



Layer-by-layer edible coatings based on mucilages, pullulan and chitosan and its effect on quality and preservation of fresh-cut pineapple (*Ananas comosus*)



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ABSTRACT

Edible coatings (ECs) based on chitosan (CH), pullulan (PU), linseed (LM), nopal cactus (NM) and aloe mucilage (AM) were applied by layer-by-layer technique to preserve the quality and prolong the shelf-life of fresh-cut pineapple. Pineapples were washed, disinfected, dried and cut into 2 cm side cubes. Fresh-cut fruit was coated by dipping using four treatments (CH + PU, CH + LM, CH + NM and CH + AM), packed into polyethylene terephthalate containers and stored for 18 d at 4 °C. Uncoated fruit was used as control. Application of layer-by-layer ECs decreased ($P < 0.05$) the weight loss, pineapple softening, and retarded the fall on total soluble solids content and color (L^* and a^*). CH + AM EC was effective in delaying ($P < 0.05$) ascorbic acid degradation. In contrast, ECs did not affect titratable acidity ($P > 0.05$). Microbiological analyses demonstrated the effectiveness ($P < 0.05$) of the layer-by-layer ECs against spoilage microorganisms, *L. monocytogenes* and *S. typhi*. CH + PU EC was the most effective in controlling microbial levels. Sensory analysis demonstrated that layer-by-layer ECs helped to preserve ($P < 0.05$) the quality properties (color, odor, flavor, texture and overall acceptance). In conclusion, layer-by-layer ECs based on CH + PU, CH + LM, CH + NM and CH + AM improved the quality and prolonged the shelf-life of fresh-cut pineapple by six days compared with control.

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1. Introduction

Production of minimally processed fresh foods has increased recently due to the consumer demand. However, production and distribution of fresh-cut fruit has been limited due to their short shelf-life. Furthermore, processing operations such as washing, sanitizing, peeling, cutting, slicing, dicing or shredding and packaging (Corbo et al., 2010) can alter the integrity, safety, and decrease the quality and shelf-life of product, thus, limiting their marketing.

Pineapple (*Ananas comosus*) is a popular tropical fruit consumed worldwide (Montero-Calderón et al., 2008; Azarakhsh et al., 2012; Gabri et al., 2014). Fresh cut-pineapple is a good source

of antioxidants (vitamin C and phenolics compounds) and is characterized by its acid taste, aroma and juiciness (Mantilla et al., 2013; Azarakhsh et al., 2012; Montero-Calderón et al., 2008). Nevertheless, fresh-cut pineapple shelf-life is short (5–7 d at 4 °C), because processing operations damage the cell membrane (Russo et al., 2014; Gabri et al., 2014; Mantilla et al., 2013), increase metabolic activities (respiration rate, enzyme activity and ethylene production) and cause deterioration (tissue softening, browning, off-flavor among others) (Azarakhsh et al., 2014). Besides, the fresh-cut fruit is susceptible to microbial spoilage because of the absence of protective peel that facilitates the microbial adhesion to tissue, which contains nutrients (vitamins, minerals, sugars and other) and pH suitable for microbial growth (Mantilla et al., 2013; Corbo et al., 2010; Gabri et al., 2014; Russo et al., 2014).

Recently, some strategies such as ozone treatments, UV light, gamma irradiation, modified atmosphere packaging, films and

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edible coatings (ECs) have been developed to improve the shelf-life of fresh-cut pineapple (Montero-Calderón et al., 2010b; Mantilla et al., 2013; Benítez et al., 2014). An EC is a thin layer of edible material (proteins, lipids and polysaccharides) applied on the food surface, usually by immersion in liquid solutions (Campos et al., 2011; Falguera et al., 2011). Through their effects, these coatings play an important role in preservation and some of their functions are to protect from mechanical, physicochemical (water loss, deterioration in texture, changes in the content of soluble solids and acids, enzymatic browning and loss of vitamin C, among others) and microbiological (helps to minimize contamination and microbial growth) damage (Oms-Oliu et al., 2008; Falguera et al., 2011).

Layer-by-layer electrostatic deposition is a process that consists in the dipping of food into solutions that contain oppositely charged compounds (Mantilla et al., 2013; Brasil et al., 2012; Sipahi et al., 2013; Arnon et al., 2015). Layer-by-layer ECs have been studied in pineapple (Mantilla et al., 2013), papaya (Brasil et al., 2012), watermelon (Sipahi et al., 2013), cantaloupe (Martíñon et al., 2014; Moreira et al., 2014) and citrus (Arnon et al., 2015). The components frequently used in these studies include chitosan, sodium alginate, pectin, carboxymethylcellulose, calcium chloride and calcium lactate. Interestingly, the effect of layer-by-layer ECs based on chitosan and mucilages has not been reported to date. In addition, the technique is based on the use of oppositely charged polyelectrolytes that bond physically and chemically (Sipahi et al., 2013). Hence, the use of nonionic polymers such as pullulan has been poorly studied.

Chitosan is a cationic polymer consisting of 1→4-linked 2-amino-2-deoxy-β-D-glucose, derived from chitin (present in the shells of crustaceans). This compound has been found to be non-toxic, biofunctional, biodegradable and biocompatible, with bacteriocidal and fungicidal properties (Helander et al., 2001; Li et al., 2006; Chung and Chen, 2008). Pullulan is a neutral polysaccharide (non-ionic), water-soluble and non-toxic, consisting of maltotriose units linked by α-1,6-glycosidic bonds. Also, it is an excellent film-forming agent (Leathers, 2003). In addition, chitosan ECs have been utilized on fruits as strawberry to reduce the microbial growth and decay. On the other hand, pullulan ECs have been effective in preserving sensory properties such as color, odor, flavor and texture (Treviño-Garza et al., 2015).

Mucilages are common constituent of plants and can be extracted from soft stems, leaves or seeds, e.g. nopal (*Opuntia ficus*), sábila (*Aloe vera*) and linseed (*Linum usitatissimum*). Aloe mucilage (AM) is a thin colorless substance obtained from the inner portion of the leaves (Pandey and Mishra, 2010). AM consist of 99.5% water and 0.5% solids, such as proteins, lipids, amino acids, vitamins, enzymes, minerals, phenolic compounds, organic acids, and polysaccharides as pectic substances (anionic) and acemannan (neutral) (Avachat et al., 2011; Silva et al., 2013; Escobedo-Lozano et al., 2015; Benítez et al., 2015). These compounds possess antibacterial (*S. aureus* and *E. coli*), antifungal (*Aspergillus*, *Cladosporium* and *Fusarium*) and antioxidant properties (due to the flavonoids, anthraquinones and tannins), among others (Pandey and Mishra, 2010; Chauhan et al., 2014; Escobedo-Lozano et al., 2015). In addition, AM has been used as an EC in grapes (Chauhan et al., 2014), apple (Song et al., 2013) and kiwifruit (Benítez et al., 2015), among others. These studies showed that *Aloe vera* ECs improve the quality of the fruit by reducing the browning, weight loss and decay. *Opuntia ficus* mucilage is obtained from nopal cladodes. According to McGarvie and Parolis (1981a, b), nopal mucilage (NM) is a complex polysaccharide (anionic) which consists of alternating galacturonic acid, linked 1→2 to rhamnose residues linked 1→4 with branching on C4. Branches are galactose residues which carry sugars such as xylose and arabinose as substituents. NM has been used as ECs in strawberry to preserve

firmness and sensory properties (Del-Valle et al., 2005). Finally, linseed mucilage (LM) is a mixture of acidic and neutral polysaccharides. According to Muralikrishna et al. (1987), LM is composed of two fractions. Acidic fraction is an anionic rhamnogalacturonan [(1→2)-linked α-L-rhamnopyranosyl and (1→4)-linked α-D-galactopyranosyluronic acid residues] with side-chains of fucose and galactose residues. Neutral fraction is an arabinoxylan [(1→4)-β-D-xylan] which arabinose and galactose residues are attached at positions O-2 and O-3. LM has been used in food industry as stabilizer and thickener agent, among others applications (Kaewmanee et al., 2014). Nevertheless, this polysaccharide has not been widely studied as an EC.

Hence, the aim of this study was to assess the effectiveness of layer-by-layer ECs based on chitosan, pullulan, and mucilages to preserve the quality and prolong the shelf-life of fresh-cut pineapple.

2. Materials and methods

2.1. Fruit

Pineapples were bought at a local market (Mercado de Abastos Estrella, San Nicolas de los Garza, Nuevo León, México) and selected based on size, color, without signs of damage or decay. Fruit with at least 12% of total soluble solids was considered as commercially ripe.

2.2. Coating materials

Linseed, nopal cactus and *Aloe vera* used for the extraction of mucilages were purchased at a local market. Chitosan (crab shell, 85% deacetylation) was obtained from Sigma-Aldrich (St. Louis MO, USA). Pullulan (90%) was supplied from Hayashibara Co. (Okayama Japan) and glycerol (99.5% purity) was purchased from Analytika®.

2.3. Mucilages extraction procedures

NM was extracted based to previous reports (Rodríguez-González et al., 2014), with some modifications. Nopal cladodes were blended for 30 min with distilled water in a ratio of 1:1 (w/v). Suspension was maintained at 90 °C for 30 min and then centrifuged at 1500 × g for 20 min. 96% ethanol was added to supernatants in a ratio of 2:1 (v/v). Precipitate mucilage was separated by centrifugation at 1500 × g for 20 min. Finally mucilage was dried for 24 h at 70 °C and pulverized to obtain fine mucilage powder.

LM was obtained as previously reported (Wang et al., 2010; Qian et al., 2012), with modifications. Linseed was suspended in distilled water in a ratio of 1:30 (w/v) and mixed by shaking for 2 h at 25 °C. The viscous suspension was filtered with a strainer and the seeds were removed. 96% ethanol was added to the suspension (2:1 v/v). Precipitated mucilage was separated by centrifugation at 1500 × g for 20 min, dried for 24 h at 70 °C and pulverized to obtain fine mucilage powder.

