

## Application of citric acid and mild heat shock to minimally processed sliced radish: Color evaluation



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### ABSTRACT

The aim of this research was to study the effects of two hurdle technologies, citric acid application (CA) at 0.3%, 0.6% and 0.9% and thermal treatments (IT) for 1, 2 and 3 min at 50 °C, on the color of radish slices over 10 d of refrigerated storage. Contribution of the hurdles and their interactions were evaluated by examining the treatment effects on the following parameters: chromatic coordinates ( $L^*$ ,  $a^*$  and  $b^*$ ) and the indices: chroma ( $\Delta C^*$ ), total color difference ( $\Delta E$ ) and Color Index ( $CI^*$ ).

The chromatic parameters for fresh radish (control samples) were  $L_0 = 69.43 \pm 0.62$ ,  $a_0 = -0.46 \pm 0.05$  and  $b_0 = 5.37 \pm 0.37$ , while the calculated color indices were chroma =  $5.39 \pm 0.36$ ,  $\Delta E = 0$  and  $CI^* = -1.19 \pm 0.17$ . Regarding control samples, the  $b^*$  values showed an increasing trend during storage, which was associated with browning of the slices. Both  $\Delta E$  and  $\Delta C^*$  values presented similar trends as reported for  $b^*$ . Based on statistical analysis of the parameters and indices tested, the single hurdle application of low citric acid concentration (0.3%) or intermediate immersion time (2 min) at 50 °C minimized the radish slices color changes during storage. However, better results were obtained when two hurdles in series were applied. According to analysis, the treatment T7 (1 min IT, 0.3% CA) was selected as the best treatment to improve the retention of typical natural color of the minimally processed sliced radishes.

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## 1. Introduction

The market for ready-to-use and ready-to-eat vegetables has increased in recent years in response to the increasing demand for fresh, healthy and convenient food (Manolopoulou and Varzakas, 2011; Geeroms et al., 2008).

Minimal processing of vegetables involves a combination of procedures, such as selection, washing, peeling, slicing and shredding, among others. Cells and membranes are damaged by processing, especially at the cut edges, leading to metabolic alterations (due to increased respiration and biochemical changes) and microbial spoilage with detrimental effects on food quality (Plaza et al., 2011; Gonzalez-Aguilar et al., 2001). Therefore, ready-to-use products usually have a shorter shelf life than whole product.

Several alternatives have been proposed to extend the shelf life of these products. Among them, controlling the temperature during distribution and storage is by far the simplest way to delay deterioration (by decreasing metabolic reaction rates) and to retain fresh product appearance (Nunes et al., 2009). However, even greater shelf life extension may be achieved by the combined application of different treatments (hurdle technology). Application of combined hurdles, at lower intensities than when they are applied individually, can improve the microbiological quality of food and reduce their impact on sensorial and nutritional quality (Ross et al., 2003; Leistner, 2000).

Enzymatic browning in fresh-cut vegetables is one of the most important phenomena that reduce shelf life extension by strongly affecting the consumer's purchase decision (Oms-Oliu et al., 2010). The disruption of cellular compartments caused by peeling and cutting allows the enzymes (such as polyphenoloxidase) and substrates (such as polyphenols) to mix and hence, to produce browning reactions (Limbo and Piergiovanni, 2007). Different treatments have been evaluated to reduce browning in fresh-cut products. The most common is to dip or immerse them in anti-browning agent solutions. For example, citric acid is

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**Table 1**

Applied treatments performed as simple and double hurdle technologies.

Citric acid (%)	Heat treatment time at 50 °C (min)			
	0	1	2	3
0	CO	T1	T2	T3
0.3	T4	T7	T10	T13
0.6	T5	T8	T11	T14
0.9	T6	T9	T12	T15

used, which lowers pH and chelates copper in the active site of the polyphenoloxidase enzyme (Limbo and Piergiovanni, 2006). In addition to chemicals, other physical treatments, like heat shock, have been successfully applied to prevent browning reactions and to extend postharvest storage of fresh-cut vegetables (Ansorena et al., 2011; Martín-Diana et al., 2006). Heat shock represses the induction of phenylalanine ammonia lyase (PAL) activity and phenolic accumulation during storage preventing tissue browning (Roura et al., 2008). Additionally, this treatment may assist in the decontamination of vegetable surfaces with higher efficiency when compared to chlorinated-water treatments (Alegria et al., 2010).

Many vegetable crops are minimally processed in Argentina. Among them, radish is gaining importance. It is used as an ingredient in mixed salads and is also considered to be an important source of medicinal and nutritional components (Lu et al., 2008). However, scarce information is found in the literature about the application of hurdle technologies to preserve minimally processed radishes during refrigerated storage.

The aim of this investigation was to determine the application effects of mild heat shock and immersion in citric acid (single and combined hurdles) on minimally processed radish to minimize color change during refrigerated storage.

## 2. Materials and methods

### 2.1. Plant material and sample preparation

Radishes (*Raphanus sativus* L.) were purchased from a local market in Mar del Plata, Argentina. They were kept at 5 ± 1 °C in darkness prior to processing. Radish roots were separated from leaves and washed in tap water to eliminate any surface contamination. Then, they were cut with a manual cutter into slices of about 3–4 mm and washed again in tap water using a ratio of sliced radish to water of 1:10 (w:w).

