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Recubrimientos comestibles y sustancias de origen natural en manzana fresca cortada: Una nueva estrategia de conservación

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EFFECT OF POLYSACCHARIDE-BASED EDIBLE COATINGS ON SHELF-LIFE OF FRESH-CUT FUJI APPLES

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ABSTRACT

The effect of alginate and gellan-based edible coatings on the shelf-life of fresh-cut Fuji apples packed in trays with a plastic film of a known permeability to oxygen (110 cm^3 O$_2$ m^{-2} bar^{-1} day^{-1}) was investigated by measuring changes in headspace atmosphere, color, firmness and microbial growth during 23 days of storage at 4 ℃. Concentration of O$_2$ and CO$_2$ in the package was measured and no significant differences between coated and uncoated fresh-cut apples were observed. Ethylene concentration in coated apples seemed to be delayed since it remained below 50 μl l^{-1} throughout the whole refrigerated storage

period, while production of this gas was detected in uncoated apples from the very initial days of storage. Coated apple wedges exhibited ethanol and acetaldehyde formation from the second week of storage indicating fermentative metabolism. Polymers were crosslinked with a calcium chloride solution, to which the antibrowning agent *N*- acetylcysteine was added, being incorporated into the coatings formulation and helping to maintain firmness and color of apples wedges during the entire storage time. The application of the edible coatings also retarded the microbiological deterioration of fresh-cut apples. Alginate and gellan edible coatings effectively prolonged the shelf-life of Fuji apples wedges by 2 weeks of storage compared with the control apple slices which showed a considerable cut surface browning and tissue softening from the very early days of storage, limiting their shelf-life to less than 4 days.

Key words: shelf-life, alginate, gellan, edible coatings, fresh-cut apples

INTRODUCTION

Overall quality and shelf life of fruits and vegetables is reduced by several factors including water loss, enzymatic browning, texture deterioration, senescence processes and microbial growth, among others. In the case of fresh-cut fruits, these events are accelerated due to lesions of tissues inflicted by peeling, slicing and cutting. Edible coatings have been used to reduce the deleterious effect brought about by minimal processing. The semipermeable barrier provided by edible coatings is aimed to extend shelf life by reducing moisture and solutes migration, gas exchange, respiration and oxidative reaction rates, as well as suppress physiological disorders on fresh-cut fruits (Wong, Camirand & Pavlath, 1994a; Baldwin, Nisperos, Chen & Hagenmaier, 1996; Park, 1999).

Edible coating, acting as a barrier to gases, is expected to generate a sort of modified atmosphere in each coated fruit piece, and along with relative humidity and optimum refrigeration temperature, contributes to achieve a reasonable shelf-life in fresh-cut products. Shelf-life extension may require delay of respiration and physiological process. Thus, the ability of films to modify gas transport has potential for applications in fresh-cut fruit and vegetables that are characterized by active metabolism even during refrigerated storage (Guilbert, Gontard & Gorris, 1996).

Alginate, a polysaccharide derived from a marine brown algae (*Phaeophyceae*) and gellan, a microbial polysaccharide secreted by the bacterium *Sphingomonas elodea* (formerly referred to as *Pseudomonas elodea*) are employed in the food industry as texturizing and

gelling agents (Mancini & McHugh, 2000; Yang & Paulson, 2000). Alginate and gellan are used as edible coatings because of their unique colloidal properties and their ability to form strong gels or insoluble polymers upon reaction with multivalent metal cations like calcium (King, 1983; Rhim, 2004).

Plasticizers like glycerol are required for polysaccharide and protein-based edible films to augment film flexibility and processability by increasing the free volume or molecular mobility of polymers reducing internal hydrogen bonding between polymer chains while increasing intermolecular spacing. Plasticizers affect the ability of the system to bind water and also generally increase film permeability to oxygen (McHugh & Krochta, 1994a, 1994b; Sothornvit & Krochta, 2000). The incorporation of lipids, either in an emulsion or as a layer coating into the films formulations, greatly improves their water vapor barrier properties (García, Martinó & Zaritzky, 2000; Yang et al. 2000).

Edible coatings may also serve as carriers of food additives such as antibrowning and antimicrobials agents, colorants, flavors, nutrients and spices (Pena & Torres, 1991; Wong, Gregorski, Hudson & Pavlath, 1996; Li & Barth, 1998; Pranoto, Salokhe & Rakshit, 2005). Sulfur-containing amino acids as *N*-acetyl-cysteine, those have been widely studied in the search for sulfite substitutes and for improving shelf-life of minimally processed apples (Molnar-Perl & Friedman, 1990; Son, Moon & Lee, 2001) can also be incorporated into coatings, and aid in prevention of enzymatic browning, as reported recently by Rojas-Graü, Sobrino-López, Tapia & Martín-Belloso (2006a).

In this work, fresh-cut Fuji apples were coated with alginate or gellan films crosslinked with calcium chloride and containing *N*-acetylcysteine as antibrowning agent, and their effect on shelf-life extension of coated apples was investigated. Effects of the coatings on gas exchange, prevention of browning, texture changes and microbial decay were evaluated.

MATERIALS AND METHODS

Materials

'Fuji' apples (*Malus domestica* Borkh) stored for 3 months under controlled atmospheres (2% O_2 and 2% CO_2 at 0 ºC) were provided by ACTEL, Lleida, Spain. Afterwards, apples were stored at 4 ± 1 ºC until processing. Food grade sodium alginate (Keltone® LV, ISP, San Diego, CA., USA) and gellan gum (Kelcogel®, CPKelco, Chicago, IL., USA) were used as the carbohydrate biopolymers for coating formulations. Glycerol (Merck, Whitehouse Station, N.J., USA) was added as plasticizer. Calcium chloride (Sigma-Aldrich Chemic, Steinhein, Germany) was used to induce crosslinking reaction. *N*-acetylcysteine (Sigma-Aldrich Chemic, Steinhein, Germany) was the added antibrowning agent. 0.025 % (w/v) of sunflower oil (La Española, Spain) with the following composition: 11g monosaturated, 30g monounsaturated and polyunsaturated 57.4g; 3.5g *omega*-3 and 55-60g *omega*-6, was used as the lipid source when emulsion films were prepared**.**

Preparation of the film forming solutions and dipping solutions

Film forming solutions were prepared by dissolving alginate (2 g/100 ml water) or gellan (0.5 g/100 ml water) powders in distilled water and heating at 70 ºC while stirring until the solution became clear. Glycerol was added as plasticizer at 1.5 g/100 ml alginate solution and 0.6 g/100 ml gellan solution, respectively. Film-forming solutions were emulsified with sunflower oil (0.025 g/100 ml film forming solution) which was dispersed using an Ultra Turrax T25 (IKA® WERKE, Germany) with a S25N-G25G device, for 5 min at 24,500 rpm, and degassed under vacuum. Emulsions were used for fruit coatings. *N*-acetylcysteine (1 g/100 ml) was added to the calcium chloride bath (2 g/100 ml water) required for the crosslinking of carbohydrate polymers. The concentrations of all ingredients used in these formulations were set up according to a previous work (Rojas-Graü, Tapia, Rodríguez, Carmona & Martín-Belloso, 2007).

Fruit coating

Apples were washed, rinsed and dried prior to cutting operations. Subsequently, apples were peeled, cored and cut into eight wedges. A maximum of 4 fruits were processed at the same time to minimize excessive exposure to aggressive conditions. The apple wedges were first dipped in water (control) or into the alginate or gellan film forming solutions for 2 minutes. Residual solutions of each polysaccharide were allowed to drip off for 1 min,

before submerging the coated fruits for 2 min in the solution of calcium chloride and *N*-acetylcysteine. Then, eight apple wedges were packaged into polypropylene trays of 500 cm^3 (Mcp Performance Plastic LTD, Kibbutz Hamaapil, Israel) and wrap-sealed using a 64 μm thickness polypropylene film with a permeability to oxygen of 110 cm^3 O_2 m^{-2} bar^{-1} day^{-1} at 23ºC and 0% RH (Tecnopack SRL, Mortara, Italy) using a MAP machine (Ilpra Foodpack Basic V/G, Ilpra, Vigenovo, Italy). Trays were filled with air; heat sealed and stored in darkness at 4 ± 1 ºC. Analyses were carried out periodically during 23 days for randomly sampled pairs of trays.

Headspace gases analysis

The atmosphere of each single tray was analyzed using a gas chromatograph equipped with a thermal conductivity detector (Micro-GC CP 2002 gas analyzer, Chrompack International, Middelburg, The Netherlands). The gaseous content of each tray was gently mixed prior to sampling and an adhesive septum was stuck to the film wrap. A 1.7 ml sample was automatically withdrawn from the headspace atmosphere. Portions of 0.25 and 0.33 ml 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, Middelburg, The Netherlands) (4 m x 0.32 mm, df = 10 mm) at 60 ºC and 100 kPa. For quantification of CO_2, ethylene (C_2H_4), acetaldehyde (C_2H_4O), and ethanol (C_2H_5OH), a Pora-PLOT Q column (Chrompack International, Middelburg, The Netherlands) (10 m x 0.32 mm, df = 10 mm), held at 70 ºC and 200 kPa, was used. Two trays were taken at each sampling time to perform the gases analysis and two replicates were carried out for each one.

Colour measurement

Cut apple surface color was directly measured with a Minolta chroma meter (Model CR-400, Minolta, Tokyo, Japan). The equipment was set up for illuminant D65 and 10º observer angle and calibrated using a standard white reflector plate. Ten replicates were evaluated for each pair of trays. Three readings were made in each replicate by changing the position of the apple wedges. Color was measured through changes in h* values. Numerical values of a* and b* parameters were employed to calculate hue angle (h^*):

$$h^* = \arctan b^*/a^*$$
<div align="right">Equation 1</div>

Firmness measurements

Apple firmness evaluation was performed using a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., England, UK) by measuring the maximum penetration force required for a 4 mm diameter probe to penetrate into apple cube of 20 mm height to a depth of 10 mm at a rate of 5 mm s^{-1}. Apple cubes, which were cut previously from apple wedges, coming in turn from ten samples randomly withdrawn from each pair of trays, were placed perpendicular to the probe so as to allow penetration in their geometric centre.

Microbiological analysis

The evolution of the microbial population of fresh-cut Fuji apples throughout storage was evaluated by the mesophilic aerobic and psychrophilic aerobic counts. A portion of 10 g of apple (taken from 8 different apple wedges) were removed aseptically from each tray and transferred into sterile plastic bags. Samples were diluted with 90 ml of saline peptone water (0.1 g peptone/100 ml water - Biokar Diagnostics, Beauvais, France + 0.85 g NaCl/100 ml water - Scharlau Chemie, S.A. Barcelona, Spain) and homogenized for 1 min in a stomacher blender (IUL Instruments, Barcelona, Spain). Serial dilutions were made and then pour plated onto plate count agar (PCA) (Biokar Diagnostics, Beauvais, France). Plates were incubated for 48h at 30 ºC to enumerated mesophilic and 5 days at 5 ºC for psychrophilic. Colonies were counted and the results expressed as CFU.g^{-1} of apples. Analyses were carried out periodically during 23 days in randomly sampled pairs of trays. Two replicate counts were performed for each tray.

Statistical analysis

Data were analyzed by analysis of variance using statistical procedures of the Statgraphics Plus V.5.1. Statistical Graphics Co., Rockville, MD, USA). Specific differences were determined by least significant difference (LSD). All comparisons were made at a 5% level of significance.

RESULTS AND DISCUSSION

Changes in headspace gas composition

A modified atmosphere can be created inside fresh fruits upon coating applications as a result of resistance to gas diffusion and reduction of respiration rate (Perez-Gago, Rojas & del Rio, 2003). Contrary to what was expected for O_2 and CO_2, no significant differences were observed between coated and uncoated apples wedges regarding the composition of these gases through the coatings along the evaluated period (Figure 1, Table 1). The permeability of the plastic film used to wrap the coated apple pieces contained in the polypropylene trays to O_2 and CO_2 was moderate (110 cm^3 O_2 m^{-2} bar^{-1} day^{-1} and 500 cm^3 CO_2 m^{-2} bar^{-1} day^{-1}) probably letting O_2 and CO_2 to pass through, preventing their accumulation in the head space and making difficult to allow an inference on the effect of the edible coatings as selective barriers to these gases.

Table 1. Variance analysis (ANOVA) of the studied parameters.

Parameters	*F-ratio*		
	Time	**Coating**	**Interaction Time-coating**
Head-space atmosphere			
O_2	724.02[*]	1.61 [n.s]	2.15 [n.s]
CO_2	852.86[*]	5.31 [n.s]	2.09 [n.s]
C_2H_4	136.89[*]	1054.88[*]	38.63[*]
Acetaldehyde	704.31[*]	741.77[*]	189.27[*]
Ethanol	248.30[*]	16.25[*]	11.74[*]
Color			
a^*	1.40 [n.s]	826.38[*]	2.27 [n.s]
h^*	1.98 [n.s]	921.08[*]	2.32 [n.s]
Texture	9.34[*]	138.35[*]	9.65[*]
Microbial growth			
Aerobic mesophilic	1613.10[*]	13733.40[*]	273.42[*]
Aerobic psychrophilic	11202.38[*]	6618.11[*]	494.27[*]

[*] $p \leq 0.05$
n.s: no significant ($p > 0.05$)

The expected trend of O_2 and CO_2 concentration along the storage time in coated fresh-cut fruits has been reported by several authors. Thus, Wong, Tillin, Hudson and Pavlath

(1994b) investigated the effect of various bilayer coatings (alginate included) on respiratory activity of coated apple pieces measuring CO_2 and ethylene production in the headspace gas composition. All the coatings studied by Wong et al. (1994b) produced a substantial rate reduction in CO_2 and ethylene, which was especially significant for the latter. The ethylene production when apple pieces were coated was a 90% lower than that observed in uncoated cut apples.
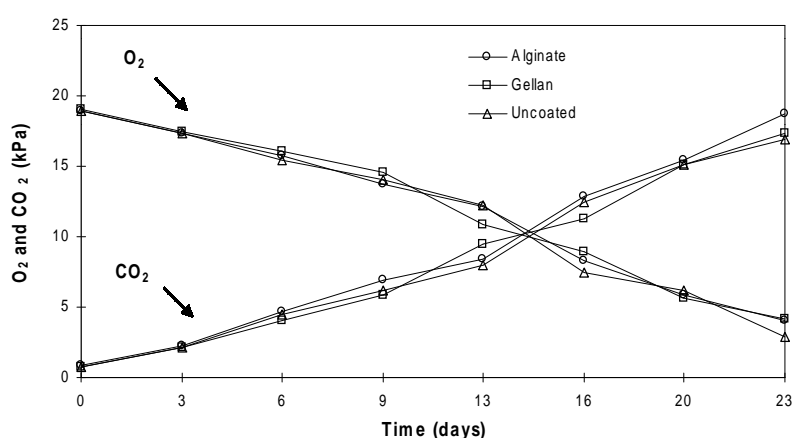

Fig. 1. O_2 and CO_2 partial pressure in packages with coated (alginate or gellan) and uncoated (control) apple wedges during storage at 4ºC. Data shown are the means (± standard deviation).

Figure 2 presents the production along the time of the other gases investigated in this study. In contrast to the behavior observed for O_2 and CO_2, clear differences are now shown between control and coated samples. This suggests the ability of the wrapping film to retain these higher molecular-weight gases (ethylene, acetaldehyde and ethanol), which is accumulated in the head space of the trays allowing sampling and detection by gas chromatography. Figure 2a shows the ethylene production of the coated and uncoated fresh-cut apples through storage. Ethylene levels varied from 7.55 µl l^{-1} to 28.25 µl l^{-1} in apples coated with alginate, and from 9.91 µl l^{-1} to 40.42 µl l^{-1} in apples coated with gellan, while in uncoated apple wedges the rise in ethylene production was from 19.16 µl l^{-1} to 154.35 µl l^{-1} at the end of the whole refrigerated storage period. The inhibitory effect of the coatings seems evident. The physiological responses elicited by the physical stress imposed by

cutting and slicing of the vegetable tissue, are well established in the literature and associated to ethylene production (Kays, 1991; Beaulieu & Baldwin, 2002). Wong et al. (1994b) employed a layer of acetylated monoglyceride (AMG) for controlling the gas diffusion through coated cut apples, and attributes the large reductions in the rates of gas evolution to this component in the formulation. The authors also used an ascorbate buffer containing calcium ions which might also contribute to inhibit respiratory activity and ethylene production.
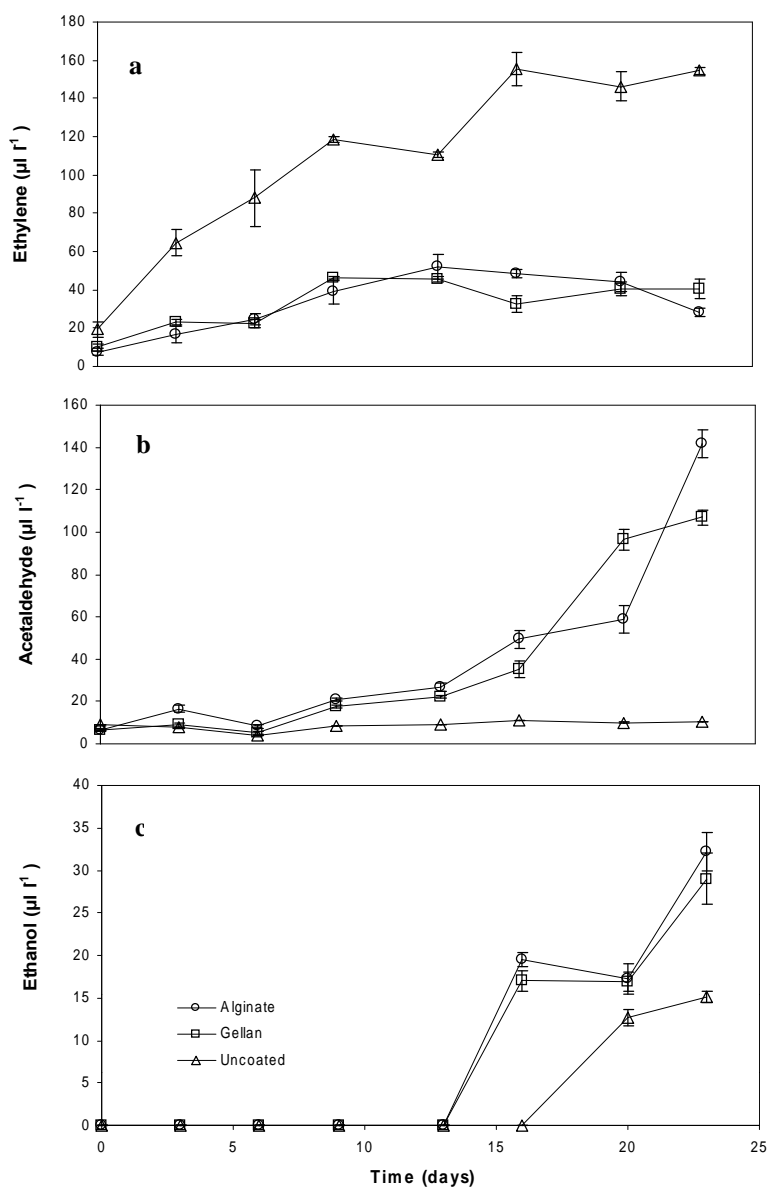
Fig. 2. Ethylene (a), acetaldehyde (b) and ethanol (c) concentration of coated (alginate or gellan) and uncoated (control) apple wedges during storage. Data shown are the means (± standard deviation).

In our case, 0.025 ml sunflower oil was incorporated by emulsification into the alginate and gellan films to improve water barrier properties, while crosslinking of the carbohydrate polymers was obtained by immersion in a calcium chloride solution; hence both sunflower oil and calcium salt might have contributed to a lower presence of ethylene in headspace gases of coated samples compared with the uncoated apples wedges. In accordance to our results, Lee, Park, Lee and Choi (2003) found a reduction of the initial respiration rate (from 44.80 to 34.95 mg $CO_2 . kg^{-1}. h^{-1}$) of fresh-cut Fuji apples coated with whey protein concentrate attributing this effect to the calcium ions contained in the film forming solution and to the oxygen barrier properties inherent to the film.

When the gas barrier created by coatings is high, an increase in the presence of some volatiles associated with anaerobic conditions can be induced (Perez-Gago et al. 2003). In this study the results of acetaldehyde and ethanol production in the coated apple wedges seem to indicate the generation of a modified atmosphere, as suggested by the lower accumulation of ethanol and acetaldehyde in the uncoated apples during refrigerated storage. The production of acetaldehyde is shown in Figure 2b. Acetaldehyde increased during storage reaching levels as high as 141.97 μl l^{-1} in coated cut apples, while in uncoated apples the production of the gas was low (10 μl l^{-1} aprox.) and was kept constant till the end of storage. Figure 2c shows the ethanol production in the coated fresh cut apples. The presence of this gas was detected (19.50 μl l^{-1}) after 15 days of storage in coated fruits, reaching values of 32.25 μl l^{-1} at the end of the storage period, while it was detected in uncoated fruits at day 20 (12.62 μl l^{-1}). The presence of ethanol after 2 weeks of storage coincides with the sudden increment of acetaldehyde in the head space of the packed coated cut apples (Fig. 2b and c). The appearance of fermentative metabolites

(acetaldehyde and ethanol) as a result of anaerobic respiration is often associated to off-flavors and its presence might be detrimental to quality (Day, 1994). Reduced internal O_2 and increased CO_2 concentration lead to anaerobic fermentation and can be brought about by fruits coatings. Edible coatings are expected to impose some restrictions to gas interchange and it is evident that the gellan and alginate coatings used in this work affect the production and the subsequent gas diffusion pattern of acetaldehyde and ethanol. Ethanol production, for instance, is an indicator of the degree of anaerobic fermentation that is taking place. Its accumulation occurs when internal atmosphere is affected by restricting gas exchange (Park, Chinnan & Shewfelt, 1994). From these results it can be inferred that O_2 and CO_2 production were also affected even if not detected by the permeability to these gases of the wrapping film that was discussed above. Soliva-Fortuny, Ricart-Coll and Martín-Belloso (2005) found in uncoated fresh-cut Golden Delicious apples packaged under 0 kPa O_2 and under 2.5 kPa O_2 + 7 kPa CO_2, and wrap-sealed with plastic films of very low oxygen permeabilities, that acetaldehyde and ethanol were produced only in small quantities during the first 3 days of storage increasing towards the end of storage regardless of the packaging conditions.

Color changes

Analysis of variance indicated that the use of edible coating in fresh-cut Fuji apples had a significant ($p \leq 0.05$) effect in the color parameter h* (Table 1). In this study, low h^* values were indicative of browning in apples wedges. Figure 3 shows that both alginate and gellan edible coatings containing *N*-acetylcysteine as antibrowning agent maintained apple wedges free from browning during 21 days of storage, demonstrating that *N*-acetylcysteine is an effective antibrowning agent to be incorporated in the formulation of edible coatings.
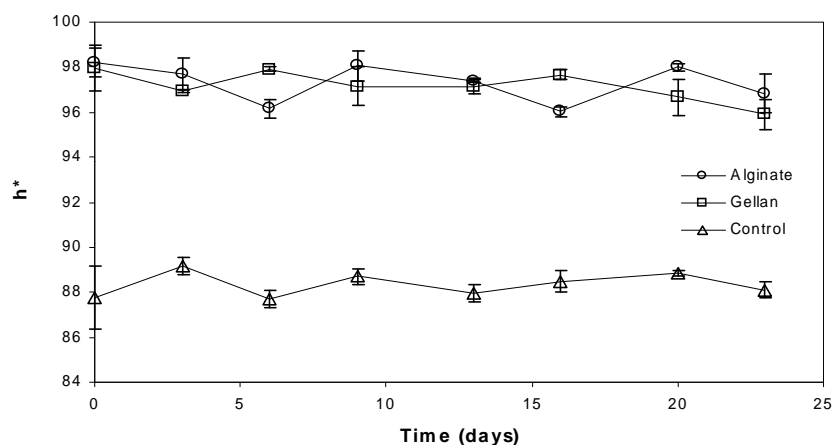
Fig. 3. Hue angle (h*) changes of coated (alginate or gellan) and uncoated (control) apple wedges during storage. Data shown are the means (± standard deviation).