AM was separated directly from the outer cortex of the leaf (Chauhan et al., 2014), then washed with sterile distilled water, and mixed in blender until a viscous solution was obtained.

2.4. Coatings solutions for layer-by-layer technique

Five different coating forming solutions were prepared in sterile distilled water until they were completely dissolved: NM (4.0% nopal mucilage plus 0.5% glycerol), LM (1.5% linseed mucilage plus 0.5% glycerol), and AM [aloe mucilage and water in a ratio of 1:1 (w/v) plus 0.5% glycerol]. Solutions based on CH and PU were elaborated as was established in our previous work (CH; 1.5%

chitosan plus 0.5% glycerol, and PU; 6.5% pullulan plus 0.5% glycerol) with modifications (without the incorporation of antimicrobial agents; Treviño-Garza et al., 2015).

2.5. Application of layer-by-layer edible coatings

Layer-by-layer technique was performed as previously reported (Mantilla et al., 2013; Brasil et al., 2012; Sipahi et al., 2013; Martiñon et al., 2014; Poverenov et al., 2014). The selected fruit was disinfected by soaking in chlorinated water (250 mg kg^{-1}) for one minute, washed with distilled water and dried with paper towels. Pineapples were then cut into 2 cm side cubes. All materials (surfaces and utensils) in contact with the fruit were previously sanitized. Fresh-cut fruit was coated using layer-by-layer technique evaluating four different treatments (two solutions per treatment were used) PU+CH, LM+CH, NM+CH and AM+CH. Pineapple cubes were dipped into each solution for 2 min (PU, LM, NM and AM) discarding the excess of coating by draining for 2 min before submerging the sample into the next solution (CH). Coated fruit was then dried inside a laminar airflow cabinet (Biobase) at room temperature for 20 min. Uncoated fresh-cut fruit was used as control. Finally, coated and uncoated samples (500 g) were packed into plastic containers (polyethylene terephthalate, 32 oz/946 mL) with lid, and stored for 18 d at 4°C (Fig. 1).

2.6. Physicochemical parameters

2.6.1. Weight loss, firmness (texture) and color

Weight loss (ten replicates for each treatment) was determined each 3 d, during 18 d of storage, using a digital balance (Mettler Toledo PG4002-S). Results were reported as percentage of weight loss, according to following equation: $\text{Weight loss (\%)} = \frac{[(\text{initial sample weight}) - (\text{final sample weight})]}{(\text{initial sample weight})} \times 100$ (Mantilla et al., 2013; Sipahi et al., 2013; Azarakhsh et al., 2014).

Firmness (expressed as N) was measured using a penetrometer (Exttech model FHT200) fitted with a 3 mm tip. Five replicates were used for each treatment.

Color measurements of fresh-cut pineapple were realized with a colorimeter (HunterLab, Colorflex[®] EZ), using the CIELAB scale, L^* ($L=0$ and $L=100$, black and white, respectively), a^* ($-a$ = green and $+a$ = red), and b^* ($-b$ = blue and $+b$ = yellow). Four replicates were used for each treatment.

2.6.2. Total soluble solids (TSS), titratable acidity (TA of citric acid), pH and ascorbic acid content

Chemical analyses (TSS, TA and pH) were carried out according to AOAC methodology. TSS were measured based on the method 932.14, using a refractometer (Exttech model RF15). TA was determined following the method 942.15. pH was measured (pH-meter, Beckman model 390) using the method 981.12 (AOAC, 1990). Finally, the determination of ascorbic acid was performed by titration with an iodine solution (0.01 mol L^{-1}) and expressed as g kg^{-1} by fresh weight. Tests were conducted in triplicate.

2.7. Microbiological parameters

Molds and yeasts, total aerobic and psychrotrophic microorganisms were enumerated in coated and uncoated samples every 3 d during the 18 d of storage. Fruit (10 g) was transferred to sterile plastic bag (Nasco Whirl-Pak[®], 18 oz/532 mL), mixed with sterile peptone solution (90 mL; 0.1% peptone and 0.85% NaCl) and homogenized for one minute. Serial dilutions were made and aliquots (one mL) of each dilution were plated onto Petri dishes containing potato dextrose agar (PDA, Bioxon) for mould and yeast enumeration, or plate count agar (PCA, Difco) for total aerobic and psychrotrophics enumeration (Mantilla et al., 2013; Sipahi et al., 2013; Martiñon et al., 2014; Poverenov et al., 2014). Plates for molds and yeasts were incubated for 5 d at 25°C , whereas the plates for total aerobic and psychrotrophic organisms were incubated for 2 d at 37°C and for 7 d at 4°C , respectively (Poverenov et al., 2014; Azarakhsh et al., 2014; Mantilla et al., 2013). After incubation, colonies were enumerated and results were expressed as colony forming units per g of fruit (CFU g^{-1}). Tests were conducted in triplicate.

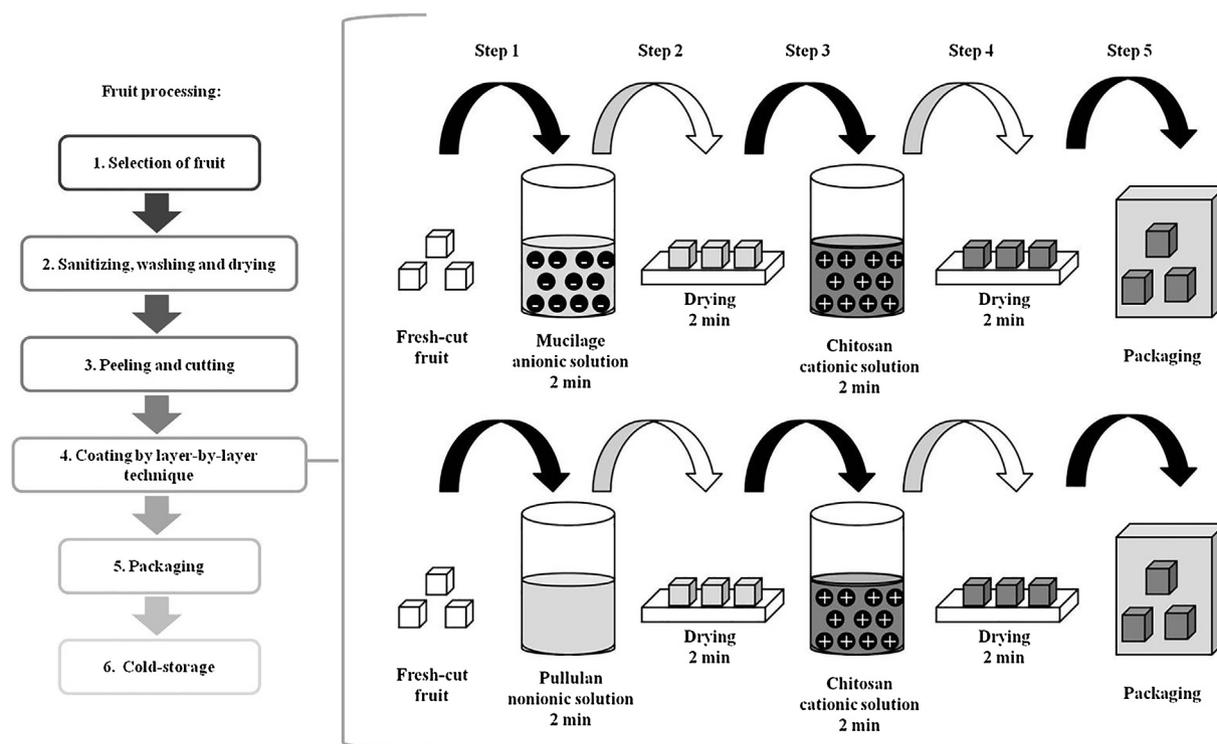


Fig. 1. Scheme of fruit processing and layer-by-layer technique.

2.7.1. Growth of pathogenic microorganisms

Listeria monocytogenes (ATCC 19114) and *Salmonella typhi* (ATCC 19430) were used in this study. Inoculum was prepared based to previous reports (; Alegre et al., 2010; Russo et al., 2014; Oliveira et al., 2014), with modifications. Bacterial strains were grown individually on brain heart infusion broth (BHI, BD Bioxon[®]) at 37 °C for 24 h, then the culture was centrifuged (2800 × g for 5 min) and resuspended in sterile saline solution (0.85% NaCl) to obtain a final concentration of 1×10^8 cells mL⁻¹. Viability of the microbial suspension was determined by plate counting on Oxford agar (Difco[™]) for *L. monocytogenes* and xylose lysine deoxycholate agar (XLD agar; Dibico) for *S. typhi*, followed by incubation at 37 °C for 2 d.

Inoculation of fresh-cut pineapple pieces (coated and uncoated) with pathogenic bacteria was carried out based on previous studies, with some modifications (; Alegre et al., 2010; Russo et al., 2014; Oliveira et al., 2014). Samples of fresh cut-pineapple (25 g) were placed in sterile plastic bags (Nasco, Whirl-Pak[®] 18 oz/ 532 mL, 11.5 × 23 cm, 0.064 mm thickness, oxygen and water vapor transmission properties of 3.76×10^{-3} cc mm⁻² day⁻¹ and 1.00×10^{-5} g mm⁻² day⁻¹, respectively) and individually inoculated with 0.1 mL of solution containing approximately 1×10^8 cells mL⁻¹ of each bacterium. The microbial load was monitored at 0, 2, 5, and 7 d on fruit stored at 4 °C. Oxford and XLD agar were used to enumerate *L. monocytogenes* and *S. typhi*, respectively. The plates were incubated for 2 d at 37 °C and the data were plotted as log CFU g⁻¹.