### 2.2. Treatments application

The samples were divided into 16 lots for the application of the different treatments: immersion in citric acid solutions and thermal treatments as single and double hurdle technologies. Citric acid treatments were carried out by immersion of radish slices in citric acid solutions of 0.3, 0.6 and 0.9% (w/w) (Merck, Argentina) for 1 min. The ratio between solids and the soaking solution was 1:10 (w:w). Thermal treatments were carried out in a thermostatically controlled water bath with recirculation (Lauda E300, Germany). The samples were placed in sterile containers and immersed in water at 50 °C for 1, 2 and 3 min. Thereafter, samples were removed from the bath and cooled immediately in cold water at 0–4 °C for 3 min. For the application of combined treatments (hurdle technology), heat treatment was carried out followed by immersion in citric acid solution. Table 1 summarizes the different treatments applied as single and double hurdle technologies.

### 2.3. Storage conditions

After treatments, 50 g of radish slices were packed in polyethylene bags (25 cm × 30 cm) of 25.4 µm thickness (with O<sub>2</sub>, CO<sub>2</sub> and water vapor transmission rates of 3.08 × 10<sup>-4</sup>, 2.05 × 10<sup>-3</sup> and 2.05 × 10<sup>-6</sup> mmol<sup>3</sup> m<sup>-2</sup> s<sup>-1</sup>, respectively, at P = 101,325 Pa, T = 25 °C).

Bags were sealed using manual equipment (HL, FS-300, Argentina). Samples were stored at 5 ± 1 °C in a refrigerated chamber (GAZA, Argentina). Two bags for each treatment were analyzed at 2, 4, 7 and 10 d of storage.

### 2.4. Color measurements

Surface color was measured with a colorimeter (Lovibond, RT Series, England). The colorimeter was standardized against a white tile ( $L^* = 97.63$ ,  $a^* = 0.3133$ ,  $b^* = 0.3192$ ). Measurements were done in triplicate over each surface sample. Color was recorded using the CIE-L\* a\* b\* uniform color space ( $L^* a^* b^*$ ), where  $L^*$  indicates lightness (whiteness or brightness/darkness),  $a^*$  indicates chromaticity on a green (−) to red (+) axis, and  $b^*$  indicates chromaticity on a blue (−) to yellow (+) axis (CIE, 1978). Numerical values  $L^*$ ,  $a^*$ ,  $b^*$  were used to estimate total color difference ( $\Delta E$ ) (Eq. (1)), chroma ( $C^*$ ) (Eq. (2)), and Color Index ( $Cl^*$ ) (Eq. (3)) according to:

$$\Delta E = \sqrt{(a^* - a_0)^2 + (b^* - b_0)^2 + (L^* - L_0)^2} \quad (1)$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

$$Cl^* = \frac{a^* \cdot 1000}{L^* \cdot b^*} \quad (3)$$

Total color difference ( $\Delta E$ ) is generally used to acknowledge the difference between two colors according to the following scale: Trace level difference  $\Delta E^* = 0$ –0.5, slight difference  $\Delta E^* = 0.5$ –1.5, noticeable difference  $\Delta E^* = 1.5$ –3.0, appreciable difference  $\Delta E^* = 3.0$ –6.0, large difference  $\Delta E^* = 6.0$ –12.0, very obvious difference  $\Delta E^* > 12.0$  (Chen and Mujumdar, 2008). Chroma ( $C^*$ ) is a measure of color intensity or saturation, which varies from dull (low value) to vivid (high value). Regarding Color Index,  $Cl^*$  values between –40 and –20 are related to blue-violet to dark green colors; between –20 and –2 to dark green and yellowish green colors; between +2 and +20 to pale yellow and deep orange colors; and between +20 and +40 to deep orange and deep red colors (Vignoni et al., 2006).

Additionally, chroma was reported as a difference ( $\Delta C^*$ ) with respect to chroma value of fresh radish at zero time.

### 2.5. Statistical analysis

Results reported in this research are LS mean values (least square mean, means estimators by the method of least squares) together with their standard deviations. Experimental data were analyzed using SAS, software version 9.0 (SAS, 1999). The General Linear Model procedure (PROC GLM) was used to carry out the Analysis of Variance (ANOVA), with confidence limits of 95%.

A statistical model was used to evaluate the effects of storage time on color indices of radish slices without any treatment (control samples, CO). For this model, the independent variable of the ANOVA was Storage Time (ST: 0, 2, 4, 7 and 10 d).

A second statistical model was used to evaluate the effects of mild heat shock treatment, as single hurdle, on color immediately after treatment as well as during storage. Thus, a two-way ANOVA was applied using the following factors as variation sources: Immersion Time (IT: 0, 1, 2 and 3 min), Storage Time (ST: 0, 2, 4, 7 and 10 d) and the interaction between them (IT \* ST). Data used

for this analysis corresponded to LS mean values of radish slices without citric acid treatment (T1, T2 and T3).

Similarly, another statistical model was used to evaluate the effects of citric acid immersion treatment, as single-hurdle, on color immediately after treatment as well as during storage. Thus, a two-way ANOVA was applied using the following factors as independent variables: citric acid concentration (CA: 0%, 0.3%, 0.6% and 0.9%), Storage Time (ST: 0, 2, 4, 7 and 10 d) and the interaction between them (CA \* ST). Data used for this analysis corresponded to LS mean values of radish slices without mild heat shock treatment (T4, T5 and T6).