The effectiveness of antibrowning agents incorporated within an edible coating has been reported by some authors. The edible coating is generally applied before the antibrowning agents so that the coating can adhere to the fruit and the antibrowning agents are incorporated in the dipping solution containing calcium for crosslinking and instant gelling of the coating (Wong et al. 1994a; Reyes, 2000; Lee et al. 2003; Rojas-Graü et al. 2007). Edible coatings have the potential to carry and hold additives as antibrowning agents on the surface of cut tissues, and in this way aid in being more effective for control of browning. Baldwin et al. (1996) found that a coating of carboxymethyl cellulose with addition of several antioxidants, including ascorbic acid, reduced browning and retarded water loss of cut apple more effectively than an aqueous solution of antioxidants. In a previous work, the effectiveness of *N*-acetylcysteine as antibrowning agent applied on aqueous solution into fresh-cut apples was demonstrated (Rojas-Graü et al. 2006a). These results show that alginate and gellan based coatings are good carriers for antibrowning agents since browning is prevented during all the storage period.

Firmness

Texture loss is the most noticeable change occurring in fruits and vegetables during prolonged storage and it is related to metabolic changes and water content (García, Martinó

& Zaritzky, 1998). According to Ponting, Jackson and Watters (1971) softening observed in fresh-cut apples may be due to the pectic acid undergoing acid hydrolysis. The firmness of uncoated apples pieces decreased from 10.19 N to 5.30 N during 23 days storage, showing a substantial softening of tissues (Fig. 4). By contrast, the use of edible coating applied on the pieces of cut apple showed a significant ($p \leq 0.05$) effect on keeping texture (Table 1). Both alginate and gellan coating showed a beneficial result on firmness retention of apple wedges during the entire storage period (Fig. 4). Hence, the use of calcium chloride for crosslinking the polymers, could minimize the softening of apple wedges. Similar results were obtained by Lee et al. (2003), who studied the effect of whey protein concentrate edible coatings in combination with antibrowning agents, on minimally processed apple slices. They found that incorporating 1% of calcium chloride within the coating formulation helped to maintain firmness of apple pieces. King and Bolin (1989), established that calcium chloride can be used as firming agent for fruit tissues by reacting with pectic acid in the cell wall to form calcium pectate, which strengthens molecular bonding between constituents of cell wall.
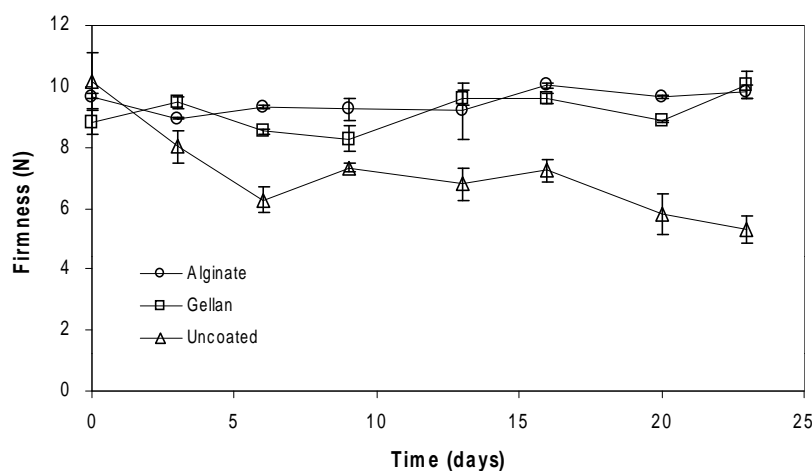

Fig. 4. Firmness changes of coated (alginate or gellan) and uncoated (control) apple wedges during storage. Data shown are the means (± standard deviation).

In addition, firmness deterioration is frequently associated with water content loss. In a previous work, Rojas-Graü et al. (2007) found that alginate or gellan edible coatings applied to fresh-cut apples were effective in controlling moisture loss when the formulation contained 0.025 ml sunflower oil/ 100 ml film forming solution. Thus, the use of sunflower oil could maintain texture due to the oil-mediated moisture retention of the coated fruit. Olivas, Rodríguez & Barbosa-Cánovas (2003) found that methylcellulose-stearic acid coating played an important role in avoiding weight loss of pear wedges, while methylcellulose coatings itself showed poor moisture barrier.

Microbiological evaluation

Significant differences ($p \leq 0.05$) between the counts of mesophilic and psychrophilic microorganisms of coated and uncoated fresh-cut apples are shown in Table 1. Figure 5 shows that edible coating applied on fresh-cut apples had a marked effect in reducing mesophilic and psychrophilic counts as compared to the uncoated apple pieces.
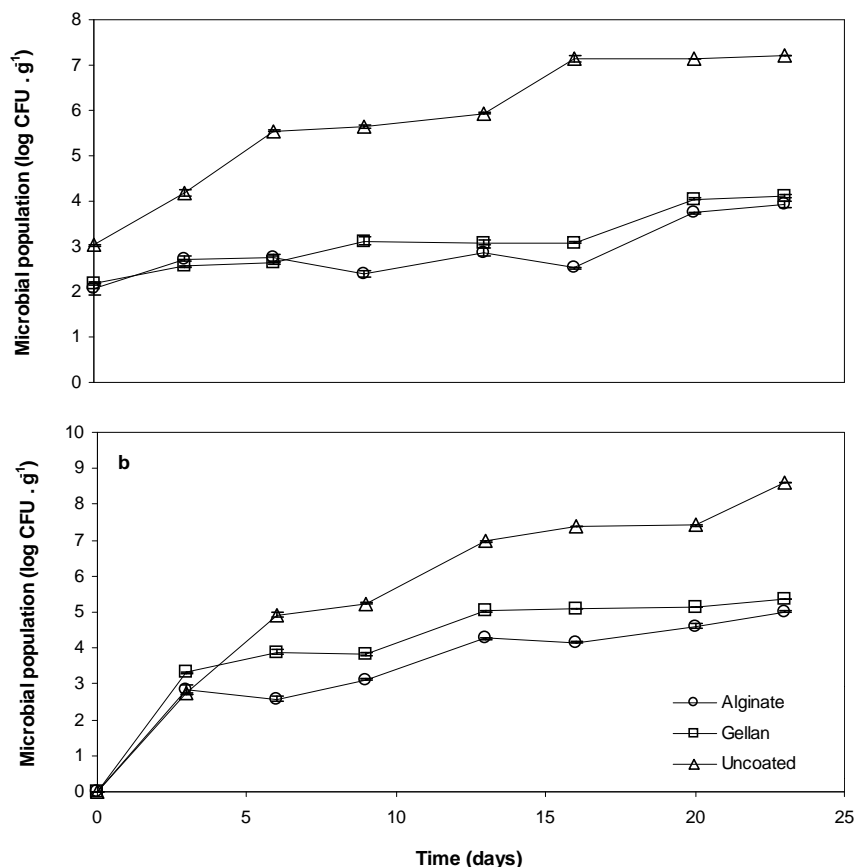
Fig. 5. Effect of alginate and gellan-based coatings on microbial growth (log CFU.g^{-1} of fruit) of apple wedges: (a) mesophilic microorganisms, (b) psychrophilic microorganisms. Data shown are the means (± standard deviation).

At the end of the 3 weeks refrigerated storage, counts of coated samples did not exceed 10^4 CFU.g^{-1} and 10^5 CFU.g^{-1} for mesophilic and psychrophilic respectively, with both types of coatings used, while uncoated apples wedges presented values as high as 10^7 CFU.g^{-1} and 10^8 CFU.g^{-1} (Fig. 5). The antibrowning agent incorporated in the coatings might have contributed to the antimicrobial effect observed here. These results are in agreement with those found by other authors who used other type of edible coatings. Lee at al. (2003) reported very similar results for minimally processed apples with various types of carbohydrate polymers and whey protein concentrate, using ascorbic acid, citric acid and oxalic acid as antibrowning agents. Howard and Dewi (1995) used an edible cellulose-based coating, on mini-peeled carrots and investigated microbial quality during storage at 2 ºC. No antibrowning agents were used. In that study, edible coating did not have any affect on microbial quality of the product since no differences with the uncoated carrots were seen. The authors, however, comment that the high relative humidity imparted by the coatings did not promote microbial growth when the counts did not exceed the limit of 10^5 cfu/g. As stated by Olivas and Barbosa-Cánovas (2005), coatings create a modified atmosphere that may change the growth rate of spoilage and pathogenic microorganisms. Since modified atmosphere may inhibit the growth of innocuous spoilage flora and encourage the growth of pathogens, the study of the development of populations of mesophilic and psychrophilic bacteria, molds and yeast during storage of fresh-cut fruits is required for microbial safety of these products.

CONCLUSIONS

Alginate and gellan edible coatings can help maintain desirable quality characteristics of fresh-cut Fuji apples. Alginate and gellan coatings significantly reduced ethylene production; however, no significant effect of coatings on respiration rates was observed probably due to the plastic wrap of moderate oxygen permeability used that did not allow accumulation of O_2 and CO_2 in the head space for sampling and detection. The coated apple wedges maintained their initial firmness and color during all refrigerated storage, corroborating that the alginate and gellan-based edible coatings are good carriers of firming agents like calcium chloride, which is used for crosslinking the polymers, and of antibrowning agents like N-acetylcysteine. From the microbiological point of view, results suggest that apples wedges coated with both alginate and gellan could have a shelf-life up to 3 weeks at 4 ºC; but, the presence of acetaldehyde and ethanol, as a result of fermentative anaerobic processes, limit their shelf-life to 2 week. Results showed that the shelf life of the coated apples was extended approximately 3 times as compared with the control which showed a considerable loss of quality from the very early days of storage, limiting their shelf-life to less of 4 days.

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MECHANICAL, BARRIER AND ANTIMICROBIAL PROPERTIES OF APPLE PUREE EDIBLE FILMS CONTAINING PLANT ESSENTIAL OILS

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ABSTRACT

Edible films, as carriers of antimicrobial compounds, constitute an approach for incorporating plant essential oils (EOs) onto fresh-cut fruit surfaces. The effect against *Escherichia coli* O157:H7 of oregano, cinnamon and lemongrass oils in apple puree film forming solution (APFFS) and in an edible film made from the apple puree solution (APEF) was investigated along with mechanical and physical properties of the films. Bactericidal activities of APFFS, expressed as BA_{50} values, (BA_{50} values are defined as percentage of antimicrobial that killed 50% of the bacteria under the test conditions) ranged from 0.019% for oregano oil to 0.094% for cinnamon oil. Oregano oil in the apple puree and in the film was highly effective against *E. coli* O157:H7. The data show that (a) the order of antimicrobial activities were: oregano oil>lemongrass oil>cinnamon oil; and (b) addition of the essential oils into film forming solution decreased water vapor permeability, increased oxygen permeability, but did not significantly alter the tensile properties of the films. These results show that plant-derived essential oils can be used to prepare apple-based antimicrobial edible films for various food applications.

Key words: Apples puree film; plant essential oils; physicochemical properties; antimicrobial activity; *Escherichia coli* O157:H7

INTRODUCTION

Epidemiological studies indicate that *Escherichia coli* serotypes are responsible for about 110,000 outbreaks of foodborne illness in the United States each year, resulting in about 110 fatalities, with the O157:H7 serotype accounting for the greatest proportion of cases (*1, 2*). These data suggest the need to protect the food against contamination as well as the consumer against infection by foodborne pathogenic bacteria.

The increase in consumption of fresh-cut produce has resulted in frequent outbreaks of illness associated with raw fruits and vegetables (*3, 4*). During minimal processing, spoilage and pathogenic microorganisms can gain access to the nutrients inside fruits and multiply (*5-7*). The presence of *Escherichia coli* O157:H7 on the surface of fruits may adversely affect the safety of fresh and fresh-cut fruit. Controlling the numbers and the growth of pathogenic bacteria is a challenging problem for the food processing industry (*8*). The use of edible films and coatings for a wide range of food products, including fresh and minimally processed vegetables and fruits, has received increasing interest because films can serve as carriers for a wide range of food additives, including antimicrobials (*9*). Incorporating antimicrobial compounds into edible films or coatings provides a novel way for enhancing the safety and shelf life of ready-to-eat foods (*10*). Essential oils have been extensively evaluated for their abilities to protect food against pathogenic bacteria contaminating apple juice (*15*) and other foods (*11*). They are also used as flavouring

agents in baked goods, sweets, ice cream, beverages, and chewing gum (*26*), and are designated as Generally Regarded as Safe (GRAS) (*11*). EOs are regarded as alternatives of chemical preservatives, and their use in foods meets the safety demands of consumers for mildly processed natural products, as reviewed by Burt (*11*). The antimicrobial activity of EOs is associated with terpenoid and phenolic components of the oils (*11*).

To assess the antimicrobial effectiveness of natural compounds and plant extracts, we previously evaluated the bactericidal activities of about 200 plant essential oils, oil compounds, phenolic compounds, and flavonoids against major foodborne pathogenic bacteria including antibiotic-resistant bacteria (*12-16*). Several of these compounds were previously also found to be active in apple juice (*15*).

Physicochemical properties of edible films (color, tensile strength, water vapor and oxygen permeability) relate to coating enhancement of mechanical integrity of foods, inhibition of moisture loss and oxidative rancidity, and final-product appearance (*17*). Combined analysis of antimicrobial and physicochemical properties is crucial for predicting the behaviour of antimicrobial edible films (*18,10*). No prior research has been reported on the antimicrobial effects against *E. coli* O157:H7 of essential oil containing apple puree edible films. The objectives of this study were (a) to determine antimicrobial activities against the *E. coli* O157:H7 of apple puree film forming solutions used in the preparation of films, and of apple pure films, both of which contain select essential oils and oil compounds, and (b) to evaluate the effects of added natural antimicrobials on changes in the physicochemical properties of the films.

MATERIALS AND METHODS

Test Compounds. Golden Delicious apple puree (38 ºBrix) (Sabroso Co., Medford, OR) was the primary ingredient in all apple-based film forming solutions (APFFS) and edible films (APEF). Glycerol was added as a plasticizing agent (Fisher Scientific, Waukesha, WI). Ascorbic acid (BASF, Mount Olive, NJ) and citric acid (Archer Daniels Midland, Decatur, IL) were utilized as browning inhibitors. High methoxyl pectin (Systems BioIndustries, Fair Lawn, NJ) was added to films to assist in film release from the cast

surface. Oregano oil (from *Origanum vulgare*), lemongrass oil (from *Cymbopogon citratus*), and cinnamon oil (from *Cinnamomum cassia*) were the EOs tested and were obtained from Lhasa Kamash Herb Co. (Berkeley, CA).

Preparation of Apple Puree Film Forming Solution (APFFS). APFFS (26% w/w) (260 g of 38 °Brix apple puree plus 700 g of 3% w/w pectin solution) was prepared by the method of McHugh and Senesi (*19*). This solution also contained 5 g of ascorbic and citric acids (0.5% w/w) and 30 g of glycerol (3% w/w). Pectin was added to increase film strength. Natural antimicrobial essential oils *(*oregano, lemongrass, and cinnamon) were then incorporated into APFFS at the following concentrations: 0 (control), 0.05%, 0.075%, 0.1% and 0.5% (w/w). These solutions were homogenized for 3 min at 12,500 rpm using a Polytron 3000 homogenizer (Kinematica, Littau, Switzerland), and then used for bactericidal studies and casting the films.

Preparation of Apple Puree Edible Film (APEF). Apple puree edible film forming solutions were prepared as described previously. Vacuum was then applied to remove bubbles. Films were then cast on 29 x 29 cm square plates, and dried at ambient conditions for ~24 h. Dried films were cut and peeled from the casting surface. These samples were used for determinations of physicochemical and antimicrobial properties of the films.

Test Buffers. Phosphate-buffered saline (PBS, pH 7.0) was prepared by mixing dibasic sodium phosphate (100 mM) and monobasic sodium phosphate (100 mM) at 2:1 ratio, diluting by half with H_2O, and adding NaCl (150 mM). For lower pH buffers (saline solutions), 2 mM citric acid-150 mM NaCl was adjusted to pH 3.3 –3.7 with 1N HCl.

Bactericidal Assays of APFFS. The source of *Escherichia coli* O157:H7 used in this study is given in ref. (12). To facilitate pipetting, the 26% APFFS solution was further diluted by ½ with pH 3.3 saline solution, v/v. This APFFS sample was used to prepare suspensions of APFFS for the assay. Oregano oil (10 µL) was added to 9.99 mL of diluted APFFS. Lemongrass oil or cinnamon oil (50 µL) was added to 9.95 mL diluted APFFS in 50 mL tubes. The tubes were warmed in a microwave oven for 10 s and then shaken to form uniform suspensions. The content of the tubes were then diluted as follows: saline solution

(500 μL) was added to five sterile 1.9 mL tubes. Serial dilutions were made starting with 1 mL of each original test solution, using 500 μL for each transfer for a total of five dilutions. Microtiter plates with 96 wells (Nalge, Rochester, NY) were prepared with saline pH 3.3 negative controls (100 μL each in 6 wells) and three test substances with five dilutions plus the test solution (100 μL each dilution per well, 6 wells). Each of these 24 wells were sampled at three time intervals: 3, 30 and 60 min at 21 ºC. Bactericidal assays were then carried out in duplicate using previously described procedures (*12,14*).

Bactericidal Activities (BA$_{50}$ Values). Bactericidal activity, defined as the percentage of test compound that kills 50% of the bacteria under the test conditions, was determined as follows. Each compound was tested at a series of dilutions. The control pH 3.3 saline diluent was matched with the pH of APFFS. The CFU values from all experiments were transferred to a Microsoft Excel 8.0 Spreadsheet. The number of CFU from each dilution was matched with the average control value to determine the percentage of bacteria killed per well. Each of the dose-response profiles (% test compound versus % bactericidal activity) was examined graphically, and the BA$_{50}$ values were estimated by linear regression. The lower the BA$_{50}$ value, the higher the antimicrobial activity.

Antimicrobial Activity of Apple Puree Edible Films (APEF). Disc inhibition zone assays were performed as a qualitative test for antimicrobial activity of the films. APEF with and without EOs (control) were aseptically cut into 12 mm diameter discs and then placed on MacConkey-Sorbitol agar (Biokar Diagnostics, Beauvais, France) plates for *E. coli* O157:H7, which had been previously spread with 0.1 mL of inoculum containing 10^5 CFU's/mL. Plates were incubated at 37 ºC for 48 h. The thickness (mm) of the inhibition zone around the film disc (colony free perimeter) was measured with a millimeter scale and the growth below the film discs (the contact area of edible film with agar surface) was visually examined. Tests were done in duplicate.

Film Thickness. Film thicknesses were measured with a micrometer IP 65 (Mitutoyo Manufacturing, Tokyo, Japan) to the nearest 0.00254 mm (0.0001 in) at five random positions around the film. The mean value was used to calculate water vapor permeability (WVP), oxygen permeability (O$_2$P), and tensile strength.

Water Vapor Permeabilities (WVP) of Films. The gravimetric Modified Cup Method based on ASTM E96-92 (*20*) was used to determine WVP. A cabinet (Thermo Electron Corp., Waltham, MA) with a variable speed fan was used to test film WVP at 25 ± 1 ℃. Fan speeds were set to achieve air velocities of 152 m/min to ensure uniform relative humidity throughout the cabinets. The cabinets were pre-equilibrated to 0% room humidity (RH) using anhydrous calcium sulphate (W.A. Hammond Drierite, Xenia, OH). Circular test cups made from polymethylmethacrylate (Plexiglas^{TM}) were used. A film was sealed to the cup base with a ring containing a 19.6 cm^2 opening using 4 screws symmetrically located around the cup circumference. Both sides of the cup contacting the film were coated with silicon sealant. Distilled water (6 mL) was placed at the bottom of the test cups to expose the film to a high percentage of RH inside the test cups. The average stagnant air gap heights between the water surface and the film were measured. Test cups, holding the films, were then inserted into the pre-equilibrated 0% RH desiccator cabinets. Steady state of water vapor transmission rate was achieved within 2 h. Each cup was weighed 8 times at 2 h intervals. Eight replicates of each film were tested. Relative humidities at the film undersides and WVPs were calculated using the WVP Correction Method (*20*).

The WVP of the films was calculated by multiplying the steady state water vapor transmission rate by the average film thickness determined as described above, and dividing by the water vapor partial pressure difference across the films:

$$WVP = \frac{(WVTR) * (thickness)}{(p_{A1} - p_{A2})} \qquad \text{Equation 1}$$

where, WVTR = water vapor transmission rate, and p_{A1} and p_{A2} = partial pressure of water vapor inside and outside the cup, respectively.

Oxygen Permeabilities (O$_2$P) of Films. An Ox-Tran 2/20 ML modular system (Modern Controls Inc., Minneapolis, MN) was utilized to measure oxygen transmission rates through the films (standard method D3985) (*21*). Oxygen transmission rates were determined at 23 ℃ and 50 ± 1% RH. Each film was placed on a stainless steel mask with an open testing area of 5 cm^2. Masked films were placed into the test cell and exposed to 98% N$_2$ + 2% H$_2$

flow on one side and pure oxygen flow on the other. The system was programmed to have a 10 h waiting period to allow the films to achieve equilibrium. Oxygen permeability was calculated by dividing O_2 transmission rate by the difference in O_2 partial pressure between both sides of the film (1 atm) and multiplying by the average film thickness measured at 5 random places. Four replicates of each film were evaluated.

Tensile Properties of Films. Standard method D882-97 (*22*) was used to measure the tensile properties of films. Films were cut into strips with a test dimension of 165 mm x 19 mm (standard method D638-02a) (*23*). Before testing, all the films were conditioned for 48 h at 23 ± 2 ℃ and 50% ± 2% RH using a saturated salt solution of magnesium nitrate (Fisher Scientific, Fair Lawn, NJ). The ends of the equilibrated strips were mounted and clamped with pneumatic grips on an Instron Universal Testing Machine (Model 55R4502, Instron, Canton, MA) with a 100 N load cell. The initial gauge length was set to 100 mm, and the films were stretched using a crosshead speed of 7.5 mm/min. Tensile properties were calculated from the plot of stress (tensile force/initial cross-sectional area) vs. strain (extension as a fraction of original length), using Series IX Automated Materials Testing System Software (Instron, Canton, MA). Fifteen specimens of each type of film were evaluated.

Statistical Analysis. Data were analyzed by one-way analysis of variance (ANOVA) using Minitab (version 13.31) software (Minitab Inc., State College, PA). Tukey's test was used to determine the differences at 5% significance level (*24*).

RESULTS AND DISCUSSION

Antimicrobial Activity of Plant Essential Oils in APFFS. Table 1 lists the experimental BA_{50} values for essential oils at three time periods, 3, 30, and 60 min. All compounds inhibited the growth of *E. col*i O157:H7. APFFS in saline pH 3.3, without EOs and containing ascorbic acid and citric acid as antibrowning agents, was not effective against the pathogen.

Oregano oil at a concentration of 0.1% in APFFS was effective at 3 min with a BA_{50} value of 0.034 (0.034% of oregano oil inhibited 50% of the *E. coli* O157:H7 after 3 min.). The activity at 30 and 60 min. was slightly greater (BA_{50} = 0.024% and 0.019%, respectively) than the activity at 3 min (Table 1). Thus, oregano oil appears to be a highly potent antimicrobial against *E. coli* O157:H7. By contrast, cinnamon oil at a concentration of 0.5% in APFFS was only effective at 30 and 60 min with BA_{50} values of 0.12 % and 0.094 %, respectively. These results indicate that cinnamon oil at a fivefold greater concentration was less effective against *E. coli* O157:H7 than oregano oil.