2.8. Sensory parameters

Sensory parameters (texture, flavor, color and acceptance) were evaluated by untrained panelists each 3 d during 18 d. Consumer panel consisted of students ($n=10$, men and women aged 20–25 years old) from our faculty. Coated and uncoated fruit was presented to consumers (in plastic containers) and assessed on a scale of 1–5 (unacceptable to excellent). Scores equal or higher to 2.5 were considered the limit of acceptability. Decay rate (sign of damage caused by mould and yeast growth on the fruit surface) was evaluated visually on a scale of 1–5, where; 1 = surface not damaged (0%) 2 = surface with slight damage (up to 25%), 3 = surface with moderate damage (25–50%), 4 = surface with severe damage (50–75%), and 5 = surface extremely damaged (75–100%). Samples with scores equal or higher to 2 were considered unacceptable. Results of sensory analysis were utilized to determine the shelf-life of product, as was established in our previous work (Treviño-Garza et al., 2015).

2.9. Statistical analyses

Data of the microbiological and physicochemical analyses were subjected to analysis of variance (ANOVA) and Tukey test. Data of sensory parameters were analyzed with Kruskal-Wallis and Mann-Whitney tests, using SPSS 17.0 software. Statistical significance was expressed at 95% level ($P \leq 0.05$).

3. Results and discussion

3.1. Physicochemical quality parameters

3.1.1. Weight loss, firmness and color

Weight loss in the fresh-cut fruit is due to mainly to water leakage. As shown in Fig. 2a, weight loss (by water leakage) increased throughout storage time in all treatments. Nevertheless, weight loss of uncoated fruit (14.15%) was higher ($P < 0.05$) at most times when compared to the coated fresh-cut fruit. After 18 d of storage, CH+NM and CH+AM ECs were the most effective to

reduce weight loss at values of 9.91 and 9.99%, respectively, followed by CH+LM and CH+PU ECs with values of 11.26% and 12.20% respectively. The beneficial effects of ECs can be due to the polymeric barrier created in the fruit surface, which reduces the water vapor transmission and therefore weight loss (Sipahi et al., 2013; Mantilla et al., 2013). Compared with values reported by Mantilla et al. (2013), who worked with multilayer-ECs (sodium alginate/pectin/calcium chloride/trans-cinnamaldehyde), the ECs based on CH+NM and CH+AM ECs showed a similar effect in reducing this parameter. The efficiency of CH+NM and CH+AM ECs is attributable to the water-binding capacity of mucilages (Gebresamuel and Gebre, 2012). In addition, CH+PU and CH+LM ECs showed higher weight loss, where the differences can be attributed to the number of layers (multilayer-ECs can further reduce the dehydration and weight loss) and type of polymers (formation of cross-linking, water-holding capacity, among others).

Firmness is another important factor for quality of fresh-cut fruit (Liu and Liu, 2014). As shown in Fig. 2, as weight loss increases, firmness decreases. Firmness of samples at day 0 (4.87–5.42 N) indicated that the coated samples presented slightly higher firmness compared to control, as previously reported by other authors (Benítez et al., 2014; Azarakhsh et al., 2014; Mantilla et al., 2013). After 3 day of storage, a significant decrease ($P < 0.05$) in firmness was observed in all treatments. However, control fruit was softer ($P < 0.05$) compared to the coated and, by day 18 of storage, control, CH+PU, CH+LM, CH+NM and CH+AM presented firmness values of 1.32, 2.60, 2.23, 2.58 and 2.61 N respectively.

In general, coated samples were firmer than control and no significant differences ($P > 0.05$) were observed between coated fruit throughout storage. According to previous reports, this “firming” effect is because of the cross-linking phenomenon of the polymers, which helps to reduce juice leakage (Brasil et al., 2012). Also layer-by-layer ECs form a physical and mechanical barrier that delays the respiratory metabolism and consequently decreases the water loss by dehydration and the degrading enzymatic activities (e.g. polyphenol oxidase) which are related to fruit softening (Brasil et al., 2012; Moreira et al., 2014). Results of firmness of fresh-cut pineapple obtained in this study are in agreement with Mantilla et al. (2013) and Azarakhsh et al. (2014) who studied the effects of multilayer-ECs (sodium alginate/pectin/calcium chloride) and, gellan and alginate-ECs, respectively. However, contrary to Benítez et al. (2013), our layer-by-layer ECs showed a positive effect in delaying fruit softening.

Regarding to color evaluations, application of layer-by-layer ECs did not affect the initial L* (74.65–78.44) and b* (38.05–43.40) values and no significant differences ($P > 0.05$) among all treatments were observed (Table 1). These values were similar to those previously reported by Benítez et al. (2014) and Azarakhsh et al. (2014). In the case of a* parameter, the initial values ranged from –1.59 to –0.88 and samples coated with CH+LM showed higher values due to the color of the coating (Table 1). Throughout storage, a significant decrease ($P < 0.05$) of L* parameter was observed in control, CH+PU and CH+NM coated samples, however, coated samples showed ($P < 0.05$) higher values (70.80 and 69.19, respectively) compared to control (62.99), which showed darkening (Table 1, Fig. 7). In contrast, L* parameter in CH+LM and CH+AM remained stable ($P > 0.05$) during the 18 d (71.45 and 72.95, respectively) similar to previous reports with alginate (Azarakhsh et al., 2014). Conversely, some studies indicate that ECs based on cassava starch (Bierhals et al., 2011) and alginate (Mantilla et al., 2013) not provided an effect on the luminosity parameter in fresh-cut pineapple. In addition, b* values decreased with time in all treatments ($P < 0.05$), although control and CH+NM coated samples showed higher values (35.51 and 35.15, respectively). According to Mantilla et al. (2013), the decrease of b*

in coated samples can be attributed to the thickness and to the polymer concentration in the coating. Finally, a^* values increased during storage time, where controls showed the higher values (5.72). In agreement with previous reports (Antonioli et al., 2003), the decrease of L^* and the increase of a^* (redness) indicates browning of the sample. As shown in Table 1 and Fig. 7 (days 6 to 18), control samples presented browned appearance (“brown spots”), while that CH+PU, CH+LM, CH+NM and CH+AM coated samples were not brown.

3.1.2. TSS, TA, pH and ascorbic acid content

TSS content is an indicator of ripeness stage since an increase is indicative of fruit ripening (Moreira et al., 2014). Initial TSS values fluctuated between 10.07 and 12.33% (Table 2), similar to those reported in previous studies (Mantilla et al., 2013; Benítez et al., 2013). During storage, TSS content increased ($P < 0.05$) in all treatments. CH+AM coated samples exhibited lower TSS values until day 15 ($P < 0.05$) compared with control (12.00 and 13.53 respectively), however, at day 18, these treatments presented

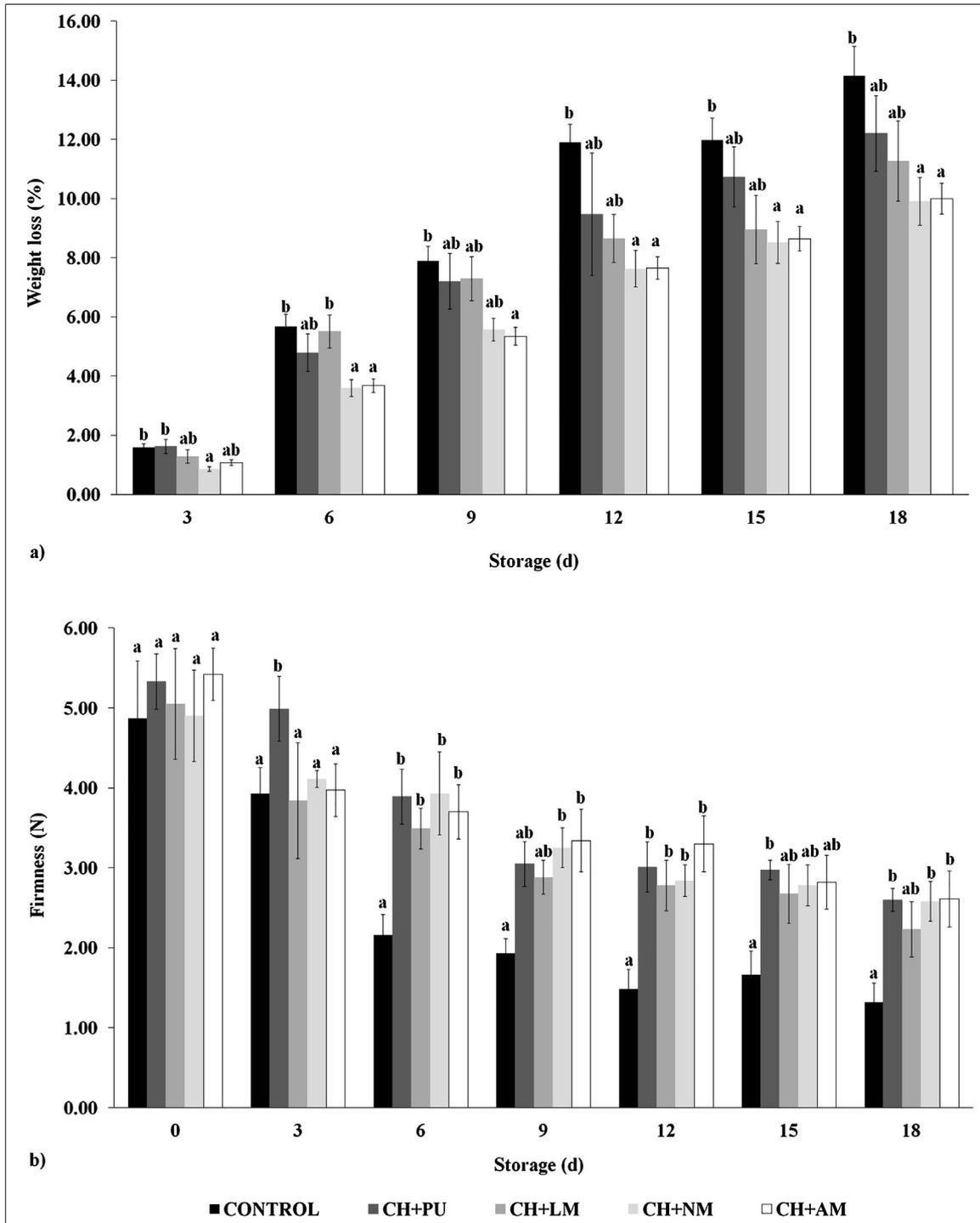


Fig. 2. Effect of layer-by-layer ECs on (a) weight loss ($n=10$) and (b) firmness ($n=5$) of fresh-cut pineapple stored for 18 d at 4°C, vertical bars represents \pm SD.

similar values (13.73 and 13.80%, respectively), whereas TSS of CH+PU, CH+LM and CH+NM treated samples ranged between 12.53–12.93%. A similar effect was reported by Chauhan et al. (2014) in grapes coated with *Aloe vera* gel. The beneficial effect of layer-by-layer CH+PU, CH+LM and CH+NM ECs to retard the changes in TSS could be due to gas barrier properties of these coatings against external environment, reducing the gas exchange, slowing down the metabolism because of the lesser amount of O₂ available and therefore delaying maturity (Moreira et al., 2014).