Finally, mild heat shock and citric acid immersion treatments were analyzed together in a complete statistical model with factors: Immersion Time (IT: 0, 1, 2 and 3 min), citric acid concentration (CA: 0, 0.3, 0.6 and 0.9%) and storage time (ST: 0, 2, 4, 7 and 10 d) taking into account double interactions (IT \* ST, CA \* ST and IT \* CA) and the triple interaction (IT \* CA \* ST). Data used for this analysis corresponded to LS mean values of radish slices treated with both mild heat shocks and citric acid treatments (T7 to T15).

For each model, differences among results obtained for different factor levels were evaluated with multiple comparisons by means of Tukey-Kramer test ( $P < 0.05$ ). For all models, PROC UNIVARIATE was applied to validate ANOVA assumptions (Kuehl, 2000).

### 3. Results and discussion

The color of food surface is the first quality parameter evaluated by consumers at the moment of purchase and is critical for product acceptance (León et al., 2006). Therefore, different color indices are required to perform a detail characterization of this quality attribute. In this context, color quality indices  $C^*$ ,  $CI^*$  and  $\Delta E$  have been characterized by a high correlation with the surface color of fruits and vegetables. Thus, these indices can be used in studies on maturation, preservation and storage of foods (Limbo and Piergiovanni, 2006; Chen and Mujumdar, 2008).

In the present research, the chromatic parameters ( $L^*$ ,  $a^*$  and  $b^*$ ) and the color quality indices ( $C^*$ ,  $CI^*$  and  $\Delta E$ ) of sliced radishes were examined during 10 d of storage.

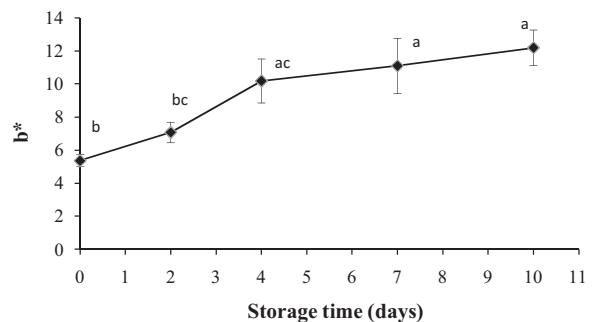
#### 3.1. Chromatic parameters and indices for fresh radish

The chromatic parameters for fresh radish (CO samples at time zero) were  $L_0 = 69.43 \pm 0.62$ ,  $a_0 = -0.46 \pm 0.05$  and  $b_0 = 5.37 \pm 0.37$ . The values of the color quality indices were  $CI^* = -1.19 \pm 0.17$ ,  $C^* = 5.39 \pm 0.36$  and  $\Delta E = 0$ . The  $L^*$  value was high, near-white in the scale, which was consistent with the visual appearance of the slices. It was further noted that  $a^*$  parameter had a value very close to zero, which is the midpoint of the color range between red and green. The  $b^*$  value was positive, indicating a tendency toward yellow. Saavedra del Aguila et al., 2008 reported similar chromatic parameters for shredded radish:  $L = 74.54$ ,  $a = -0.55$  and  $b = 3.90$ .

It may also be noted that the initial value of chroma (which was used as a reference to calculate the  $\Delta C^*$ ) had a value almost identical to the  $b^*$  parameter, which indicated that this particular index was strongly influenced by the  $b^*$  parameter (Eq. (2)). Regarding  $CI^*$  value, it presented a negative and low value, indicating a slight yellowness of the slices, which was not perceived by the human eye (Vignoni et al., 2006).

#### 3.2. Color evolution of control samples during the storage

Based on the statistical analysis applied to control samples,  $L^*$  and  $a^*$  parameters did not present significant changes during storage. Although some fluctuations in those values were observed, significant differences were not detected ( $P > 0.05$ ). On the other hand, an increment in  $b^*$  value was observed along storage (Fig. 1).

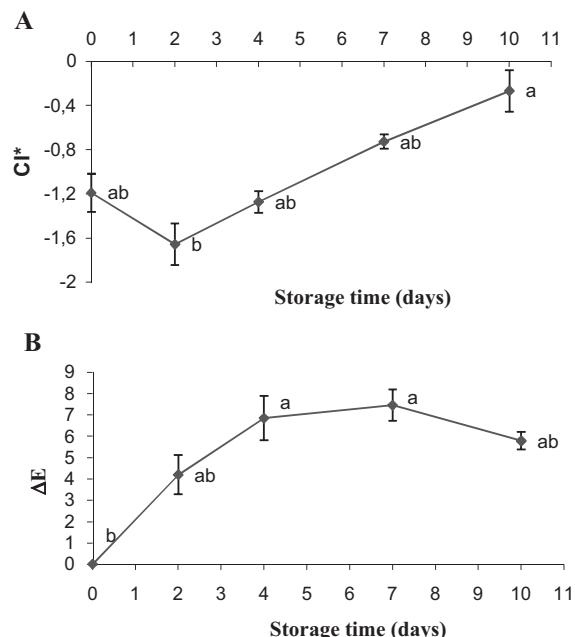


**Fig. 1.** Evolution of  $b^*$  for control samples during storage. Values are mean  $\pm$  s.d. Values with the same letter are not significantly different ( $P < 0.05$ ),  $n = 3$ .

