# INACTIVATION OF *LISTERIA MONOCYTOGENES*, *SALMONELLA ENTERITIDIS* AND *ESCHERICHIA COLI* O157:H7 AND SHELF LIFE EXTENSION OF FRESH-CUT PEARS USING MALIC ACID AND QUALITY STABILIZING COMPOUNDS

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## ABSTRACT

*Inactivation of* Listeria monocytogenes, Salmonella enteritidis *and* Escherichia coli *O157:H7 and shelf life extension of fresh-cut pears using malic acid (MA) and quality stabilizing compounds (N-acetyl-L-cysteine, glutathione and calcium lactate; CGLW) were investigated. Trays of treated fresh-cut pears were wrap sealed with a thick polypropylene film (64 μm) semipermeable to water vapor, $O_2$ and $CO_2$, and stored at 5C for 30 days. Changes in headspace gas, firmness and color of the fresh-cut pears were also determined. Large reductions of* L. monocytogenes *(6.57 $log_{10}$ cfu/g)*, S. enteritidis *(6.60 $log_{10}$ cfu/g) and* E. coli *O157:H7 (2.62 $log_{10}$ cfu/g) just after processing were achieved in those fresh-cut pears dipped in CGLW + MA. Microbiological shelf life of pear pieces dipped in CGLW + MA was extended by more than 21 days in comparison with those cut pears immersed in water used as control sample. Lower consumption of $O_2$ and production of $CO_2$, ethylene and ethanol of fresh-cut pears dipped in CGLW + MA were also observed. In addition, the color and firmness of pear pieces in CGLW + MA were maintained by more than 21 days in comparison with control samples. In conclusion, the combination of MA with quality stabilizing compounds can be a good alternative for assuring the safety and quality of fresh-cut pears.*

## PRACTICAL APPLICATIONS

The use of natural substances generally recognized as safe (GRAS) such as malic acid and N-acetyl-L-cysteine, glutathione and calcium lactate as

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antimicrobials and quality stabilizing compounds, respectively, can result suitable to fresh-cut products industry, since they can assure the safety and quality of those products, while improving their sensory attributes and maintaining the fresh-like and healthy properties of these products greatly demanded by the consumers.

## INTRODUCTION

Consumption of ready-to-eat fresh fruits has rapidly increased in the last years (Corbo *et al.* 2000) due to their characteristics of freshness, low caloric contents, commodity to be used and an active promotion of fruits and vegetables as basic components of a healthy diet. Nevertheless, it is well known that minimally processed fruits and vegetables are generally more perishable than raw materials. Injury stresses due to operations such as peeling, cutting, shredding, slicing, etc. lightly increase tissue respiration and lead to various biochemical deteriorations such as browning, off-flavor development and texture breakdown which decrease the fresh-cut fruit quality (Varoquaux and Wiley 1994; Martín-Belloso *et al.* 2006). Moreover, minimal processing may increase the microbial spoilage of the product (Pittia *et al.* 1999) as well as the consumer risk to acquire diseases because the pathogenic flora can transfer from the skin to the flesh of the fruit. In fact, the number of outbreaks and cases by consumption of fresh-cut fruits and derivatives has increased in recent years. Eswaranandam *et al.* (2004) indicated that apple snack trays, assorted processed fruits and vegetables, and cut cantaloupe melon were recalled for possible contamination with *Listeria monocytogenes.* Likewise, Fan *et al.* (2005) reported one recall associated with fresh-cut apples due to a possible contamination with that microorganism. *Escherichia coli* O157 : H7 and *Salmonella* have been responsible for outbreaks associated with pear and melon fresh fruit, as well as apple juice and cider in recent years (Besser *et al.* 1993; Powell and Luedtke 2000; USFDA 2001; Harris *et al.* 2003; CDC 2007).

Refrigerated storage as well as modified atmospheres (typically reduced oxygen) have been commonly used to extend the shelf life of fresh-cut fruits. However, the growth of some pathogenic bacteria such as *L. monocytogenes* and *E. coli* O157:H7 in fresh-sliced cactus pears stored in both air and modified atmosphere at 4C have been reported (Corbo *et al.* 2005). Likewise, survival and/or growth of *E. coli* O157:H7, *Salmonella* serovars and *L. monocytogenes* in fresh-cut apples stored at different temperatures have been also reported (Fisher and Golden 1998; Liao and Sapers 2000; Lanciotti *et al.* 2003).

Numerous preservation strategies to avoid quality loss of fresh-cut fruits, such as the use of additives (Raju and Bawa 2006) added direct or indirectly on

the fruits to reduce: (1) enzymatic browning, such as ascorbic acid, sodium erythorbate and 4-hexylresorcinol, as well as sulphydryl-containing amino acids and peptides as cysteine and glutathione (Ahvenainen 1996; Laurila *et al.* 1998; Sapers and Miller 1998; Rojas-Graü *et al.* 2006b); (2) softening such as calcium chloride and lactate (Luna-Guzmán and Barrett 2000; Gorny *et al.* 2002; Johnston *et al.* 2002), and (3) microbiological spoilage and risk such as citric and malic acid (MA), lemon juice, hexanal, (E)-2-hexenal, hexyl acetate (Brul and Coote 1999; Pittia *et al.* 1999; Lanciotti *et al.* 2003; Derrickson-Tharrington *et al.* 2005; Raybaudi-Massilia *et al.* 2007, 2009), have been proposed in recent years. However, effective strategies to avoid those phenomena and reduced health risk at the same time in different fresh-cut fruit have not been extensively studied (Raybaudi-Massilia *et al.* 2007, 2009).

The objectives of this study were to evaluate the effectiveness of a combination of MA, N-acetyl-L-cysteine, glutathione and calcium lactate (CGLW) applied through dipping treatments to inactivate *L. monocytogenes*, *S. enteritidis* and *E. coli* O157:H7 populations inoculated in fresh-cut pears as well as to evaluate its influences on the shelf life of the product.

## MATERIALS AND METHODS

### Pears

Winter pears (*Pyrus communis* L.) cv. *Flor de Invierno*, partially ripe (commercial ripeness), were provided by ACTEL, Lleida, Spain. A flesh characterization was carried out following the official methods of fruit juices and other vegetables and derivatives (BOE 1988). Total acidity expressed as grams of citric acid/100 mL (0.35 ± 0.02), pH (Crison 2001 pH-meter, Crison Instruments S.A., Barcelona, Spain) (4.31 ± 0.04) and soluble solids content expressed as % (Atago RX-1000 refractometer; Atago Company Ltd., Tokyo, Japan) (14.3 ± 0. 21) were the evaluated parameters.

### Dipping Treatments

Two aqueous solutions containing: (1) N-acetyl-L-cysteine (Acros Organics, NJ) at 1% (w/v), reduced glutathione (Acros Organics, Morris Plains, NJ) at 1% (w/v), calcium lactate pent-hydrate (Scharlau Chemie S.A., Barcelona, Spain) at 1% (w/v) into sterile distilled water (CGLW), and (2) CGLW plus DL-MA (Scharlau Chemie S.A.) at 2.5% (w/v) (CGLW + MA) were prepared to avoid physicochemical and microbiological spoilage of fresh-cut pears. In addition, sterile distilled water (W) was used as control treatment. Concentrations of those substances were selected according to

previous studies (Gorny *et al.* 2002; Oms-Oliu *et al.* 2006; Rojas-Graü *et al.* 2006b; Raybaudi-Massilia *et al.* 2007, 2009). The temperature of dipping solutions containing or not stabilizing substances and/or MA was room temperature (18C).