TA of fresh-cut pineapples (ranged between 0.62–0.73%, Table 2) was not affected ($P > 0.05$) by layer-by-layer ECs. TA values found in this study were similar to those previously reported for fresh pineapple (Bierhals et al., 2011; Montero-Calderón et al., 2008; Gabri et al., 2014). Throughout storage, TA decreased in all treatments (Table 2), however, no significant differences ($P > 0.05$) were found among coated and uncoated samples by the end of storage (0.42–0.49%). The decrease of this parameter is due to the transformation of acids in the fruit ripening process (Benítez et al., 2014).

Regarding to pH, application of CH+PU and CH+AM ECs did not affect ($P > 0.05$) the pH of the fruit (3.65–3.66) and the values obtained were similar to the control samples (pH=3.67). Nevertheless, CH+LM and CH+NM coated fresh-cut fruit presented a slightly higher pH value (3.75–3.76; Table 2) due to the pH of the coating forming solutions (LM=5.96, NM=4.99 and CH=4.00, data not shown), however these values were within a range reported for the fresh-cut pineapple (Montero-Calderón et al., 2008; Benítez et al., 2014; Russo et al., 2014). In fact, by day 18 of storage, pH values of CH+LM and CH+NM coated samples were higher (3.90–3.93; $P < 0.05$) when compared with control, CH+PU and CH+AM coated samples (3.77–3.85, Table 2). In general, there was a tendency to increase the pH values ($P < 0.05$) in all treatments, this behavior could be related with the decrease in the citric acid content of the fruit (Benítez et al., 2014).

Ascorbic acid content of coated and control samples ranged between 0.34–0.40 g kg⁻¹ (Table 2), these were in agreement with previous reports (Bierhals et al., 2011; Montero-Calderón et al., 2010a), however higher values have been reported by Mantilla et al. (2013). These differences could be attributed at factors such as cultivar, harvest season, and packaging conditions, among others (Liu and Liu 2014; Montero-Calderón et al., 2008). In agreement with previous reports, during storage, a decrease in ascorbic acid content was found in all treatments. CH+PU, CH+LM and CH+NM ECs did not prevent the loss of ascorbic acid and no significant differences ($P > 0.05$) with control were found by the end of storage (Mantilla et al., 2013). In contrast, CH+AM coated samples had higher ($P < 0.05$) ascorbic acid content at day 18 (0.22 g kg⁻¹) as previously found in kiwifruit coated with *Aloe vera* (Benítez et al., 2015).

3.2. Microbiological quality parameters

Microbiological evaluations showed that the application of layer-by-layer ECs reduced ($P < 0.05$) microbial levels. Molds and yeast counts at day 0 ranged between 1.08–2.68 log CFU g⁻¹ for coated samples and 3.07 log CFU g⁻¹ for control; the number of total aerobic microorganisms fluctuated between 1.36–1.60 log CFU g⁻¹ and 1.63 CFU g⁻¹, for coated and controls, respectively (Table 3). This reduction observed in coated samples can be attributed to the barrier formed around the fruit surface and to the antimicrobial activity of the polymers (Sipahi et al., 2013; Mantilla et al., 2013; Song et al., 2013). No psychrotrophic microorganisms were detected at the initial evaluation (Table 3).

During storage, microbial load increased significantly ($P < 0.05$) in all treatments and, by the end of storage, molds and yeasts, total aerobic and psychrotrophic microorganisms counts were higher for control than for the coated fruit (Table 3). On day 18, levels of yeast and molds ranged from 3.17–5.04 log CFU g⁻¹ for coated and 6.62 log CFU g⁻¹ for control fruit, total aerobic microorganisms

Table 1
Effect of layer-by-layer edible coatings on color of fresh-cut pineapple stored for 18 d at 4 °C.

Storage (d)	Control	CH+PU	CH+LM	CH+NM	CH+AM
L* (luminosity)					
0	^a 74.65 ¹ (0.44) ^C	^a 78.44 (1.07) ^B	^a 75.00 (1.50) ^A	^a 76.12 (1.07) ^B	^a 75.63 (0.99) ^A
3	^a 75.79 (0.97) ^{BC}	^a 77.01 (0.63) ^B	^a 73.30 (1.73) ^A	^a 71.89 (2.61) ^{AB}	^a 76.86 (0.80) ^A
6	^a 71.29 (1.61) ^{ABC}	^a 76.27 (1.62) ^{AB}	^a 73.89 (0.61) ^A	^a 72.43 (1.75) ^{AB}	^a 75.72 (0.85) ^A
9	^a 65.24 (2.61) ^{AB}	^b 73.99 (0.91) ^{AB}	^b 74.92 (0.39) ^A	^{ab} 71.81 (1.18) ^{AB}	^b 74.11 (2.28) ^A
12	^a 63.25 (3.25) ^A	^{bc} 74.79 (0.85) ^{AB}	^{bc} 73.01 (1.10) ^A	^{ab} 67.01 (1.85) ^A	^c 75.76 (0.72) ^A
15	^a 63.01 (2.38) ^A	^b 73.48 (0.61) ^{AB}	^b 71.34 (0.90) ^A	^{ab} 68.82 (1.74) ^{AB}	^b 73.41 (1.59) ^A
18	^a 62.99 (2.01) ^A	^b 70.80 (2.20) ^A	^b 71.45 (0.83) ^A	^{ab} 69.19 (1.64) ^{AB}	^b 72.95 (0.45) ^A
a* (green-red)					
0	^{ab} -1.37 ¹ (0.15) ^A	^{ab} -1.41 (0.16) ^A	^b -0.88 (0.09) ^A	^a -1.59 (0.14) ^A	^{ab} -1.17 (0.11) ^A
3	^c 0.67 (0.11) ^B	^{ab} -1.02 (0.12) ^{AB}	^{ab} -0.69 (0.22) ^A	^b -0.42 (0.14) ^B	^a -1.11 (0.11) ^{AB}
6	^c 1.43 (0.13) ^{BC}	^a -0.54 (0.12) ^B	^b 0.54 (0.11) ^B	^a -0.26 (0.09) ^B	^a -0.70 (0.09) ^B
9	^b 2.34 (0.26) ^{CD}	^a 0.35 (0.08) ^C	^a 0.52 (0.13) ^B	^a 0.56 (0.17) ^C	^a 0.27 (0.03) ^C
12	^b 2.98 (0.11) ^D	^a 0.32 (0.05) ^C	^a 0.61 (0.07) ^B	^a 0.83 (0.23) ^{CD}	^a 0.48 (0.11) ^{CD}
15	^b 3.78 (0.42) ^D	^a 0.76 (0.16) ^D	^a 0.74 (0.06) ^B	^a 1.18 (0.23) ^{CD}	^a 0.52 (0.10) ^{CD}
18	^b 5.72 (0.64) ^E	^a 1.08 (0.09) ^D	^a 1.04 (0.19) ^B	^a 1.56 (0.13) ^D	^a 0.74 (0.11) ^D
b* (blue-yellow)					
0	^a 42.40 ¹ (3.65) ^{AB}	^a 40.82 (2.55) ^C	^a 38.05 (1.67) ^{AB}	^a 43.40 (2.07) ^C	^a 40.64 (1.68) ^D
3	^a 44.45 (0.25) ^A	^a 37.77 (1.86) ^{BC}	^a 39.62 (2.78) ^B	^a 42.76 (1.36) ^{BC}	^a 38.39 (1.38) ^{CD}
6	^a 40.17 (1.47) ^{AB}	^a 35.92 (1.55) ^{ABC}	^a 36.00 (0.34) ^{ABC}	^a 40.84 (1.61) ^{ABC}	^a 37.79 (0.74) ^{BCD}
9	^b 39.52 (0.27) ^{AB}	^a 34.57 (0.88) ^{ABC}	^a 32.30 (0.10) ^{AB}	^b 39.05 (0.71) ^{ABC}	^a 33.43 (0.45) ^{AB}
12	^{bc} 38.25 (0.96) ^{AB}	^a 30.62 (1.05) ^A	^a 30.68 (0.84) ^A	^{bc} 36.51 (1.55) ^{ABC}	^{ab} 34.26 (0.77) ^{ABC}
15	^b 36.46 (1.76) ^{AB}	^{ab} 31.74 (0.63) ^{AB}	^a 30.98 (0.27) ^A	^{ab} 35.61 (1.34) ^{AB}	^{ab} 32.61 (0.79) ^A
18	^b 35.51 (0.18) ^A	^a 30.82 (0.96) ^{AB}	^a 30.31 (1.00) ^A	^b 35.15 (0.59) ^A	^a 30.06 (0.28) ^A

¹Standard deviation (n = 4).

^{a,b}Means within a row which do not have a common superscript letter, are significantly different ($P < 0.05$).

^{A,B}Means within a column which do not have a common superscript letter, are significantly different ($P < 0.05$).

Table 2

Effect of layer-by-layer edible coatings on chemical parameters of fresh-cut pineapple stored for 18 d at 4 °C.

