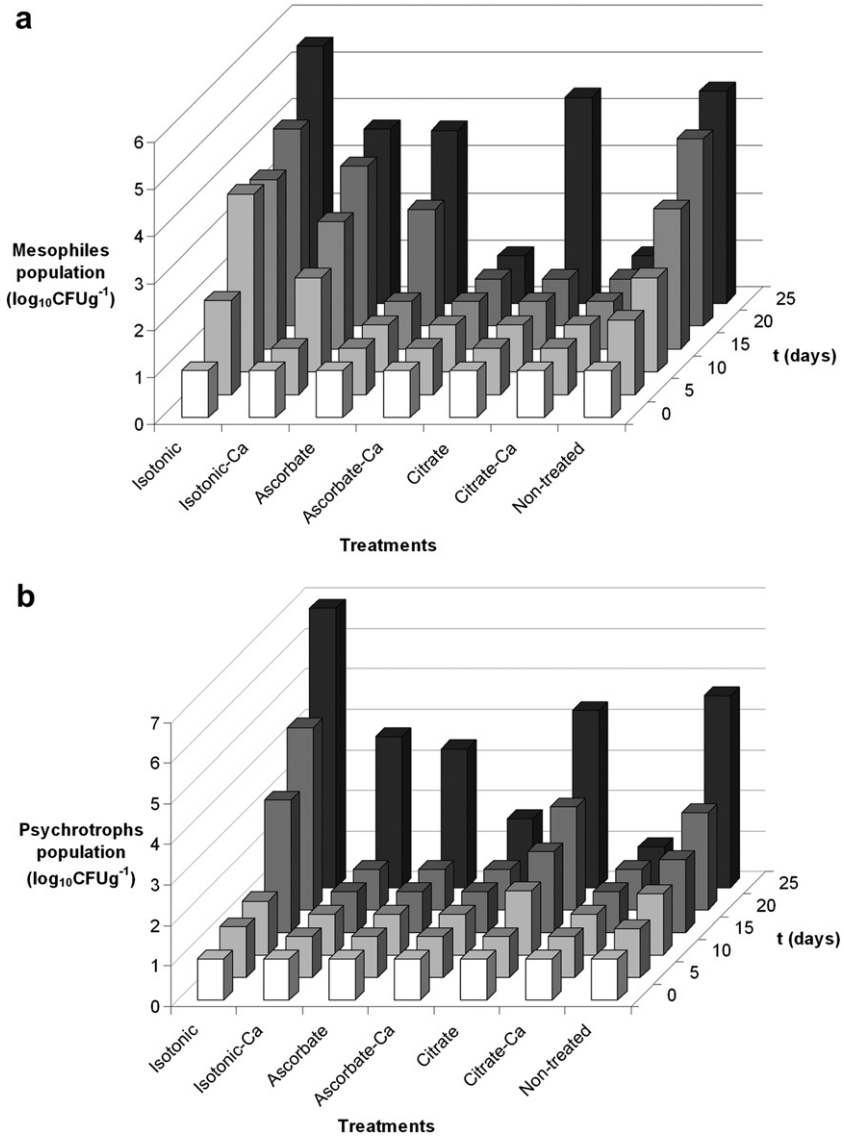

Fig. 4. Mesophilic ($LSD^{(*)} = 0.14$) (a) and psychotrophic (LSD = 0.109) (b) aerobic counts in packaged pear samples during cold storage at 4 °C. (*) Least significant difference.

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reducing the respiration quotient has also been observed in other fruits like mango (Torres et al., 2008).

A significant negative correlation (99% significance level) between O_2 consumption and acetaldehyde and ethanol production was found for every treatment. This agrees with the results reported by Agar, Massantini, Hess-Pierce, and Kader (1999) for MP kiwifruit. These authors concluded that the O_2 concentration during storage is a key factor in the accumulation of fermentative products.