## Strains and Inoculum Preparation

Strains of *L. monocytogenes* 1.131 (CECT 932) and *E. coli* O157:H7 (CECT 4267) from the Spanish Type Culture Collection (University of Valencia, Valencia, Spain), and *S. enteritidis* 1.82 (NCTC 9001) from the National Collection of Type Culture (Central Public Health Laboratory, London, U.K.) were maintained in tryptone soy agar (Biokar Diagnostics, Beauvais, France) slants at 5C until its use. Stock cultures of *L. monocytogenes* and *E. coli* O157:H7 were grown in tryptone soy broth (TSB) (Biokar Diagnostics) with 0.6% yeast extract (Biokar Diagnostics), whereas, *S. enteritidis* was cultured in TSB. *Escherichia coli* O157:H7 and *S. enteritidis* were incubated at 37C with continuous agitation for 11 h at 120 rpm, while *L. monocytogenes* was incubated at 35C with continuous shaking for 15 h at 200 rpm to obtain cells in early stationary growth phase. The maximum growth for *L. monocytogenes*, *S. enteritidis* and *E. coli* O157:H7 was $10^9$ colony forming units/milliliter (cfu/mL). Concentrations were then adjusted to $10^8$ cfu/mL using saline peptone water (Biokar Diagnostics) at 0.1% plus sodium chloride (Scharlau Chemie S.A.) at 0.85%.

## Pears Processing and Packaging

For challenge studies, pears were sanitized, washed, dried and cut as suggested by Raybaudi-Massilia *et al.* (2007, 2009). The cut pears were divided into three parts and then dipped for 2 min in each dipping solution (CGLW, CGLW + MA, W) with a ratio fruit: solution equal to 1:2 and constant agitation using a magnetic stirrer. After natural draining for 2 min, 10 cylinders of pears with a total weight of 50 g were placed into polypropylene trays of $173 \times 129 \times 35$ mm (Ilpra Systems España, S.L., Barcelona, Spain). Afterward, the pear pieces were inoculated uniformly by spreading 500 μL of *L. monocytogenes*, *S. enteritidis* or *E. coli* O157:H7 stock cultures ($10^8$ cfu/mL) over its entire upper surface with a sterile micropipette. The trays were then wrap sealed with a 64-μm thick polypropylene film with a water vapor permeability of 142.86 fmol/s/m$^2$ k/Pa$^{-1}$ at 38C and 90% relative humidity (RH), $O_2$ permeability of 52.38 fmol/s/m$^2$ kPa$^{-1}$ at 23C and 0% RH, and $CO_2$ permeability of 2.38 fmol/s/m$^2$ kPa$^{-1}$ at 23C and 0% RH (Tecnopack SRL, Mortara, Italy) using a vacuum-compensated packaging machine (ILPRA Food Pack Basic V/6, Ilpra S. CP. Vigevono, Italy). Trays were sealed in air and stored in refrigeration at 5C for 30 days. On the other hand, 50 g of

non-inoculated fresh-cut pears processed in the same way as previously indicated were packed, sealed and stored at 5C for 30 days for shelf life and sensory studies.

Trays of fresh-cut pears dipped in each solution (CGLW, CGLW + MA, W) for microbial challenge studies (20 trays for *L. monocytogenes*, 20 trays for *S. enteritidis* and 20 trays for *E. coli* O157:H7) and shelf life estimation (10 trays for physicochemical determinations and 10 trays for microbiological analysis) were prepared to be analyzed at 0, 7, 14, 21 and 30 days of refrigerated storage. In addition, 12 trays (six for 0 day and six for 15 days of storage) were prepared to evaluate the sensory characteristics.

### *L. monocytogenes*, *S. enteritidis* and *E. coli* O157:H7 Survival

Fifty grams of inoculated fresh-cut pears were diluted with 450 mL of buffered peptone water (pH 7.2) (Biokar Diagnostics) and homogenized in a masticator (IUL Instruments, Barcelona, Spain) for 1 min. Serial dilutions from first dilution were prepared and spread at reason of 0.1 mL on Palcam, Hektoen and MacConkey-Sorbitol agar plates in duplicate for *L. monocytogenes*, *S. enteritidis* and *E. coli* O157:H7 counts, respectively. The plates were incubated for 24–48 h at 35–37C. Culture media were provided by Biokar Diagnostics.

### *L. monocytogenes*, *S. enteritidis* and *E. coli* O157:H7 Recovery

Injured cells of *L. monocytogenes*, *S. enteritidis* and *E. coli* O157:H7 from fresh-cut pears dipped in CGLW + MA were recovered using buffered peptone water (pH 7.2) and maintained for 20 min at 35–37C. Subsequently, an aliquot of 0.1 mL was spread plated on Palcam, Hektoen and MacConkey-Sorbitol agars for *L. monocytogenes*, *S. enteritidis* and *E. coli* O157:H7 counts, respectively. Those plates were then incubated for 24–48 h at 35–37C. The employed recovery medium was selected according to Liao and Fett (2005), whereas recovery time (20 min) was selected, taking into account the generation time of each microorganism from growth curves previously made in the laboratory (data not shown), where repairing of injured cell without cellular multiplication was assumed.

### Mesophilic, Psychrophilic and Yeast and Molds Counts

Mesophilic and psychrophilic microorganism counts in non-inoculated fresh-cut pears were made according to the ISO 4833:1991 (1991) guideline using plate count agar (Biokar Diagnostics) and pour plate method. The plates of psychrophilic microorganisms were incubated at 5C for 10–14 days, whereas mesophilic microorganisms were incubated at 35C for 48 h. On the

other hand, yeasts and molds counts were made according to the ISO 7954:1987 (1987) guideline using chloramphenicol glucose agar (Biokar Diagnostics) and spread plate method. The plates were incubated at room temperature (18C) for 3–5 days. All the microbial counts were expressed as $\log_{10}$ cfu/g and the reported values are the mean of four determinations $\pm$ SD.

## Headspace Gases Analysis

Gas composition of the package headspace was determined using a Micro-GP CP 2002 gas analyzer (Chrompack International, Middelburg, Netherlands) equipped with a thermal conductivity detector. A sample of 1.7 mL was automatically withdrawn from the headspace atmosphere. Portions of 0.25 and 0.33 µL were injected for $O_2$ and $CO_2$ determination, respectively. The $O_2$ content was analyzed with a CP-Molsieve 5Å packed column (Chrompack International) (4 m $\times$ 0.32 mm, df = 10 mm) at 60C and 100 kPa. On the other hand, a Pora-PLOT Q column (Chrompack International) (10 m $\times$ 0.32 mm, df = 10 mm) was held at 70C and 200 kPa for $CO_2$, ethylene and ethanol quantification. A pair of trays randomly taken was analyzed in duplicate for each dipping condition at 0, 7, 14, 21 and 30 days. Thus, the reported values are the mean of four determinations $\pm$ SD.