Storage (d)	Control	CH + PU	CH + LM	CH + NM	CH + AM
TSS (%)					
0	^c 12.33 (0.07) ^A	^b 10.67 (0.15) ^A	^a 10.07 (0.07) ^A	^{ab} 10.47 (0.07) ^A	^b 10.73 (0.07) ^A
3	^c 12.07 (0.24) ^A	^{bc} 11.47 (0.18) ^B	^a 10.07 (0.07) ^A	^b 11.33 (0.07) ^B	^a 10.67 (0.07) ^A
6	^c 12.87 (0.37) ^B	^{ab} 11.27 (0.07) ^B	^a 10.93 (0.07) ^B	^{bc} 12.07 (0.07) ^C	^a 11.13 (0.07) ^{AB}
9	^c 13.07 (0.00) ^{BC}	^{ab} 11.60 (0.07) ^B	^a 11.23 (0.12) ^{BC}	^b 12.00 (0.34) ^C	^{ab} 11.80 (0.00) ^{BC}
12	^d 13.53 (0.07) ^{CD}	^{bc} 12.47 (0.07) ^C	^a 11.00 (0.07) ^B	^c 12.73 (0.00) ^D	^b 12.27 (0.18) ^C
15	^c 13.53 (0.00) ^{CD}	^a 12.13 (0.07) ^C	^a 11.93 (0.13) ^{CD}	^b 12.73 (0.07) ^D	^a 12.00 (0.07) ^C
18	^b 13.80 (0.07) ^D	^a 12.93 (0.12) ^D	^a 12.53 (0.07) ^D	^a 12.93 (0.13) ^D	^b 13.73 (0.07) ^D
TA (% citric acid)					
0	^a 0.62 (0.05) ^{CD}	^a 0.66 (0.02) ^B	^a 0.62 (0.02) ^{AB}	^a 0.62 (0.02) ^B	^a 0.73 (0.02) ^C
3	^a 0.65 (0.01) ^D	^a 0.64 (0.03) ^B	^a 0.67 (0.01) ^B	^a 0.57 (0.04) ^{AB}	^a 0.63 (0.04) ^{BC}
6	^b 0.61 (0.01) ^{CD}	^{ab} 0.58 (0.01) ^{AB}	^a 0.52 (0.01) ^A	^a 0.51 (0.01) ^{AB}	^{ab} 0.60 (0.03) ^{AB}
9	^{ab} 0.54 (0.02) ^{BC}	^b 0.58 (0.02) ^{AB}	^{ab} 0.53 (0.01) ^{AB}	^a 0.50 (0.01) ^A	^b 0.59 (0.02) ^{AB}
12	^a 0.49 (0.03) ^{AB}	^b 0.59 (0.02) ^{AB}	^{ab} 0.51 (0.02) ^A	^{ab} 0.51 (0.01) ^{AB}	^{ab} 0.54 (0.01) ^{AB}
15	^a 0.46 (0.03) ^{AB}	^a 0.50 (0.02) ^A	^a 0.50 (0.01) ^A	^a 0.48 (0.00) ^A	^a 0.52 (0.01) ^{AB}
18	^a 0.42 (0.02) ^A	^a 0.49 (0.00) ^A	^a 0.47 (0.05) ^A	^a 0.48 (0.02) ^A	^a 0.49 (0.02) ^A
pH					
0	^a 3.67 (0.01) ^{AB}	^a 3.65 (0.01) ^A	^b 3.75 (0.00) ^A	^b 3.76 (0.01) ^A	^a 3.66 (0.00) ^A
3	^b 3.68 (0.01) ^{AB}	^d 3.78 (0.01) ^C	^d 3.79 (0.00) ^{AB}	^c 3.75 (0.00) ^A	^a 3.65 (0.00) ^A
6	^a 3.64 (0.01) ^A	^b 3.72 (0.00) ^B	^c 3.79 (0.01) ^{AB}	^d 3.83 (0.00) ^B	^b 3.74 (0.02) ^B
9	^a 3.69 (0.01) ^{ABC}	^c 3.81 (0.00) ^{CDE}	^b 3.74 (0.01) ^A	^c 3.81 (0.01) ^B	^c 3.78 (0.01) ^{BC}
12	^a 3.73 (0.01) ^{BCD}	^b 3.80 (0.01) ^{CD}	^c 3.85 (0.02) ^{BC}	^c 3.89 (0.01) ^C	^{ab} 3.76 (0.01) ^{BC}
15	^a 3.75 (0.02) ^{CD}	^{bc} 3.84 (0.01) ^{DE}	^c 3.87 (0.01) ^C	^c 3.91 (0.03) ^C	^{ab} 3.78 (0.00) ^{BC}
18	^a 3.77 (0.01) ^D	^{ab} 3.85 (0.03) ^E	^b 3.90 (0.02) ^C	^b 3.93 (0.02) ^C	^a 3.80 (0.02) ^C
Ascorbic acid (g Kg⁻¹)					
0	^a 0.37 (0.01) ^C	^a 0.34 (0.02) ^B	^a 0.36 (0.01) ^C	^a 0.39 (0.04) ^C	^a 0.40 (0.02) ^C
3	^a 0.30 (0.03) ^{BC}	^a 0.35 (0.04) ^B	^a 0.30 (0.02) ^{BC}	^a 0.35 (0.03) ^C	^a 0.39 (0.03) ^C
6	^a 0.31 (0.03) ^{BC}	^a 0.34 (0.02) ^B	^a 0.28 (0.02) ^{ABC}	^a 0.34 (0.01) ^C	^a 0.36 (0.01) ^{BC}
9	^a 0.24 (0.02) ^{AB}	^a 0.33 (0.02) ^B	^a 0.29 (0.05) ^{ABC}	^a 0.32 (0.01) ^{BC}	^a 0.30 (0.01) ^{AB}
12	^a 0.20 (0.02) ^A	^b 0.28 (0.00) ^{AB}	^{ab} 0.26 (0.01) ^{AB}	^b 0.30 (0.00) ^{BC}	^b 0.27 (0.02) ^A
15	^a 0.19 (0.00) ^A	^{ab} 0.24 (0.02) ^{AB}	^a 0.18 (0.01) ^A	^{ab} 0.23 (0.02) ^{AB}	^b 0.26 (0.02) ^A
18	^a 0.17 (0.00) ^A	^a 0.18 (0.01) ^A	^{ab} 0.18 (0.01) ^A	^{ab} 0.19 (0.01) ^A	^b 0.22 (0.01) ^A

¹Standard deviation (n = 3).^{a,b}Means within a row which do not have a common superscript letter, are significantly different ($P < 0.05$).^{A,B}Means within a column which do not have a common superscript letter, are significantly different ($P < 0.05$).**Table 3**

Effect of layer-by-layer edible coatings on microbial growth of fresh-cut pineapple stored for 18 d at 4 °C.

Storage (d)	Control	CH + PU	CH + LM	CH + NM	CH + AM
Molds and yeasts (log CFU g⁻¹)					
0	^c 3.07 ¹ (0.04) ^A	^a 1.43 (0.38) ^A	^b 2.46 (0.05) ^A	^a 1.08 (0.18) ^A	^{bc} 2.68 (0.05) ^A
3	^c 3.58 (0.04) ^B	^a 2.39 (0.08) ^B	^a 2.46 (0.12) ^A	^b 2.89 (0.06) ^B	^b 2.69 (0.09) ^A
6	^b 4.50 (0.01) ^C	^a 2.57 (0.23) ^B	^a 2.98 (0.25) ^B	^a 2.96 (0.16) ^B	^a 3.00 (0.04) ^B
9	^d 4.89 (0.02) ^D	^a 2.67 (0.06) ^{BC}	^c 3.35 (0.04) ^B	^b 3.11 (0.03) ^B	^c 3.28 (0.02) ^C
12	^d 5.56 (0.03) ^E	^a 2.81 (0.13) ^{BC}	^c 4.12 (0.06) ^C	^b 3.44 (0.10) ^C	^b 3.48 (0.04) ^D
15	^d 6.59 (0.01) ^F	^a 2.84 (0.18) ^{BC}	^c 4.42 (0.18) ^C	^b 3.98 (0.04) ^D	^{bc} 4.14 (0.02) ^E
18	^e 6.62 (0.05) ^F	^a 3.17 (0.03) ^C	^d 5.04 (0.07) ^D	^b 4.03 (0.00) ^D	^c 4.63 (0.02) ^F
Total aerobic counts (log CFU g⁻¹)					
0	^b 1.63 ¹ (0.58) ^A	^a 1.36 (0.10) ^A	^b 1.60 (0.00) ^A	^{ab} 1.48 (0.00) ^A	^{ab} 1.52 (0.07) ^A
3	^d 2.55 (0.64) ^B	^a 1.30 (0.00) ^A	^{bc} 1.56 (0.70) ^A	^c 1.60 (0.00) ^A	^b 1.48 (0.00) ^A
6	^d 3.29 (0.65) ^C	^a 1.73 (0.46) ^B	^c 2.42 (0.66) ^B	^{ab} 1.90 (0.87) ^{AB}	^{bc} 2.16 (0.28) ^B
9	^c 3.38 (0.18) ^C	^a 2.16 (0.14) ^C	^c 3.08 (0.02) ^C	^{ab} 2.33 (0.03) ^{BC}	^b 2.49 (0.10) ^B
12	^c 3.98 (0.52) ^D	^a 2.59 (0.11) ^D	^b 3.54 (0.11) ^D	^a 2.70 (0.01) ^C	^a 2.56 (0.24) ^B
15	^c 4.12 (0.05) ^D	^a 3.25 (0.12) ^E	^{bc} 3.91 (0.06) ^E	^{ab} 3.46 (0.40) ^D	^a 3.25 (0.24) ^C
18	^c 4.67 (0.05) ^E	^a 3.58 (0.04) ^F	^b 4.04 (0.02) ^E	^{ab} 3.84 (0.20) ^D	^b 3.99 (0.01) ^D
Psychotropic (log CFU g⁻¹)					
0	^a ND ^A	^a ND ^A	^a ND ^A	^a ND ^A	^a ND ^A
3	^a 1.00 ¹ (0.00) ^B	^a ND ^A	^a 1.00 (0.00) ^B	^a 1.00 (0.00) ^B	^a ND ^A
6	^c 2.55 (0.09) ^C	^a ND ^A	^b 1.79 (0.25) ^C	^b 1.49 (0.43) ^B	^b 1.26 (0.24) ^B
9	^d 3.15 (0.45) ^D	^a 1.00 (0.00) ^B	^c 2.75 (0.15) ^D	^b 1.42 (0.10) ^B	^b 1.50 (0.17) ^B
12	^c 3.57 (0.03) ^E	^a 2.10 (0.17) ^C	^b 2.84 (0.10) ^D	^b 2.68 (0.07) ^C	^a 1.97 (0.07) ^C
15	^e 4.05 (0.07) ^F	^a 2.63 (0.02) ^D	^d 3.50 (0.11) ^E	^b 2.88 (0.83) ^C	^c 3.18 (0.06) ^D
18	^d 4.18 (0.07) ^F	^a 2.44 (0.25) ^D	^c 3.79 (0.05) ^E	^b 3.12 (0.05) ^C	^c 3.63 (0.01) ^E

¹Standard deviation (n = 3).^{a,b}Means within a row which do not have a common superscript letter, are significantly different ($P < 0.05$).^{A,B}Means within a column which do not have a common superscript letter, are significantly different ($P < 0.05$).