3.4. Microbiological analysis

Fig. 4 shows the average value of populations of mesophilic and psychrotrophic aerobic microorganisms in MP pears during storage at 4 °C. The ANOVA results showed to be significant the factors of treatment, storage time and the interaction. These microorganism counts were under the legally required levels for minimally processed vegetables for 25 storage days (around 10^7 cfu g^{-1}). These values can reach levels of up to 10^7 cfu g^{-1} when the postharvest conditions and those during the processing of the fruits are inadequate (Nguyen & Carlin, 1994). Similar results have been reported for minimally processed melon, kiwi, papaya and pineapple (O'Connor-Shaw, Roberts, Ford, & Nottingham, 1994) and vacuum impregnated melon (Trujillo, Lopez, Tavera, Tapia, & Cava, 2001) for 11 and 16 storage days, respectively. On the other hand, no concentrations higher than 10^1 cfu g^{-1} were detected in mould and yeast counts during storage (data not shown).

Samples impregnated with isotonic solution and non-treated samples showed the highest ($p < 0.05$) level of mesophilic, and psychrotrophic microbial growth throughout time. On the contrary, samples impregnated with antibrowning solutions showed very low growth and great stability during the first 15 days of storage. The antimicrobial activity of ascorbic acid and citrate compounds reported by some authors (Brul & Coote, 1999; Helander, Wright-von, & Mattila-Sandholm, 1997; Shah & Nath, 2008) could explain the observed results.

Adding calcium slowed down bacterial growth significantly ($p < 0.05$). This could be attributed not only to an increase in the resistance of the cellular tissue to bacterial infection when calcium is present, but also to the antimicrobial effect attributed to the lactate ion, dependent on the pH (Saftner, Bai, Abbott, & Lee, 2003; Shelef, 1994).

3.5. Sensory evaluation

Results of the sensory evaluation were analysed through a multifactor analysis of variance taking into account the effect of treatment, panellist and test session, and the interaction between these factors. For all the sensory parameters evaluated, no significant effect ($p > 0.05$) of panellist and test session was found. Panellist did not detect significant differences ($p > 0.05$) in the juiciness and firmness of different the samples. Nevertheless, samples impregnated with ascorbate (both with and without calcium) were considered to be more transparent and sweet, less brownish and acid than those treated with citrate or isotonic solution. Aroma and flavour of samples impregnated with the solutions containing the different antibrowning agents were considered less intense than those impregnated with the isotonic solution (reference). The presence or absence of calcium lactate in the impregnated samples did not modify significantly ($p < 0.05$) the aroma or flavour of samples, which corroborates that this salt does not modify or change taste and flavour of fruits. Luna-Guzman and Barrett (2000) found similar results during the sensory evaluation of minimally processed cantaloupe melon treated with calcium lactate. Regarding the overall preference of samples, those impregnated with ascorbic solution (both with and without calcium) were preferred by the panellists.

4. Conclusion

The VI treatments which best maintain the quality of the pear throughout storage and prolong the product's shelf life in terms of the preservation of colour and mechanical properties of the samples were those carried out with ascorbate solutions containing calcium lactate. This treatment also led not only to a very small alteration of the samples in terms of ethanol and acetaldehyde generation but also to a low bacterial growth, so fermentation process occurred only due to fruit metabolism. The shelf life limiting time for storage of ascorbate-calcium treated samples was 20 days, on the basis of the microbial counts and mechanical response, since the other changes were not so relevant for product quality.

In general, calcium lactate was not observed to have a significant effect on colour development, but supposed a better preservation of the mechanical response of the samples during storage, thus probably limiting the fermentative routes and the microbial growth during storage. Treatments with 4-HR enhanced browning reactions when compared with the treatment carried out without an antibrowning agent, whereas treatments with citrate and EDTA were less effective at controlling browning. The sensory evaluation showed that the use of calcium lactate do not modify the aroma or flavour of samples and, those impregnated with both ascorbic solutions, with and without calcium lactate, were the most preferred by the panellist.

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