## Color Determination

Color of the fresh-cut pears was measured in a pair of trays for each dipping condition randomly chosen using a tri-stimulus Minolta CR-400 Chroma Meter (Konica Minolta Sensing, Inc., Osaka, Japan) using the illuminant D75 and observation angle of 10°, calibrated with a standard white plate ($Y = 94.00$, $x = 0.3158$, $y = 0.3322$). Three readings of $L^*$ (lightness), $a^*$ (green chromaticity) and $b^*$ (yellow chromaticity) coordinates were recorded for each pear cylinder. A total of 10 cylinders of fresh-cut pears were analyzed by pair of tray. Therefore, the reported values are the mean of 30 determinations $\pm$ SD. Numerical values of $a^*$ and $b^*$ parameters were employed to calculate the hue angle ($h°$) as follows (Eq. 1):

$$h° = \arctan \frac{b^*}{a^*} \tag{1}$$

## Firmness Determination

Firmness measurement was performed in 10 cylinders coming from a pair of fresh-cut pears trays for each dipping condition using a TA-TX2 Texture Analyzer (Stable Micro Systems Ltd., Surrey, England) at the following

conditions: pretest speed: 2 mm/s, test speed: 5.0 mm/s, post-test speed: 5.0 mm/s, and penetration distance: 10 mm. The resistance of penetration was measured as the necessary strength for a cylindrical probe of 4 mm of diameter and plane basis to penetrate a cylindrical sample of pear flesh of 2.00 cm height. Five pieces of each tray were analyzed. The reported values are the mean of 10 measurements ± SD.

## pH Determination

The pH of fresh-cut pears immersed in different dipping solutions was determined weekly using a pH-meter (Crison Instruments) for evaluating the influence of the added substances over this parameter as well as possible changes throughout the storage time. The reported values are the mean of five determinations (each one in duplicate) made throughout the storage time ($n = 10$) ± SD.

## Sensory Evaluation

Fresh-cut pears dipped in CGLW, CGLW + MA and W were used to carry out sensory analyses in a similar way as Raybaudi-Massilia *et al.* (2007). Samples of fresh-cut pears from dipping treatments were prepared to be evaluated just after processing ($t = 0$ day) and just after 15 days of storage at 5C. Thirty volunteers aged between 20 and 50 years who like and eat pear frequently did make the sensory tests. Twelve pear cylinders (two per dipping condition and per storage time) were randomly given to the panelists. Samples were coded with three-digit numbers and panelists scored acceptability of odor, color, taste, acidity and firmness characteristics on a structured 10-cm hedonic scale labeled from "extremely unpleasant" (0) to "extremely pleasant" (10). A glass containing potable water and pieces of nonsalted cracker were provided to panelists for eliminating the residual taste between samples.

## Predictive Modeling and Statistical Analysis

Growth of mesophilic, psychrophilic and yeasts and molds populations in non-inoculated fresh-cut pears were modeled according to the Gompertz equation modified by Zwietering *et al.* (1990) (Eq. 2), whereas the microbiological shelf life (*SL*) was calculated through Eq. (3), considering as maximum limit of mesophilic aerobic total count at expiry date $10^7$ cfu/g according to Spanish regulation for hygienic processing, distribution and commerce of prepared meals (BOE 2001). This limit was also considered for psychrophilic and yeast and mold populations.

$$Y = k + A \cdot \exp\{-\exp[(\mu_{max} \cdot 2.7182/A)(\lambda - t) + 1]\} \tag{2}$$

$$SL = \lambda - \dfrac{A\left\{\ln\left[-\ln\left(\dfrac{\log(10^7) - k}{A}\right)\right] - 1\right\}}{\mu_{max} \cdot 2.7182} \tag{3}$$

where $Y$ is the count of microorganisms ($\log_{10}$ cfu/g) for a given time, $k$ is the microorganism initial count estimated by the model ($\log_{10}$ cfu/g), $A$ is the maximum microorganism growth attained at the stationary phase ($\log_{10}$ cfu/g), $\mu_{max}$ is the maximal growth rate ($\Delta \log_{10}$ [cfu/g]/day), $\lambda$ is the lag time (days), $t$ is the storage time (days) and $SL$ is shelf-life time.

The experiments were carried out twice and a multifactor analysis of variance with posterior multiple range test was used to find significant differences ($P < 0.05$) among storage time and dipping condition on microbiological counts, headspace gas, firmness, color and sensory evaluation profile.

## RESULTS

### Effect of Dipping Condition and Storage Time on Pathogenic Bacteria Inoculated in Fresh-Cut Pears

Treatment condition noticeably influenced pH values of fresh-cut pears (Table 1). In addition, the populations of *L. monocytogenes*, *S. enteritidis* and *E. coli* O157:H7 were significantly ($P < 0.05$) affected by dipping conditions

TABLE 1.
pH VALUES OF FRESH-CUT PEARS INOCULATED OR NOT WITH *LISTERIA MONOCYTOGENES*, *SALMONELLA ENTERITIDIS* AND *ESCHERICHIA COLI* O157:H7 AND DIPPED IN AQUEOUS SOLUTIONS OF QUALITY STABILIZING COMPOUNDS WITH AND WITHOUT MALIC ACID

| Dipping condition | pH* | | | |
|---|---|---|---|---|
| | Non-inoculated | Inoculated with *L. monocytogenes* | Inoculated with *S. enteritidis* | Inoculated with *E. coli* O157:H7 |
| CGLW | 4.84 ± 0.07 | 4.75 ± 0.09 | 4.56 ± 0.14 | 4.61 ± 0.11 |
| CGLW + MA | 4.4 ± 0.4 | 4.0 ± 0.4 | 4.2 ± 0.4 | 4.0 ± 0.3 |
| W | 4.75 ± 0.14 | 4.65 ± 0.02 | 4.63 ± 0.02 | 4.70 ± 0.18 |

* Means of values obtained in five determinations in duplicate made throughout storage time ($n = 10$) ± SD.
CGLW, dipped in aqueous solution of N-acetyl-L-cysteine at 1% (w/v), glutathione at 1% (w/v) and calcium lactate at 1% (w/v); CGLW + MA, dipped in CGLW + malic acid at 2.5% (w/v); W, dipped in sterile distilled water.

immediately after processing ($t = 0$ day). Fresh-cut pears dipped in CGLW and W and then inoculated with *L. monocytogenes*, *S. enteritidis* or *E. coli* O157:H7 did not show significant changes ($P > 0.05$) in bacterial counts after processing ($t = 0$ day), whereas those fresh-cut pears dipped in CGLW + MA exhibited significant decreases ($P < 0.05$) in those populations (Fig. 1).

Characteristic colonies of *L. monocytogenes* and *S. enteritidis* were not recovered from fresh-cut pears treated with CGLW + MA after a recovery step in buffered peptone water for 20 min at 35–37C. Therefore, more than five log reductions of those pathogens could be reached after processing ($t = 0$ day). Nevertheless, a higher acid resistance of *E. coli* O157:H7 population to MA was observed, since a survival fraction of 3.84 $\log_{10}$ cfu/g of this microorganism was detected in those fresh-cut pears immersed in CGLW + MA just after processing (Fig. 1c).

Populations of *L. monocytogenes*, *S. enteritidis* and *E. coli* O157:H7 inoculated in fresh-cut pears immersed in different dipping conditions showed distinct behavior throughout the storage time. A slight growth of *L. monocytogenes* on fresh-cut pears dipped in CGLW (from 6.57 to 7.81 $\log_{10}$ cfu/g) or W (from 6.57 to 7.60 $\log_{10}$ cfu/g) was observed after 7 days of storage (Fig. 1a). On the other hand, fresh-cut pears dipped in CGLW + MA reduced the population of *L. monocytogenes* to undetectable levels just after processing, and its growth was not detected throughout the storage time. In contrast, populations of *S. enteritidis* and *E. coli* O157:H7 on fresh-cut pears were significantly reduced ($P < 0.05$) throughout the storage time at 5C, irrespective of the dipping solution used (Fig. 1b,c). A higher survival rate of the microorganisms was observed in those fresh-cut pears dipped in W, followed by those immersed in CGLW, whereas in CGLW + MA, populations of *S. enteritidis* and *E. coli* O157:H7 were undetectable from 0 to 14 days, respectively (Fig. 1b,c). Characteristic colonies of *E. coli* O157:H7 in fresh-cut pears dipped in CGLW + MA were not found after a recovery step at $t \geq 14$ days of storage; thus, more than five log reductions of this microorganism were achieved.