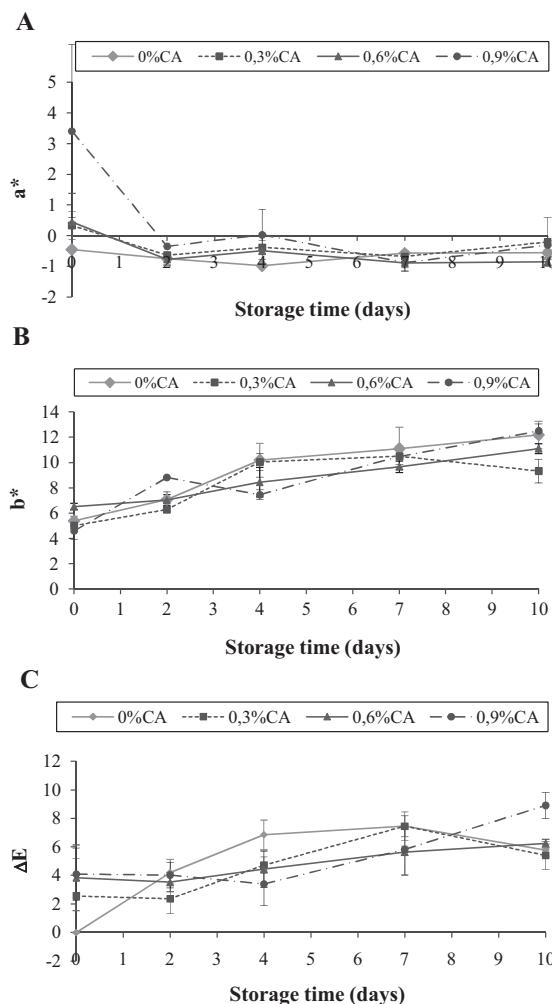
This increase was observed for four days, reaching values close to twice the reported value for fresh radish. After that,  $b^*$  values remained constant until the end of storage. It was observed that the white part of the slices turned to yellowish brown with increasing storage time. These changes in  $b^*$  values could be related to browning reactions observed in the non-treated samples due to endogenous enzymes (e.g. polyphenoloxidase, PPO). The quinones formed by means of PPO reactions could take part in secondary reactions bringing about the formation of dark secondary products (Rocha and Morais, 2003). Comparable results were reported by Saavedra del Aguila et al., 2008 working with shredded radish during storage at 5 °C.

Changes in chroma ( $\Delta C^*$ ) and  $\Delta E$  presented the same tendency as that reported for the  $b^*$  parameter. That trend could be explained by the predominant weight of the  $b^*$  parameter compared to the small values of  $a^*$  and  $L^*$  by means of Eqs. (1) and (2). Schreiner et al., 2003 reported that in 'Nevadar' radish, postharvest color changes ( $\Delta C^*$ ) could be correlated to changes in soluble and insoluble pectic substances, total glucosinolates and alkenyl glucosinolates. These compounds are related to the sensory quality of radishes not only by means of color but also texture.

Fig. 2 shows the results for  $CI^*$  and  $\Delta E$ . Color Index ( $CI^*$ ) presented negative values with a minimum on the second day of



**Fig. 2.** Evolution of  $CI^*$  (A) and  $\Delta E$  (B) for control samples during storage. Values are mean  $\pm$  s.d. Values with the same letter are not significantly different ( $P < 0.05$ ),  $n = 3$ .



**Fig. 3.** Effects of mono-hurdle citric acid application on  $a^*$  (A),  $b^*$  (B) and  $\Delta E$  (C) during storage. Values are mean  $\pm$  s.d.,  $n=3$ .

storage. Instead,  $\Delta E$  showed positive values, with a maximum detected on day seven of storage, corresponding to a large difference in color with respect to the fresh radish slices. At the end of the storage, large difference in color with respect to fresh samples was observed. Similar results were reported by Chen and Mujumdar, 2008.

### 3.3. Treatments applied as mono-hurdles

#### 3.3.1. Effect of citric acid immersion

The statistical model used to evaluate the effects of citric acid immersion treatments (CA) together with storage time (ST) on  $L^*$  parameter of radish slices yielded a significant CA \* ST interaction ( $P=0.007$ ). This result indicated that the behavior of  $L^*$  during storage was dependent on the citric acid concentration. A slight decrease in the  $L^*$  value was observed when low CA concentration were used. However, this parameter increased with high CA concentrations, indicating an increase in the brightness of slices. Despite this, significant differences were not detected in  $L^*$  values at the end of storage (10 d) for samples treated with different concentrations of CA (data not shown).