ND = Not detected.

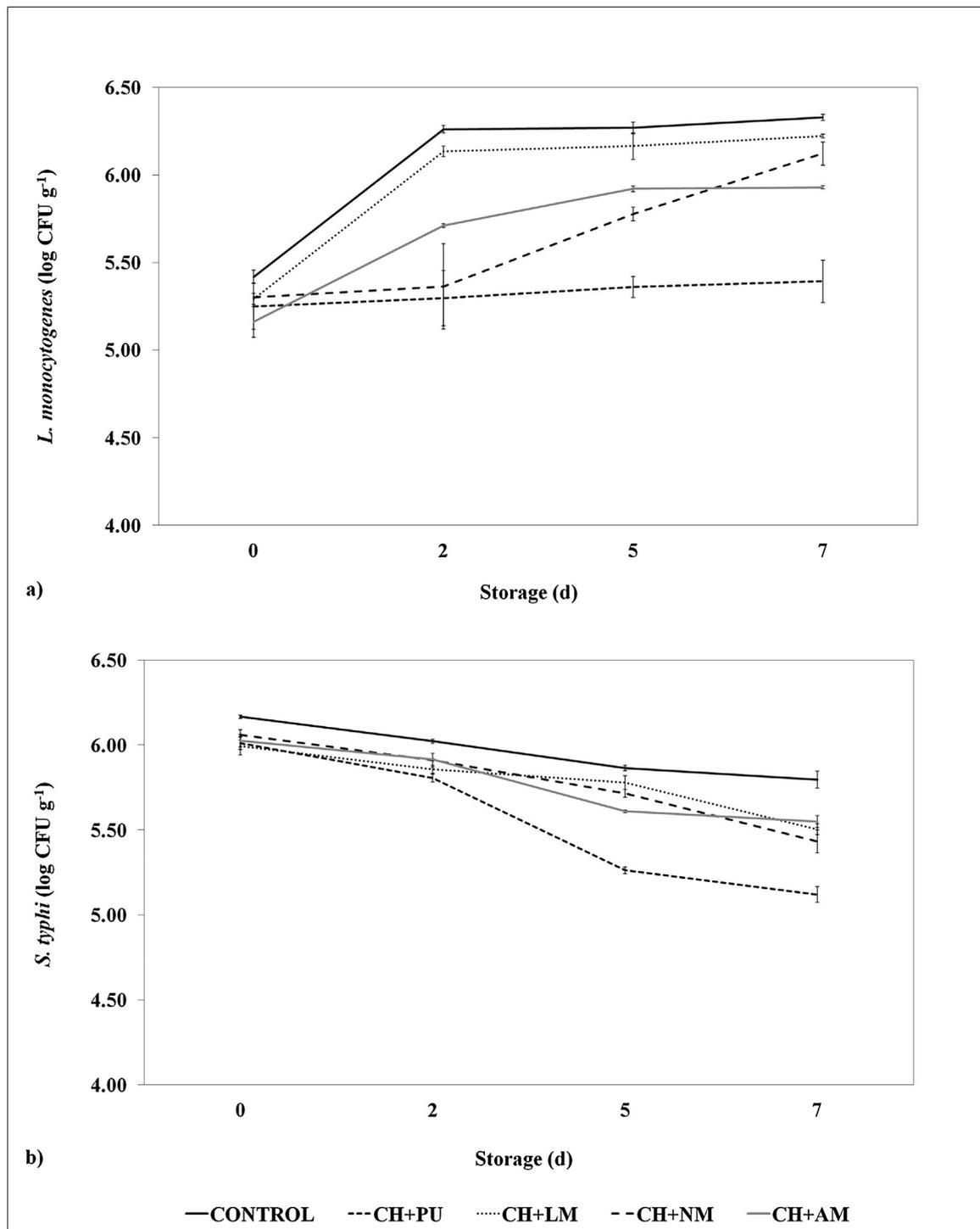


Fig. 3. Effect of layer-by-layer ECs on survival and growth of (a) *L. monocytogenes* and (b) *S. typhi* on fresh-cut pineapple after 7 d of storage at 4°C, vertical bars represents \pm SD (n=3).

ranged between 3.58–4.04 log CFU g⁻¹ (coated) and 4.67 log CFU g⁻¹ (control), and psychrotrophic microorganisms ranged between 2.44–3.79 log CFU g⁻¹ (coated) and 4.18 log CFU g⁻¹ (control).

Covers composed of CH+NM, CH+LM and CH+AM reduced microbial counts, similar to previously reported with chitosan-based ECs in cantaloupe (Moreira et al., 2014) and *Aloe vera*-based ECs in apple slices (Song et al., 2013), grapes (Chauhan et al., 2014) and kiwifruit (Benítez et al., 2015). In addition, to our knowledge, there have been no previous studies about microbiological growth

in fresh-cut fruit coated with NM and LM, and, the reason may be due to these polymers have not been widely studied as coatings.

Moreover, CH+PU was the most effective treatment to reduced microbial load, this can be attributed to the lack of interaction of the pullulan with the positively charged amino group of chitosan (Kandemir et al., 2005). According to previous reports (Helander et al., 2001; Liu et al., 2004; Li et al., 2006, 2007; Chung and Chen 2008), the amino group (NH₃⁺) of chitosan is responsible of its antimicrobial character. Free amine groups can interact with negatively charged molecules (e.g. phosphoryl groups in cell

membranes) present in some microorganisms, this interaction leading to the leakage of intracellular constituents and cell death. Hence, binding of these groups with anionics compounds (e.g. some proteins, inorganic polyelectrolytes or polymers such as alginate and pectin, among others) can reduce the antimicrobial activity (Chung and Cheng, 2008).

Although multilayer-ECs based on polymers with opposite charges are effective to improve the microbiological quality (Brasil et al., 2012; Martiñon et al., 2014; Moreira et al., 2014; Sipahi et al., 2013; Mantilla et al., 2013), our results suggest that the use of neutral polymers such as pullulan, could represent good alternative for bi or multilayer ECs; the absence of electrostatic interactions (formation of polyelectrolyte complexes) allows chitosan to maintain its antimicrobial activity. In comparison with results of Mantilla et al. (2013), in this study CH+PU ECs showed lower efficacy to reduce total aerobic, and similar activity in reducing psychrotrophic microorganisms; however it exhibited higher activity to reduce molds and yeasts growth in fresh-cut pineapples. In addition, CH+LM, CH+NM and CH+AM presented similar activity to reduce molds and yeasts, and lower ability to reduce psychrotrophic and total aerobic microorganisms. These differences may be associated with the polymer type, the number of layers and the incorporation of antimicrobial compounds (trans-cinnamaldehyde) into the formulation.

3.2.1. Growth of pathogenic microorganisms

Pathogenic bacteria can grow in fruit as melon, pear, apple (Oliveira et al., 2014), peach (Alegre et al., 2010), watermelon,

papaya (Penteado and Leitão, 2004) and pineapple (Russo et al., 2014). On the other hand, chitosan has antimicrobial activity against *L. monocytogenes* and *S. typhi* (Helander et al., 2001; Li et al., 2006). By contrast, the mucilages evaluated have no activity against these microorganisms. NM showed a slight activity against *L. monocytogenes* (data not shown).

As shown in Fig. 3a, initial *L. monocytogenes* counts ranged between 5.16–5.42 log CFU g⁻¹. During storage time, microbial load increased significantly ($P < 0.05$) in most treatments. However, *L. monocytogenes* was unable to grow under the CH+PU treatment (5.39 log CFU g⁻¹). When compared to the control (6.33 log CFU g⁻¹), growth of this microorganism at day 7 was reduced by CH+AM (5.93 log CFU g⁻¹) and slightly by CH+NM (6.12 log CFU g⁻¹) and CH+LM (6.22 log CFU g⁻¹). These results showed that fresh-cut pineapple can be an adequate substrate for growth of this microorganism even at low temperatures, and the ECs can reduce or inhibit growth (Penteado and Leitão, 2004; Russo et al., 2014).