## Microbial Stability of Non-inoculated Fresh-Cut Pears

The growth of mesophilic and psychrophilic microorganisms, as well as yeasts and molds in non-inoculated fresh-cut pears dipped in CGLW, CGLW + MA and W stored at 5C for 30 days, was successfully modeled with the Gompertz's equation modified by Zwietering *et al.* (1990) (Eq. 2), since the coefficients of correlation ranged from 97.88 to 99.97% (Table 2). The behavior of those microbial populations under different treatment conditions is shown in Fig. 2, and parameters of Gompertz such as $K$, $A$, $\mu_{max}$ and $\lambda$ were provided in Table 2.
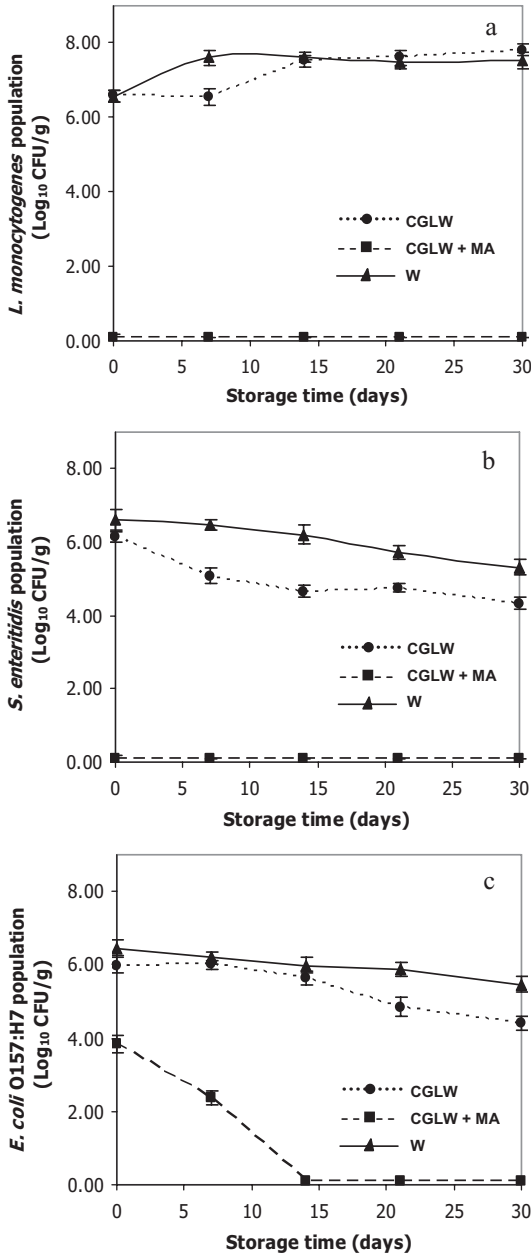
FIG. 1. BEHAVIOR OF *LISTERIA MONOCYTOGENES* (a), *SALMONELLA ENTERITIDIS* (b)
AND *ESCHERICHIA COLI* O157 : H7 (c) IN FRESH-CUT PEARS INOCULATED AFTER
DIPPING IN AQUEOUS SOLUTIONS OF N-ACETYL-L-CYSTEINE AT 1% (W/V),
GLUTATHIONE AT 1% (W/V) AND CALCIUM LACTATE AT 1% (W/V) (CGLW), CGLW
PLUS DL-MALIC ACID 2.5% (W/V) (CGLW + MA), AND DISTILLED WATER (W) PACKED
IN AIR AND STORED AT 5C DURING 30 DAYS
Values are means of four determinations ± SD.

TABLE 2.
GOMPERTZ PARAMETERS TO DESCRIBE THE GROWTH OF MESOPHILIC, PSYCHROPHILIC, YEASTS AND MOLDS POPULATIONS AND TO PREDICT THE MICROBIOLOGICAL SHELF LIFE OF NON-INOCULATED FRESH-CUT PEARS DIPPED IN AQUEOUS SOLUTIONS OF QUALITY STABILIZING COMPOUNDS WITH AND WITHOUT MALIC ACID

| Population | Dipping condition | $R^2$ | MSE | Gompertz parameters* | | | | Shelf-life† (days) |
|---|---|---|---|---|---|---|---|---|
| | | | | $k$ | $A$ | $\mu_{max}$ | $\lambda$ | |
| Mesophilic | CGLW | 99.71 | 0.014 | 2.40 ± 0.21 | 4.93 ± 0.29 | 0.279 ± 0.019 | 0.51 ± 0.23 | 19.31 |
| | CGLW + MA | 97.88 | 0.065 | 1.72 ± 0.29 | 3.8 ± 0.4 | 0.22 ± 0.04 | 2.02 ± 0.43 | >30 |
| | W | 99.97 | 0.002 | 2.79 ± 0.10 | 5.75 ± 0.13 | 0.319 ± 0.007 | 0.04 ± 0.02 | 8.92 |
| Psychrophilic | CGLW | 99.72 | 0.008 | 2.13 ± 0.12 | 3.44 ± 0.15 | 0.32 ± 0.03 | 1.3 ± 0.9 | >30 |
| | CGLW + MA | 99.77 | 0.005 | 1.12 ± 0.07 | 3.21 ± 0.12 | 0.189 ± 0.011 | 2.7 ± 0.7 | >30 |
| | W | 99.96 | 0.002 | 2.99 ± 0.06 | 5.63 ± 0.13 | 0.273 ± 0.006 | 0.7 ± 0.5 | 10.26 |
| Yeasts and molds | CGLW | 99.59 | 0.019 | 2.55 ± 0.27 | 5.0 ± 0.4 | 0.248 ± 0.019 | 0.20 ± 0.07 | 17.52 |
| | CGLW + MA | 99.74 | 0.007 | 1.81 ± 0.16 | 3.55 ± 0.21 | 0.234 ± 0.016 | 0.23 ± 0.0.2 | >30 |
| | W | 99.88 | 0.005 | 2.95 ± 0.16 | 4.48 ± 0.19 | 0.392 ± 0.026 | 0.54 ± 0.08 | 11.15 |