Based on statistical analysis,  $a^*$  parameter did not show significant interaction between factors under consideration, but both ST ( $P<0.05$ ) and CA ( $P<0.0001$ ) resulted significant. Radish slices subjected to immersion in acid treatments (Fig. 3A) showed increments in  $a^*$  values with respect to fresh samples (at time zero). In

particular, the highest CA concentration (0.9%) showed the highest value for this parameter, with significant differences regarding the other concentrations ( $P<0.0001$ ). The increase in  $a^*$  values indicated a change in the green-red scale with a tendency toward redder values. Those changes were associated with the observed redness of the white part of the slices, caused by a possible diffusion of the skin pigments (anthocyanins). Amodio et al. (2011) observed a similar effect when applying citric acid to minimally processed artichokes. Patil et al., 2009 also indicated that the radish skin (epidermal tissue) was an important source of anthocyanins, pigments that change their color with pH. After two days of storage, a decrease in  $a^*$  value was observed in all samples, then it remained constant up to the end of storage.

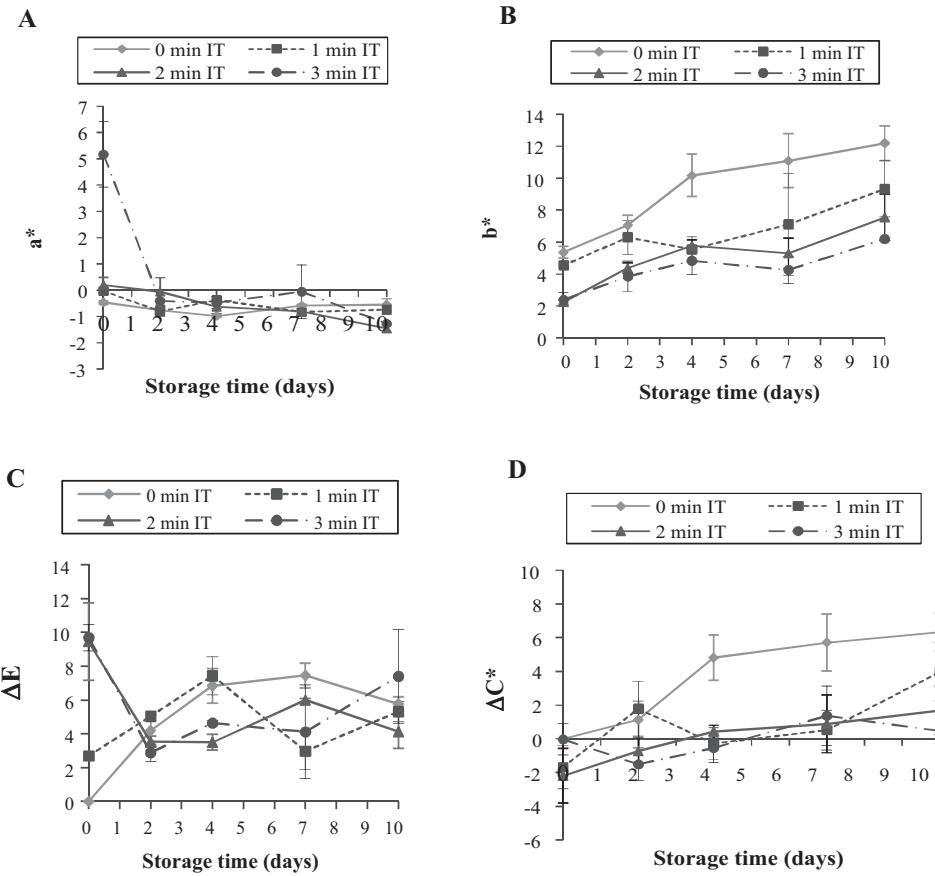
Moreover, the  $CI^*$  index showed a similar behavior to that of the  $a^*$  parameter throughout storage (data not shown).

The statistical model used to evaluate the effect of CA as a single hurdle together with ST on  $b^*$  parameter indicated a significant interaction ( $P=0.001$ ) between factors considered in the analysis. As in the case of  $L^*$ , that result implied that the behavior of  $b^*$  values during storage was also dependent on the citric acid concentration used in the immersion treatment. All samples treated with different CA concentrations showed an increase in  $b^*$  values during storage (Fig. 3B). However, as can be observed, the different treatments with different acid concentrations presented dissimilar profiles. At zero time, the hurdle 0.6% CA showed an increase in the  $b^*$  value compared to control samples as well as the other concentrations, but significant differences were not obtained ( $P>0.05$ ) at the end of storage. During the first days of storage, increments detected in  $b^*$  values were higher for samples treated with 0.9% of citric acid than those obtained for the other concentrations. At the end of the storage, the lowest  $b^*$  value was found in radish slices treated with the lowest concentration of citric acid ( $P>0.05$ ).

Results of statistical analysis applied to  $\Delta E$  values were comparable to those found for  $L^*$  and  $b^*$  parameters, with a significant interaction ( $P=0.001$ ) between factors considered in the analysis. Fig. 3C shows the evolution of  $\Delta E$  for samples treated only with citric acid as a preservation treatment. At the beginning of the storage, a change in total color difference was detected in all samples after treatment. Citric acid leads to a reduction in the pH of the solution. Some authors reported that pH changes increase the values of  $\Delta E$  and chroma in Chinese red radish due to modifications of anthocyanins and glucosinolates (Jing et al., 2012). While control samples presented an increase in  $\Delta E$  from the beginning of the storage, radish slices treated with 0.3% CA did not show any significant change until the second day of storage. After that, an increment was detected up to day seven. Then, changes were not observed in  $\Delta E$  until the end of storage. Radish slices treated with 0.6% citric acid solution showed a more stable behavior, without changes in  $\Delta E$  during the entire period of storage. Finally, samples treated with the highest citric acid concentration did not show any color change until day four of storage. After that, significant increases were detected in this parameter. Thus, the highest value was evidenced at the end of the storage for the highest CA concentration, indicating large differences with respect to fresh samples ( $\Delta E$  close to 9).