Table 1 also shows that the activity of lemongrass oil against *E. coli* O157:H7 at a concentration of 0.5 % in APFFS is similar to that of cinnamon oil. The BA_{50} value of 0.059 at 60 min means that 0.059% of lemongrass oil inhibited 50% of the bacteria under the test conditions. Compared to oregano oil, it took about five times higher concentration of cinnamon or lemon grass oils to achieve the same activity against *E. coli* O157:H7.

Table 1. Bactericidal Activities (BA_{50} Values) of Essential Oils against *E. coli* O157:H7 in Apple Puree Film Forming Solution (APFFS)[a] Incubated for 3, 30, and 60 min at 21 °C.

essential oil (% w/w) in 50% APFFS[a]	BA_{50} value for *E. coli* O157:H7 [b]		
	3 min	30 min	60 min
oregano oil, 0.1%	0.034 ± 0.01	0.024 ± 0.007	0.019 ± 0.004
cinnamon oil, 0.5%	> 0.34[c,]	0.12	0.094 ± 0.04
lemon grass oil, 0.5%	0.28 ± 0.03	0.078 ± 0.02	0.059 ± 0.005

[a] APFFS is 50% apple puree film formula suspension in saline pH 3.7 buffer.
[b] Average values and standard deviations of two replicates of BA_{50} values.
[c] > signifies that less than 50% of bacteria were killed at the highest dose used.

It is also instructive to compare the activities of the same compounds in the APFFS to activities previously observed in "cloudy" apple juice (*15*). The comparison against *E. coli* shows that (a) the BA_{50} values at 60 min and 21 °C for oregano oil, cinnamon oil, and lemongrass oil (0.019, 0.094, and 0.059%, respectively) in apple puree were similar to those in apple juice following incubation at 37 °C for 60 min; and (b) although *E. coli*

O157:H7 resists inactivation in acidic pH (*25*), the low pH of both apple juice (~3.7) and the apple puree (~3.3) does not seem to contribute significantly to the antimicrobial effects of the test substances.

Antibacterial Activity of Plant Essential Oils in APEF. Antibacterial activities of APEF with EOs are shown in Table 2. The inhibitory activities were based on the measurement of clear inhibition zones surrounding film disks. If a surrounding clear zone were not present, it was assumed that the compound was not inhibitory and the diameter was assigned as zero. APEF without EOs served as control to determine any possible antimicrobial effect of the normal film. The control film did not inhibit *E. coli* O157:H7.

Table 2. Antibacterial Activity of Essential Oils Incorporated into Apple Puree Edible Films against *E. coli* O157:H7.

essential oil type	concentration	*E. coli* O157:H7	
	(% w/w)	inhibitory zone (mm)[a]	inhibitory effect
oregano oil	0 (control)	0	-
	0.05	< 1	+
	0.075	1.2	+
	0.1	1.4	+
cinnamon oil	0 (control)	0	-
	0.05	0	+
	0.075	< 1	+
	0.1	< 1	+
	0.5	1.1	+
lemongrass	0 (control)	0	-
	0.05	0	-
	0.075	0	-
	0.1	<1	+
	0.5	1.2	+

[a] Values are measurements of thickness (mm) of inhibitory zone (colony free perimeter).
+: represents an inhibitory effect; -: represents no inhibitory effect.

As with APFFS, in APEFs, oregano oil was once again the most effective compound against *E. coli* O157:H7. The inhibitory zone increased with increasing concentration of

oregano oil in the films (Table 2). Figure 1 shows the inhibitory effect of APEF against *E. coli* O157:H7 with 0.1 % oregano oil and without the oil.
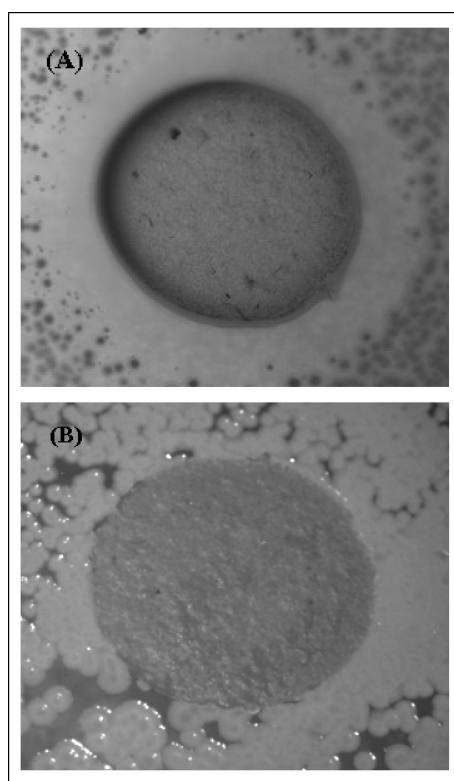
Figure 1. Inhibitory zone (*E. coli* O157:H7 colony free perimeter) of apple-puree edible films containing 0.1% oregano oil (A) compared to control without oregano oil (B).

The figure shows that the film without the oil had no effect on the bacteria. By contrast, a film containing 0.1% oregano oil completely killed all the surrounding bacteria. Previously, it was suggested that diffusion of antimicrobials from a film disc depends on the size, shape, polarity of the diffusing molecule, the chemical structure of the film, and the degree of molecular crosslinking (*10*). We do not know whether these factors also govern antimicrobial properties of fruit and vegetable films.

By contrast, inhibition of *E. coli* O157:H7 by cinnamon oil and lemongrass oil at concentrations of <0.5 % in the films was lower than those observed with oregano oil

(Table 2). However, the inhibitory effect increased when the concentration of both EOs (cinnamon and lemongrass) was increased to 0.5%. Compared to oregano oil, it took about a five times higher concentration of cinnamon or lemongrass oils to achieve the same activity against *E. coli* O157:H7. As cinnamon oil is added to numerous commercial foods (*27*), for its pleasant flavor, and is GRAS-listed (*28*), the compound merits use in antimicrobial edible films.

Water Vapor Permeability

McHugh (*29*) developed the first edible films made from fruit purees, characterizing their permeability properties. Because apple-based edible films were not very good moisture barriers, addition of lipids was necessary to improve the water barrier properties. In this study, it was observed that all the WVP values decreased when the fraction of the hydrophobic compounds (EOs) increased. This effect was more prominent with oregano oil. At the maximum concentration (0.1% w/v), oregano oil induced a significant ($p<0.05$) decrease in film water vapor permeability (Table 3).

Water vapor transfer generally occurs through the hydrophilic portion of the film and therefore depends on the hydrophilic-hydrophobic ratio of the film constituents (*30*). Because each hydrophobic substance has unique physico-chemical properties, films based on lipids have variable behavior against moisture transfer. In this study, the behavior observed in films containing different essential oils might be due to differences in the hydrophobicity/hydrophilicity parts of each molecule. (*31*). Our results suggest that oregano oil may have a dual benefit. It can be used to both impart antimicrobial activities and to enhance barrier properties of the films.

Oxygen Permeability

APEF is a good oxygen barrier, exhibiting values of 22.64 ± 1.28 cm^{3}µm/m^2-d-kPa. This result agrees with earlier observations (*29*). The oxygen permeability values increased with increasing amounts of EOs in the films. Hence, a significant difference ($p<0.05$) was observed with oregano oil (0.1% w/v), reaching values of 38.12 ± 0.80 cm^{3}µm/m^2-d-kPa compared to the control value of 22.64 ± 1.28 cm^{3}µm/m^2-d-kPa (Table 3). Nonpolar materials such as lipids act as excellent moisture barriers, but are less effective gas barriers.

McHugh and Krochta (*18*) indicated that films containing lipids exhibit relatively poor oxygen barrier properties. The chemical nature of oil plays a major role in the barrier properties of edible films.

Table 3. Effect of Concentration (% w/w) of Essential Oils on Water Vapor Permeability (WVP) and Oxygen Permeability (O_2P) Properties of Apple Puree Edible Films.

essential oil	essential oil concentration (% w/w)	thickness[A] (mm)	RH inside cup[AB] (%)	WVP[AB] (g-mm/kPa-h-m^2)	O_2P^A (cm^3-μm/m^2-d-kPa)
control	0	0.153^{ab}	62.6^{ab}	7.04 ± 0.63^b	22.64 ± 1.28^a
oregano oil	0.05	0.142^{ab}	60.3^a	7.06 ± 0.18^b	32.82 ± 0.79^c
	0.075	0.152^{ab}	63.7^b	6.67 ± 0.57^{ab}	33.72 ± 0.80^c
	0.1	0.135^a	62.8^{ab}	6.17 ± 0.56^a	38.12 ± 0.80^d
lemongrass oil	0.05	0.140^{ab}	61.7^{ab}	6.71 ± 0.33^{ab}	28.92 ± 1.57^{bc}
	0.075	0.148^{ab}	63.4^{ab}	6.59 ± 0.58^{ab}	26.83 ± 0.84^b
	0.1	0.150^{ab}	62.5^{ab}	6.92 ± 0.29^{ab}	34.24 ± 1.55^{cd}
	0.5	0.150^{ab}	63.7^b	6.62 ± 0.86^{ab}	30.25 ± 0.78^{bc}
cinnamon oil	0.05	0.155^b	61.4^{ab}	7.48 ± 0.42^b	30.52 ± 0.79^c
	0.075	0.145^{ab}	62.0^{ab}	6.83 ± 0.41^{ab}	28.21 ± 0.63^{bc}
	0.1	0.143^{ab}	62.2^{ab}	6.74 ± 0.83^{ab}	31.72 ± 0.99^c
	0.5	0.152^{ab}	63.3^{ab}	6.82 ± 0.85^{ab}	32.08 ± 0.50^c

[A] Thickness and RH data are mean values. WVP and O_2P data are mean values ± standard deviations.

[B] Relative humidity at the inner surface and WVP values were corrected for stagnant air effects using the WVP Correction Method (*16*).

[a,b,c,d] Means in same column with different letters are significantly different (p<0.05).

Tensile Properties

Tensile strength expresses the maximum stress developed in a film during tensile testing (*32*). The tensile properties of the APEF with and without EOs are summarized in Table 4. Lemongrass oil and cinnamon oil had different effects on the tensile strength of the films. Compared to the control films without added EOs, increasing the concentration of lemongrass oil from 0.05 to 0.1% w/w caused a significant reduction (*p*<0.05) in tensile strength. By contrast, addition of the same amount of cinnamon oil increased the tensile

strength (Table 4).   The difference between the two films may due to difference in polarities of the added compounds. High concentrations (0.5% w/w) of these oils added tensile strength to control films. These results agree with those obtained by Pranoto (*9*) for physical and antibacterial properties of an alginate edible film containing garlic oil.

Table 4. Effect of Concentration (% w/w) of Essential Oils on the Tensile Properties of Apple Puree Edible Films.

essential oil	essential oil concentration (% w/w)	tensile strength[A] (MPa)	elongation[A] (%)	elastic modulus[A] (MPa)
control	0	0.64 ± 0.017^{b}	25.4 ± 2.1^{ab}	5.06 ± 0.54^{ab}
oregano oil	0.05	0.61 ± 0.04^{b}	27.4 ± 2.2^{b}	5.47 ± 0.64^{b}
	0.075	0.61 ± 0.03^{b}	27.4 ± 3.7^{b}	4.41 ± 0.40^{ab}
	0.1	0.62 ± 0.02^{b}	26.5 ± 2.0^{b}	4.73 ± 0.32^{ab}
lemongrass oil	0.05	0.65 ± 0.03^{b}	25.8 ± 1.0^{ab}	5.18 ± 0.33^{ab}
	0.075	0.57 ± 0.06^{ab}	24.2 ± 2.6^{ab}	5.58 ± 1.03^{b}
	0.1	0.54 ± 0.02^{a}	23.5 ± 1.5^{ab}	4.01 ± 0.70^{a}
	0.5	0.63 ± 0.02^{b}	24.8 ± 1.9^{ab}	4.49 ± 0.53^{a}
cinnamon oil	0.05	0.72 ± 0.05^{c}	25.2 ± 2.6^{ab}	7.60 ± 0.59^{c}
	0.075	0.79 ± 0.09^{c}	26.1 ± 2.2^{b}	7.00 ± 1.03^{c}
	0.1	0.75 ± 0.05^{c}	25.3 ± 2.6^{ab}	5.65 ± 0.37^{b}
	0.5	0.61 ± 0.04^{ab}	22.6 ± 1.8^{a}	4.04 ± 0.39^{a}

[A] Tensile strength, elongation and elastic modulus data are mean values ± standard deviations.
  a,b,c Means in same column with different letters are significantly different at p<0.05.

Elongation at break is a measure of the film's stretchability prior to breakage (*33*). Generally, the presence of essential oils did not significantly change the elongation of the films. However, at a level >0.5% (w/w), cinnamon oil significantly reduced (*p*<0.05) the elongation at break value (Table 4).

No significant differences were observed in the elastic modulus between films with and without oregano or lemongrass oils (*p*<0.05). However, a significant increase in the elastic modulus of the film was noted at a concentration of 0.075% cinnamon oil (Table 4). In

related studies, Zivanovic (*34*) noted a decrease in tensile strength and an increase in elongation of chitosan films enriched with essential oils. Begin and Van Calsteren (*35*) noted that an increased molecular weight of the counter ion resulted in thicker and more elastic but less strong chitosan films.

CONCLUSION

The antimicrobial activity of oregano oil in apple puree edible films and film forming solutions against *E. coli* O157:H7 was significantly greater than the activities of cinnamon oil and lemongrass oil. There was no adverse effect of additives on water vapor permeability properties. The antimicrobial films showed good oxygen permeability. The tensile strength of the films containing certain levels of antimicrobials did not differ significantly from control films without added antimicrobials. The antimicrobial activity data obtained with the APFFS (BA_{50} values) can serve as a guide for selection of appropriate levels of plant compounds for incorporation into antimicrobial edible films. Our ultimate goal is to develop commercially viable technologies for the manufacture of fruit- and vegetable-based films incorporating antimicrobial phytochemicals in an efficient manner. Future research could be conducted to evaluate the sensory aspects of using these natural essential oil compounds in edible films and coatings, as well as to characterize their stability.

ACKNOWLEDGMENTS

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EFFECTS OF PLANT ESSENTIAL OILS AND OIL COMPOUNDS ON MECHANICAL, BARRIER AND ANTIMICROBIAL PROPERTIES OF ALGINATE-APPLE PUREE EDIBLE FILM

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ABSTRACT

We evaluated mechanical, barrier and antimicrobial properties of 0.1-0.5% suspensions of the following essential oils (EOs)/oil compounds (OCs) against the foodborne pathogen *Escherichia coli* O157:H7 in alginate-apple puree edible film (AAPEF): oregano oil/carvacrol; cinnamon oil/cinnamaldehyde; and lemongrass oil/citral. The presence of plant antimicrobials did not significantly affect water vapor and oxygen permeabilities of the films, but did significantly modify tensile properties. Antimicrobial activities of solutions used to prepare edible films (AAPFFS) were also determined. The results obtained demonstrate that carvacrol exhibited the strongest antimicrobial activity against *E. coli* O157:H7. The data show that the antimicrobial activities were in the following order: carvacrol > oregano oil > citral > lemongrass oil > cinnamaldehyde > cinnamon oil. These studies show that plant-derived essential oils and their constituents can be used to prepare apple-based antimicrobial edible films for food applications.

Keywords: alginate film; apple puree; plant essential oils; mechanical properties, barrier properties; antimicrobial activity; *Escherichia coli* O157:H7.

INTRODUCTION

Edible films can improve shelf life and food quality by serving as selective barriers to moisture transfer, oxygen uptake, lipid oxidation, and losses of volatile aromas and flavors (Kester & Fennema, 1986). Their use is gaining importance in food protection and preservation due to the fact that they provide advantages compared to films made from synthetic materials (Weber, Haugaard, Festersen, & Bertelsen, 2002; Tharanathan, 2003). Potential properties and applications of edible films and coatings have been extensively reviewed (Min, Harris, Han, & Krochta, 2005; Serrano, Valverde, Guillen, Castillo, Martínez-Romero, & Valero, 2006; Bravin, Peressini, & Sensidoni, 2006; Jagannath, Nanjappa, Das Gupta, & Bawa, 2006).

McHugh, Huxsoll and Krochta (1996) developed the first edible films made from fruit purees. They found that apple-based edible films are excellent oxygen barriers, but not very good moisture barriers. Addition of hydrocolloids such as alginate may improve the barrier and tensile properties of fruit-based films (Mancini and McHugh, 2000). Novel films can be developed by combining fruit purees with various gelling agents. Alginate, a polysaccharide extracted from marine brown algae (*Phaeophyceae*), is a common type of gelling agent employed in the food industry (Mancini et al., 2000; Yang & Paulson, 2000). This polysaccharide is of interest as a potential film or coating component because of its unique colloidal properties. These include thickening, stabilizing, suspending, film forming, gel producing, and emulsion stabilizing properties (King, 1983; Rhim, 2004).

Interest in antimicrobial films has risen recently due to increased consumption of fresh-cut produce. Such consumption has resulted in occasional outbreaks of illness associated with contaminated fruits and vegetables (Brackett, 1999; Thunberg, Tran, Bennett, Matthews, & Belay, 2002). During minimal processing, spoilage and pathogenic microorganisms can contaminate fruits (Del Rosario & Beuchat, 1995; Thunberg et al., 2002). For example, the presence of *Escherichia coli* O157:H7 on fruit surfaces may adversely affect the safety of fresh and fresh-cut fruit. The use of edible films and coatings for food products, including fresh and minimally processed fruits and vegetables, is of interest because films and coatings can serve as carriers for a wide range of food additives, including antimicrobials (Pranoto, Salokhe, & Rakshit, 2005).

Plant essential oils (EOs) and oil compounds (OCs) have been previously evaluated for their ability to protect food against pathogenic bacteria contaminating apple juice (Friedman, Henika, Levin, & Mandrell, 2004) and other foods (Burt, 2004). However, little published data exist on the incorporation of EOs and OCs into edible films. EOs are also used as flavouring agents in baked goods, sweets, ice cream, beverages, and chewing gum (Fenaroli, 1995) and are designated as Generally Regarded as Safe (GRAS) (Burt, 2004).

A complete analysis of both antimicrobial and physicochemical properties is important for predicting the behaviour of antimicrobial edible films in food systems (McHugh & Krochta, 1994a; Cagri, Ustunol, & Ryser, 2001). This is the first study to investigate the antimicrobial effects of alginate-apple puree edible films containing EOs or OCs against *E. coli* O157:H7. The objectives of this study were (a) to evaluate the effects of adding alginate and natural antimicrobials on the mechanical and barrier properties of the films, and (b) to determine antimicrobial activities against the *E. coli* O157:H7 of alginate-apple puree film forming solutions and alginate-apple puree films containing a variety of EOs and OCs.

MATERIALS AND METHODS

Test compounds. Food grade sodium alginate (Keltone® LV, ISP, San Diego, CA., USA) and Golden Delicious apple puree (38 °Brix) (Sabroso Co., Medford, OR) were the primary ingredients in all alginate/apple puree-based films. Glycerol (Fisher Scientific, Waukesha, WI) was added as a plasticizing agent and *N*-acetylcysteine (Sigma-Aldrich Chemical Co., Steinhein, Germany) was used as a browning inhibitor. The following EOs and OCs were obtained from Lhasa Kamash Herb Co. (Berkeley, CA): oregano, lemongrass, and cinnamon cassia. Citral and cinnamaldehyde were purchased from Sigma Chemical Co. (Milwaukee, WI). Carvacrol was a gift of Millennium Chemical Co. (Jacksonville, FL).

Preparation of alginate-apple puree film forming solution (AAPFFS). A 26% w/w AAPFFS was formed by combining 260 g of 38 °Brix apple puree with 715 g of 2% w/w alginate solution, 10 g of N-acetylcysteine (1% w/w) and 15 g of glycerol (1.5% w/w) using a modified method of McHugh and Senesi (2000). Natural antimicrobial EOs and

OCs were then incorporated into the AAPFFS at the following concentrations: 0 (control), 0.1% w/w (oregano oil and carvacrol) and 0.5% w/w (lemongrass oil, citral, cinnamon oil and cinnamaldehyde). These solutions were homogenized for 3 min at 12,500 rpm using a Polytron 3000 homogenizer (Kinematica, Littau, Switzerland) and then used for the bactericidal studies and casting the films.

Preparation of alginate-apple puree edible film (AAPEF). AAPFFS was prepared as described previously and then, vacuum was applied to remove bubbles. Films were cast on level 29 x 29 cm square plates and dried at ambient conditions for ~24 h. Dried films were cut and peeled from the casting surface. These film samples were used for determinations of barrier, mechanical and antimicrobial properties of the films.

Film thickness. Film thickness was measured with a micrometer IP 65 (Mitutoyo Manufacturing, Tokyo, Japan) to the nearest 0.00254 mm (0.0001 in) at five random positions around the film. The mean value was used to calculate water vapor permeability (WVP), oxygen permeability (O_2P), and tensile strength.

Water vapor permeability (WVP) of films. The gravimetric Modified Cup Method based on ASTM E96-92 (McHugh, Avena-Bustillos, & Krochta, 1993) was used to determine WVP. A cabinet with a variable speed fan was used to test film WVP. Cabinet temperature of 25 ± 1 ºC was maintained in a Forma Scientific reach-in incubator (Thermo Electron Corp., Waltham, MA). Fan speeds were set to achieve air velocities of 152 m/min to ensure uniform relative humidity throughout the cabinets. Cabinets were pre-equilibrated to 0% room humidity (RH) using anhydrous calcium sulphate (W.A. Hammond Drierite, Xenia, OH). Circular test cups made from polymethylmethacrylate (Plexiglas[TM]) were used. A film was sealed to the cup base with a ring containing a 19.6 cm^2 opening using 4 screws symmetrically located around the cup circumference. Both sides of the cup contacting the film were coated with silicon sealant. Distilled water (6 mL) was placed in the bottom of the test cups to expose the film to a high percentage RH inside the test cups. Average stagnant air gap heights between the water surface and the film were measured. Test cups holding films were then inserted into the pre-equilibrated 0% RH desiccator cabinets. Steady state of water vapor transmission rate was achieved within 2 h. Each cup was weighed 8 times at 2 h intervals. Eight replicates of each film were tested. Room humidities

at the film undersides and WVPs were calculated using the WVP Correction Method (McHugh et al., 1993).

The WVP of the films was calculated by multiplying the steady state water vapor transmission rate by the average film thickness determined as described above and dividing by the water vapor partial pressure difference across the films:

$$WVP = \frac{(WVTR)(thickness)}{(p_{A1} - p_{A2})} \qquad \text{Equation 1}$$

where WVTR = water vapor transmission rate and p_{A1} and p_{A2} = water vapor partial pressure inside and outside the cup, respectively.

Oxygen permeability (O$_2$P) of films. An Ox-Tran 2/20 ML modular system (Modern Controls Inc., Minneapolis, MN) was utilized to measure oxygen transmission rates through the films according to standard method D3985 (ASTM, 1995). Oxygen transmission rates were determined at 23 ℃ and 50 ± 1% RH. Each film was placed on a stainless steel mask with an open testing area of 5 cm^2. Masked films were placed into the test cell and exposed to 98% N$_2$ + 2% H$_2$ flow on one side and pure oxygen flow on the other. The system was programmed to have a 10 h waiting period to allow the films to achieve equilibrium. Oxygen permeability was calculated by dividing O$_2$ transmission rate by the difference in O$_2$ partial pressure between both sides of the film (1 atm) and multiplying by the average film thickness measured at 5 random places. Four replicates of each film were evaluated.