* Values are means ± SE.
† Predicted values from Gompertz's equation (Eq. 3) using $10^7$ cfu/g as maximal limit for microbiological shelf-life.
CGLW, dipped in an aqueous solution of N-acetyl-L-cysteine at 1% (w/v), glutathione at 1% (w/v) and calcium lactate at 1% (w/v); CGLW + MA, dipped in CGLW + malic acid at 2.5% (w/v); W, dipped in sterile distilled water; $k$, initial bacterial counts estimated by the model ($\log_{10}$ cfu/g); $A$, maximum microorganisms growth attained at the stationary phase ($\log_{10}$ cfu/g); $\mu_{max}$, maximal growth rate ($\Delta \log_{10}$ [cfu/g/day]; $\lambda$, Lag time (days); $R^2$, correlation coefficient (%); MSE, mean Square Error.
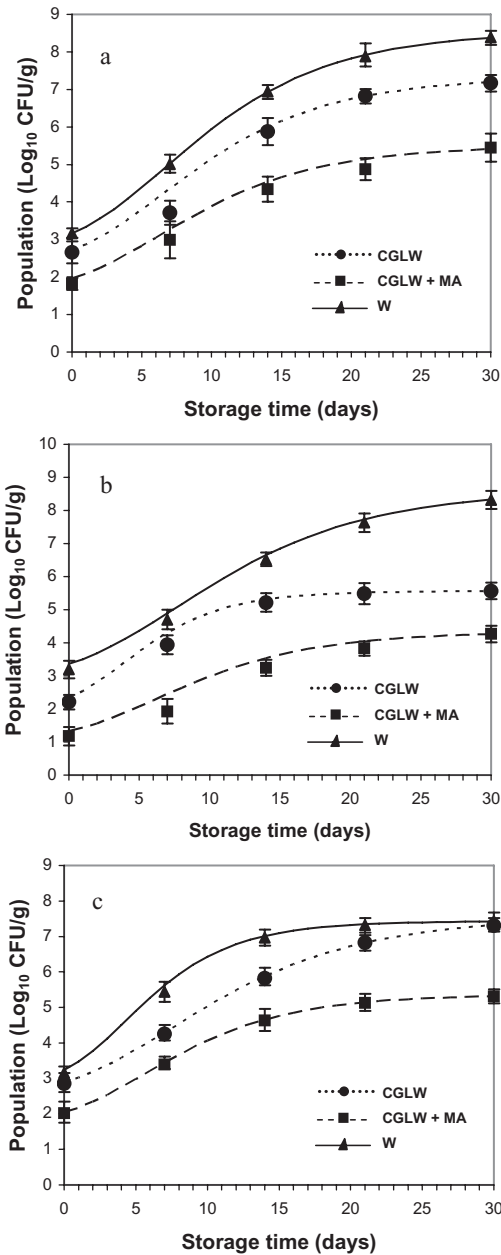
FIG. 2. INFLUENCE OF THE DIPPING CONDITION AND STORAGE TIME ON MESOPHILIC
BACTERIA (a), PSYCHROPHILIC BACTERIA (b), YEASTS AND MOLDS (c) GROWTH
($\log_{10}$ cfu/g) IN NON-INOCULATED FRESH-CUT PEARS DIPPED IN AQUEOUS SOLUTIONS
OF N-ACETYL-L-CYSTEINE AT 1% (W/V), GLUTATHIONE AT 1% (W/V) AND CALCIUM
LACTATE AT 1% (W/V) (CGLW), CGLW PLUS DL-MALIC ACID 2.5% (W/V) (CGLW + MA)
AND DISTILLED WATER (W) PACKED IN AIR AND STORED AT 5C DURING 30 DAYS
Values are means of four determinations ± SD.

Significant differences ($P < 0.05$) for each population (mesophilic, psychrophilic and yeasts and molds) were detected among dipping conditions. In addition, a statistically significant increase ($P < 0.05$) of the native flora in fresh-cut pears was observed throughout the storage time, irrespective of the dipping condition used (Fig. 2).

Mesophilic population ranged from 3.16 to 8.37 $\log_{10}$ cfu/g in fresh-cut pears dipped in W, from 2.72 to 7.19 $\log_{10}$ cfu/g in CGLW and from 1.97 to 5.40 $\log_{10}$ cfu/g in CGLW + MA (Fig. 2a) after 30 days of storage at 5C. Therefore, the mesophilic growth was more affected in fresh-cut pears dipped in CGLW and CGLW + MA than in those dipped in W (Fig. 2). Populations of mesophilic microorganisms reached a level of $10^7$ cfu/g in those fresh-cut pears dipped in W, CGLW and CGLW + MA after 8.92, 19.31 and >30 days of refrigerated storage, respectively (Table 2).

Quality stabilizing substances added into dipping solutions could delay the proliferation of the mesophilic and psychrophilic microorganisms and yeast and molds populations in comparison with those samples immersed in W, since lower $A$ and $\mu_{max}$ values as well as longer $\lambda$ were observed. Nevertheless, those parameters were even more affected with the addition of MA into the dipping solution (Table 2).

Shelf life of fresh-cut pears dipped in CGLW was mainly limited by yeasts and molds population (17.52 days) rather than mesophilic (19.31 days) and psychrophilic (>30 days) populations (Table 2). On the other hand, fresh-cut pears dipped in CGLW + MA kept the microbial growth below the permitted maximum limit for more than 30 days. Those results demonstrated that stabilizing and MA had a bactericidal effect which favored the yeast and molds growth in cut pears because of a lower competition among bacteria and yeasts and molds for the nutrients. Despite this, a more prolonged shelf life was accomplished with the addition of stabilizing compounds plus MA to the dipping solutions.

## Headspace Gas Changes

Decreases in the oxygen concentrations and increases in the carbon dioxide, ethanol and ethylene production in fresh-cut pears were observed. The changes were significantly affected ($P < 0.05$) by the dipping condition and storage time. A progressive decrease in the oxygen concentration of fresh-cut pears dipped in W was observed from $t = 0$ day until 30 days of storage (Fig. 3a). On the other hand, fresh-cut pears immersed in CGLW or CGLW + MA kept the oxygen concentration during the first 7 days of storage and then progressively decreased throughout the time (Fig. 3a). A higher carbon dioxide and ethanol production was also observed throughout the storage time in fresh-cut pears dipped in W than in those pear pieces immersed in CGLW or CGLW + MA (Fig. 3b,c).
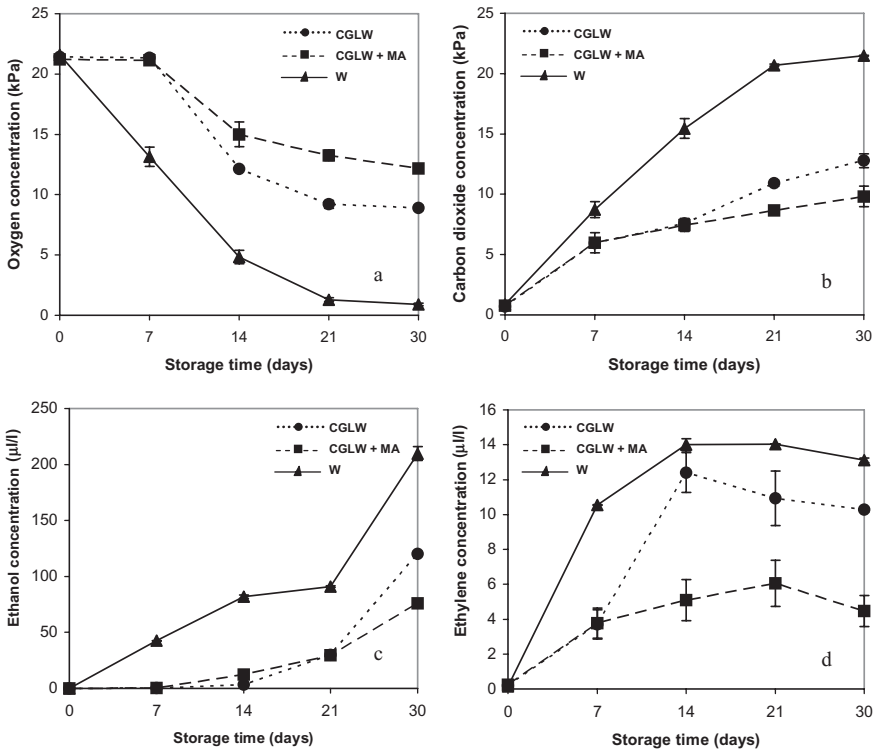
FIG. 3. CHANGES IN OXYGEN (a), CARBON DIOXIDE (b), ETHANOL (c) AND
ETHYLENE (d) CONCENTRATION IN HEADSPACE OF TRAYS WITH FRESH-CUT
PEARS DIPPED IN AQUEOUS SOLUTIONS OF N-ACETYL-L-CYSTEINE AT 1% (W/V),
GLUTATHIONE AT 1% (W/V) AND CALCIUM LACTATE AT 1% (W/V) (CGLW),
CGLW PLUS DL-MALIC ACID 2.5% (W/V) (CGLW + MA) AND DISTILLED WATER (W)
PACKED IN AIR AND STORED AT 5C DURING 30 DAYS
Values are means of four determinations $\pm$ SD.