Statistical analysis of  $\Delta C^*$  values showed that only ST was a significant factor ( $P<0.0001$ ), indicating that application of different citric acid concentrations did not affect the chroma of samples (data not shown).

According to the parameters and indices previously analyzed, immersion of radish slices in solutions with a low concentration of citric acid (0.3%) as a single hurdle was a useful treatment to minimize undesirable color changes during storage. Citric acid is one of the control agents of the cell respiratory metabolism, which contributes to better preservation of the product. In this sense, a decrease in cell metabolism could be the main cause of the



**Fig. 4.** Effects of mono-hurdle mild heat shock on  $a^*$  (A),  $b^*$  (B),  $\Delta E$  (C) and  $\Delta C^*$  (D) during storage. Values are mean  $\pm$  s.d.,  $n=3$ .

natural color retention of sliced radish. Comparable results were obtained by other authors working with shredded radish and beetroot (Saavedra del Aguila et al., 2008; Vitti et al., 2005).

### 3.3.2. Effect of mild heat treatment

In general, the statistical model used to evaluate the effect of mild heat shock treatment (IT) as a single-hurdle together with storage time (ST) showed significant interactions ( $P<0.0001$ ) for almost all indices under study (except for  $b^*$ ), indicating that the behavior of those indices during storage was dependent on the thermal treatment applied. In particular, the model presented significant results for IT and ST ( $P<0.0001$ ) when analyzing  $b^*$ . Therefore, both the mild heat treatment and the storage time led to changes in  $b^*$  values. However, the pattern of change during storage was similar for the different treatments.

Regarding  $L^*$  parameter, thermal treatments applied for 2 and 3 min significantly reduced  $L^*$  values at the beginning of storage ( $P=0.0099$  and  $P=0.0398$ , respectively), indicating that these treatments decreased the sample brightness. On the other hand,  $L^*$  values did not change immediately after treatment in the samples immersed for 1 min. Throughout storage, differences in the behavior were observed but without a defined pattern (data not shown). At the end of storage, values of  $L^*$  resulted into 68.48, 63.55 and 62.13 for samples treated for 1, 2 or 3 min, respectively.

Fig. 4A presents the values obtained for the  $a^*$  parameter. Radish slices subjected to mild heat shock treatments for 1 or 2 min did not show changes in  $a^*$  values, immediately after treatment. During storage, only a slight decrease was detected in these samples, without significant differences among control and treated samples. However, when the treatment time was extended to 3 min,  $a^*$  values significantly increased ( $P<0.0001$ ). This behavior could be

associated with the redness of the slices caused by the pigment diffusion favored by the time/temperature (3 min/50 °C) combination. After that, a significant decrease in  $a^*$  values was detected (until day two of storage) probably due to the degradation of the diffused pigments. Then,  $a^*$  values continued to decline but at a significantly lower rate than during the first days and without significant differences with respect to control samples or those samples treated for 1 or 2 min.

Fig. 4B shows the evolution of  $b^*$  parameter during storage. As previously discussed, both single factors IT and ST resulted significant in the statistical analysis, but without interaction between them. After the mild heat shock treatment a decrease in  $b^*$  values was observed. After that, all samples registered an increase in this parameter, maintaining differences detected initially along storage. The increase was associated with browning phenomena occurring during storage. The highest immersion time (3 min) had lower values of the parameter  $b^*$ .

Fig. 4C shows the evolution of  $\Delta E$  during storage for samples treated with mild heat shocks. Similar to the changes observed in  $L^*$ ,  $a^*$  and  $b^*$  parameters, after mild heat treatments, large color differences ( $\Delta E$  higher than 6) were obtained with immersion time of 2 or 3 min. Samples immersed in hot water (50 °C) for 1 min, also presented noticeable color differences ( $\Delta E$  higher than 2) with respect to control samples. During storage, color changes took place but without a defined pattern. After 10 d of storage, the  $\Delta E$  in samples treated for 3 min still showed the highest difference with respect to fresh radish. Samples treated with mild heat shock for 2 min presented the lowest color difference with respect to fresh samples.

Fig. 4D shows  $\Delta C^*$  evolution during storage in samples treated with mild heat shocks. Chroma did not show any significant change immediately after treatment application. During storage, different

behavior was observed for  $\Delta C^*$  of samples immersed in water at 50 °C with different immersion times. Samples treated for 3 min presented variable behavior in  $\Delta C^*$ , reaching at the end of storage similar values without significant differences compared to fresh slices. On the other hand, samples treated for 1 min showed a similar behavior to control samples with increments in this index during storage. Samples treated for 2 min presented an intermediate behavior, with a final value significantly lower than those detected for control samples or those treated only for 1 min, but without differences compared to those treated for 3 min.