Furthermore, viable counts of *Salmonella* (starting at 5.99–6.17 log CFU g⁻¹) during storage decreased significantly ($P < 0.05$) in all treatments (Fig. 3b). At days 5 and 7, CH+PU coated fruit showed the lower microbial load ($P < 0.05$); with values of 5.26 and 5.12 log CFU g⁻¹, respectively. In addition, microbial load of fruit coated with CH+NM, CH+LM and CH+AM ranged between 5.43–5.55 log CFU g⁻¹, while control samples presented the higher load ($P < 0.05$) with values of 5.80 CFU g⁻¹, by the end of storage. These results showed that *Salmonella* can survive but not grow at 4 °C in fresh-cut pineapple. Also, our results confirm that layer-by-layer ECs

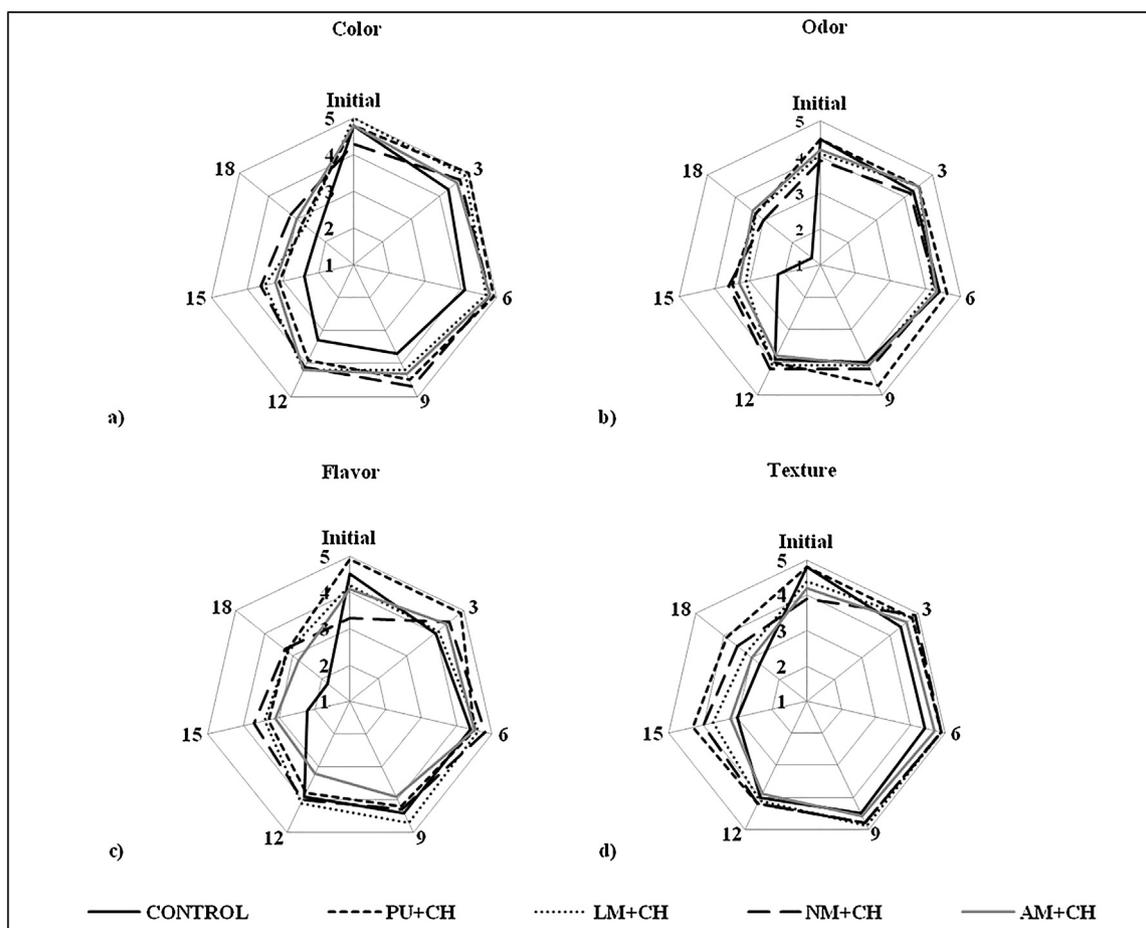


Fig. 4. Effect of layer-by-layer ECs on sensory quality parameters (a) color, (b) odor, (c) flavor and (d) texture of fresh-cut pineapple stored for 18 d at 4 °C. Scale 1–5 (unacceptable to excellent) (n = 10).

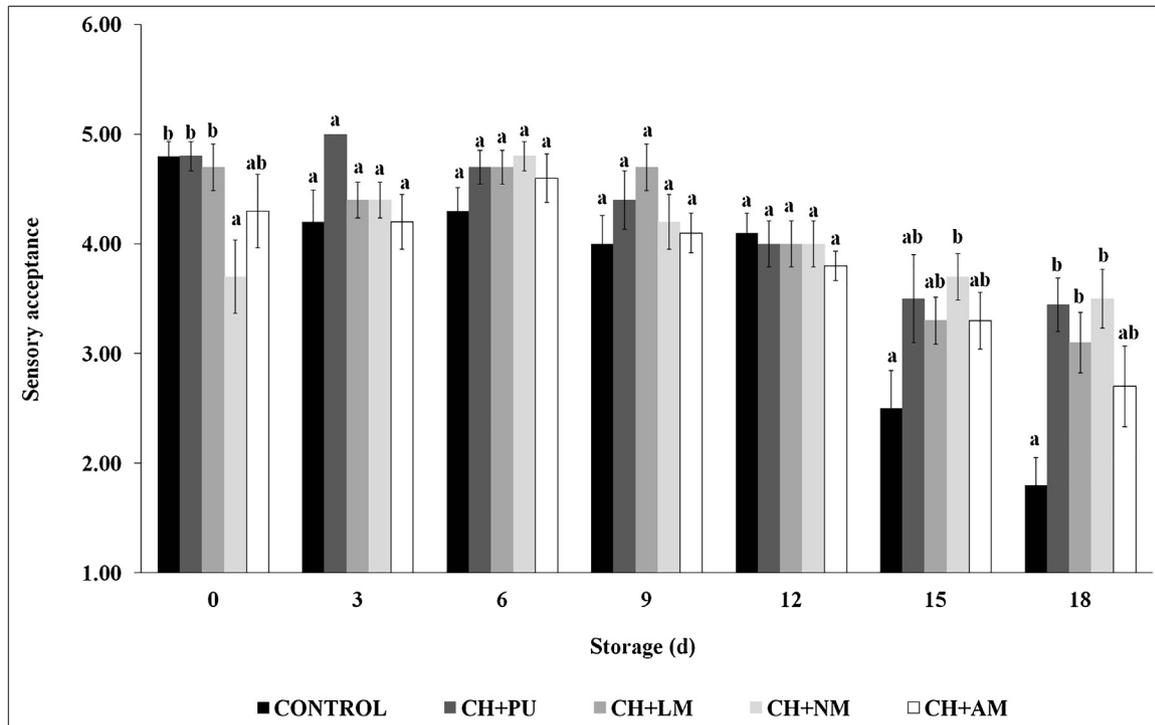


Fig. 6. Effect of layer-by-layer ECs on sensory overall acceptance of fresh-cut pineapple stored for 18 d at 4 °C, vertical bars represents \pm SD (n=10).

based on ionic and nonionic polymers, such as CH+PU, are effective to reduce the growth of pathogenic microorganism.

3.3. Sensory quality parameters

Sensory properties are a very important characteristic for coated fruit, and the materials used as coatings can impact in the

typical attributes of the product (Azarakhsh et al., 2014). Initial (day 0) sensory properties (color, odor and texture) of coated and uncoated fresh-cut fruit presented scores of good to excellent (4.30–5.00, 3.90–4.50, 3.90–4.80, respectively, Fig. 4). This indicates that initially the ECs did not affect these sensory characteristics, contrary to the data reported by Mantilla et al. (2013). Flavor evaluations ranged from 3.30 to 4.90, where the

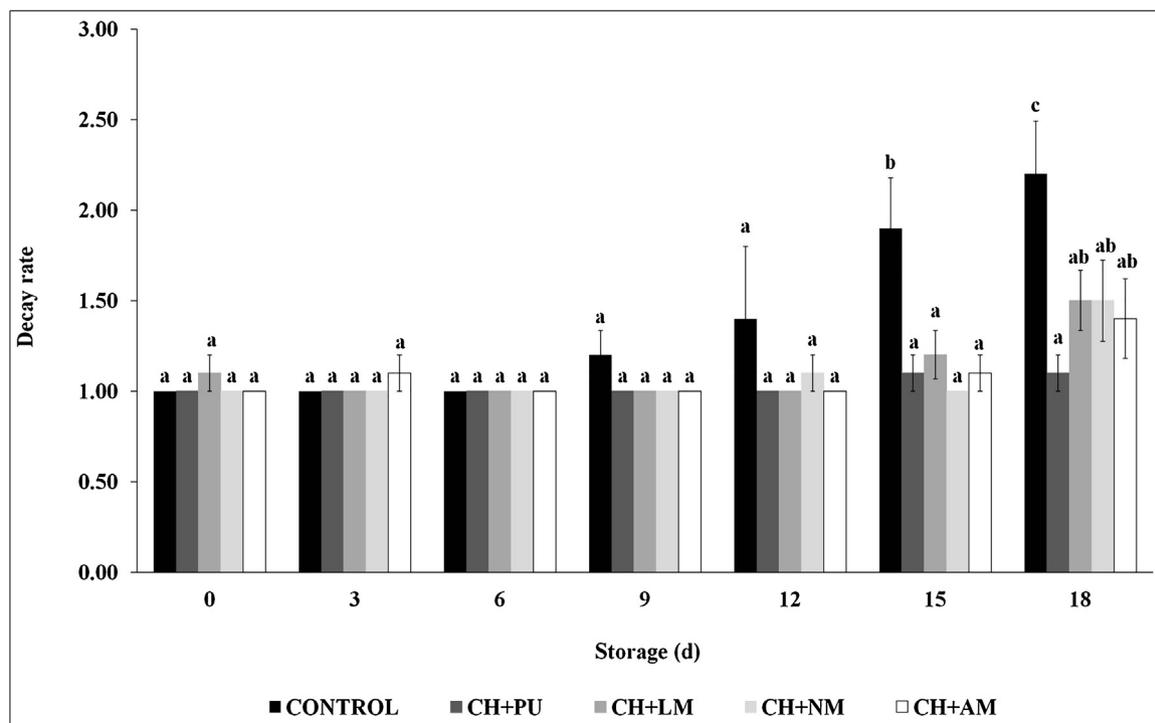


Fig. 5. Effect of layer-by-layer ECs on sensory decay rate of fresh-cut pineapple stored for 18 d at 4 °C. Scale 1–5 (0–100% damage), vertical bars represents \pm SD (n=10).