Likewise, a significant ($P < 0.05$) increase of ethylene production during
the storage of fresh-cut pears was found, irrespective of the dipping condition.
In addition, ethylene production was influenced by the dipping condition as
smaller amounts of ethylene (Fig. 3d) were detected in the packages with
fresh-cut pears dipped in CGLW + MA (5.09 µL/L) and CGLW (12.4 µL/L) in
comparison with those cut-pears dipped in W (14.0 µL/L) at 14 days of
storage. From that time, ethylene concentration was maintained in fresh-cut
pears dipped in W, slightly decreased in fresh-cut pears dipped in CGLW
(10.27 µL/L), and continued to increase until 21 days (6.06 µL/L) and then
decreased (4.47 µL/L) (Fig. 3d) in fresh-cut pears immersed in CGLW + MA.

## Color Changes

Storage time did not have a significant effect ($P > 0.05$) on the lightness ($L*$) of fresh-cut pears; nevertheless, it was noticeably affected ($P < 0.05$) by the dipping condition. Higher values of lightness were found in fresh-cut pears dipped in CGLW or CGLW + MA than in those cut-pears immersed in W (Fig. 4a). On the other hand, both dipping condition and storage time influenced significantly ($P < 0.05$) the hue angle ($h°$). Hence, higher values of $h°$ just after processing ($t = 0$ day) were observed in fresh-cut pears dipped in CGLW + MA (100.35) or CGLW (99.87) in comparison with cut pears dipped in W (90.26) (Fig. 4b). In addition, a significant ($P < 0.05$) decreasing of $h°$ was observed after 14 days of storage in all dipping conditions, being more noticeable in fresh-cut pears dipped in W (Fig. 4b).

## Firmness Changes

Significant differences ($P < 0.05$) in fresh-cut pears firmness were observed among dipping conditions, showing fresh-cut pears immersed in CGLW and CGLW + MA higher values of firmness than those dipped in W at the sample preparation day ($t = 0$ day) (Fig. 5). On the other hand, changes in firmness of fresh-cut pears were negligible throughout the storage time when CGLW or CGLW + MA were used as dipping treatments; instead, when W was used, a decrease started at day 7 of storage (Fig. 5).

## Sensory Evaluation of Fresh-Cut Pears

Taste and acidity of fresh-cut pears dipped in CGLW and CGLW + MA were evaluated at 0 and 15 days of storage, whereas these attributes in fresh-cut pears immersed in W were not carried out at 15 days due to its high microbiological load. Fresh-cut pears dipped in CGLW + MA received the greater scores for those attributes (Fig. 6).

Significant differences ($P < 0.05$) in fresh-cut pears color dipped in W and those dipped in CGLW and CGLW + MA were detected from the same day of the preparation of samples ($t = 0$ day), with fresh-cut pears dipped in CGLW and CGLW + MA being the best accepted by panelists (Fig. 6). However, statistically significant differences ($P < 0.05$) in odor, color, firmness, taste and acidity among the same dipping treatment conditions were not detected by the panelists at 0 and 15 days of storage at 5C. Sensory results are in accordance with those found by instrumental determination, as lower lightness ($L*$) and $h°$ values were found in fresh-cut pears dipped in W in comparison with those found in fresh-cut pears immersed in CGLW + MA and CGLW from the sample preparation day (Fig. 4). In contrast, firmness differences among dipping conditions were not detected by the panelists (Fig. 6), whereas instrumentally, those differences were found (Fig. 5).
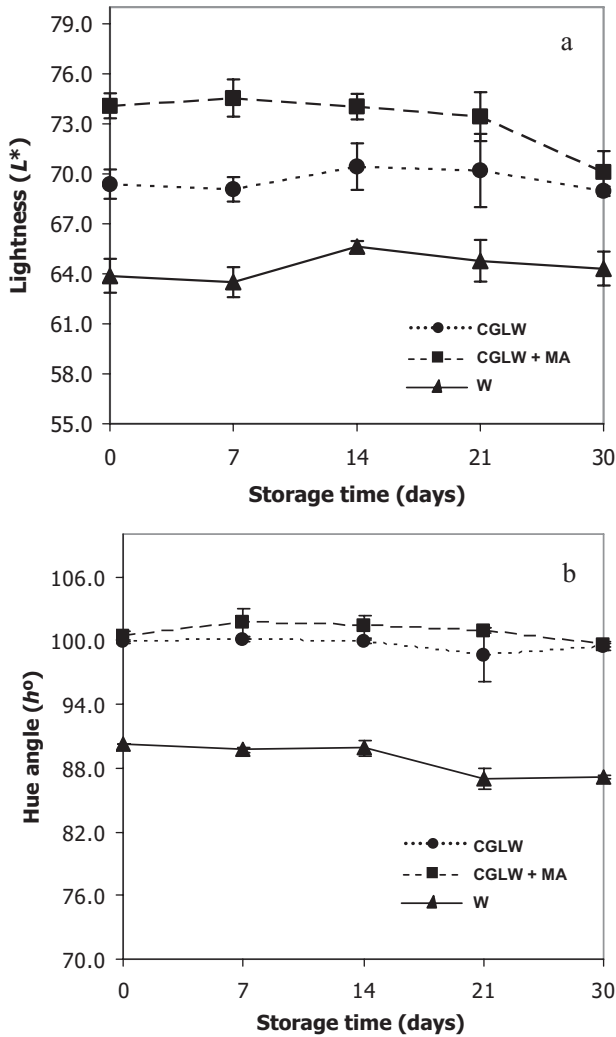
FIG. 4. CHANGES IN LIGHTNESS (*L*\*) (a) AND HUE ANGLE (*h*°) (b) OF FRESH-CUT
PEARS DIPPED IN AQUEOUS SOLUTIONS OF N-ACETYL-L-CYSTEINE AT 1% (W/V),
GLUTATHIONE AT 1% (W/V) AND CALCIUM LACTATE AT 1% (W/V) (CGLW), CGLW
PLUS DL-MALIC ACID 2.5% (W/V) (CGLW + MA) AND DISTILLED WATER (W) PACKED
IN AIR AND STORED AT 5C DURING 30 DAYS
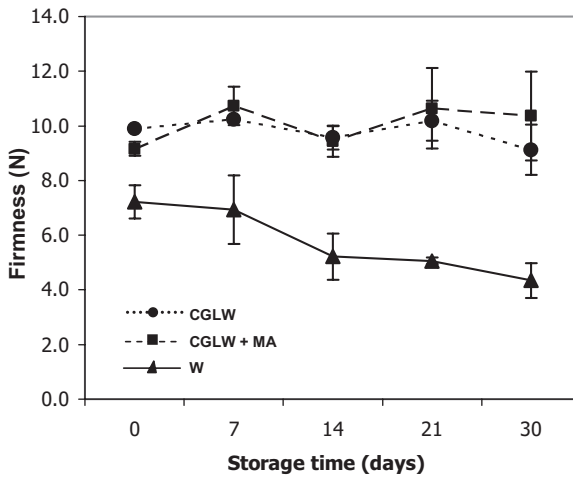Values are means of 30 determinations ± SD.