Finally, an analogous trend was shown for the single hurdle with citric acid. The  $CI^*$  presented a pattern similar to that for the corresponding  $a^*$  parameter (data not shown).

According to the parameters and indices analyzed, it can be said that an intermediate IT of 2 min at 50 °C was suitable to minimize color changes in radish slices during storage. Similar results were found by other authors working with apples and asparagus (Rocha and Morais, 2003; Siomos et al., 2010). In particular, Siomos et al., 2010 observed that asparagus retained the initial white color due to the inhibition of anthocyanin synthesis in the peel after application of citric acid. Moreover, Goyeneche et al., 2013 found that at 50 °C approximately 20% of polyphenoloxidase was inactivated by means of thermal treatment, decreasing enzymatic browning. Probably other enzymes involved in color and texture changes, like peroxidase or PAL, could be inhibited by a mild heat shock contributing to the radish color retention. The effect of mild heat treatment on the PAL-enzyme inhibition was previously reported for minimally processed lettuce (Pereyra et al., 2005).

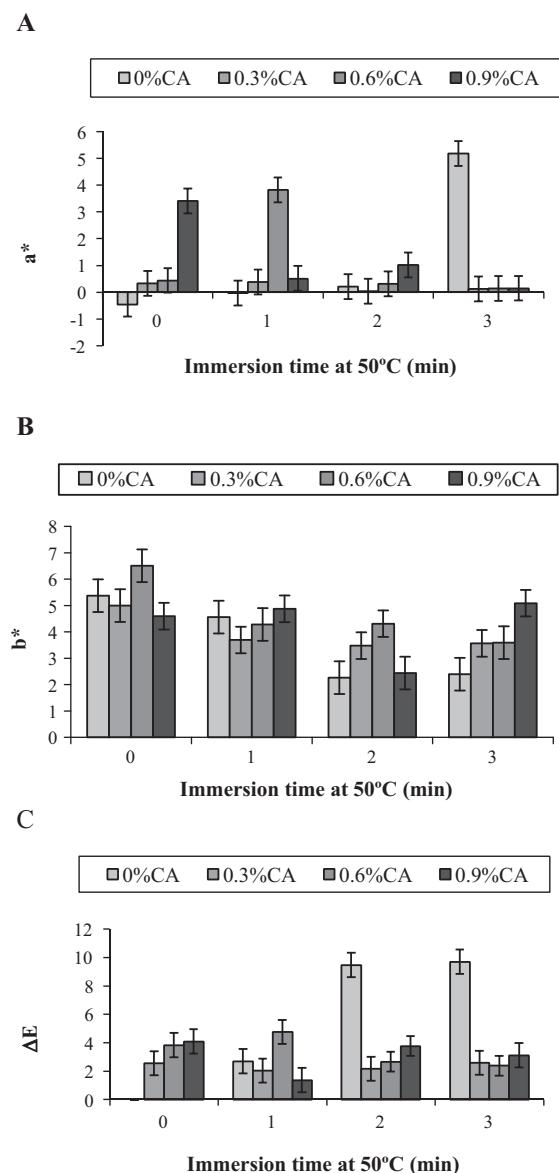
#### 3.4. Treatments applied as double-hurdles

The complete model with the three factors (IT, CA and ST) showed a significant triple interaction for all the parameters and indices evaluated ( $P < 0.0001$ ). In order to simplify the analysis, the parameters and indices were evaluated for each storage time. The model was reduced to a factorial model with two factors: IT and CA, and the subsequent interaction IT\*CA. Thus, in this section only analysis for zero and ten days of storage was presented. All indices presented significant double interaction between the factors ( $P < 0.05$ ), except for  $\Delta C^*$  at day zero. For this particular index, the mathematical model did not fit, indicating that there was not a defined pattern for its behavior.

At day zero, only  $L^*$  values for treatment T13 (0.3% CA/3 min IT) were lower than the corresponding values of control samples, indicating a darkening of the slices. The other  $L^*$  values were similar or higher to the corresponding values of control samples, which indicated an increase of samples brightness. At the end of storage, all samples showed similar behavior regarding the  $L^*$  parameter.

Changes in the  $a^*$  parameter, immediately after treatment, are presented in Fig. 5A. A significant increase in the value of this parameter was detected in samples treated with 0.6% CA and 1 min IT (T8). This behavior was also observed for  $\Delta E$  (Fig. 5C) and  $CI^*$  (data not shown). This increase was associated with the redness observed in samples, probably caused by the skin pigments diffusing into the slice. At the end of storage, samples treated with the combination of hurdles IT = 3 min/CA = 0.6% (T14) presented the highest  $a^*$  (Fig. 6A),  $\Delta E$  (Fig. 6C) and  $\Delta C^*$  (Fig. 6D) values. Thus, these combinations were not found to be suitable to minimize color change in minimally processed sliced radish.