Tensile properties of films. Standard method D882-97 (ASTM, 1997) was used to measure tensile properties of films. Films were cut into strips with a test dimension of 165 mm x 19 mm according to standard method D638-02a (ASTM, 2002). All films were conditioned for 48 h at 23 ± 2 ℃ and 50% ± 2% RH before testing using a saturated salt solution of magnesium nitrate (Fisher Scientific, Fair Lawn, NJ). The ends of the equilibrated strips were mounted and clamped with pneumatic grips on an Instron Model 55R4502 Universal Testing Machine (Instron, Canton, MA) with a 100 N load cell. The initial gauge length was set to 100 mm and films were stretched using a crosshead speed of 7.5 mm/min. Tensile properties were calculated from the plot of stress (tensile force/initial

cross-sectional area) vs. strain (extension as a fraction of original length), using Series IX Automated Materials Testing System Software (Instron, Canton, MA). Fifteen specimens of each type of film were evaluated.

Source of bacteria. The Food and Drug Administration (FDA) provided *Escherichia coli* O157:H7 (strain SEA18B88; our file, strain RM1484). This strain was isolated from apple juice associated with an outbreak of human infection (Friedman, Henika, & Mandrell, 2002).

Test buffers. Phosphate-buffered saline (PBS, pH 7.0) was prepared by mixing dibasic sodium phosphate (100 mM) and monobasic sodium phosphate (100 mM) at 2:1 ratio, diluting by half with H_2O (v/v), and adding NaCl (150 mM). For lower pH buffers, 2 mM citric acid-150 mM NaCl was adjusted to pH 3.3 –3.7 (saline solutions).

Preparation of samples for bactericidal assays of AAPFFS. To facilitate pipetting, the 26% AAPFFS solution was further diluted by ½ with pH 3.3 saline solution, v/v. This AAPFFS sample was used to prepare suspensions for the assay. Oregano oil or carvacrol (10 μL) was added to 9.99 mL of diluted AAPFFS. Lemongrass oil, citral, cinnamon oil or cinnamaldehyde (50 μL) was added to 9.95 mL diluted AAPFFS in 50 mL tubes. The tubes were warmed in a microwave oven for 10 s and then shaken to form uniform suspensions. The content of the tubes were then diluted as follows: saline solution (500 μL) was added to five sterile 1.9 mL tubes. Serial dilutions were made starting with 1 mL of each original test solution, using 500 μL for each transfer for a total of five dilutions. Microtiter plates with 96 wells (Nalge, Rochester, NY) were prepared with saline pH 3.3 negative controls (100 μL each in 6 wells) and three test substances with five dilutions plus the test solution (100 μL each dilution per well, 6 wells). These 24 wells were sampled at three time intervals: 3, 30 and 60 min at 21 ºC.

Bactericidal assays of AAPFFS. A previously described assay (Friedman et al., 2002; Friedman et al., 2004) was used with some modifications. *E. coli* O157:H7 bacteria streaked on Luria-Bertani (LB) agar plates (Difco Inc., Sparks, MD) were subcultured and incubated for 16-18 h at 37 ºC. LB broth cultures were prepared by harvesting a few

isolated colonies from the plates with a sterile loop and suspending them into 5 mL LB broth in 15 mL sterile plastic tubes. The capped tubes were incubated with agitation at 37 ºC for 18 h. Bacterial suspensions were prepared for growth of ~100-200 CFU per lane on the square plates with grids used for counting. A sample (1 mL) of an 18 h LB broth culture of *E. coli* O157:H7 was added to a 1.9 mL microfuge tube. The bacteria were pelleted by centrifugation in a microfuge (15,800 *g*) for 1 min. After the supernatant was removed, 1 mL of sterile PBS (phosphate buffered saline, pH 7.0) was added to the pellet. The pellet was resuspended by gentle aspiration in and out of a transfer pipette. The sample's optical density at 620 nm was adjusted by ¼ dilution with PBS to *ca*. 0.4. The suspension (20 μL) was added to PBS (980 μL). The resulting suspension (80 μL) was then added to 5 mL saline solution pH 3.3, vortexed, and poured into a sterile, plastic Petri dish. The suspensions (50 μL) were drawn with a multichannel Eppendorf pipette and added to six microtiter plate wells. This was repeated until all of the 24 prepared wells were inoculated. The inoculated microtiter plates were sampled three times (3, 30, and 60 min) at 21 ºC without agitation. At the end of each incubation time, aliquots (10 μL) from each of six wells were drawn with an Eppendorf multichannel pipette for spotting of six 10-μL drops at the top of a square LB agar Petri plate. The plates were tilted before spotting to avoid coalescence of drops and tapped gently to facilitate movement of the liquid to the bottom. They were then placed uncovered for 10 min in a biological safety hood until dry, recovered, and incubated overnight at 37 ºC. Each well with test solution (150 μL) plus bacteria contained ~1,500 to 3,000 cells. Experiments were done in duplicate.

Bactericidal activities (BA$_{50}$ Values). Bactericidal activities, defined as the % of test compound that kills 50% of the bacteria under the test conditions, were determined as follows. Each compound was tested at a series of dilutions. The control pH 3.3 saline diluent was matched with pH of AAPFFS. The CFU values from all experiments were transferred to a Microsoft Excel 8.0 Spreadsheet. The number of CFU from each dilution was matched with the average control value to determine the percent of bacteria killed per well. Each of the dose-response profiles (% test compound versus % bactericidal activity) was examined graphically and the BA$_{50}$ values were estimated by a linear regression. The lower the BA$_{50}$, the higher the bactericidal activity was observed.

Antimicrobial activity of alginate-apple puree edible films (AAPEF). Disc inhibition zone assays were performed as a qualitative test for antimicrobial activity of the films. AAPEF with and without EOs and OCs were aseptically cut into 12 mm diameter discs and then placed on MacConkey-Sorbitol agar (Biokar Diagnostics, Beauvais, France) plates for E. coli O157:H7, which had been previously spread with 0.1 mL of inoculum containing 106 CFU/mL of tested bacterium. Plates were incubated at 37 ºC for 48 h. The thickness (mm) of the inhibition zone around the film disc (colony free perimeter) was then measured and the growth below the film discs (the contact area of edible film with agar surface) was examined visually. Tests were done in duplicate.

Statistical analysis. Data were analyzed by one-way analysis of variance (ANOVA) using Minitab version 13.31 software (Minitab Inc., State College, PA). Tukey test was used to determine the difference at 5% significance level (SAS, 1999).

RESULTS AND DISCUSSION

Barrier and mechanical properties.

Water vapor permeability. WVP is a measure of the facility with which a material can be penetrated by water vapor (Cagri et al, 2001). Polysaccharide edible films tend to be poor moisture barriers because of their hydrophilic nature (Kester et al., 1986; García, Martinó, & Zaritzky, 1998). The incorporation of lipids, either in an emulsion or as a layer coating into the films formulations, greatly improves their water vapor barrier properties (García, Martinó, & Zaritzky, 2000; Yang et al., 2000).

In the present study, WVP properties were not affected by the incorporation of EOs and OCs into the film, presumably because these EOs consist mostly of terpene-like compounds, not lipids. However, a slight decrease in WVP was observed after incorporation of 0.5% w/w cinnamaldehyde (Table 1). Hernández, (1994) indicated that water vapor transfer generally occurs through the hydrophilic portion of the film and depends on the hydrophilic-hydrophobic ratio of the film components.

Oxygen permeability. Oxygen permeability of the AAPEF with and without EOs and OCs are summarized in Table 1. The O_2P of the alginate-apple puree film was 10.20 ± 0.91 cm^{3}µm/m^2-d-kPa indicating that this film is a good oxygen barrier. This value is two times lower than that of an apple puree-pectin film (22.64 ± 1.28 cm^{3}µm/m^2-d-kPa) we observed

in a previous study (McHugh et al., 1996; Rojas-Graü, Avena-Bustillos, Friedman, Henika, Martín-Belloso, & McHugh, 2006). This difference is hypothesized to be caused by the effect of the type of carbohydrate used in the formulation (McHugh et al., 1996). Addition of antimicrobial agents did not affect the oxygen permeability of the films. Compared to the control films, a slight decrease in O_2P of the films was observed with lemongrass oil and citral (0.5% w/w) (Table 1).

Table 1. Effect of concentration (% w/w) and type of plant essential oils/oil compounds on water vapor permeability (WVP) and oxygen permeability (O_2P) properties of alginate-apple puree edible films.

Essential oil and oil compounds (% w/w)	Thickness[A] (mm)	RH inside cup[AB] (%)	WVP[AB] (g-mm/Kpa-h-m^2)	Oxygen permeability[A] (cm^{3}μm/m^2-d-kPa)
Control (0)	0.119 ± 0.004^{NS}	65.03 ± 1.81^a	4.95 ± 0.43^a	10.20 ± 0.91^a
Oregano oil (0.1)	0.118 ± 0.007	63.46 ± 0.65^a	5.25 ± 0.33^a	11.00 ± 0.92^a
Carvacrol (0.1)	0.117 ± 0.008	64.11 ± 0.86^a	5.02 ± 0.22^a	10.89 ± 0.76^a
Lemongrass oil (0.5)	0.122 ± 0.006	65.70 ± 1.77^{ab}	4.91 ± 0.40^a	9.38 ± 0.32^b
Citral (0.5)	0.118 ± 0.004	63.87 ± 0.89^a	5.12 ± 0.13^a	9.94 ± 0.15^{ab}
Cinnamon oil (0.5)	0.117 ± 0.008	64.77 ± 0.79^a	4.90 ± 0.27^a	10.50 ± 0.62^a
Cinnamaldehyde (0.5)	0.118 ± 0.009	67.10 ± 0.80^b	4.37 ± 0.54^b	11.03 ± 0.70^a

[A] Thickness and RH data are mean values. WVP and O_2P data are mean values $\pm$ standard deviations.
[B] Relative humidity at the inner surface and WVP values were corrected for stagnant air effects using the WVP Correction Method (McHugh et al., 1993).
[NS] Not significantly different
[a,b] Means in same column with different letters are significantly different ($p<0.05$).

Tensile properties. Tensile strength, elongation, and elastic modulus are parameters that relate mechanical properties of films to their chemical structures (McHugh and Krochta, 1994b). Tensile strength expresses the maximum stress developed in a film during tensile testing (Gennadios, Brandenburg, Park, Weller, & Testin, 1994). Incorporation of EOs and OCs caused a significant reduction ($p<0.05$) in tensile strength of the films (Table 2). This effect was more pronounced in films containing oregano oil and carvacrol, which displayed

lower values of tensile strength 2.47 ± 0.37 and 2.58 ± 0.37 MPa, respectively. Elongation at break is a measure of the film's stretch ability prior to breakage (Krochta and DeMulder-Johnston, 1997). The percent elongation of control AAPEF was 51.06% and increased in all films containing EOs and OCs, reaching a maximum value of 58.33% with carvacrol (Table 2). The elastic modulus of AAPEF (7.07 ± 1.09 MPa) was significantly greater than most of the films containing antimicrobial agents (Table 2). No significant differences were observed in the elastic modulus between films with and without cinnamon oil or cinnamaldehyde ($p < 0.05$).

Table 2. Effect of concentration (% w/w) and type of plant essential oils/oil compounds on the tensile properties of alginate-apple puree edible films.

Essential oil and oil compounds (% w/w)	Tensile strength[A] (MPa)	Elongation[A] (%)	Elastic modulus[A] (MPa)
Control (0)	2.90 ± 0.52[a]	51.06 ± 3.89[a]	7.07 ± 1.09[a]
Oregano oil (0.1)	2.47 ± 0.37[b]	56.96 ± 3.86[b]	5.75 ± 0.96[b]
Carvacrol (0.1)	2.58 ± 0.37[b]	58.33 ± 4.66[b]	5.96 ± 1.12[b]
Lemongrass oil (0.5)	2.56 ± 0.46[b]	55.95 ± 5.55[ab]	6.02 ± 1.07[b]
Citral (0.5)	2.52 ± 0.44[b]	57.38 ± 5.71[b]	6.46 ± 1.27[ab]
Cinnamon oil (0.5)	2.84 ± 0.48[ab]	57.88 ± 5.37[b]	6.86 ± 1.16[a]
Cinnamaldehyde (0.5)	2.75 ± 0.42[ab]	55.50 ± 7.40[ab]	6.77 ± 0.87[a]

[A] Tensile strength, elongation, and elastic modulus data are mean values ± standard deviations.
[a,b] Means in same column with different letters are significantly different at $p < 0.05$

Antimicrobial properties.

Antimicrobial activity of plant essential oils and oil compounds in AAPFFS. The experimental BA_{50} values for EOs and OCs at three time periods, 3, 30, and 60 min are show in Table3. All compounds inhibited the growth of *E. coli* O157:H7. AAPFFS in saline pH 3.3 without EOs or OCs and containing *N*-acetylcysteine as an antibrowning agent was not effective against the pathogen.

Table 3. Bactericidal activities (BA_{50} values) of plant essential oils/oil compounds against *E. coli* O157:H7 in alginate-apple puree film forming solution (AAPFFS)[a] incubated for 3, 30, and 60 min at 21 ℃.

Oil/oil compound (% w/w) in 50% AAPFFS[a]	BA_{50} value for *E. coli* O157:H7 [b]		
	3 min	30 min	60 min
Oregano oil (0.1)	0.025nd	0.010±0	0.012±0
Carvacrol (0.1)	0.020±0.0007	0.011±0.001	0.011±0.0007
Lemongrass oil (0.5)	>0.34[c]	0.066±0.01	0.059±0.006
Citral (0.5)	>0.34[c]	0.093±0.02	0.057±0.0007
Cinnamon oil (0.5)	>0.34[c]	0.16±0.08	0.087±0.05
Cinnamaldehyde (0.5)	>0.34[c]	0.11±0	0.086±0.03

[a] AAPFFS is 50% apple puree film formula suspension in saline pH 3.7 buffer.
[b] BA_{50} = Average values and standard deviations of two replicates of BA_{50} values
[c] >: less than 50% of bacteria were killed at the highest dose used.
nd: no detected

Table 3 shows that carvacrol at a concentration of 0.1% w/w in the AAPFFS was effective at 3 min with a BA_{50} value of 0.020 (0.020% of carvacrol inhibited 50% of the *E. coli* O157:H7 after 3 min). The activity at 30 min was twice as great ($BA_{50} = 0.011\%$) than at 3 min; at 60 min, it was the same ($BA_{50} = 0.011\%$) as at 30 min. Carvacrol appears to exhibit high antimicrobial effects against *E. coli* O157:H7. Similar behaviour was observed with oregano oil. The BA_{50} values of oregano oil against *E. coli* O157:H7 at 3, 30, and 60 min were 0.025, 0.010, and 0.012%, respectively, only slightly higher (the activity was lower) than those mentioned for carvacrol. The antimicrobial activity of oregano oil can be accounted for by its content of carvacrol. The antibacterial properties of carvacrol are associated with their lipophilic character, leading to accumulation in membranes and to subsequent membrane-associated events such as energy depletion (Sikkema, De Bont, & Poolman, 1995). Previously it was shown by HPLC that oregano oil contains about 86% carvacrol (Friedman, et al., 2004). Carvacrol, the major component of oregano oil, is designated as Generally Regarded as Safe (GRAS) (Dingman, 2000).

The activity of lemongrass oil at concentrations of 0.5% in the AAPFFS against *E. coli* O157:H7 were similar to those of citral (Table 3). Compared to carvacrol and oregano oil, it took about five times more of citral and lemongrass oil to achieve the same activity against *E. coli* O157:H7. On the other hand, cinnamaldehyde at a concentration of 0.5% w/w in the

AAPFFS was only effective at 30 and 60 min with BA_{50} values of 0.11 and 0.086%, respectively (Table 3). These results indicate that cinnamaldehyde at a fivefold greater concentration was less effective than carvacrol. The activity of cinnamon oil against *E. coli* O157:H7 at a concentration of 0.5% in the AAPFFS was of the same order as that observed with cinnamaldehyde (Table 3). These results were expected in view of the fact that cinnamon oil contains about 85% of cinnamaldehyde (Friedman et al., 2004).

Antimicrobial activity of plant essential oils and oil compounds in AAPEF. Table 4 shows the results of antimicrobial activities of the films containing the essential oils and oil compounds. The listed inhibitory activities were estimated from measurement of clear inhibition zones surrounding the film disks. If a surrounding clear zone was not present, it was assumed that the compound was not inhibitory and the diameter was assigned as zero. AAPEF without EOs and OCs served as a control to determine any possible antimicrobial effect of the film without additives. The control film did not inhibit the *E. coli* O157:H7.

The results show that all films containing added essential oils and oil compounds significantly inhibited the growth of *E. coli* O157:H7. As expected, AAPEF containing carvacrol was the most effective (greater surrounding clear zone) against *E. coli* O157:H7 (Table 4). Inhibition of *E. coli* O157:H7 by lemongrass oil/citral and cinnamon oil/cinnamaldehyde at 0.5% w/w in the films was lower than that observed with oregano oil/carvacrol (Table 4). Compared to carvacrol, it took about five times more cinnamaldehyde and citral to achieve the same activity against *E. coli* O157:H7. Because cinnamon oil is present in numerous commercial foods (Friedman, Kozuekue, & Harden, 2000), has a pleasant taste, and is GRAS-listed (Adams et al., 2004), the compound merits use as an antimicrobial in edible films.

Table 4. Antibacterial activity of plant essential oils/oil compounds incorporated into alginate-apple puree edible films against *E. coli* O157:H7.

Essential oil and oil compounds	Concentration (% w/w)	*E. coli* O157:H7	
		Inhibitory zone (mm)[a]	Contact area [b]
Control	0	0	-

Oregano oil	0.1	1.2	+
Carvacrol	0.1	1.6	+
Lemongrass oil	0.5	1.0	+
Citral	0.5	1.2	+
Cinnamon oil	0.5	< 1	+
Cinnamaldehyde	0.5	1.0	+

[a]Values are measurements of thickness (mm) of inhibitory zone (colony free perimeter).
[b]Contact area is the part of agar on Petri dish directly underneath film pieces. +: represents an inhibitory effect; -: represents no inhibitory effect.

CONCLUSIONS

There was no adverse effect of the additives on water vapor and oxygen permeabilities. Tensile properties; however, were significantly affected by addition of EOs and OCs. The antimicrobial activity of oregano essential oil and of carvacrol in alginate-apple puree edible films and film forming solutions against *E. coli* O157:H7 was significantly greater than the activities of lemongrass oil, citral, cinnamon oil, and cinnamaldehyde. The antimicrobial data obtained with the alginate-apple puree forming solution can serve as a guide for selection of appropriate levels of plant compounds for incorporation into antimicrobial edible films. Incorporating EOs and OCs into edible films provides a novel way to enhance the safety and shelf-life in food systems.

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APPLE PUREE-ALGINATE EDIBLE COATING AS CARRIER OF ANTIMICROBIAL AGENTS TO PROLONG SHELF LIFE OF FRESH-CUT APPLES

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ABSTRACT
Edible coatings with antimicrobial agents can extend the shelf-life of fresh-cut fruit. The effect of lemongrass, oregano oil and vanillin incorporated in apple puree-alginate edible coatings, on the shelf-life of fresh-cut Fuji apples, was investigated. Coated apples were packed in air filled polypropylene trays and wrapped with polypropylene film. Changes in headspace atmosphere, color, firmness, sensory quality and microbial growth were measured during 21 days storage at 4 ºC. A significant reduction in the rates of O_2 depletion and CO_2 evolution was observed in samples containing high concentrations of essential oils (EOs). Ethylene production in the coated apples remained below 50 µl l^{-1}, while production of this gas increased continuously in uncoated apples and those coated without EOs during storage. Apples coated with apple puree-alginate exhibited ethanol and acetaldehyde formation in the first week. Coatings with calcium chloride and *N*-acetylcysteine helped to maintain firmness and color, while lemongrass containing coatings induced severe texture softening. Vanillin containing coatings (0.3% w/w) were the most effective in terms of sensory quality after 2 weeks storage. All antimicrobial coatings significantly inhibited the growth of psychrophilic aerobes, yeasts and molds. The antimicrobial effect of EOs against *L. innocua* inoculated into apple pieces before coating was also examined. Lemongrass (1.0 and 1.5% w/w) and oregano oil containing coatings (0.5% w/w) exhibited the strongest antimicrobial activity against *L. innocua* (4 log reduction).

Keywords: edible coating, alginate, antimicrobials, *Listeria innocua*, shelf-life, fresh-cut apples
INTRODUCTION
Minimally processed fruits go through preparation steps such as peeling, cutting or slicing, to increase their functionality and maintain their fresh-like properties. Minimal processing alters the integrity of fruit and induces wounding stress and spoilage. Also, the presence of microorganisms on the surface of fruit may compromise the safety of fresh-cut fruit (Del Rosario and Beuchat, 1995; Thunberg et al., 2002). Controlling fresh quality and growth of spoilage and pathogenic bacteria is a challenging problem for the fresh-cut fruit industry.

Edible coatings, containing antimicrobial agents, are gaining importance as potential treatments to reduce the deleterious effects imposed by minimal processing on fresh-cut fruit (Alzamora and Guerrero, 2003; Burt and Reinders, 2003). The use of edible coatings for a wide range of food products, including fresh and minimally processed fruit, has received increased interest, because coatings can serve as carriers for a wide range of food

additives, including antibrowning agents, colorants, flavors, nutrients, spices and various antimicrobials that can extend product shelf-life and reduce the risk of pathogen growth on food surfaces (Baldwin et al., 1996; Wong et al., 1996; Cagri et al., 2004; Pranoto et al., 2005). Incorporating antimicrobial compounds into edible films or coatings provides a novel way to improve the safety and shelf life of ready-to-eat foods (Cagri et al., 2004). Some of the more commonly used antimicrobials include benzoic acid, sorbic acid, lysozyme, lactoferrin, bacteriocins (nisin and pediocin) and plant-derived secondary metabolites, such as essential oils and phytoalexins.

Vanillin is a plant essential oil fraction that has been used recently as a bacteriostatic rather than a bactericidal agent in fresh-cut apples (Rupasinghe et al., 2006). EOs have also been evaluated for their ability to protect food against pathogenic bacteria in contaminated apple juice (Friedman et al., 2004; Raybaudi-Massilia et al., 2006) and other foods (Burt, 2004). These EOs are designated as Generally Regarded as Safe (GRAS) (Burt, 2004), and used as flavouring agents in baked goods, sweets, ice cream, beverages, and chewing gum (Fenaroli, 1995). These compounds can be added to edible films and coatings to modify flavor, aroma, and odor, as well as to introduce antimicrobial properties (Cagri et al., 2004). EOs are regarded as alternatives to chemical preservatives, and their use in foods meets the demands of consumers for minimally processed natural products, as reviewed by Burt (2004).

Little published data exist on the incorporation of plant essential oils into edible films and coatings. No prior research has been reported on the incorporation of natural plant extracts into edible coatings applied on fresh-cut fruits. McHugh et al. (1996) developed the first edible films made from fruit purees which were shown to be a promising tool for improving quality and extending shelf-life of minimally processed fruits (McHugh and Senesi, 2000; Salcini and Massantini, 2005). Rojas-Graü et al. (2006) recently investigated the effect of plant essential oils on antimicrobial and physical properties of apple puree edible films. Alginate-apple puree films, containing plant essential oils, have not been explored as edible coatings previously. The objectives of this work were 1) to study the effects of natural antimicrobial agents, incorporated in fruit puree-polysaccharide edible coatings, on native psychrophilic aerobic bacteria, yeasts, molds and inoculated *Listeria innocua* in fresh-cut

Fuji apples, and 2) to characterize the physicochemical properties of the coatings as they relate to fruit quality and shelf-life.

