INFLUENCE OF CALCIUM LACTATE AND MODIFIED ATMOSPHERE ON
RESPIRATION RATE, OPTICAL AND MECHANICAL PROPERTIES OF SLICED
PERSIMMON

ABSTRACT

The aim of this study was to evaluate the effect of a modified atmosphere (5% and 10% of \( \text{CO}_2 \)) and calcium lactate treatment on the respiratory metabolism of minimally processed persimmon. A static system to measure changes in the composition of the headspace was used. Composition, texture and colour were also analysed. Persimmon slices were evaluated immediately after the washing treatment and after the \( \text{O}_2 \) composition had decreased to 17% to avoid changes in the metabolic pathway. All samples were stored at 4°C. The results showed that modified atmosphere did not affect compositional properties, although there was a slight increase in pH values at the end of each treatment. Calcium lactate treatment reduced the respiration rate, in terms of \( \text{O}_2 \) in samples kept in air. Additionally, a calcium lactate effect was immediately observed on mechanical properties after the washing stage. On the other hand, luminosity and \( b^* \) coordinate decreased in unwashed and calcium lactate samples kept in 5% \( \text{CO}_2 \).

Keywords: fresh-cut persimmon, modified atmosphere, calcium lactate, respiration rate, physicochemical properties

INTRODUCTION

The demand for minimally processed products has increased due to many factors such as the reduction in time required to prepare meals, the smaller size of families and the growing concern about fresh and healthy foods. Therefore the sector of minimally processed fruits and vegetables is at its peak.
Minimally processed products, which are also called ready-to-used, fresh-cut or IV gamme products, are fruits and vegetables that have been exposed to certain mechanical processes such as washing, peeling, coring, slicing and cutting before packaging (Izumi & Watada, 1994; Barry-Ryan & O’Beirne, 1998; Degl’Innocenti et al., 2007). However, these products are more perishable than intact fruits or vegetables because of physical stress (Watada et al., 1996). Texture and appearance are two important factors which have a great influence on consumers’ acceptability (Toivonen & Brummel, 2008). It is also essential to prevent the development of strange flavours and to guarantee microbiological stability.

There are numerous researchers that have studied different methods to extend and improve the shelf life of fresh-cut products (Montero-Calderón et al., 2008; Aguayo et al., 2008; Rojas–Graü et al., 2007; Marrero & Kader, 2006; Soliva-Fortuny et al., 2004; Luna–Guzmán & Barrett, 2000). In fact, modified atmosphere packaging (MAP) is one of the most widely used conservation techniques. MAP involves the modification of the composition of the atmosphere inside the package, which is achieved as a result of the interplay between the respiration of the product and the transfer of gases through the packaging material (Fonseca et al., 2002; Sandhya, 2010). This interaction leads to a decrease in the O$_2$ concentration and an increase in CO$_2$ inside the container (Fonseca et al., 2002). If the permeability of the packaging material is suitable for the respiration of the product, the product will have a longer shelf life (Sandhya, 2010). Nevertheless, the CO$_2$ concentration inside the package might not achieve the required level, meaning that it cannot be used as either a fungicide or bactericide (Serrano et al., 2008). In fact, Soliva-Fortuny et al. (2004) studied the microbial shelf life and certain biochemical changes in apple cubes under different MAP conditions, observing that microbial growth was partially inhibited, which led them to conclude that poor O$_2$ and/or high CO$_2$ atmospheres had neither bactericidal nor fungicidal effects. Therefore the combination of MAP and the application of some substances such as antioxidants, calcium salts,
antimicrobial agents etc. could also be useful for extending the shelf life of fresh-cut fruits or vegetables. In this regard, calcium salts have been used to maintain the mechanical properties of minimally processed products (Silveira et al., 2011; Alandes et al., 2009; Martín-Diana et al., 2006). Furthermore, the antimicrobial effect of calcium lactate has been evidenced by many researchers (Aguayo et al., 2008; Torres et al., 2008; Moraga et al., 2009). Thus, the use of this salt together with modified atmosphere packaging could maintain the shelf life of minimally processed products from a sensorial, physicochemical and microbial point of view.