FIG. 5. CHANGES IN FIRMNESS (N) OF FRESH-CUT PEARS DIPPED IN AQUEOUS SOLUTIONS OF N-ACETYL-L-CYSTEINE AT 1% (W/V), GLUTATHIONE AT 1% (W/V) AND CALCIUM LACTATE AT 1% (W/V) (CGLW), CGLW PLUS DL-MALIC ACID 2.5% (W/V) (CGLW + MA) AND DISTILLED WATER (W) PACKED IN AIR AND STORED AT 5C DURING 30 DAYS
Values are means of 10 determinations ± SD.

## DISCUSSION

Immersion of fresh-cut pears in CGLW and W did not affect populations of *L. monocytogenes*, *S. enteritidis* and *E. coli* O157:H7 inoculated just after processing. This behavior was also reported by DiPersio *et al.* (2003), who indicated that populations of *Salmonella* inoculated in apple slices were not significantly ($P > 0.05$) reduced by immersion in W. However, those results differed from those found by Derrickson-Tharrington *et al.* (2005) who indicated a reduction about 0.9–1.0 log cfu/g of *E. coli* O157:H7 in fresh-sliced apples immersed in water for 10 min. This decrease is due probably to the processing and inoculation method used by those latter authors, as fresh-sliced apples were first inoculated with *E. coli* O157:H7 and then immersed in water. This fact suggests that microbial reduction could be caused by rinsing fresh-cut fruit in water. On the other hand, a higher reduction of *E. coli* O157:H7 (2.62 $\log_{10}$ cfu/g) in fresh-cut pears just after processing using CGLW + MA was found in our study in comparison with those reported by Derrickson-Tharrington *et al.* (2005) (0.9–1.3 $\log_{10}$ cfu/g) using acidic dipping solutions containing 2.8% ascorbic acid, 1.7% citric acid or 50% commercial lemon juice with or without preservatives (0.9–1.3 $\log_{10}$ cfu/g).
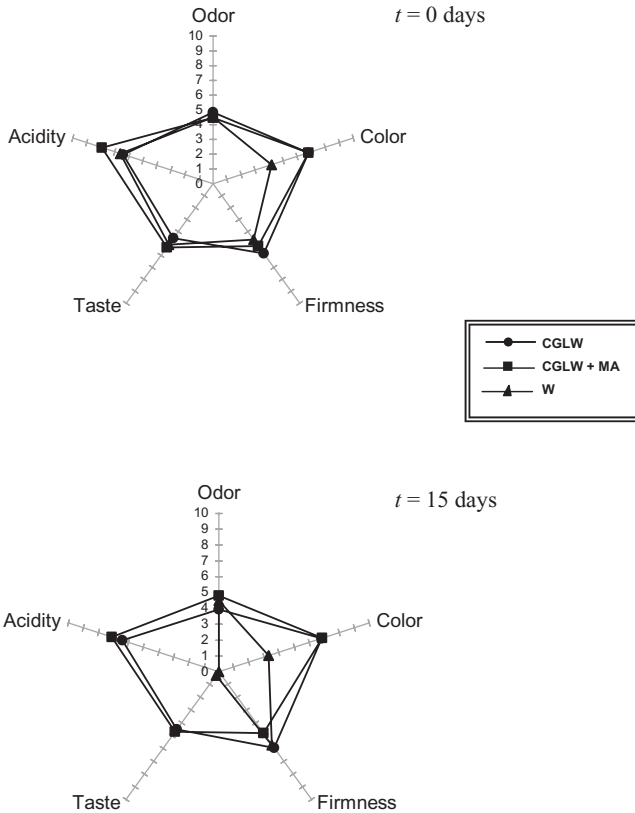
FIG. 6. INFLUENCE OF THE STORAGE TIME ON SENSORY CHARACTERISTIC OF
FRESH-CUT PEARS DIPPED IN AQUEOUS SOLUTIONS OF N-ACETYL-L-CYSTEINE AT
1% (W/V), GLUTATHIONE AT 1% (W/V) AND CALCIUM LACTATE AT 1% (W/V) (CGLW),
CGLW PLUS DL-MALIC ACID 2.5% (W/V) (CGLW + MA) AND DISTILLED WATER (W)
PACKED IN AIR AND STORED AT 5C DURING 15 DAYS
Values are means of 30 determinations.

Those differences could be a consequence of the kind of acid and concentration used.

Among the pathogenic microorganisms studied, *E. coli* O157:H7 resulted more resistant to MA than *L. monocytogenes* and *S. enteritidis*, as a higher survival rate of the first was observed just after processing ($t = 0$ day). Similar acid resistance of *E. coli* O157:H7 has been observed by other researchers (Benjamin and Datta 1995; Derrickson-Tharrington *et al.* 2005; Ingham *et al.* 2006). In such sense, Lin *et al.* (1996) indicated that three systems are involved in the acid tolerance of *E. coli* O157:H7, including an

acid-induced oxidative system, an acid-induced arginine-dependent system and a glutamate-dependent system.

MA is an organic acid of low lipid solubility (Leo *et al.* 1971) and, consequently, its entrance to the cell could be limited, as the cell membrane has low permeability to polar compounds (Lücke 2003). Therefore, its anti-microbial effect could be explained by a decrease in the medium pH as suggested by Beuchat and Golden (1989). However, some authors have found that effectiveness of the organic acids can vary depending on its molecular weight. Eswaranandam *et al.* (2004) indicated that smaller undissociated molecules of malic (134.09 Dalton) and lactic (90.08 Dalton) acids may enter more easily into the bacterial cells in comparison with citric (192.13 Dalton) and tartaric (150.09 Dalton) acids, which may not enter the inside of the cell effectively. Lou and Yousef (1999) indicated that the antimicrobial action of several organic acids is attributed to cytoplasm acidification, as undissociated organic acids can pass through the cell membrane and dissociate inside the cytoplasm, and interfere with metabolic processes of the microbial cell.

Nonetheless, our results demonstrated that not only does MA have a powerful bactericidal effect, but also, stabilizing substances such as CGLW incorporated into the dipping solution showed an antimicrobial effect. This effect could be observed on populations of *S. enteritidis* and *E. coli* O157:H7 throughout storage time on fresh-cut pears dipped in CGLW in comparison with W, being more significant for *S. enteritidis* population than *E. coli* O157:H7 (Fig. 1b,c), whereas population of *L. monocytogenes* was not significantly affected by those substances (Fig. 1a).

Reduction in counts of *S. enteritidis* and *E. coli* O157:H7 throughout storage in fresh-cut pears dipped in W suggests that other factors such as storage temperature (5C), competition of the native microflora or headspace gas composition of the trays might influence the pathogen survival. In this way, Raybaudi-Massilia *et al.* (2007) reported significant changes ($P < 0.05$) in headspace gas composition of fresh-cut apples dipped in W. These authors observed a meaningful decrease of $O_2$ and increase of $CO_2$ in those fresh-cut apples dipped in W during 30 days of storage at 5C. However, it is well known that *Salmonella* species and *E. coli* O157:H7 are facultative anaerobic micro-organisms, and thus, headspace gas composition could not be the main cause of reduction of those populations. Therefore, we consider that storage temperature and competition of the native microflora might be the main factors that caused the reduction on *Salmonella* and *E. coli* O157:H7 counts in fresh-cut pears dipped in W through storage, as Liao and Sapers (2000) reported a reduction of *Salmonella* Chester population in apple disks stored at 8C after 3 days of storage, and Raybaudi-Massilia *et al.* (2007) noted a significant growth of mesophilic, psychrophilic and yeasts and molds populations on fresh-cut apples during the storage. On the other hand, growth of *L.*

*monocytogenes* in fresh-cut pears dipped in W throughout the storage time was observed in comparison with the others pathogens, thus demonstrating the psychrotrophic nature of this microorganism (Lou and Yousef 1999).