MATERIALS AND METHODS

Materials. Apple puree (38 ºBrix) (Indulleida S.A., Lleida, Spain) and food grade sodium alginate (Keltone® LV, ISP, San Diego, CA, USA) were the primary ingredients in all coating solutions. Glycerol (Merck & Co. Inc., Whitehouse Station, NJ, USA) was added as plasticizer. Calcium chloride (Sigma-Aldrich Corp. Steinhein, Germany) was used to induce crosslinking. *N*-acetylcysteine (Sigma-Aldrich Corp. Steinhein, Germany) acted as a browning inhibitor. Oregano and lemongrass oils (Aceites Esenciales Dicana S.A., Barcelona, Spain), and vanillin (Scharlau Chemie S.A., Barcelona, Spain) were the antibrowning agents and active compounds tested.

Preparation of the coating solutions. Coating solutions (CS) were prepared by mixing apple puree (26% w/w) with an alginate solution (2% w/w) which was previously prepared by dissolving alginate in distilled water and heating at 70 ºC while stirring until the solution became clear. CS also contained glycerol (1.5% w/w). EOs and active compounds were then incorporated into CS at the following concentrations: 0% (control), oregano (0.1% and 0.5% w/w), lemongrass (1% and 1.5% w/w) or vanillin (0.3% and 0.6% w/w), respectively. These solutions were homogenized for 3 min at 12,500 rpm using an Ultra Turrax T25 (IKA® WERKE, Germany) homogenizer with a S25N-G25G device, and degassed under vacuum. *N*-acetylcysteine (1% w/v) was added to a calcium chloride bath (2% w/v) to crosslink the carbohydrate polymers. These formulations were chosen in accordance with previous work (Rojas-Graü et al., 2006; 2007).

Fruit coating. 'Fuji' apples (*Malus domestica* Borkh) used in this study were purchased in a local market and stored at 4 ± 1 ºC before processing. Apples were washed, rinsed and dried prior to cutting operations. Subsequently, apples were cut into cylinders 1.4 cm diameter by 2 cm height. The apple pieces were first dipped into CS (with or without EOs) for 2 minutes. Residual solution was allowed to drip off for 1 min, before submerging the

cylinders for 2 min in the solution of calcium chloride and *N*-acetylcysteine. Next, apple pieces were packaged into polypropylene trays (Mcp Performance Plastic LTD, Kibbutz Hamaapil, Israel) and wrapped using a 64 μm thickness polypropylene film with a permeability to oxygen of 110 $cm^3.O_2.m^{-2}.bar^{-1}.day^{-1}$ at 23 ℃ and 0% RH (Tecnopack SRL, Mortara, Italy) using a MAP machine (Ilpra Foodpack Basic V/G, Ilpra, Vigenovo, Italy). Trays were filled with air, heat sealed, and stored in darkness at 4 ± 1 ℃. Analyses were carried out periodically during 21 days storage in randomly sampled pairs of trays.

Headspace gas analysis. The atmosphere of each single tray was analyzed using a gas chromatograph equipped with a thermal conductivity detector (Micro-GC CP 2002 gas analyzer, Chrompack International, Middelburg, The Netherlands). A sample of 1.7 mL was automatically withdrawn from the headspace atmosphere. Portions of 0.25 and 0.33 mL were injected for O_2 and CO_2 determination, respectively. The O_2 content was analyzed with a CP-Molsieve 5 Å packed column (4 m x 0.32 mm, df = 10 mm) (Chrompack International, Middelburg, The Netherlands) at 60 ℃ and 100 kPa. For quantification of CO_2, ethylene (C_2H_4), and ethanol (C_2H_5OH), a Pora-PLOT Q column (10 m x 0.32 mm, df = 10 mm) (Chrompack International, Middelburg, The Netherlands), held at 70 ℃ and 200 kPa, was used. Two trays were taken at each sampling time to perform the gas analysis and two replicates were carried out for each one.

Color measurement. Fresh-cut apple surface color was directly measured with a CR-400 Minolta chroma meter (Minolta, Inc., Tokyo, Japan). Color was measured using the CIE L^*, a^*, b^* coordinates. Illuminant D65 and 10º observer angle were used. The instrument was calibrated using a standard white reflector plate. Ten pieces were evaluated for each pair of trays. Three readings were made in each replicate by changing the position of the apple pieces. Numerical values of a^* and b^* parameters were employed to calculate hue angle: $h^* = \tan^{-1} (b^*/a^*)$.

Firmness measurement. Apple firmness evaluation was performed using a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., England, UK) by measuring the maximum penetration force required for a 4 mm diameter probe to penetrate into an apple cylinder of 20 mm height and 15 mm diameter to a depth of 10 mm at a rate of 5 $mm.s^{-1}$. Apple

cylinders were placed perpendicular to the probe so as to allow penetration in their geometric centre.

Sensory evaluation. Sensory quality of coated apple pieces was evaluated at 1, 7 and 14 days of storage. For the hedonic tests, the consumers evaluated four pieces of fresh-cut apples: coated without EOs (control), and coated with EOs [vanillin (0.3%), lemongrass (1%) or oregano oil (0.1%)]. Thirty persons, aged between 20 and 45 years old and who eat apples frequently, were recruited among students and personnel of the Department of Food Technology, University of Lleida. The order of the samples was randomized for each consumer. They were asked to evaluate the samples on a 10 cm non-structured linear scale with anchor points at each end. The attributes evaluated were: color by visual observation under white lightning, texture by biting with front teeth, taste by masticating, and overall preference; where 0 indicated extreme dislike and 10 indicated extreme like. The judges' average response was calculated for each attribute.

Microbiological analysis

Preparation of bacterial culture. Since it is important to know the effect of coatings on pathogenic microorganisms relevant to fresh-cut fruit, as well as on the native psychrophilic aerobic bacteria, yeasts, and molds, an experiment was designed to evaluate the antimicrobial effect of coatings containing EOs on *Listeria innocua* inoculated (as surrogate of the pathogenic *Listeria monocytogenes*) on apple pieces. *L. innocua* (Laboratoire de Re´pression des Fraudes, Montpellier, France) was maintained in tryptone soy agar (TSA) (Biokar Diagnostics, Beauvais, France) slants at 5 ºC. Stock culture of *L. innocua* was grown in TSB + 0.6% of yeast extract (Biokar Diagnostics, Beauvais, France) at 35 ºC for 15 h and 200 rpm (cell in early stationary phase). The maximum level reached for the microorganism was 6.5×10^9 colony forming units per milliliter ($CFU.ml^{-1}$). Concentration was then adjusted to 10^8 $CFU.ml^{-1}$ using saline peptone water with 0.1% peptone (Biokar Diagnostics, Beauvais, France) and 0.85% NaCl (Scharlau Chemie, S.A., Barcelona, Spain).

Apple inoculation. Ten grams of Fuji apple were inoculated with 100 µl of an appropriately diluted culture so as to contain 10^6 CFU.ml^{-1}of *Listeria innocua*. The inoculated pieces were dried for 0.5 h and then coated. The coating forming solutions were prepared and applied using the methodology described previously. Coated apples were packed and stored as explained above.

Incidence of *Listeria* sp. in fresh cut apple. Occurrence of *Listeria* in non-inoculated samples was studied according to the ISO 11290-1 guideline working with Fraser Half Broth (FHB) (Biokar Diagnostics, Beauvais, France) as pre-enrichment medium and Fraser Complete Broth (FCB) (Biokar Diagnostics, Beauvais, France) as enrichment medium, with posterior streaking on Oxford and Palcam agar (Biokar Diagnostics, Beauvais, France).

Psychrophilic aerobic bacteria, yeasts/molds, and *Listeria innocua* counts. Apple pieces (10 g) were removed aseptically from each tray and transferred into sterile plastic bags. Samples were diluted with 90 ml of saline peptone water (0.1% peptone - Biokar Diagnostics, Beauvais, France + 0.85% NaCl - Scharlau Chemie, S.A. Barcelona, Spain) and homogenized for 1 min in a stomacher blender (IUL Instruments, Barcelona, Spain). Serial dilutions were made and then poured onto plate count agar (PCA) (Biokar Diagnostics, Beauvais, France) and chloramphenicol glucose agar (CGA) to quantify psychrophilic aerobic bacteria, yeasts and molds. Samples were spread on plates of Palcam agar (Biokar Diagnostics, Beauvais, France) to count *L. innocua*. Plates were incubated for 10-14 days at 5 °C to enumerate psychrophilic aerobic bacteria, 3 days at 25 °C to enumerate yeasts and molds, and 48 h at 30 °C to quantify *L. innocua*. Colonies were counted and the results expressed as CFU.g^{-1} of apples. Analyses were carried out periodically over 21 days of storage in randomly sampled pairs of trays. Two replicate counts were performed for each tray. Determinations of psychrophilic aerobic bacteria, yeasts and molds, and *Listeria innocua* immediately after coating (time zero) and at 4, 7, 10, 14, 17 and 21 days of storage were made.

Statistical analysis

Data were analyzed by one-way analysis of variance using statistical procedures of the Statgraphics Plus V.5.1. (Statistical Graphics Co., Rockville, MD, USA). Specific differences were determined by least significant difference (LSD) and Tukey's tests. All comparisons were made at the 5% level of significance.

RESULT AND DISCUSSION

Changes in headspace gas composition

Intact commodities continue respiring through the skin after harvesting. If disruption of the skin occurs by cutting, the rate of respiration is accelerated and characteristic flavor and aroma compounds in fruits are modified (Beaulieu and Baldwin, 2002). Edible coatings are capable of producing a modified atmosphere on coated fruits by isolating the coated product from the environment, acting as a barrier to oxygen, carbon dioxide and water vapor and decreasing the rate of respiration (Olivas and Barbosa-Cánovas, 2005).

In this study, the effects of the edible coatings containing EOs on respiratory activity (O_2 and CO_2), ethylene, acetaldehyde and ethanol production of fresh-cut Fuji apple pieces was evaluated by analysis of headspace gas in polypropylene packaging during 21 days of refrigerated storage.

Figures 1A and 1B show that the type and concentration of EOs affected significantly ($p \leq 0.05$) the headspace gas concentration of both O_2 and CO_2 in packaged fresh-cut coated apples during the evaluated period. A very slight decrease was observed in O_2 headspace concentration in apple pieces coated with CS containing lemongrass oil (1 and 1.5% w/w) and at higher concentration of oregano oil (0.5% w/w), while O_2 concentration in the other coated samples decreased reaching levels as low as 3.54 % with uncoated fresh-cut apples (Figure 1A). Similarly CO_2 concentrations in samples with high levels of EOs (1% and 1.5% lemongrass; 0.5% oregano oil) were unaffected during storage time, while a substantial increase in CO_2 concentration was observed in the rest of the samples (Figure 1B). The pattern exhibited by the coatings with high concentrations of EOs suggests that reduction in the rate of CO_2 evolution and O_2 depletion is affected by the plant essential oils

incorporated into the alginate-apple puree coating. In previous work, it was observed that dried films made of alginate-apple puree and containing natural essential oils showed higher oxygen permeability than those edible films based only on alginate (Olsen et al., 2006).

The trend of O_2 depletion and CO_2 evolution in coated fresh-cut fruits has been reported by several authors. Thus, Wong et al. (1994) investigated the effect of various bilayer coatings on respiratory activity of coated apple pieces measuring CO_2 production in the headspace gas composition. All coatings studied by the authors (alginate included) produced a substantial rate reduction in this gas, compared to uncoated cut apples. They suggest that the diffusion of the headspace O_2 to the tissue was inhibited by the high O_2 resistance of the coating. However, Olivas et al. (2005) indicated that coatings with selective permeability to gases are capable of decreasing the interchange of O_2 and CO_2 between coated fruit and the environment, slowing down the metabolism by decreasing internal O_2 concentration and increasing CO_2 concentration.
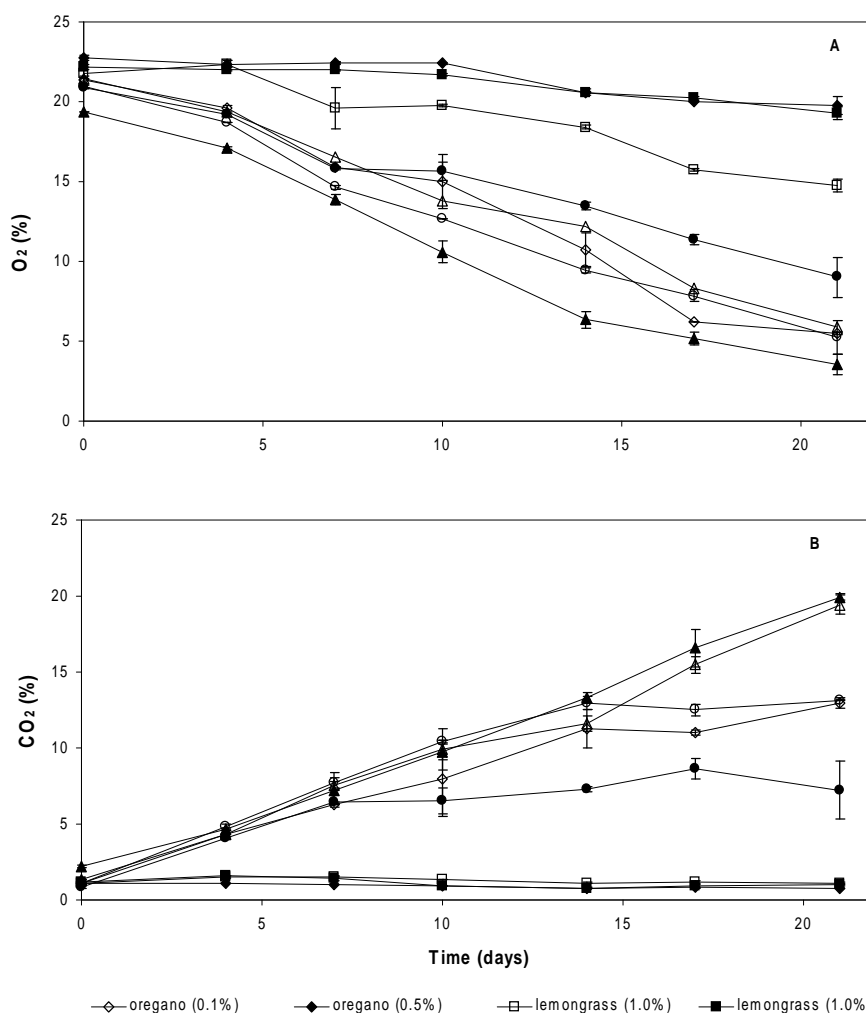

-◇- oregano (0.1%)　　-◆- oregano (0.5%)　　-□- lemongrass (1.0%)　　-■- lemongrass (1.0%)

Figure 1. O_2 and CO_2 headspace gas concentration in trays containing coated (with or without EOs) and uncoated apple pieces during storage at 4ºC. Data shown are the means (± standard deviation).

The physiological responses elicited by the physical stress imposed by cutting and slicing the vegetable tissue, are well established in the literature and associated to ethylene production (Kays, 1991; Beaulieu et al., 2002). Ethylene evolution of the coated and uncoated fresh-cut apples observed in this study is shown in Figure 2A. All samples coated with alginate-apple puree and containing EOs exhibit a constant low level in ethylene production. No statistical differences ($p > 0.05$) were observed between samples coated with different concentrations of the same compound. However, the results are particularly different in apple pieces without coating or edible coating without EOs, where ethylene production increased steadily reaching values of 230.07 and 132.38 μl l^{-1} respectively, at the end of the refrigerated storage period (Figure 2A). The inhibitory effect of the coatings with EOs seems evident. This agrees with results reported by Wong et al. (1994) in which a substantial rate reduction (90%) of ethylene was observed in fresh-cut apple coated with bilayer coatings of buffered polysaccharide/lipid. Authors attributed this reduction primarily to the diffusion barrier properties of the lipid layer and secondarily to the inhibitory effect of the ascorbate buffer which contained calcium ions. In our case, EOs

were incorporated by emulsification into the apple puree-alginate coating solutions to improve antimicrobial properties, while crosslinking of the carbohydrate polymers was obtained by immersion in a calcium chloride solution; hence both components might have contributed to the decrease in ethylene production found in this study. Additionally, Lee et al. (2003) found a reduction of the initial respiration rate (from 44.80 to 34.95 mg CO_2 . kg $^{-1}$. h $^{-1}$) of fresh-cut Fuji apples coated with whey protein concentrate attributing this effect to the calcium ions contained in the film forming solution and to the oxygen barrier properties inherent to the film.

Reduced interchange of gases between fruits and their immediate surroundings due to the use of coatings can drastically reduce oxygen levels and alter the respiratory metabolism in such a way to produce anaerobiosis and fermentation, resulting in off-flavors caused by accumulation of ethanol and acetaldehyde (Alonso and Alique, 2004). In this study, the production of acetaldehyde and ethanol in apple pieces coated with apple puree-alginate coatings indicates the creation of an anaerobic modified atmosphere, as suggested by the lower accumulation of ethanol and acetaldehyde in the headspace of polymeric packages containing the uncoated fresh-cut apples during refrigerated storage (Figure 2B and C).
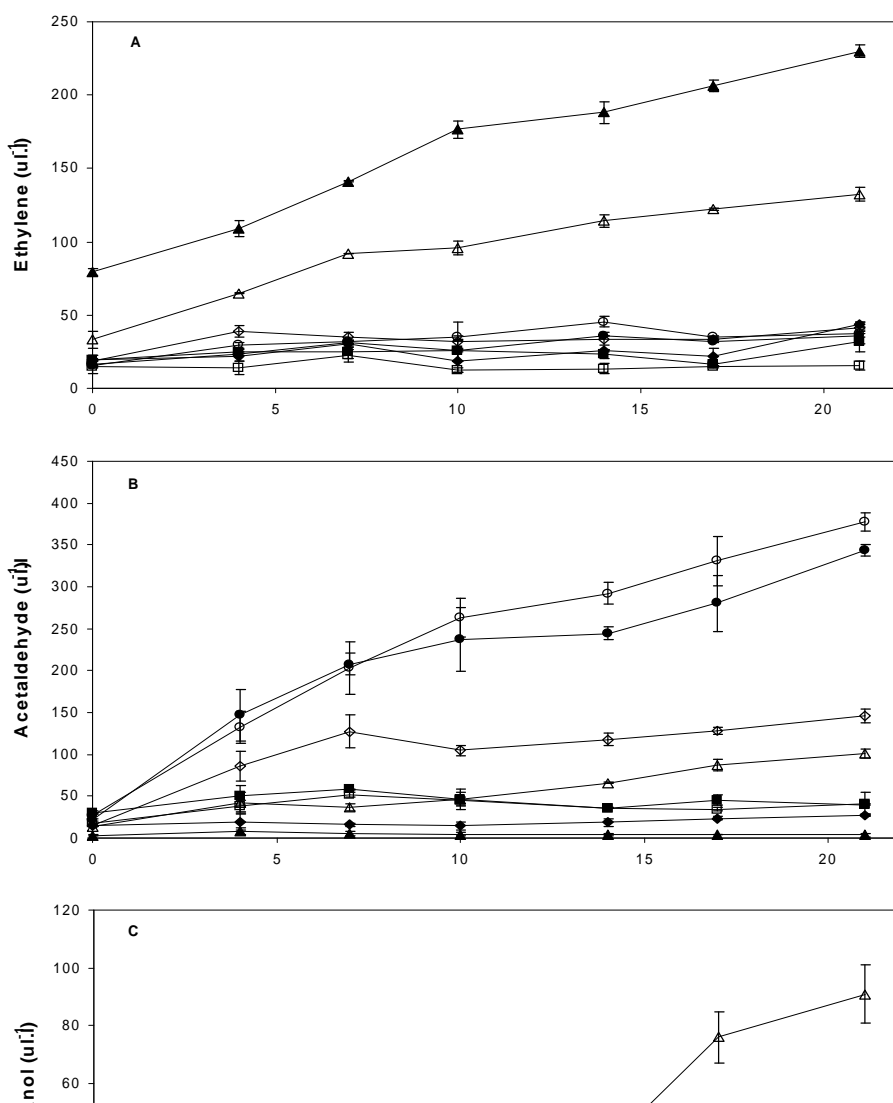
Figure 2. Ethylene (a), acetaldehyde (b) and ethanol (c) headspace gas concentration in trays containing coated (with or without EOs) and uncoated apple pieces during storage at 4ºC. Data shown are the means (± standard deviation).

The acetaldehyde evolution of coated and uncoated fresh-cut apples is shown in Figure 2B. The type of EOs incorporated into alginate-apple puree coatings affected significantly ($p \leq 0.05$) the acetaldehyde production. Acetaldehyde concentrations of vanillin coated fresh-cut apples increased during storage reaching levels as high as 377.47 and 344.00 µl l^{-1} in samples containing vanillin at 0.3 and 0.6% w/w respectively; while in the rest of coated apples the production of the gas was low and constant until the end of storage. These differences can be attributed to the different nature of the antimicrobials incorporated in CS. Coatings with antimicrobial agents of lipophilic nature such as oregano and lemongrass seemed to offer a major resistance to diffusion of acetaldehyde from the interior of the pieces of fruits towards the head space of the package, as compared to apple pieces coated with formulations in which vanillin (not lipid crystalline compound) had been incorporated. It can be inferred that the concentration of this gas inside the coated fruit is higher than detected in the polymeric package headspace.

Ethanol is an indicator of the degree of anaerobic fermentation that is taking place and its accumulation occurs when internal atmosphere is affected and gas exchange is restricted (Park et al., 1994). Figure 2C shows the ethanol production in coated and uncoated fresh-cut apples. The presence of this gas was detected after 1 week of storage in apple pieces with the coatings that showed lesser gas barrier properties (vanillin 0.3 and 0.6% w/w, oregano oil 0.1% and lemongrass 1%), reaching values of 43.23 µl l^{-1} at the end of the storage period. However, in coated apple pieces containing high levels of lemongrass and oregano oil in its formulation, ethanol was detected at day 10, and values did not exceed 26 µl l^{-1} after 21 days of storage (Figure 2C). It is important to highlight the fact that ethanol production increased rapidly after 14 days of storage in packages of apple pieces coated with apple puree-alginate coatings without EOs reaching values of 90.98 µl l^{-1} at the end of the refrigerated storage (Figure 2C) indicating a shift to anaerobic respiration due to the low oxygen permeability of the apple-alginate coating. The appearance of these fermentative metabolites (ethanol and acetaldehyde) as a result of anaerobic respiration is often associated to off-flavors and its presence might be detrimental to quality (Day, 1994). Edible coatings are expected to impose some restrictions to gas interchange and it is evident that the edible coatings containing EOs used in this work affected positively the production and the subsequent gas diffusion pattern of acetaldehyde and ethanol.

Color changes

The use of antimicrobial edible coatings had a significant ($p \leq 0.05$) effect in the color parameters L^*, a^*, b^* and h* of fresh-cut Fuji apples (Table 1). From the 7th day of storage, L^* values tended to decrease in all coated samples, except in those containing lemongrass (1.0% w/w) and vanillin (0.6% w/w), which kept constant L^* values throughout storage. Color of uncoated fresh-cut apples darken rapidly and was not measured during storage.

The a^* value is a measure of greenness and is highly related to color changes of apple flesh (Goupy et al., 1995). Apple puree-alginate coatings containing EOs produced a modification of the a^* coordinate compared to the control (without EOs) (Table 1). This difference was especially apparent in samples coated with solutions containing lemongrass. External color of apples coated with lemongrass (1.0% and 1.5% w/w) tended to greenish as indicated by the decrease of a^* values (Table 1), but internal color was seriously affected, leading to darken from the first days of storage.