Additionally, persimmon production has greatly increased in recent decades due to the use of techniques to remove astringency, which keeps the fruit’s texture firm. In fact, the production of persimmon in the Ribera del Xúquer area (Valencia, Spain) was multiplied by 140 from 1992 to 2002 (Llácer & Badenes, 2002). Moreover, according to GVA, 2013 the average of production between 2002 and 2011 was 49.312 tonnes, while 134.600 tonnes were produced in 2012, showing a continuous growing (GVA, 2013). Consequently, there is an important surplus of this product and different types of marketing of minimally processed product have been relied on to improve its distribution and consumption.

Therefore, the aim of this study was to evaluate the effect of both a CO$_2$-rich atmosphere and the application of lactate calcium on the composition, respiration rate, colour and texture properties of fresh-cut persimmon.

**MATERIALS AND METHODS**

**Raw materials**

For the purpose of carrying out the required experiments, fruits of persimmon (*Diospyros kaki*) of the variety “Rojo Brillante” were used. They were stored for 24 h at 4 °C before
being processed. Fruits were selected according to their ripening stage, colour and
general appearance in order to optimize homogeneity of the samples.

Treatment of persimmon samples

After selecting the persimmon they were washed in tap water with commercial sodium
hypochlorite (Amukina, Laboratories Angelini, Farma-Lepori, Barcelona, Spain) using
the recommended dose: 0.02 (v/v) for 1 min. The samples were then dried with
absorbent paper and the fruits were cut in slices which were approximately 1.5 cm thick.
All the samples were mixed to randomize the variability provided by the raw material.
The persimmon slices were dipped into a solution of calcium lactate pentahydrate (2%)
(Panreac, Barcelona, Spain) in a 3:1 (L/kg) water:fruit ratio for 5 min. The samples were
then drained for 5 min. Cut fruit that was not dipped and cut fruit dipped only in tap water
were used as controls.

Application of modified atmosphere

300 g of persimmon slices were packaged in glass jars (1.937 L). Prior to storage at 4°C, the headspace composition of the glass jars was modified by a gas mixer (WITT-KM-
ME 100-3 GB. WITT-GASETECHNICK, Witten Germany). The gas mixer was connected
to a valve at the top of the glass jars. The mixture of gas was circulated for 3 min, and
the valves were then closed, following which the composition of the headspace was
immediately measured. The atmosphere composition studied was 5% CO₂+20% O₂ and
10% CO₂+20% O₂ (balanced with N₂), and both of these were compared to the
composition of the air.

Analytical determinations
The persimmon slices were evaluated immediately after the washing treatment, and also when the O$_2$ composition decreased to 17% (48-72 h), in order to prevent changes in metabolic behaviour from a lower availability of oxygen.

Moisture content, soluble solids content pH, water activity and titratable acidity

Moisture content was determined by drying the fruit to constant weight at 60 °C in a vacuum oven at 10 kPa (adaptation of method 934.06 AOAC, 2000). Soluble solids were measured in previously homogenized samples using a refractometer (Zeiss, ATAGO model NAR-3T, Japan). Water activity (a$_w$) was measured with a hygrometer (Fast-lab, GBX, France). pH was obtained directly from the homogenized sample by means of a pH-meter (“Seven Easy” METTLER TOLEDO - United States) with a contact electrode. Titratable acidity was determined by potentiometric titration with 0.1 N NaOH (Panreac, Barcelona, Spain) of up to pH 8.1-8.2. Distilled water (40 mL) was added to the homogenized sample (9 g). Results were expressed as g of malic acid per 100 g of sample.

Determination of respiration rate

A closed system was chosen to measure the respiration rate. Persimmon slices (about 300 g) were placed in 1.937 L hermetic glass jars with a septum in the lid for sampling the gas in the headspace at different times. The jars were stored in a temperature controlled chamber (P Selecta, Hot-Cold M 4000668, Barcelona, Spain). Gas sampling was carried out every 30 or 60 min by means of a needle connected to a gas analyser (PBI Dansensor-CheckMate 9900 O$_2$/ CO$_2$, Ringsted, Denmark). Experimental points were considered in the time range where a linear relationship was observed between gas concentration and time. This means that no changes in the respiration pathway of
the samples occurred in this period, meaning that changes in the composition of the headspace did not lead to notable alterations in their metabolism.