Concerning parameter  $b^*$  (Fig. 5B), there were no significant differences between control and the different treatments at day zero, indicating that initially, selected hurdles had no effect on this parameter. Comparable results were found by others researchers working with fruits and vegetables (Rocha and Morais, 2003; Mazzeo et al., 2011). After 10 d of storage, all samples presented  $b^*$  values lower than control (Fig. 6B), except for T12 (0.9% CA/2 min

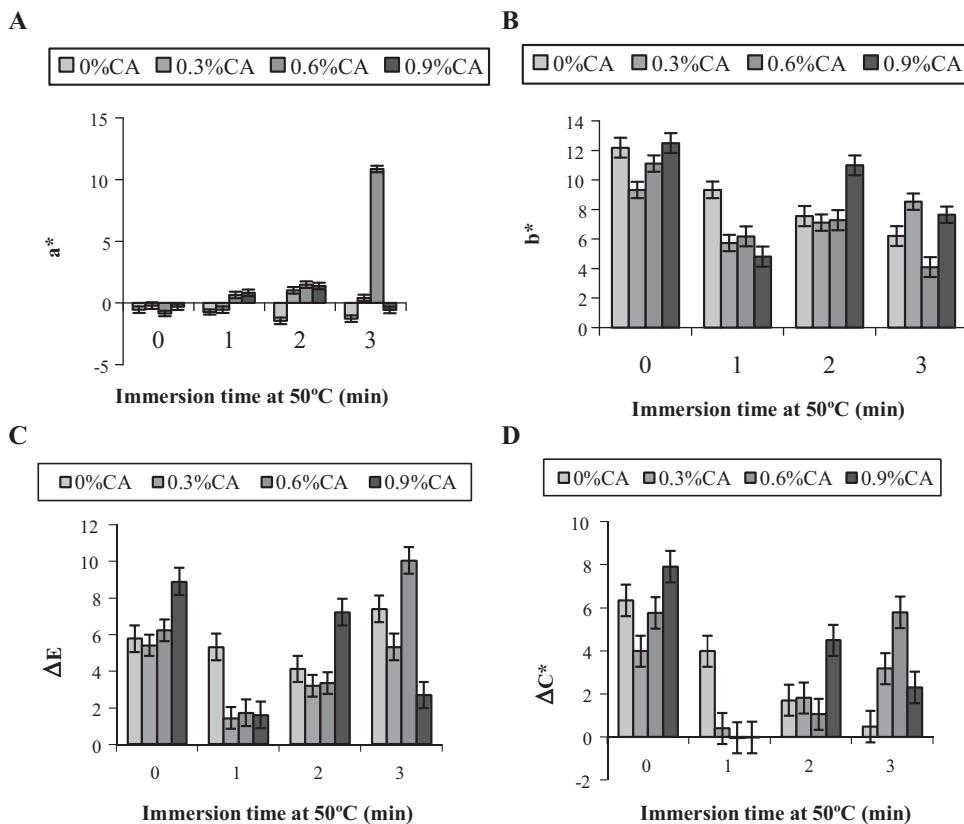


**Fig. 5.** Immediate effects of treatments on  $a^*$  (A),  $b^*$  (B) and  $\Delta E$  (C) for double-hurdles at day zero. Values are mean  $\pm$  s.d.,  $n=3$ .

IT), which did not present significant differences with respect to control samples ( $P > 0.05$ ). At day zero,  $\Delta E$  and  $CI^*$  did not present significant differences between control samples and those with double hurdles ( $P > 0.05$ ).

After 10 d of storage, the hurdles combination T12 and T14 presented maximum  $\Delta E$  values (Fig. 6C) indicating large differences with respect to control samples and the other treatments ( $P < 0.05$ ). Similar behavior was observed in Fig. 6D for  $\Delta C^*$ . Because of those results, these combinations were not suitable for minimizing color changes in minimally processed sliced radish.

From global analysis of immediate effect of mild heat shock and citric acid treatments on radish color parameters  $L^*$ ,  $a^*$  and  $b^*$ , and  $\Delta C^*$ ,  $CI^*$  and  $\Delta E$ , as well as their behavior during refrigerated storage, the combined treatment of 1 min at 50 °C/0.3% CA (T7) was selected as most capable of improving retention of the typical natural color of minimally processed radish slices stored at 5 °C for 10 d. It was observed that the highest concentration of citric acid combined with long immersion time in the thermal bath (T15) did not present any significant improvement over the milder treatments.



**Fig. 6.** Effects of double-hurdles on  $a^*$  (A),  $b^*$  (B),  $\Delta E$  (C) and  $\Delta C^*$  (D) after 10 days of storage. Values are mean  $\pm$  s.d.,  $n=3$ .

In general, the application of two hurdles in series improved color preservation of the radish slices compared to the application of a single hurdle. In fact, the application of mild heat treatment was more effective than a single immersion in citric acid solution at any concentrations. Thus, thermal inactivation and modification of pH are effective methods for browning prevention since PPO activity is highly dependent of these parameters (Goyeneche et al., 2013). Moreover, thermal and pH stability of anthocyanins and glucosinolates (chemical compounds presents in radishes) could contribute to retain typical product color (Jing et al., 2012).

#### 4. Conclusions

The results of this investigation indicate that immersion in citric acid solutions and application of mild heat shock treatments applied as hurdle technologies in series modified the color of sliced radish along 10 d under refrigeration. Based on statistical analysis, the combined treatment of mild heat shock (1 min/50 °C) and the immersion in a solution of 0.3% CA (T7) was selected as most capable of improving retention of the fresh color of radish slices during entire storage. The rapid discoloration due to browning that occurs on radish surfaces after slicing during minimal processing and storage can be diminished by the use of these hurdle technologies.

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