Regarding the yellowness values, a significant decrease in b^* values in samples coated with apple puree-alginate coatings was observed during storage. The abrupt decrease in $b*$ values in samples containing 1.5% of lemongrass, which fluctuated between 28.52 and 21.77 during the period of storage, was particularly apparent. After one week of storage, b^* values of all coated samples decreased with storage time (Table 1).

The hue value (h^*) represents true color, which is effective for visualizing the color appearance of food products (McGuire, 1992). ANOVA revealed that time of storage did not affect significantly ($p>0.05$) the change of h^* values observed in fresh-cut apples coated with alginate-apple puree. In contrast, the type of coating influenced significantly the tonality of coated cut apples. The maximum values of h^* were obtained in fresh-cut apples coated with lemongrass and the lowest were observed in apple coated without incorporation of EOs (Table 1). This was expected since the main variation of parameter a^* (h^* values are calculated from numerical values of a^* and b^*) was observed in samples coated with lemongrass and without EOs.

Table 1. Changes in the color parameters of apple pieces coated with alginate-apple puree (with or without EOs) during storage.

Time (days)	Without EOs	Oregano oil		Lemongrass		Vanillin	
	0	0.1	0.5	1	1.5	0.3	0.6
L*							
0	63.05 ± 0.10 [a, A]	65.46 ± 0.46 [a, AB]	67.87 ± 0.66 [a, B]	65.84 ± 1.19 [a, AB]	69.15 ± 2.25 [a, B]	66.67 ± 1.19 [a, B]	66.33 ± 2.10 [a, B]
7	55.01 ± 1.64 [b, A]	60.30 ± 2.01 [b, BC]	61.37 ± 0.47 [b, BC]	64.40 ± 0.05 [a, C]	62.33 ± 0.11 [b, BC]	59.59 ± 0.89 [b, B]	64.12 ± 0.03 [a, B]
14	55.02 ± 0.41 [b, A]	59.50 ± 0.25 [b, B]	58.53 ± 2.52 [b, AB]	63.79 ± 0.61 [a, BC]	63.73 ± 0.14 [b, BC]	57.25 ± 2.08 [b, AB]	64.21 ± 0.43 [a, B]
21	52.61 ± 0.50 [b, A]	58.50 ± 0.28 [b, B]	58.93 ± 0.47 [b, B]	64.79 ± 1.74 [a, C]	63.71 ± 0.75 [b, C]	59.63 ± 0.51 [b, BC]	59.05 ± 1.65 [b, C]
a*							
0	-1.96 ± 0.10 [a, C]	-2.88 ± 0.07 [a, B]	-2.47 ± 0.17 [a, BC]	-3.25 ± 0.35 [a, B]	-4.21 ± 0.61 [a, A]	-2.52 ± 0.18 [a, BC]	-2.67 ± 0.04 [a, BC]
7	-1.67 ± 0.19 [ab, C]	-2.42 ± 0.33 [a, B]	-2.02 ± 0.22 [ab, C]	-3.83 ± 0.01 [a, A]	-3.47 ± 1.12 [ab, A]	-2.21 ± 0.09 [a, BC]	-2.54 ± 0.07 [a, B]
14	-1.15 ± 0.57 [b, A]	-2.34 ± 0.22 [a, C]	-1.44 ± 0.12 [ab, C]	-3.33 ± 0.46 [a, B]	-3.08 ± 0.78 [b, BC]	-2.09 ± 0.35 [a, C]	-2.74 ± 0.40 [a, BC]
21	-0.91 ± 0.33 [b, C]	-2.14 ± 0.16 [a, B]	-0.68 ± 0.23 [b, C]	-3.09 ± 0.06 [a, A]	-3.09 ± 0.29 [b, A]	-2.12 ± 0.06 [a, B]	-1.96 ± 0.01 [b, B]
b*							
0	26.30 ± 0.15 [a, AB]	24.55 ± 1.29 [a, A]	23.92 ± 0.44 [a, A]	25.70 ± 1.13 [a, A]	28.52 ± 0.24 [a, B]	25.56 ± 0.27 [a, A]	25.83 ± 2.15 [a, AB]
7	20.76 ± 1.52 [b, B]	22.61 ± 0.32 [a, B]	21.19 ± 1.39 [b, B]	26.87 ± 0.85 [a, A]	26.02 ± 0.13 [a, A]	22.97 ± 0.67 [b, B]	24.37 ± 0.11 [b, AB]
14	22.33 ± 0.93 [b, B]	19.50 ± 0.12 [b, C]	19.35 ± 0.63 [b, C]	26.36 ± 0.39 [a, A]	22.66 ± 1.09 [b, B]	21.65 ± 0.14 [b, BC]	23.52 ± 0.64 [b, B]
21	21.04 ± 0.65 [b, B]	20.26 ± 1.13 [b, B]	20.96 ± 0.87 [b, B]	25.21 ± 1.87 [a, A]	21.77 ± 0.77 [b, B]	21.50 ± 0.52 [b, B]	22.73 ± 1.12 [b, A]
h*							
0	94.27 ± 0.24 [ab, B]	96.70 ± 0.51 [a, A]	95.89 ± 0.51 [a, AB]	97.20 ± 0.46 [a, A]	98.40 ± 1.12 [a, A]	95.63 ± 0.46 [a, AB]	95.92 ± 0.41 [a, AB]
7	94.58 ± 0.20 [a, AB]	96.10 ± 0.74 [a, A]	93.90 ± 0.58 [a, B]	98.11 ± 0.23 [a, A]	97.58 ± 2.46 [a, A]	95.50 ± 0.39 [a, AB]	95.94 ± 0.19 [a, AB]
14	92.98 ± 1.58 [b, A]	96.86 ± 0.81 [a, B]	95.97 ± 0.84 [a, B]	97.20 ± 1.10 [a, B]	97.79 ± 2.30 [a, B]	95.50 ± 0.94 [a, B]	96.66 ± 1.15 [a, B]
21	92.49 ± 0.98 [b, A]	96.27 ± 1.46 [a, B]	91.87 ± 0.71 [b, A]	96.99 ± 0.39 [a, B]	98.10 ± 1.03 [a, B]	95.62 ± 0.28 [a, B]	94,92 ± 0.23 [a, B]

Data shown are the means (± standard deviation). Means for the same parameter in the same column (a-b) or in the same line (A-C) with different letters are significantly different (p≤0.05), according to ANOVA and the Tukey´s test.

In general, the lowest changes in color parameters of fresh-cut apples were observed when vanillin (0.3% and 0.6% w/w) was incorporated into the coating formulation (Table 1). Similar result was obtained by Rupasinghe et al. (2006) who observed that incorporation of vanillin into NatureSeal[TM] solution did not modify the color of fresh-cut apples during the storage period.

On the other hand, all coating formulations used in this work contained N-acetylcysteine an antibrowning agent which helped to keep the apple pieces free from browning during the entire period of storage. By contrast, fresh-cut apples without coatings showed high positive value of a^* (1.34) and low h^* value (87.50) in the first hour of storage, which is an index of browning. These results demonstrated that *N*-acetylcysteine is an effective antibrowning agent to be incorporated in the formulation of edible coatings. Edible coatings have the potential to carry and hold additives as antibrowning agents on the surface of cut tissues, aiding to an effective control of browning. In a previous work, the effectiveness of *N*-acetylcysteine as antibrowning agent incorporated into alginate coating (without apple puree) prevented browning of coated fresh-cut apples (Rojas-Graü et al., 2007).

Texture changes

The texture of the fruits is likely to soften during storage due to several factors, including loss in cell turgidity pressure, loss of extracellular and vascular air and the degradation of the cell wall and consequent loss of water by the cell breakdown (Martínez-Ferrer et al., 2002).

Incorporation of EOs into formulations used to cover the apple pieces had a significant effect ($p \leq 0.05$) on the firmness of the fruit. Figure 3 shows the firmness evolution of uncoated and coated apple pieces during 21 days of storage. It can be observed that, samples coated with apple puree-alginate coatings containing vanillin (0.3 and 0.6% w/w), oregano (0.1% w/w) and without EOs, maintained their firmness during the entire refrigerated storage period. Furthermore, apple pieces coated with vanillin containing coatings achieved the highest firmness values (Figure 3). These results are in agreement with those of Rupasinghe et al. (2006) who observed that incorporation of vanillin 12mM into the NatureSeal[TM] solution did not affect the firmness of 'Empire' and 'Crispin' apples slices. However, for uncoated apples, the initial texture (7.60 N) declined in a gradual manner from the beginning of storage and ending in values as low as 4.03 N (Figure 3).
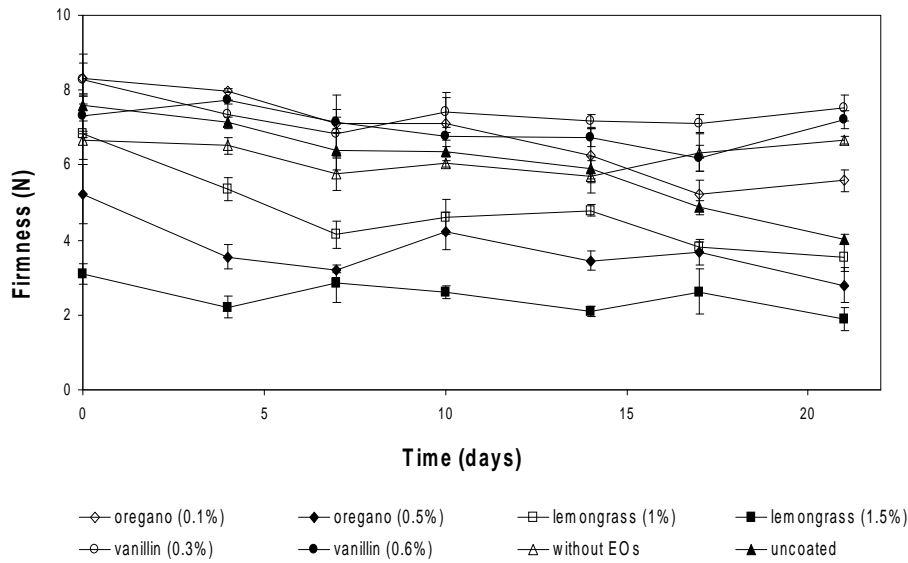
Figure 3. Firmness changes of coated (with or without EOs) and uncoated apple pieces during storage. Data shown are the means (± standard deviation).

Texture enhancers such as calcium chloride may be added to edible coatings to improve fruit quality during storage by inhibition of loss of firmness of minimally processed fruits (Olivas et al., 2005). In previous work, we could observe that 2% alginate coating had a beneficial result on firmness retention of Fuji apple wedges during 21 days of storage, attributing this result to the use of calcium chloride for crosslinking the polymers, which could minimize the softening of apple wedges. This agrees with results obtained in this study. Similar results were obtained by Lee et al. (2003) who demonstrated that incorporation of calcium chloride (1%) within the coating formulation helped to maintain firmness of apple pieces with whey protein concentrate edible coatings. The effect of calcium chloride as firming agent has been extensively established in the literature. King and Bolin (1989) established that calcium chloride can be used as firming agent for fruit tissues since it reacts with pectic acid in the cell wall to form calcium pectate, which strengthens molecular bonding between constituents of cell wall.

In spite of the excellent firming properties of calcium, apple pieces coated with apple puree-alginate coatings containing 1.5% w/w lemongrass and 0.5% w/w oregano oil experienced an abrupt decrease in the initial firmness values during the first hours after coating (3.08 and 5.22 N, respectively), indicating a rapid degradation of the texture. This effect was more pronounced in fresh-cut apples coated with CS containing 1.5% of lemongrass, which reached values as low as 1.9 N (Figure 3). Possibly, these samples resulted in more severe softening than the rest of coated apples because of the low pH (3.52) of the CS containing this EO, while pH in the rest of CS oscillated between 4.2 and 4.7. Lee et al. (2003) reported a severe softening of fresh-cut Fuji apple coated with carrageenan coating solution containing ascorbic acid and citric acid. Authors attributed this loss of firmness to the lower pH of the coating solution. Ponting et al. (1971) indicated that softening observed in fresh-cut apples may be due to the pectic acid undergoing acid hydrolysis.

Sensory evaluation

Edible coatings that have little or no taste are desirable to prevent detection during consumption (Contreras-Medellin and Labuza, 1981). However, incorporation of natural antimicrobial agents into alginate-apple puree edible coatings could change the original flavors of foods due to the strong flavours associated with them. In this study, the use of EOs had a significant effect ($p \leq 0.05$) in all evaluated sensory attributes of coated fresh-cut apples.

Figure 4 shows the color, texture, odor, taste and overall preference scores for fresh-cut apple coated with alginate-apple puree with or without EOs. It can be observed that, initially, cut fruits with alginate-apple puree coating containing vanillin (0.3% w/w) and coated samples without EOs had higher scores (>6) than samples containing lemongrass (1% w/w) or oregano oil (0.1% w/w) for all the sensory attributes evaluated. Coated apples pieces without EOs showed the highest overall preference throughout the period of evaluation, indicating that alginate-apple puree formulation by itself did not modify the sensory attributes of the fresh apple. By contrast, the lowest overall preference was initially observed in samples containing oregano oil in its formulation, although these samples maintained higher sensory scores for color. In spite of the low concentration used of oregano oil (0.1% w/w), some consumers detected a residual aromatic herbal taste which diminished the overall preference of these samples. Contrary to our results, Roller and Seedhar (2002) reported that fresh cut kiwifruit and honeydew melon treated with 1 mM of carvacrol (active compound of oregano oil) has been found to delay spoilage without causing adverse sensory changes.

As can be seen in figure 4, after the first week of storage the scores of sensory attributes of samples containing oregano oil were relatively the same, in contrast with the scores showed by samples containing lemongrass (1% w/w), which were strongly affected by the incorporation of this essential oil. The main sensory characteristics damaged by the use of lemongrass oil were texture and color (Figure 4), which in the case of texture showed the lowest values in agreement with instrumental data (Figure 3). With respect to color, the incorporation of lemongrass affected negatively the original color of apples, especially internal color, which tended to darken in the first days of storage. Both negative effects caused that sample coated with lemongrass were rejected by the consumers after 7 days of storage. It is important to highlight the fact that as fresh-cut apple color was directly measured in the surface, the internal differences observed by consumers might have gone undetected instrumentally (Table 1). On the other hand, the use of vanillin in the coating formulation did not affect the typical color of the apple samples, maintaining its color scores during the entire period of evaluation (Figure 4). Leúnda et al. (1999) analyzed the color changes in minimally processed apples treated with vanillin and/or potassium sorbate at room temperature. They observed that apple containing only vanillin, exhibited the minor change for more than 90 days.

In addition, taste evaluation indicated that only fresh-cut apples coated with vanillin incorporated into the formulation, or without EOs (control) obtained acceptable scores after 7 days of storage, whereas the rest

of the samples were below the limit of liking. Although oregano oil exhibited the strongest antimicrobial activity, its taste was not totally compatible with apple pieces, and its use is perhaps more appropriate in other type of foods such as meat, poultry or fish products. Control samples (without EOs) after 14 days of storage were only evaluated by consumers in terms of firmness, odor and color characteristics (obtaining high scores), since microbiological counts (next section) of these samples rendered high microbiological counts.
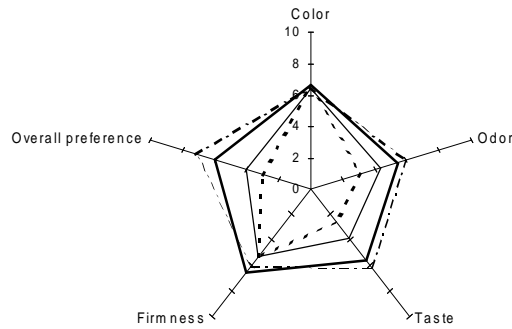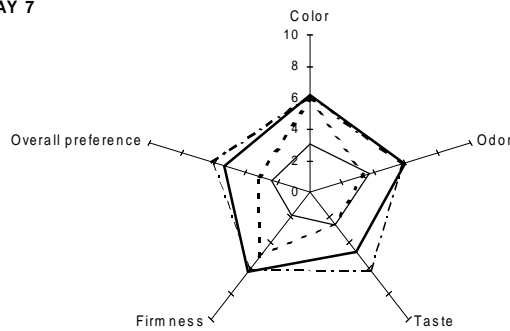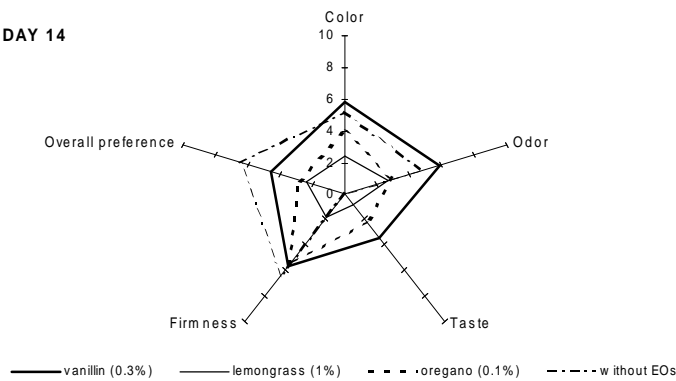
Figure 4. Evolution of sensory attributes of coated fresh-cut Fuji apple containing or not antimicrobial agents (EOs) after 1, 7 and 14 days of storage.

As yet, no consumer acceptance tests or other sensory studies have been published on any foods with antimicrobial coatings. However, sensory studies have been conducted on related food products such as fruit puree containing some natural antimicrobials. For instance, Cerrutti and Alzamora (1996) evaluated the sensory properties of fruit purees (including apple puree) containing different concentrations of vanillin. They reported that purees had a pleasant vanillin flavor while maintaining the actual taste of the fruit, in agreement with our results.

Microbiological analysis

Fresh-cut fruits are a fertile environment for microorganisms to grow due to the high amount of moisture and sugar present on their surface. In addition, coatings applied on fresh-cut fruits create an internal modified atmosphere that may change the growth rate of spoilage and pathogenic microorganisms (Olivas et al., 2005). Since modified atmosphere may inhibit the growth of innocuous spoilage flora and encourage the growth of pathogens, the study of the development of populations of psychrophilic bacteria, molds and yeast during cold storage of fresh-cut fruits is required for assuring microbial safety of these products.

Psychrophilic aerobic bacteria. Significant differences ($p \leq 0.05$) between the counts of psychrophilic microorganisms of fresh-cut apples coated with or without EOs (control) were observed. Figure 5A shows that alginate-apple puree edible coatings containing EOs applied on fresh-cut apples had a marked effect in reducing psychrophilic counts as compared to the control. Total colony counts of apple pieces coated without EOs increased approximately 5.5 log cycles from day 0 to day 21 of storage, reaching values as high as 10^7 CFU.g^{-1}. No significant differences were observed in uncoated apple pieces, where the levels of psychrophilic aerobic bacteria reached values of 10^8 CFU.g^{-1}, indicating that coating itself did not reduce survival of these microorganisms.
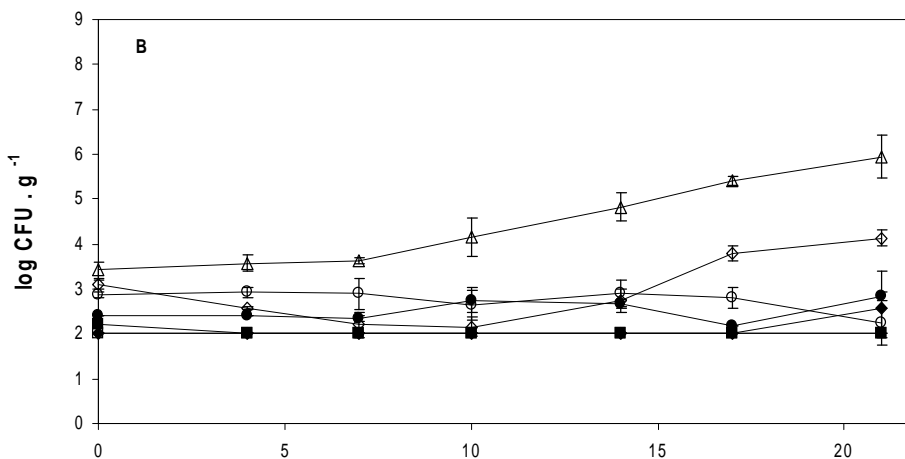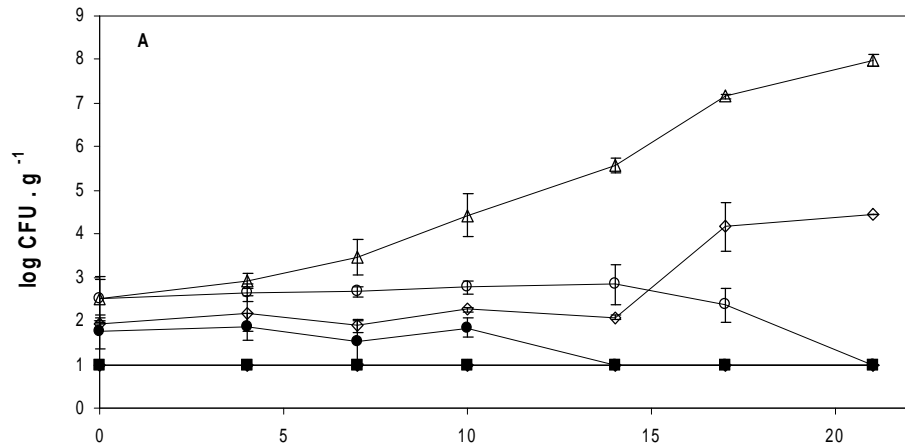
Figure 5. Effect of antimicrobial agents (EOs) into alginate-apple puree edible coating on microbial growth (log CFU.g^{-1} of fruit) of apple pieces: (A) psychrophilic microorganisms, (B) yeasts/molds. Data shown are the means ($\pm$ standard deviation).

Regarding the action of the antimicrobial coatings on psychrophilic counts, no colonies were detected (detection limit, 1.0 log CFU.g^{-1}) in samples coated with lemongrass (1.0% and 1.5% w/w) and oregano oil (0.5% w/w) coatings (Figure 5A). The effectiveness of these natural antimicrobials seemed to have been favoured by the low pH of the coating forming solutions. As mentioned before, pH ($<$ 3.6) of coatings containing lemongrass (1.0 and 1.5%) and oregano oil (0.5%) was low compared with pH of the other CS (about 4.5), resulting in a severe softening of these samples (Figure 4). Skandamis and Nychas (2000) indicated that the susceptibility of bacteria to the antimicrobial effect of essential oils appears to increase with a decrease in the pH of the food. Generally, the lower the pH, the more effective essential oils and their components are (Burt, 2004).

On the other hand, samples coated with vanillin and 0.1% oregano oil presented a lower initial concentration of psychrophilic bacteria with respect to control samples, as indication of a bacteriostatic effect throughout the first 14 days of storage. After this period, a light increment in psychrophilic aerobic bacteria concentration of samples coated with 0.1% oregano CS was observed. However, in all cases, counts of samples with antimicrobial coatings did not exceed 10^4 CFU.g^{-1} at the end of the period of storage (Figure 5A).

Incorporation of natural antimicrobial agents into edible coatings applied on fresh-cut products has not been widely studied. Rupasinghe et al. (2006) studied the effect of 12 mM of vanillin incorporated into a commercial antibrowning dipping solution (NatureSeal™) on apple slices stored during 19 days at 4ºC. They observed that 12 mM of vanillin was effective in reducing the total microbial load on fresh-cut apples ('Empire' and ´Crispin') obtaining a percent of inhibition of 37% and 66%, respectively. Lanciotti et al. (1999) suggested that the addition of citrus essential oils to fresh sliced fruit mixture (apple, pear, grape, peach and kiwifruit) inhibited the proliferation of naturally occurring microbial population.