CH+NM coated fruit showed the lower initial scores ($P < 0.05$) because the consumers detected the NM flavor (Fig. 4c). Similar results were reported by Del-Valle et al. (2005) when analyzed ECs made with cactus-mucilage applied to strawberry. Initial overall acceptance fluctuated from 3.7 to 4.8; the higher values were obtained by CH+PU ECs and control; and again, CH+NM coat presented the lower scores ($P < 0.05$) (Fig. 6). All treatments presented scores of 1 on the initial decay rate (Fig. 5), indicating no apparent damage by molds.

Throughout storage, in terms of color, scores decreased in all treatments, especially in uncoated fruit ($P < 0.05$), and by day 18, coated samples presented the higher scores ($P < 0.05$; 2.7–3.2) when compared to control (2.3) where consumers identified mainly darkening and browning (Figs. 4 a and 7). Browning is mainly due to the oxidative degradation of ascorbic acid, and the action of the enzyme polyphenol oxidase on phenolic substrates, the result of these reactions is the generation of a brown coloration in the fruit (Oms-Oliu et al., 2008). Small changes in color parameters (a^* coordinate) are a good indicator of the absence of oxidative and enzymatic browning (Brasil et al., 2012). These results support observations in the color evaluations (Table 1) and also demonstrate the beneficial effect of layer-by-layer ECs (can act

as an oxygen barrier) to preserve this parameter. These results agree with studies conducted in pineapple (Mantilla et al., 2013), papaya (Brasil et al., 2012) and cantaloupe (Martíñon et al., 2014).

Odor remained stable during 12 d in all treatments, nevertheless, on days 15 and 18, control presented the lowest scores ($P < 0.05$; 2.20 and 1.30, respectively) probably due to microbial growth on the fruit surface (which cause off-odors) such as previously reported Martíñon et al. (2014) in fresh-cut cantaloupe. Also, formation of undesirable off-odors and off-flavors can be associated to ripening (sugar accumulation and consumption organic acids), degradation enzymatic, and production of volatile compounds thorough storage (Montero-Calderón et al., 2008, 2010b). Moreover, CH+PU, CH+NM, CH+AM and CH+LM coated samples presented the higher scores ($P < 0.05$; 3.00–3.40), preserving the original pineapple's odor (Fig. 4b), in agreement with previous reports (Benítez et al., 2013; Martíñon et al., 2014).

Flavor produced by CH+NM ECs in samples during the initial evaluation was not detected in subsequent analyses (Fig. 4c). On days 15 and 18, flavor scores decreased significantly ($P < 0.05$), however, coated fresh-cut pineapples exhibited the higher scores (2.80–3.30) compared to control (1.80). In general, the causes of off-flavor can be related to the microbial proliferation, sugars

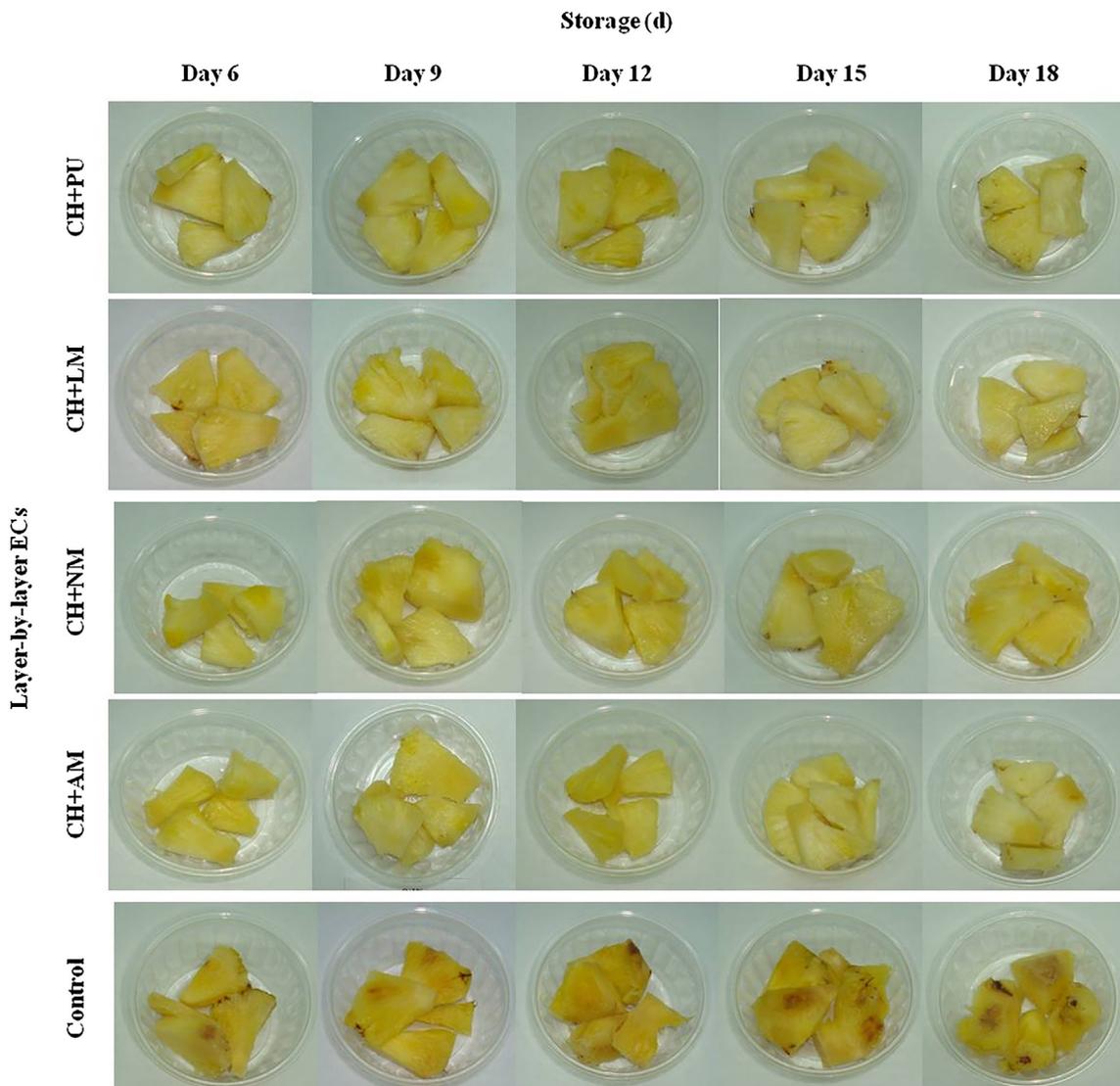


Fig. 7. Effect of layer-by-layer ECs on appearance of fresh-cut pineapple after 6, 9, 12, 15 and 18 d of storage at 4°C.

accumulation and increase of pH caused by transformation of organic acids (Montero-Calderón et al., 2008; Mantilla et al., 2013; Poverenov et al., 2014; Benítez et al., 2014).

Texture remained constant during 12 d, and declined thereafter from day 15 (Fig. 4d). By the end of storage, control samples presented the lower ($P < 0.05$) scores (2.70), whereas coated samples showed values from 3.00 to 3.89. The fresh-cut fruit softening may be due to the degradation of cell wall (due to enzymatic processes e.g. polyphenol oxidase and peroxidase), microbial growth (production of microbial hydrolytic enzymes), surface dehydration and the increase in respiration rates (Brasil et al., 2012; Moreira et al., 2014; Benítez et al., 2014; Liu and Liu, 2014); these processes support the observations found in texture, firmness, weight loss and microbiological evaluations (Fig. 2a,b and Table 3).

Moreover, decay rate remained constant for 9 d, however after day 12 of storage significant differences ($P < 0.05$) among treatments were observed (Fig. 5). By day 18, decay rate slightly increased in CH+LM, CH+NM, CH+AM coated fruit (1.40–1.50) while CH+PU coated samples remained constant ($P > 0.05$; 1.00–1.10, surface not damage) during all storage period. By the end of storage, uncoated fruit presented the higher decay rate scores [$P < 0.05$; 2.20, surface with slight damage (up to 25%)], indicating that ECs helped to delay damage by molds (Mantilla et al., 2013; Moreira et al., 2014; Brasil et al., 2012; Sipahi et al., 2013).

Finally, in terms of sensorial overall acceptance, the CH+PU, CH+NM, CH+LM and CH+AM coated fresh-cut pineapple was accepted by consumers during 18 d, and no significant differences ($P < 0.05$) were found between the different coatings by the end of storage. In contrast, control samples were accepted only for 12 d (Fig. 6). In comparison with data reported by Mantilla et al. (2013), the layer-by-layer ECs in this study extended the sensory acceptance by 6 more days.

4. Conclusions

Layer-by-layer ECs based on mucilages, pullulan and chitosan, helped to preserve the quality and increase the shelf-life of fresh-cut pineapple (*Ananas comosus*) by reducing the weight loss, pineapple softening, and by delaying alteration of TSS content and color (L^* and a^*). ECs application did not affect TA. CH+AM ECs were effective in reducing ascorbic acid degradation. CH+PU and CH+AM ECs did not affect the pH, and, samples coated with CH+LM, CH+NM presented only a slight increment in the pH throughout storage. Microbiological analyses demonstrated the effectiveness of the layer-by-layer ECs against spoilage microorganisms, *L. monocytogenes* and *S. typhi*. CH+PU EC was the most effective in controlling microbial levels. Finally, sensory evaluations demonstrated that all ECs also helped to preserve quality properties such as color, odor, flavor, texture, overall acceptance and delayed signs of decay. In general, layer-by-layer ECs based on CH+PU, CH+LM, CH+NM and CH+AM improved the quality and prolonged the shelf-life of fresh-cut pineapple for 6 days compared with control. Thus, it can be concluded that layer-by-layer ECs have potential application in the food industry to preserve the overall quality and extend the shelf-life of the fresh-cut pineapple.

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