The respiration rate \( (R_i, \text{mL} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}) \) of the samples in terms of \( \text{CO}_2 \) emission and \( \text{O}_2 \) consumption was determined from the slope of the fitted linear equation, according to Eq. (1), where \( y_i \) is the gas concentration (\%\( \text{O}_2 \), \%\( \text{CO}_2 \)) at time \( t \), \( i \) being \( \text{O}_2 \) or \( \text{CO}_2 \), \( m \) is the mass of the fresh samples and \( V \), the volume (mL) of headspace.

\[
y_{it} = y_{ito} + 100 \times R_i \times \frac{m}{V} \times t \tag{Eq. 1}
\]

**Analysis of optical parameters**

The colour of the persimmon samples was measured by means of a spectrocolorimeter Minolta (CM-3600 d, Tokyo, Japan) with a window of 7 mm in diameter. The colour was analysed immediately after the dipping stage and at the end of each experiment. CIE-\( L^*a^*b^* \) coordinates were obtained using D65 illuminant and \( 10^\circ \) observer as reference system. It was measured in triplicate and four areas of the persimmon sliced were taken for the purpose of determining their colour. Furthermore, the difference in colour (\( \Delta E \)) as compared to initial colour values was estimated using the following equation (2):

\[
\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \tag{Eq. 2}
\]

**Measurement of mechanical properties**

Mechanical properties were analysed using a texture analyser (TA.XT2 Texture Analyzer Aname, Stable Micro Systems, Haslemere, England) by means of a puncture test (5 mm diameter punch) to achieve a relative deformation of 95% at a speed of 1.5 mm/s. The parameters analysed were: maximum force (F, N) and the distance at which the
maximum force took place (d, mm). The analysis was performed immediately after the washing stage and at the end of each experiment. The parameters were measured in triplicate and four areas of the sliced persimmon sliced were taken to determine their mechanical properties.

**Statistical analysis**

An ANOVA analysis using Statgraphics Centurion Software was performed to evaluate the effect of process variables (percentage of CO\(_2\) in the composition of the headspace gas and dipping treatment) on the results obtained with a significant level of 95%.

**RESULTS AND DISCUSSION**

**Evolution of the compositional properties**

Table 1 shows water and soluble solid mass fraction, pH and titratable acidity for each treatment and % of CO\(_2\) applied, both after the dipping treatment (initial conditions) and when the O\(_2\) composition decreased to 17% (final conditions). A simple ANOVA was performed to analyse the differences in compositional properties immediately after the dipping treatment and at the end of the trial.

The soaking treatment led to an increase in water mass fraction as compared to unwashed samples due to the entrance of water into the matrix of the fruit, and also to a decrease in soluble solid mass fraction. In general, the initial values for these two compositional properties were maintained at the end of each trial. The values relating to water activity were also steady. The initial and final water activity averages were 0.984 (±0.006) and 0.982 (±0.005) respectively. These values were similar to others reported in previous studies (Castelló et al.; 2006; Igual et al., 2008). On the other hand, there was a slight increase in pH values in all samples that were exposed to CO\(_2\)-rich
atmospheres. This behaviour was also observed in samples treated with calcium lactate, which were also preserved in an air composition atmosphere. Wright & Kader (1997a) studied the effect of controlled atmosphere storage on fresh-cut persimmon and strawberry. Generally, pH values of both persimmons and strawberries stored under various atmospheres tended to increase. In addition, Holcroft & Kader (1999) investigated the effects of low O\(_2\) atmospheres alone, or in combination with 20 kPa CO\(_2\) on `Selva´ strawberries. They observed that both external and internal tissue pH increased over the 10-day storage period. However, Li & Kader (1989) did not observe any significant effect on pH in `Selva´ strawberries stored in a controlled atmosphere. In relation to titratable acidity, the control samples were slightly higher than control samples for non astringent persimmon (0.117±0.008 g of malic acid per 100 g of sample) obtained by Vázquez-Gutiérrez et al. (2012). Moreover, there was a decrease in titratable acidity of water treated samples which were stored in air. This might be due to the advance in the ripening process.