Yeasts and molds. Antimicrobial coatings not only inhibited psychrophilic microorganisms, but also yeast and molds present in fresh-cut Fuji apple (Figure 5B). Yeasts and molds were not significantly reduced by the coating without incorporation of EOs ($p \leq 0.05$). In alginate-apple puree coatings containing 1.0% and 1.5% w/w of lemongrass, and 0.5% of oregano oil, yeasts and molds were not detected (detection limit,

2.0 log CFU.g^{-1}) on apple pieces during the entire period of storage. However, yeasts and molds counts reached 4.1 log CFU.g^{-1} in cut apples with 0.1% w/w of oregano oil-coating after 21 of storage. As observed in psychrophilic counts, the effectiveness of oregano oil (0.1% w/w) decreased during storage time, while yeasts and molds counts increased in the last days of storage. The growth rate of yeasts and molds was inhibited in samples coated with the maximum concentration of oregano oil (0.5% w/w) and vanillin in both concentrations used (0.3 and 0.6% w/w) not exceeding 3 log CFU.g^{-1}at the end of storage. Microbiological criteria (IFST, 1999) for nonthermal processed fruits indicated that a maximum yeast count of 6 log CFU.g^{-1}is considered acceptable at any point in the shelf-life of a fruits product. In this work, yeast counts did not exceed these levels at any time during storage.

Incidence of Listeria innocua. *Listeria* species were not detected in non-inoculated apple pieces after applying the ISO 11290-1 guideline.

Survival of inoculated L. innocua in fresh-cut apple. Effects of antimicrobial coatings on *L. innocua* counts during refrigerated storage are shown in Figure 6. Results showed that the use of alginate-apple puree coatings with EOs reduced significantly ($p \leq 0.05$) the microorganism levels in fresh-cut apple as compared to apple pieces coated without incorporation of EOs. A slight decrease in initial counts of *L. innocua* (6.8 CFU.g^{-1}) was observed in control samples (coating without EOs), reaching counts of 6.2 CFU.g^{-1}after 21 days of storage. This result shows that alginate-apple puree edible coating per se was not active against *L. innocua*. As expected, antimicrobial activity was stronger at the higher concentrations of EOs. In fact, Figure 6 shows that alginate-apple puree coating containing lemongrass (1.0 and 1.5% w/w) and oregano oil (0.5% w/w) were the most effective against the growth of *L. innocua*, reducing the number of colonies to below the limit of detection (2.0 log CFU.g^{-1}) in the first week of storage. Upon enrichment of 10 g of cut apples in peptone saline water by 45 min before spread plate on Palcam agar plates, all samples rendered negative results, indicating that *L. innocua* was eliminated by the antimicrobial coatings.
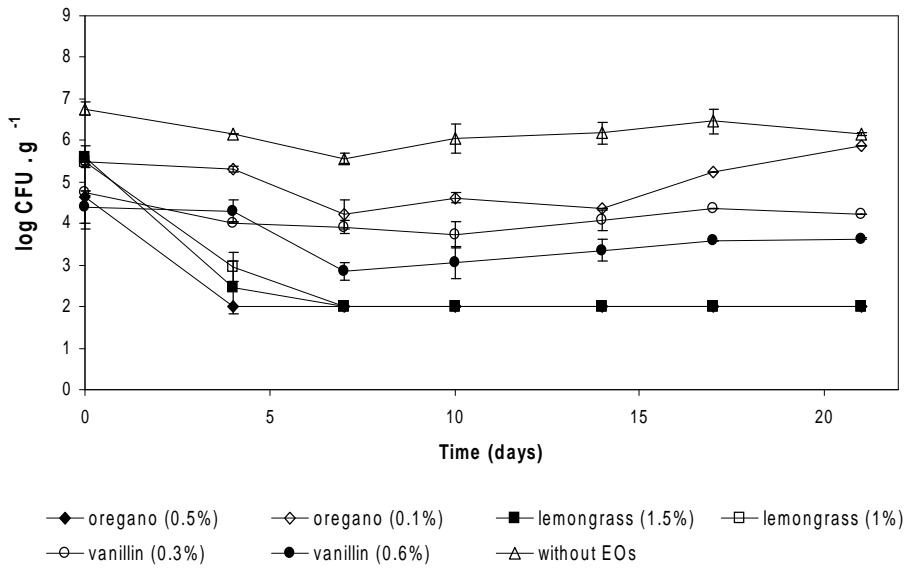
Figure 6. Effect of antimicrobial agents (EOs) into alginate-apple puree edible coating on the microbial counts of *Listeria innocua* inoculated on apple pieces (log CFU.g^{-1} of fruit). Data shown are the means (± standard deviation).

In all cases, it was observed a reduction of initial counts of inoculated *L. innocua* (10^6 CFU.g^{-1}), immediately after application of coating with EOs. For instance, initial counts of *L. innocua* in apple pieces coated with alginate-apple puree containing vanillin (0.3 and 0.6% w/w) decreased approximately 3.0 log cycles during the early hours of storage with respect to initial levels of inoculum. By contrast, the initial reduction of *L. innocua*-inoculated in samples coated with solution coating-oregano oil 0.1% w/w was approximately of 1.6 log cycle, reducing its growth rates until values of 4.4 log CFU.g^{-1} after 2 week of storage. The effect of oregano oil (0.1% w/w) into coating seemed temporary because the counts of *L. innocua* reached similar values that control sample at the end of storage (6 log CFU.g^{-1}) (Figure 6). A previous work showed that 0.1% w/w of oregano oil incorporated into alginate-apple puree film forming solution was an effective antimicrobial agent (Rojas-Graü et al., 2006). In this work, a higher concentration of this compound (0.5% w/w) was necessary to obtain similar results because its effectiveness decreased when it was applied into a coating. Dawson et al. (2002) indicated that antimicrobial films are often more effective in inhibiting target microorganism when applied to nutrient media than to real food systems.

The antimicrobial activity of some EOs including oregano oil, lemongrass oil and vanillin is assigned to a number of small terpenoid and phenolic compounds, which also in pure form have been shown to exhibit antibacterial or antifungal activity (Burt, 2004; Friedman et al., 2004).

CONCLUSIONS

Apple puree-alginate edible coatings were successfully formulated with the addition of EOs such as lemongrass, oregano oil and vanillin and resulted in a variety of beneficial effects on the shelf-life of

fresh-cut Fuji apples. Several of the tested antimicrobial coatings reduced ethylene production in coated fresh-cut apples. The calcium chloride and *N*-acetylcysteine containing apple puree-alginate coatings helped to maintain firmness and color. Coatings with EOs seemed to effectively inhibit the growth of *L. innocua* inoculated on apple pieces as well as psychrophilic aerobic bacteria, yeasts and molds. Considering the adverse effects of lemongrass in the color and texture of coated fresh-cut apple, future research is needed on others EOs that are compatible with fruit sensory characteristics.

ACKNOWLEDGMENTS

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La obtención de manzanas frescas cortadas lleva consigo una serie de operaciones, tales como el pelado, descorazonado y cortado, las cuales inflingen daños en los tejidos desencadenando cambios de textura, color, sabor y olor en el alimento, todo en detrimento directo de la calidad del producto fresco cortado. Además, deben estudiarse factores como la variedad del fruto, el estado de madurez, las condiciones de almacenamiento, entre otras, para alcanzar el procesamiento óptimo en frutas cortadas. Aplicar métodos que de manera individual o combinado frenen el deterioro impuesto por todos estos factores, constituye una de las principales líneas de investigación en el área de conservación de productos frescos cortados.

Teniendo en cuenta este planteamiento, se realizaron estudios preliminares que permitieron determinar el estado óptimo de madurez para el procesamiento de manzana Fuji, así como el efecto de distintos tratamientos para la conservación del color, estableciendo de esta manera las condiciones óptimas para su procesado mínimo. Una vez determinadas las características de la fruta a procesar y las concentraciones de los antioxidantes, se procedió a evaluar el empleo de recubrimientos comestibles como método de conservación en manzana fresca cortada. Inicialmente, se procedió a formular y estudiar la capacidad de estos recubrimientos para formar una matriz estructural, caracterizando sus propiedades de barrera y de transporte de aditivos. Una vez establecida la mejor formulación, se procedió entonces a evaluar la capacidad de estos recubrimientos para adherirse a los trozos de fruta, ejercer como barrera a los gases y al vapor de agua, y servir de transporte para agentes antioxidantes y antimicrobianos. Finalmente se realizó una valoración del período de vida útil de los trozos de manzana recubiertos, basado en los cambios fisiológicos, físico-químicos, sensoriales y microbiológicos experimentados por la fruta.

A.- EFECTO DEL EMPLEO DE ANTIOXIDANTES DE ORIGEN NATURAL EN EL MANTENIMIENTO DE LA CALIDAD DE MANZANA FRESCA CORTADA

Cambios en el color

El efecto de diferentes agentes antioxidantes de origen natural [ácido ascórbico, 4-hexylresorcinol (4-HR), glutatión y N-acetilcisteína] y del tiempo de almacenamiento en el color de rodajas de manzana Fuji se evaluó mediante un diseño de superficie de respuesta, el cual permitió optimizar la concentración de antioxidantes necesaria para obtener los mínimos cambios en las coordenadas L^*, a^*, b^* y en los parámetros colorimétricos tono (h^*) y diferencia de color (ΔE^*).

Cuando se evaluó la luminosidad de los trozos de manzana, sin aplicación de antioxidantes, se observó una disminución de este parámetro desde 76,4±0,7 hasta 66,44±0,3 al cabo de 14 días de almacenamiento, siendo el tiempo de almacenamiento el único factor que tuvo un efecto significativo sobre el mismo. Un efecto similar fue observado por Son et al., (2001) quienes emplearon N-acetilcisteína (1%) o glutatión (1%) como agentes antioxidantes. Con respecto al parámetro a^*, se observó que la presencia de 4-HR, así como el tiempo de almacenamiento jugaron un papel importante en la variación de este parámetro. Aunque el uso de este antioxidante resultó tener un efecto beneficioso en el mantenimiento de a^*, su concentración no debería exceder de 0,7% p/v ya que se observó que concentraciones mayores inducirían la presencia de coloraciones oscuras en los trozos de manzana. Por su parte, tanto el uso individual como combinado de N-acetilcisteína y glutatión, mantuvieron los valores iniciales de a^* relativamente constantes durante todo el período de almacenamiento, siendo necesarias

concentraciones alrededor del 1% p/v cuando se emplearon de forma individual y de 0,6% p/v cuando se usaron mezclas de ambos compuestos. Como indicativos del pardeamiento, además de los cambios de L^* y a^* también se tomaron en cuenta las variaciones de h^* y ΔE^*, ya que los cambios en la coordenada b^* no fueron significativos.

El tono de las muestras de manzana sin tratar descendió bruscamente desde el primer día de almacenamiento, alcanzando valores tan bajos como $63,87\pm1,15$ al final de este período, evidenciándose un intenso pardeamiento en las muestras sin tratar. Dichas reacciones de pardeamiento pudieron ser inhibidas por el uso de N-acetilcisteína, el cual mantuvo altos valores de h^* durante todo el tiempo de almacenamiento, coincidiendo con los resultados obtenidos para el parámetro a^* y demostrando su efectividad como agente antioxidante.

El tiempo de almacenamiento jugó un papel determinante en las diferencias de color observadas en las rodajas de manzana. No obstante, con el uso de 4-HR y N-acetilcisteína se logró obtener la menor diferencia entre el color inicial y final de las muestras tratadas, siendo esta diferencia menor a medida que incrementaba la concentración de los antioxidantes en la solución. La mínima diferencia de color después de 14 días de almacenamiento se obtuvo con el uso de 0,6 % p/v de 4-HR; sin embargo fueron necesarias concentraciones ligeramente superiores de N-acetilcisteína (0,75% p/v) para obtener resultados similares.

Es importante destacar que bajo las condiciones de trabajo establecidas en esta investigación, el uso de ácido ascórbico no produjo ningún efecto significativo en el color de las rodajas de manzana. No obstante, se observó un gradual oscurecimiento en aquellas muestras donde el ácido ascórbico fue empleado como tratamiento protector.

Evolución de la actividad enzimática

Con la finalidad de inhibir la actividad de las enzimas polifenol oxidasa (PPO) y peroxidasa (POD) presentes en trozos de manzana fresca cortada, se evaluó el efecto de algunos antioxidantes de origen natural (4-HR, glutatión y N-acetilcisteína) comparado con el uso de ácido ascórbico, evaluando además el posible efecto sinérgico obtenido por la combinación de ambos compuestos.

La actividad de la enzima PPO tendió a aumentar con el tiempo. Sin embargo el uso de 1% de N-acetilcisteína o glutatión causó la mayor inhibición de la enzima, observándose una menor actividad al final del período de almacenamiento. Oms-Oliu, et al. (2006) observaron resultados similares, reportando una inhibición total de la enzima PPO en trozos de pera tratados con ambos compuestos. Algunos autores han señalado que los agentes antioxidantes que contienen grupos sulfuro, como es el caso de N-acetilcisteína y glutatión, no causan por sí mismos la inhibición de las enzimas PPO, aunque estos compuestos producen una aparente inhibición de la actividad enzimática debido a su habilidad para conjugarse con productos primarios de la oxidación, actuando de forma competitiva con la PPO (Molnar-Perl y Friedman, 1990, Richard-Forget et al., 1992; Billaud et al., 2004).

Por el contrario, la mayor actividad enzimática de la PPO se observó desde el primer día de almacenamiento en trozos de manzana tratados con 1% de ácido ascórbico alcanzando valores superiores a 1,5 después de dos semanas. De acuerdo con nuestros resultados, Eissa, et al. (2006) reportaron que el ácido ascórbico fue el que exhibió la menor inhibición de la actividad PPO en rodajas de manzana Red Delicious tratadas con diferentes compuestos antioxidantes. Aunque la efectividad del ácido ascórbico en

el control del pardeamiento enzimático ha sido demostrada en numerosas investigaciones, se ha observado que su efecto es temporal. Algunos autores atribuyen la temporalidad del efecto del ácido ascórbico a que éste se consume durante las reacciones antioscurecimiento, siendo oxidado irreversiblemente cuando reacciona con pigmentos intermedios, enzimas endógenas y metales como el cobre (Luo y Barbosa-Canovas, 1997; Ozoglu y Bayindirly, 2002).

Cuando se evaluó la efectividad de los agentes antioxidantes en combinación con ácido ascórbico se observó una ligera mejoría en el control de la PPO, aunque la actividad enzimática en estas muestras aumentó al final del almacenamiento. La mayor efectividad fue observada con el uso combinado de ácido ascórbico y 4-HR, aunque solo inhibió la actividad enzimática PPO durante los primeros días de almacenamiento. En concordancia con los resultados obtenidos, Luo y Barbosa-Cánovas, (1997) señalaron que el uso combinado de 4-HR y ácido ascórbico mejoró notablemente la inhibición del pardeamiento enzimático en rodajas de manzana Fuji.

Por el contrario, la actividad de la enzima POD durante todo el período de almacenamiento se vio ralentizada cuando se emplearon combinaciones de ácido ascórbico al 1% con 4-HR, N-acetilcisteína o glutatión. Sin embargo, el uso individual de dichos compuestos no ejerció ningún efecto significativo en el control de la actividad enzimática POD observándose un incremento gradual durante el almacenamiento, siendo más acusado en rodajas de manzanas tratadas con 1% de ácido ascórbico. Al igual que lo observado con la enzima PPO, el uso de ácido ascórbico no ejerció ningún efecto inhibidor de la actividad enzimática.

B.- EFECTO DEL ESTADO DE MADUREZ EN LA CALIDAD DE MANZANA FRESCA CORTADA

Con el fin de poder establecer las condiciones óptimas de procesamiento mínimo para conservar manzana Fuji fresca cortada, se partió de una materia prima en tres estados de madurez diferentes, clasificada según su nivel de firmeza en: verde (79±3,2 N), verde-madura (67±3,2 N) y madura (56±2,2 N) correspondientes a un temprano, intermedio y avanzado estadio de madurez.

Cambio en la composición de los gases del espacio de cabeza

La composición gaseosa del espacio de cabeza en los envases durante el almacenamiento se vio afectada por el estado de madurez de las frutas, especialmente cuando se encontraban envasadas bajo una atmósfera modificada (2,5 kPa O_2 + 7 kPa CO_2). En estas condiciones se observó un descenso brusco de las concentraciones iniciales de oxígeno (O_2), alcanzado valores por debajo de 1 kPa después de 1 semana de almacenamiento. Aunque las concentraciones de O_2 también descendieron en muestras envasadas en aire, dicho consumo también disminuyó de manera gradual durante todo el período de almacenamiento, manteniendo concentraciones por encima de los 5 kPa en rodajas de manzana procesadas en un estado de madurez intermedio y avanzado.

El grado de madurez de la fruta en el momento del procesamiento y el tipo de atmósfera empleada para su envasado afectó significativamente la producción de dióxido de carbono (CO_2). En cambio, el uso de N-acetilcisteína y ácido ascórbico como agentes antioxidantes no ejercieron ningún efecto en la evolución

de este gas. Es importante destacar que la producción de CO_2 durante el almacenamiento fue menor en rodajas de manzanas procesadas en un estado de madurez intermedio, lo que sugiere su uso para obtener un producto de mayor calidad y vida útil. Mantener una moderada concentración de CO_2 dentro del envase es importante con el fin de evitar posibles reacciones adversas en la fruta durante el período de almacenamiento. Lakakul et al., (1999) destacaron la importancia de mantener los niveles de O_2 justo por encima del umbral de fermentación y los de CO_2 por debajo del límite que pudiera causar cambios degradativos en la atmósfera de cabeza del envase.

Por otro lado, la concentración de etileno se vio afectada por el estado de madurez, el tratamiento protector usado en el mantenimiento del color y por las condiciones de almacenamiento. Las rodajas de manzana poco maduras produjeron menos etileno que aquéllas procesadas en un estado de madurez más avanzado, dado a que, como es sabido, la tasa de producción de etileno es mucho mayor a medida que avanza el estado de madurez del fruto. Por otro lado, la producción de etileno fue mucho más intensa en aquellas muestras envasadas bajo una atmósfera inicial de aire, dónde la concentración del gas alcanzó los 60 ppm después de 1 semana de almacenamiento. Por el contrario, las concentraciones de este gas en los envases que contenían una atmósfera inicial baja en O_2, fueron bastante inferiores, observándose un máximo de concentración de 35ppm después de la primera semana de almacenamiento, el cual constituye aproximadamente la mitad del gas detectado en muestras envasadas bajo una atmósfera no modificada. Gil et al., (1998) observaron una inhibición completa de la producción de etileno en manzana Fuji fresca cortada envasada en ausencia de O_2, atribuyendo esta inhibición a los requerimientos de O_2 necesarios para la síntesis de etileno. Es conocido que la producción de etileno en frutos frescos es estimulada cuando el tejido sufre algún tipo de daño, conduciendo a una acumulación de este gas en el espacio de cabeza e induciendo, en muchos casos, efectos indeseables (Watada y Qi, 1999). La aplicación de agentes antioxidantes inmediatamente después del troceado de las manzanas, creó un efecto protector en las mismas que se tradujo en una menor producción de etileno, siendo ésta inferior en muestras tratadas con N-acetilcisteína que en aquellas tratadas con ácido ascórbico, manteniéndose esta tendencia durante el almacenamiento.

El estado de madurez de la materia prima utilizada también influyó significativamente en la presencia de etanol en el espacio de cabeza de los envases. Se observó una ligera pero progresiva acumulación de etanol en todas las muestras durante las primeras 3 semanas de almacenamiento, momento a partir del cual se produjo un repentino aumento en la concentración de este gas, especialmente cuando las manzanas utilizadas se encontraban en un avanzado estado de madurez. Este aumento en los niveles de etanol coincide con concentraciones bajas de O_2 y altas de CO_2 observadas a partir de la tercera semana de almacenamiento en aquellos envases que contenían manzanas en un estado de madurez avanzado, sugiriendo la creación de condiciones anaeróbicas y por ende el desarrollo de olores y sabores desagradables. Pesis (2005), señaló que el incremento de la respiración anaeróbica en frutas sobre-maduras se debe a una reducción de la actividad mitocondrial en sus tejidos, posiblemente como consecuencia de algún daño en la membrana, imposibilitando a las células para producir suficiente energía.

También se observó que la interacción entre el estado de madurez de la materia prima y el uso de antioxidantes afectó significativamente la producción de etanol, detectándose un repentino incremento en la concentración de este gas a partir del día 30 de almacenamiento, en rodajas de manzanas maduras y tratadas con ácido ascórbico. Las bajas concentraciones de O_2 presente en los envases que contenían dichas muestras, después de la tercera semana de almacenamiento, fue posiblemente lo que desencadenó el incremento en la producción de este gas. Soliva-Fortuny et al., (2002) señalaron que bajas concentraciones de O_2 pueden conducir a la producción de metabolitos fermentativos tales como el etanol, el cual es responsable de la producción de olores y sabores extraños.

Cambios en el color

La evolución del color observado en las rodajas de manzana cortada fue determinada por cambios en la luminosidad (L^*) y el tono (h^*) de las muestras durante el almacenamiento. Ambos parámetros estuvieron afectados por el estado de madurez, la atmósfera de envasado y el uso de antioxidantes.

Las manzanas procesadas en un estadio temprano e intermedio de madurez mantuvieron mejor su luminosidad inicial. De hecho, las rodajas de manzana que se encontraban en un estado de madurez avanzado experimentaron un brusco descenso en la luminosidad durante los 43 días de almacenamiento, independientemente de las condiciones de envasado. Además se observó que la atmósfera de envasado (2,5 kPa O_2 + 7 kPa CO_2) empleada en este estudio, mantuvo los valores iniciales de L^* sin grandes cambios durante el almacenamiento, comparado con las envasadas en aire. Soliva-Fortuny et al., (2002) reportaron un descenso similar en los valores de L^* durante las primeras semanas de almacenamiento en trozos de manzana Golden Delicious almacenada bajo una atmósfera inicial de aire. Además del efecto de la atmósfera de almacenamiento, se observó un importante descenso de la luminosidad en muestras tratadas con ácido ascórbico, evidenciándose desde las primeras horas de almacenamiento valores de L^* inferiores (aprox. 72) comparado con los valores iniciales observados en muestras tratadas con N-acetilcisteína (aprox. 76).

En general, a medida que aumentó el grado de madurez en la fruta, mayor fue el cambio experimentado por h^* durante el almacenamiento. Las manzanas procesadas en un avanzado estado de madurez, tratadas con ácido ascórbico y almacenadas bajo una atmósfera inicial de aire sufrieron un gran descenso de este parámetro, alcanzando valores tan bajos como 83,80±0,07 después de 43 días de almacenamiento. Por el contrario, aquellas muestras tratadas con N-acetilcisteína mantuvieron su tonalidad inicial (aprox. 100) durante todo el período de almacenamiento, independientemente del estado de madurez o las condiciones de envasado. Con estos resultados se corroboró la efectividad de N-acetilcisteína en el control del pardeamiento enzimático, comparado con ácido ascórbico, el agente antioxidante más tradicionalmente usado en la industria alimentaría.

Cambios en la textura

Como era de esperar, el estado de madurez de la fruta en el momento del procesamiento influyó significativamente en la evolución de la firmeza durante el almacenamiento. A mayor grado de madurez,

más intenso fue el ablandamiento observado en los tejidos. Las rodajas de manzana procesadas en un estadio temprano e intermedio de la madurez mantuvieron sin cambios apreciables su textura inicial (aprox. 9 y 7 N, respectivamente). Sin embargo aquellas procesadas en un estado de madurez más avanzado experimentaron un acelerado ablandamiento después de su procesamiento, alcanzando valores tan bajos como 1,9 N al final del almacenamiento. Estas reacciones de deterioro coinciden con las altas concentraciones de etileno detectadas en rodajas de manzanas procesadas en un estado de madurez de consumo. Gorny et al., (2002) señalaron que el ablandamiento tisular de las frutas durante el proceso de maduración es potenciado por la presencia de etileno, el cual se ha demostrado que interacciona en el metabolismo de la pared celular. Además, la pérdida acelerada de firmeza en manzana madura se vio favorecida por la presencia de ácido ascórbico, el cual probablemente gracias a su bajo pH favoreció la degradación de la textura. Estos resultados coinciden con los obtenidos por Ponting et al., (1972), quienes reportaron que el uso de soluciones ácidas, como la del ácido ascórbico, redujeron significativamente la firmeza de rodajas de manzana Golden Delicious.