The mesophilic flora detected in fresh-cut pears without substances (W) at day 0 appeared to be constituted by bacteria more than yeasts and molds, because counts of mesophilic and psychrophilic populations were higher than counts of yeasts and molds (Fig. 2). Jay *et al.* (2005) and Brackett (2001) reported that fruits may be able to support the bacteria, yeasts and molds growth due to their nutrient contents; however, the pH of the majority fruits may favor the growth of the latter microorganisms, with the exception of pears, which sometimes can undergo spoilage by bacteria as *Erwinia* spp. On the contrary those fresh-cut pears dipped in CGLW or CGLW + MA at day 0 appeared to be constituted in its majority by yeasts and molds (Fig. 2). Antibacterial effect, more than antifungal, of MA by reduction in pH of fresh-cut pears and stabilizing compounds would explain the differences found among the predominant flora in fresh-cut pears immersed in W and those dipped in CGLW and CGLW + MA.

Significant increases ($P < 0.05$) of mesophilic, psychrophilic and yeast and molds populations in fresh-cut pears were observed throughout the storage time, irrespective of the treatment condition. However, the growth of those populations was limited in those fresh-cut pears immersed in CGLW and CGLW + MA as consequence of added substances. A similar increase of the native flora in fresh-cut "Conference" pears throughout the storage time was reported by Soliva-Fortuny and Martín-Belloso (2003) and Soliva-Fortuny *et al.* (2004).

Results obtained in this study have demonstrated that substances (CGLW) used for avoiding the loss of quality of the fresh-cut pears from a physicochemical point of view had an additional antimicrobial effect. However, that antimicrobial effect was smaller than the effect caused by the addition of MA to the dipping solution. Jay *et al.* (2005) reported that, although the antioxidant agents are used in foods primarily to prevent the auto-oxidation of lipids, some of them, such as butylated hydroxyanisole, butylated hydroxytoluene, ethylenediaminetetraacetic acid, sodium citrate, lauric acid, monolaurin, etc. have showed to possess antimicrobial activity against a wide range of microorganisms.

About headspace gas composition of the fresh-cut pears trays, the consumption of oxygen and carbon dioxide production observed in those pear pieces treated with CGLW, CGLW + MA or W could be consequence of the respiration rate of the tissue, which continues being a living tissue even after cutting. This fact could also be due to the respiration rate of the native microbial flora, which goes on increasing throughout the storage time. Similar behavior in the headspace gases into trays has also been reported by

Soliva-Fortuny *et al.* (2004) in fresh-cut "Conference" pears. The decrease of oxygen levels into the fresh-cut pears trays treated with W leads to an anaerobic transformation of the pear sugars into alcohol (ethanol) and $CO_2$ by the native microflora, thus increasing the concentration of those gases into the trays (Fig. 3). However, a lower consumption of oxygen and lower carbon dioxide and ethanol production throughout the storage time in those fresh-cut pears dipped in CGLW and CGLW + MA was observed as an effect of the antimicrobial action of MA and stabilizing substances on the native microflora.

Ethylene production into trays demonstrates that pear pieces are living tissues and continue ripening after cutting, following a classic behavior of climacteric fruit (Wills *et al.* 1998). Moreover, during the pear processing, a tissue mechanical stress is produced, and in consequence, production of ethylene by the fruit could be induced (Pech *et al.* 2003).

The color of the fresh-cut pears was kept to the acceptable levels throughout the storage time regardless of the treatment used. However, pear pieces treated with CGLW + MA or CGLW showed higher lightness and $h°$ values than those fresh-cut pears dipped in W. The observed reduction in the color of fresh-cut pears without substances (W) could be caused by injury stress during processing, which produces a cellular decompartmentalization or delocalization of enzymes and substrates leading to various biochemical reactions that cause deteriorations such as browning, off-flavors and texture breakdown (Varoquaux and Wiley 1994). Reductions of lightness in fresh-cut pears caused by dipping conditions or storage time have also been reported by other researchers in different varieties of pears (Dong *et al.* 2000; Gorny *et al.* 2002; Soliva-Fortuny *et al.* 2002a; Oms-Oliu *et al.* 2006).

The results obtained in this study demonstrated the effectiveness of dipping the fresh-cut pears in solutions containing N-acetyl-L-cysteine and glutathione to control browning in the same way that Molnar-Perl and Friedman (1990) did in apples and potatoes, Rojas-Graü *et al.* (2006a,b) in fresh-cut "Fuji" apple and Oms-Oliu *et al.* (2006) in fresh-cut "Flor de invierno" pear. Molnar-Perl and Friedman (1990), Richard *et al.* (1991) and Richard-Forget *et al.* (1992) indicated that cysteine, N-acetyl-L-cysteine and glutathione can prevent browning by competitive reaction with polyphenol oxidase, reacting with the intermediate quinones to form stable colorless compounds. In addition, we found that incorporation of MA to the dipping solution helped to maintain better the color of the fresh-cut pears during storage time, thus demonstrating its antioxidant capacity. In this sense, Meyer *et al.* (2002) indicated that organic acids as citric, tartaric and malic may also prevent the browning of food by acting as metal chelators.

With regard to the firmness of fresh-cut pears, we observed that those pear pieces dipped in solutions containing calcium lactate (CGLW or CGLW + MA) better maintained the firmness in comparison with those pear

pieces dipped in W. It is well known that calcium plays a special role in maintaining the cell wall structure by interacting with the pectic acid to form calcium pectate, and its use is highly recommendable to minimize physiological disorders (Poovaiah 1986). Calcium chloride and calcium lactate have been used as antisoftening agents in cut apples, pears and melon with good results (Ponting *et al.* 1972; Dong *et al.* 2000; Luna-Guzmán and Barret 2000; Gorny *et al.* 2002; Soliva-Fortuny *et al.* 2002b,c, 2004; Rojas-Graü *et al.* 2006a). Nonetheless, some researchers have indicated that cut fruit treated with calcium lactate showed better flavor than those treated with calcium chloride (Ponting *et al.* 1972; Luna-Guzmán and Barret 2000).

## CONCLUSIONS

A combination of quality stabilizing compound such as CGLW + MA was effective in reducing populations of *L. monocytogenes* and *S. enteritidis* in fresh-cut pears by more than five log cycles just after processing, and just after 14 days of refrigerated storage for *E. coli* O157:H7. This fact demonstrates that, among the pathogenic microorganisms studied, *E. coli* O157:H7 was the most resistant to that dipping treatment; therefore, this pathogen may be considered as target microorganism in this kind of product. In addition, this combination of substances was also able to inhibit the native microflora growth.

Although the quality stabilizing substances used in this study were shown to possess antimicrobial activity against both pathogenic and spoilage microorganisms in fresh-cut pears, when MA was added to the dipping solution, the greatest bactericidal effect on these microorganisms was observed.

Physicochemical parameters such as color, firmness and gas composition of the headspace of fresh-cut pears dipped in CGLW + MA or CGLW were better maintained than in those fresh-cut pears immersed in W.

The results obtained in this research demonstrate that the safety and quality of fresh-cut pears could be assured by the use of CGLW + MA as dipping treatment in the fruit industry.

## ACKNOWLEDGMENTS

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