**Respiration rate**

Figure 1 shows respiration rates in terms of O\(_2\) consumption and CO\(_2\) production for each of the treatments performed and CO\(_2\) concentrations used. In stored air, calcium lactate treated samples had lower respiration rates than other samples. Albors et al. (2008) also observed that persimmon slices, which were washed in ascorbic acid and calcium lactate solution, had lower respiration rates than unwashed and water washed sliced persimmon on the 4\(^{th}\) day of storage. This decrease has also been noticed in other studies that have used this calcium salt. Thus, Luna-Guzmán & Barret (2000) studied the effect of calcium lactate (alone or in combination with heat) on the respiration (in terms of CO\(_2\)) of melon cylinders. As a result, CO\(_2\) production was not increased until day 6 for all samples. Nevertheless, calcium lactate, water dipped and heat treated melon showed lower CO\(_2\) levels than untreated samples. Silveira et al. (2011) also observed that calcium
propionate, tartrate, lactate, ascorbate and chloride treated melon had lower respiration rates than the rest of the salts studied at the end of their shelf-life. On the other hand, O\(_2\) consumption rate was reduced in the un-dipped samples in a CO\(_2\)-rich atmosphere. Li & Kader (1989) measured respiration rate in terms of O\(_2\) consumption in `Selva´ strawberries held in CO\(_2\)-rich atmospheres (air +10%, 15% or 20% CO\(_2\)). The respiration rate was lower in CO\(_2\)-rich atmospheres than when the fruit was kept in air. In this research, the use of high CO\(_2\) atmospheres led to an increase in oxygen consumption in washed samples, especially with calcium lactate treatment. The combination of calcium lactate and a high percentage of CO\(_2\) could reverse the retardation effect on the respiration rate in comparison to calcium lactate treatment in air. The CO\(_2\) production rate was significantly decreased in all treatments at high concentrations of CO\(_2\). This behaviour could be explained by the high susceptibility of cut persimmon fruit to rich-CO\(_2\) atmospheres which completely collapse the release of this gas in its metabolic pathways.

**Evolution of the optical properties**

Table 2 shows the initial colour analysis before storage. Neither a* nor b* coordinates showed significant differences. However, there was a small difference in luminosity (L*) depending on the treatment used before storing. Thus, these results showed that all optical properties were uniform. Furthermore, dipping the fruit in water or calcium lactate did not change its initial colour. The initial value for luminosity was similar to the results obtained by Albors et al. (2008). However a* and b* coordinates were slightly higher in this study than those reported by Albors et al. (2008).

Figure 2 shows graphic L*-a* and chromatic plane b*-a* as a function of the dipping treatment and CO\(_2\) concentration in the glass container at the end of the trial as a function of the treatment and the concentration of CO\(_2\) in the glass container both at the beginning and at end of the experiment. In the graphic L*-a*, luminosity decreased in unwashed
and calcium lactate samples stored in a 5% CO$_2$ atmosphere. Samples washed with tap water which were stored in 10% CO$_2$ also showed lower luminosity. Regarding the a* coordinate, un-dipped and water dipped samples stored in air, turned a reddish colour. This could be due to the fact these samples had ripened more. Furthermore, water washed samples had a lower titratable acidity, which reinforces this theory. However, calcium lactate samples did not show this behaviour under the same conditions. It is possible that calcium had had a slowing effect on the synthesis of carotenoids. On the other hand, CO$_2$-rich atmospheres kept a* coordinate values. High CO$_2$ atmospheres could have also had slowing effect on the synthesis of carotenoids. However, Wright & Kader (1997b) reported that atmospheres with 12% CO$_2$ and 2% O$_2$ kept better the values of retinol equivalent in comparison with slices of persimmon stored under 2% O$_2$ or air. In addition, 5% CO$_2$ atmospheres reduced the b* coordinate in undipped and calcium lactate samples. The decrease in b* coordinate values along with a lower luminosity in these samples could be related to the browning phenomena. It is possible that persimmon slices were subjected to some kind of stress under these conditions which could cause browning reactions. In fact, Kader & Ben-Yehoshua (2000) reported that the oxidation of phenolic compounds by polyphenol oxidase resulted from loss of compartmentalization within the cells when exposed to physical and/or physiological stresses. Nevertheless, a higher percentage of CO$_2$ could have counteracted the polyphenoloxidase action.

Colour differences at the end of each test are shown in figure 3. Un-dipped and calcium lactate persimmon slices stored under 5% CO$_2$ atmosphere showed the most significant changes in colour. The effect of calcium was also observed by Martin-Diana et al., (2005) who reported that high concentrations of calcium lactate in lettuce gave higher colour variation than samples treated at low or intermediates concentrations. The possible increase in the synthesis of carotenoids was not significantly reflected in samples packaged in an air composition.
Evolution of the mechanical properties

Figure 4 shows values of maximum force and distance of persimmon slices both at the beginning, and also at the end of the experiment before the O\textsubscript{2} composition in the headspace dropped to values lower than 17% O\textsubscript{2}. Calcium lactate treated samples showed the highest values of maximum strength immediately after the immersion stage. This increase was maintained throughout the study. The maintenance of the cell wall structure mainly depends on the calcium binding pectic components of the middle lamella (Grant et al., 1973; Poovaiah, 1986; Quiles et al., 2004). Calcium salts make it possible to maintain the mechanical properties of minimally processed products (Martin-Diana et al., 2006; Rico et al., 2007). On the other hand, the firmness of un-dipped samples did not significantly vary under any conditions studied. Samples stored in 10% CO\textsubscript{2} presented great differences most likely due to the variability of the samples. In fact, there were no differences between the initial and final values for each treatment. In relation to persimmon slices stored in 5% CO\textsubscript{2}, washed samples showed a significant increase (\(\alpha <0.01\)) in maximum force at the end of storage. An improvement in the fruits’ firmness has been observed in many research studies analysing CO\textsubscript{2}-rich atmospheres (Li & Kader, 1989; Harker et al., 2000). In contrast, Wright & Kader (1997ab) observed that in the case of sliced persimmon which was stored in a different controlled-atmosphere, their firmness tended to decrease for all the treatments studied. In this study, higher firmness was shown in calcium lactate samples stored in 10% CO\textsubscript{2}. There were no differences in the distance at which maximum force took place.

CONCLUSIONS

Calcium lactate reduced the respiration rate, in terms of O\textsubscript{2} in samples stored in air. However, a high concentration of CO\textsubscript{2} in the headspace atmosphere could have counteracted the calcium lactate slowing effect on respiratory metabolism. Additionally,
the calcium lactate treatment slowed down the reddish appearance of samples and improved the firmness of persimmon slices.

REFERENCES


soluble compounds and textural properties of persimmon “Rojo Brillante”. Food Research International, 47(2), 218-222.


**Figure caption**

**Figure 1.** Respiration rate, in terms of O$_2$ consumption and CO$_2$ emission, for each treatment applied and under different % CO$_2$ in the composition of the headspace.

**Figure 2.** Graphic of L$^*$-a$^*$ and chromatic plane representation (b$^*$-a$^*$) of sliced persimmon for each treatment applied and under different % CO$_2$ in the composition of the headspace. Dark symbols (t=0) correspond to initial values.

**Figure 3.** Colour differences of sliced persimmon for each treatment applied and under different % CO$_2$ in the composition of the headspace.

**Figure 4.** Maximum force expressed in Newton (N) and the distance (d) at which it occurs in millimetres in sliced persimmon for each treatment applied and under different % CO$_2$ in the composition of the headspace.
Tables

**Table 1.** Values for water and soluble solid mass fractions, pH and titratable acidity of sliced persimmon for each treatment at the beginning of the storage (t=0) and at the end storage (t=f).

**Table 2.** Initial values for luminosity (L*) and a* and b* coordinates of sliced persimmon before the modification of the composition of the headspace.
Figure 1

- Unwashed
- Tap water
- Calcium lactate
Figure 2
Figure 3
Figure 4
Table 1.

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Parentheses indicate standard deviation
*Indicates a significant difference (p-value<0.05)
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Parentheses indicate standard deviation
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