C.- PELÍCULAS Y RECUBRIMIENTOS COMESTIBLES

Una vez establecido el tipo y concentración de antioxidantes a emplear, así como el estado óptimo de madurez para la conservación de manzana Fuji, se emplearon recubrimientos comestibles (RC) de base polisacárida como método de conservación alternativo, estableciendo su capacidad para transportar aditivos y sus propiedades de barrera. Los ensayos iniciales se centraron en optimizar diferentes formulaciones de películas comestibles (PC) y RC elaborados a partir de alginato o gelano, basados en su capacidad como soporte de agentes antioxidantes y de barrera al vapor de agua. En una segunda fase, se planteó estudiar la incorporación de diferentes agentes antimicrobianos, así como puré de manzana como nuevo ingrediente funcional. Para ello fue necesario hacer ensayos preliminares de las PC con el fin de establecer el posible efecto que ejercerían los nuevos ingredientes en la formulación final. Así se evaluó, individualmente, PC elaboradas a partir de puré de manzana o bien constituidas por una mezcla de alginato y puré de manzana, ambas conteniendo aceites esenciales, teniendo en cuenta sus propiedades de barrera, mecánicas y antimicrobianas. Finalmente, y de acuerdo con los resultados obtenidos en estos ensayos previos, se procedió a la aplicación de un RC constituido por alginato, puré de manzana, antioxidantes y antimicrobianos de origen natural, sobre trozos de manzana Fuji, evaluando su vida útil a través de cambios en la actividad respiratoria, color, textura, crecimiento microbiano y finalmente sus características sensoriales.

C.1.- Recubrimientos y películas comestibles a base de alginato y gelano. Incorporación de agentes antioxidantes.

Se utilizó un diseño de superficie de respuesta con el fin de optimizar las concentraciones de glicerol y antioxidantes a ser incorporados en recubrimientos de alginato (2% p/v) o gelano (0,5% p/v), basando

dicha optimización en cambios de resistencia al vapor de agua y cambios en el color superficial de trozos de manzana Fuji recubiertos. Una vez optimizadas estas concentraciones, se evaluó la incorporación de aceite de girasol con el fin de mejorar las propiedades de barrera impuestas por los recubrimientos.

Resistencia al vapor de agua (RVA)

En general, los valores de RVA observados en trozos de manzana recubiertos con alginato (entre 13,50 y 15,70 s/cm) y gelano (entre 13,10 y 14,60 s/cm) fueron en ambos casos superiores a los observados en muestras sin recubrir, donde en promedio se observó una RVA de 12,50 s/cm. La presencia de glicerol como plastificante dentro de las formulaciones ejerció un efecto significativo en los valores de RVA, tendiendo estos a aumentar a medida que se incrementaba la concentración del plastificante en la formulación. En el caso de manzanas cortadas recubiertas con alginato, la mayor RVA fue observada cuando el glicerol estuvo presente en una concentración no superior al 1,75% p/v, cantidad a partir de la cual los valores de RVA empezaron a disminuir. En el caso de trozos de manzana recubiertos con gelano se observó el mismo comportamiento que en el caso del alginato, con la diferencia de que la máxima RVA se obtuvo con una concentración de glicerol del 0,63% p/v. Los agentes plastificantes se emplean generalmente para aumentar la flexibilidad en películas y recubrimientos comestibles, pero la concentración en la que se use debe ser controlada, ya que por su naturaleza hidrofílica ocasiona, en muchos casos, un descenso en la fuerza de tensión de la película y un aumento en la permeabilidad a los gases y al vapor de agua. García et al., (1998) demostraron que un incremento en las concentraciones de agentes plastificantes, tales como glicerol o sorbitol, descendieron la permeabilidad al vapor de agua de recubrimientos elaborados a partir de almidón.

Por otro lado, la incorporación de antioxidantes como N-acetilcisteína y glutatión también afectaron significativamente los valores de RVA. Se observó en trozos de manzana recubiertos con alginato un aumento de la RVA a medida que incrementaba la concentración de N-acetilcisteína hasta valores cercanos al 1% p/v en su formulación, concentración a partir de la cual causó el efecto contrario. Sin embargo, la incorporación de N-acetilcisteína en recubrimientos de gelano ejerció el efecto inverso, observándose una disminución de la RVA con el incremento de este antioxidante en la formulación. La incorporación de compuestos hidrofílicos, tales como antioxidantes y plastificantes, tiene un efecto directo en las propiedades de permeabilidad al vapor de agua de los RC. El comportamiento observado en los trozos de manzana recubiertos con alginato o gelano conteniendo compuestos antioxidantes corroboró este hecho. Tal como señaló Ayrancy et al. (2004) muchos de los agentes antioxidantes incorporados en RC son compuestos hidrofílicos los cuales pudieran ocasionar un aumento de la permeabilidad al vapor de agua y acelerar la pérdida de peso del alimento donde se apliquen. Adicionalmente, la interacción entre el agente plastificante y los antioxidantes en recubrimientos de alginato también resultó significativa, siendo necesarias concentraciones de glicerol alrededor de 1,75% p/v y de N-acetilcisteína o glutatión alrededor del 1% p/v para alcanzar los valores más altos de RVA en recubrimientos de alginato. No se observó ninguna interacción significativa en los recubrimientos de gelano.

Evolución del color. Efectividad de los recubrimientos como portadores de antioxidantes

Los principales cambios observados en el color de trozos de manzana recubiertos con alginato o con gelano se debieron únicamente a la presencia o no de los antioxidantes dentro de la formulación, ya que el uso de glicerol no mostró ningún efecto significativo en la evolución de los parámetros a^* y h^*. N-acetilcisteína incorporada en recubrimientos de alginato o gelano, produjo el mayor efecto individual en la prevención del pardeamiento enzimático durante el período de estudio (48 horas en condiciones atmosféricas) quedando evidente la capacidad de este compuesto para prevenir el oscurecimiento cuando es incorporado dentro de un RC. Por su parte, la efectividad de glutatión como agente antioxidante también fue observada en recubrimientos de alginato, aunque su efectividad fue ligeramente menor. Sin embargo, se observó una interacción significativa entre N-acetilcisteína y glutatión, independientemente del recubrimiento empleado, siendo necesario concentraciones alrededor de 1% p/v de cada compuesto para mantener los trozos de fruta libre de pardeamiento. Molnar-Perl y Friedman (1990) demostraron que la combinación de N-acetilcisteína y glutatión a una concentración de 25 y 20 mM previnieron el oscurecimiento de rodajas de manzana Golden y Red Delicious.

La efectividad de ambos agentes antioxidantes en el mantenimiento del tono de los trozos de manzana recubiertos coincidió con la observada para el parámetro a^*. En este caso, los valores más altos de h^*, alrededor de 100, fueron obtenidos a medida que se aumentaban las concentraciones de N-acetilcisteína o glutatión en ambos RC. Este resultado contrasta con el repentino descenso de h^* observado en trozos de manzana sin recubrir (control), el cual descendió de $97,82\pm0,21$ a $89,87\pm0,31$ en sólo 48 horas, dejando en evidencia la efectividad de N-acetilcisteína y glutatión en el mantenimiento del color de trozos de manzana.

Existen varios métodos para la aplicación de agentes antioxidantes en el recubrimiento de frutas cortadas. Los agentes antioxidantes pueden ser incorporados directamente dentro de la formulación del RC por inmersión de los trozos de fruta en una solución previa al recubrimiento, o formando parte de una segunda solución la cual es aplicada después del recubrimiento principal, tal como es el caso de soluciones de calcio necesarias para el entrecruzamiento de algunos RC. En este trabajo se confirmó que la incorporación de antioxidantes en la solución de cloruro de calcio empleada para la gelificación de RC elaborados a base de alginato y gelano, funcionan eficientemente en la prevención del pardeamiento, confirmándose además, la capacidad de dichos RC como portadores de aditivos. De acuerdo con Lee, et al. (2003) y Reyes (2000), los RC son aplicados normalmente antes que los antioxidantes, asegurando la adhesión del recubrimiento sobre la fruta y la portabilidad de estos compuestos en la capa más propensa al pardeamiento.

Efecto de la adición de lípidos a los recubrimientos de alginato y gelano

Cuando se estudiaron las propiedades de barrera al vapor de agua se observó que, aunque la incorporación de determinadas cantidades de glicerol y de compuestos antioxidantes incrementó la RVA, estos valores fueron aún bajos comparado con los observados en muestras sin recubrir. Diferentes autores han señalado que las matrices formadas a partir de compuestos polisacáridos, no proveen una efectiva protección frente a la pérdida de agua (Ben-Yehoshua et al., 1985; Kester y Fennema, 1986; Wong et al., 1994). Debido a la naturaleza hidrófila, tanto de ambos recubrimientos como de los agentes plastificantes y antioxidantes, se obtuvieron valores bajos de RVA, por lo que se hizo imprescindible añadir otro compuesto que ayudará a mejorar las propiedades de barrera de los RC. Con esta premisa, y teniendo en

cuenta el hecho de que los lípidos mejoran las propiedades de barrera al agua, se decidió añadir diferentes concentraciones de aceite de girasol (AG) en aquellas formulaciones que habían mostrado los valores más altos de RVA y el menor índice de pardeamiento, es decir: 1,5% p/v glicerol + 1% p/v de N-acetilcisteína en recubrimientos de alginato y 0,6% p/v de glicerol + 1% p/v de N-acetilcisteína en recubrimientos de gelano. Después de la adición de AG en las formulaciones escogidas, se evidenció un incremento de la RVA en todas las muestras en comparación con aquellas sin recubrir (12,15 s/cm), alcanzando valores tan altos como 19,60 y 27,60 s/cm en recubrimientos de alginato y gelano, respectivamente, conteniendo ambos 0,025% p/v de AG. Aunque ambos valores de RVA fueron incrementados, el mayor aumentó de resistencia se observó en recubrimientos de gelano. También se observaron diferencias visuales en el grosor de los recubrimientos formados sobre los trozos de manzana, por lo que se decidió realizar una evaluación instrumental del grosor de ambos recubrimientos.

Con el fin de determinar el efecto de la incorporación de aceite de girasol (0,025; 0,05 y 0,125%) en la permeabilidad al vapor de agua (PVA) de películas de alginato y gelano, se realizaron ensayos complementarios en PC conteniendo la misma formulación elegida anteriormente. La PVA se determinó sólo en aquéllas PC que una vez formadas no mostraron una presencia visible de aceite en su superficie y que además se despegaron fácilmente sin romperse. De acuerdo con estos criterios de selección, se evaluaron PC que contenían las menores concentraciones de aceite de girasol (0,025 en alginato y 0,025 y 0,05% en gelano) quedando descartada en ambos casos aquellas que contenían una concentración de 0,125%, ya que mostraron una excesiva exudación del aceite. Se observó que la incorporación de 0,025% de aceite de girasol en la formulación de alginato no causó una mejora en las propiedades de permeabilidad de la película, registrándose valores muy similares al control, alrededor de 1,04 y 1,08 g-mm / kPa-h-m^2, respectivamente. Por su parte, la PVA de películas de gelano conteniendo diferentes concentraciones de aceite de girasol (0,025 y 0,05%) en su formulación fueron similares (0,80 y 0,77 g-mm / kPa-h-m^2, respectivamente). Sin embargo, cuando las permeabilidades de las PC de gelano conteniendo aceite de girasol se compararon con la PVA del control (sin aceite), se observaron valores de PVA de 0,96 g-mm / kPa-h-m^2, quedando en evidencia una disminución de este parámetro de aprox. 20% e indicando una mejora de la PVA. Yang y Paulson (2000) reportaron también una disminución de la PVA en películas de gelano, de 1,5 a 1,2 g-mm / kPa-h-m^2 cuando se incorporó ácido palmítico y esteárico dentro de su formulación. Coincidiendo con los resultados de RVA obtenidos en recubrimientos de alginato y gelano, la menor PVA fue observada en películas de gelano.

Evaluación microscópica de los recubrimientos

Mediante la evaluación microscópica se pudo observar que tanto los recubrimientos de alginato como los de gelano se encontraban bien adheridos cubriendo los trozos de manzana de una forma homogénea. Sin embargo, tal como se observaba visualmente, los recubrimientos de gelano resultaron ser más gruesos (155,75±13,30 µm) comparados con los recubrimientos de alginato (132,45±20,48 µm), lo que de algún modo justificó los valores más altos de RVA observados en recubrimientos de gelano.

Estudio de vida útil

Se realizó un seguimiento de la vida útil de manzanas Fuji cortadas y recubiertas con las formulaciones optimizadas de alginato y gelano, estudiando los cambios en la respiración, color, textura y estabilidad microbiológica de trozos de fruta almacenados bajo refrigeración en bandejas plásticas recubiertas con plástico de permeabilidad media al oxígeno (110 cm^3 O^2 m^{-2} bar^{-1} day^{-1}).

Cambios en la composición gaseosa del espacio de cabeza

El efecto que tuvo la aplicación de recubrimientos comestibles elaborados a base de alginato o gelano en el intercambio gaseoso de trozos de manzana se evaluó siguiendo la evolución de oxígeno, dióxido de carbono, etileno, acetaldehído y etanol en la atmósfera del espacio de cabeza durante 23 días de almacenamiento. Entre los resultados obtenidos, destacó la evolución del O_2 y el CO_2, ya que no se observaron diferencias significativas en la concentración de estos gases en muestras cubiertas o sin recubrir. Peréz-Gago et al., (2003) señalaron que la aplicación de un RC permite la creación de una atmósfera modificada dentro de la fruta como resultado de la resistencia a la difusión de los gases, traduciéndose en una disminución de la tasa respiratoria. En nuestra investigación, la similitud de los resultados obtenidos en la respiración de trozos de manzanas recubiertos y sin recubrir se atribuyó a la permeabilidad del plástico usado para sellar las bandejas donde fueron almacenadas las muestras, el cual probablemente permitió el paso del O_2 y el CO_2 hacia el exterior del envase, imposibilitando medir el cambio en la composición gaseosa del espacio de cabeza y así poder detectar el posible efecto ejercido por los RC como barrera selectiva a estos gases.

Sin embargo, el mayor peso molecular de los otros gases estudiados (etileno, acetaldehído y etanol) probablemente hizo posible su acumulación y detección en el espacio de cabeza de los envases, observándose diferencias en su evolución durante el almacenamiento, dependiendo de la presencia y tipo de RC. Los niveles de etileno en trozos de manzanas sin recubrir alcanzaron valores tan altos como 154,35 μl l^{-1} al final del período de almacenamiento, mientras que en trozos recubiertos con alginato o gelano alcanzaron valores bastante inferiores 28,25 y 40,42 μl l^{-1}, respectivamente. El descenso en la producción de etileno en las muestras recubiertas pudo deberse a la composición de los RC empleados. La presencia de AG en la formulación y de calcio en la solución formadora de entrecruzamiento, pudo ser la causa del aumento de la permeabilidad a este gas. Wong et al., (1994), atribuyó el descenso en la tasa de producción de etileno en manzana cortada a los componentes presentes en su formulación, una primera capa de monoglicérido acetilado seguida de una inmersión en una solución conteniendo iones de calcio.

Aunque una de las principales aplicaciones de los RC en productos frescos es la de controlar el intercambio gaseoso del producto, no es recomendable el uso de RC que presenten una barrera demasiado alta a los gases, ya que podría inducir un incremento en la presencia de algunos compuestos volátiles asociados con condiciones anaeróbicas. En este estudio se observó que la aplicación de RC sobre trozos de manzana creó una atmósfera modificada en el producto, lo cual se evidenció por la poca acumulación de acetaldehído y etanol en muestras sin recubrir. La concentración de acetaldehído incrementó continuamente durante el período de almacenamiento alcanzando valores de 141,97 μl l^{-1} y 106,88 μl l^{-1} en trozos de manzanas recubiertos con alginato y gelano, respectivamente, mientras que en trozos sin

recubrir la concentración de este gas fue muy inferior (10 µl l^{-1}), manteniéndose sin grandes cambios hasta el final del almacenamiento. Por su parte, la presencia de etanol fue evidente a partir de la segunda semana en manzanas recubiertas, alcanzando una concentración máxima de 32,25 µl l^{-1} al final del almacenamiento, mientras en trozos de manzana sin recubrir se detectó un primer incremento (12,62 µl l^{-1}) después de la tercera semana de almacenamiento. La presencia de etanol a partir de la segunda semana de almacenamiento coincidió con el súbito incremento de acetaldehído en el espacio de cabeza de los envases conteniendo trozos de manzana recubiertos, reduciendo la vida útil de este producto a 2 semanas. Day, (1994) señaló que la presencia de metabolitos fermentativos tales como acetaldehído y etanol, los cuales son resultado de la respiración anaeróbica, están frecuentemente asociados con la presencia de sabores desagradables en los alimentos, influyendo directamente sobre la calidad final del producto.

Evolución del color

Como ya se observó en el estudio previo de alginato y gelano, estos recubrimientos fueron eficientes transportadores de agentes antioxidantes por un período de 48 horas en las condiciones extremas a las que fueron sometidos los trozos de manzana recubiertos. La efectividad de los RC como soporte de antioxidantes por un período más largo de tiempo se evaluó mediante los cambios experimentados en los parámetros colorimétricos a* y h* durante 23 días de almacenamiento. Los trozos de manzana recubiertos con ambos RC no mostraron ningún cambio sustancial en los valores iniciales de a* y h*, manteniendo los trozos de fruta libre de pardeamiento durante todo el período de almacenamiento (Figura 1) y demostrando que N-acetilcisteína es un efectivo agente antioxidante que puede ser incorporado dentro de formulaciones de alginato y gelano en el recubrimiento de frutas cortadas. Baldwin et al., (1996), observó una reducción considerable del pardeamiento en manzana cortada cuando incorporó diferentes agentes antioxidantes dentro de una formulación de carboximetilcelulosa, que cuando estos agentes fueron aplicados en una solución acuosa, quedando en evidencia la capacidad de los RC de transportar y mantener aditivos.
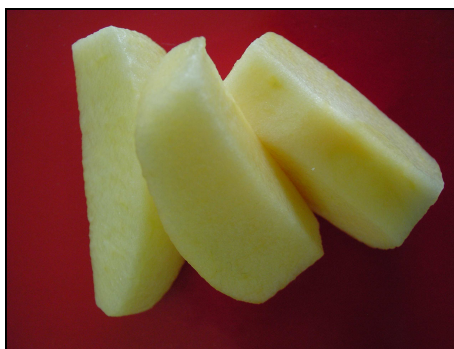

Figura 1.- Trozos de manzana Fuji recubiertos con una mezcla de gelano (0,5%), glicerol (0,6%), aceite de girasol (0,025%) y N-acetilcisteína (1 %) como agente antioscurecimiento después de 23 días de almacenamiento.

Cambios en la firmeza

La aplicación de RC ejerció un efecto determinante en la evolución de la textura de las manzanas cortadas. Las muestras sin RC experimentaron una progresiva degradación de la textura alcanzando valores de 5,30N frente a los 10,19N iniciales. Por el contrario, la aplicación de recubrimientos de alginato y gelano ejercieron un efecto positivo en el mantenimiento de la firmeza de los trozos de manzana, manteniendo sus valores de firmeza inicial durante todo el período de almacenamiento. Como se mencionó anteriormente, las propiedades gelificantes del alginato y del gelano se deben a su capacidad para formar enlaces con iones divalentes como el calcio, por lo que el empleo de una solución de cloruro de calcio (2%) fue necesario para lograr el entrecruzamiento y formación estable de los recubrimientos. El calcio es conocido por ser un agente reafirmante del tejido en frutas, y aunque en este trabajo se empleó como coadyuvante en la formación de los RC, también ejerció un efecto benéfico en el mantenimiento de la firmeza de los trozos de manzana. Adicionalmente, y como ya es sabido, la pérdida de firmeza de los tejidos está frecuentemente asociada con la pérdida del contenido de agua, por lo que la incorporación de aceite de girasol en la formulación de recubrimientos de alginato y gelano evitó la pérdida excesiva de agua en la fruta y por ende la pérdida de firmeza, algo que ya se había observado anteriormente por el aumento en los valores de RVA.

Estabilidad microbiológica

La aplicación de recubrimientos de alginato y gelano en trozos de manzana inhibió el crecimiento microbiano durante todo el período de almacenamiento, observándose un crecimiento inferior a 10^4 $UFC.g^{-1}$ y 10^5 $UFC.g^{-1}$ para microorganismos mesófilos y psicrófilos, respectivamente, comparado con los altos recuentos observados en muestras sin recubrir, los cuales alcanzaron valores tan altos como 10^7 $UFC.g^{-1}$ y 10^8 $UFC.g^{-1}$ al final de tres semanas de almacenamiento. Con el fin de asegurar la estabilidad y seguridad de los productos frescos cortados es necesario en primer lugar controlar el crecimiento de la flora nativa de la fruta fresca. Los RC crean una atmósfera modificada que puede cambiar la tasa de crecimiento de microorganismos, controlando el crecimiento de microorganismos inocuos propios de la fruta, así como evitar el crecimiento de patógenos (Olivas y Barbosa-Cánovas, 2005).

C.2.- Película comestible a base de puré de manzana. Incorporación de aceites esenciales.

Las películas comestibles como soporte de aceites esenciales (AEs) constituyen una nueva posibilidad para la incorporación de compuestos antimicrobianos en la superficie de alimentos tales como carnes, embutidos, frutas enteras y más recientemente en frutas frescas cortadas. Pero la efectividad de aplicar una PC en cualquier tipo de alimentos está estrechamente relacionada con la naturaleza de los materiales utilizados en su elaboración y de la interacción de ésta con el alimento, por lo que determinar sus propiedades de barrera, mecánicas y antimicrobianas es importante para predecir el comportamiento del RC en el alimento. Aunado a esto, la incorporación de puré de manzana en la formulación confiere propiedades nutricionales y sensoriales adicionales, tanto a la película como a los RC.

Propiedades antimicrobianas

La efectividad de los AEs como agentes antimicrobianos fue estudiada tanto en la solución formadora de películas (SFP) como en las PC una vez formadas. En ambos casos, la efectividad de los AEs fue probada frente al crecimiento del microorganismo patógeno *E. coli* O157:H7.

Actividad antimicrobiana de los AEs en las SFPs

Cuando fueron evaluadas las SFPs conteniendo los AEs, se observó que todos los compuestos estudiados (aceites esenciales de orégano, canela y hierba de limón) inhibieron el crecimiento de *E. coli* O157:H7. En comparación se observó que en ausencia de AEs, y a pesar de la incorporación de ácido ascórbico y ácido cítrico como antioxidantes en la SFP, el crecimiento de *E. coli* O157:H7, fue inevitable. Dentro de los AEs, el de orégano fue el que ejerció un mayor efecto antimicrobiano. De hecho, una concentración de 0,1% p/v de este aceite fue efectiva frente al patógeno con solo 3 min de contacto entre el compuesto y el microorganismo, mostrando un valor de BA_{50} de 0,034 (0,034% del aceite de orégano inhibió el 50% de *E. coli* O157:H7 después de 3 min a 21°C). La actividad del compuesto después de 30 y 60 min de exposición fue ligeramente mayor, arrojando valores de BA_{50} de 0,024% y 0,019%, respectivamente. Nuestros resultados coinciden con diferentes estudios que señalan al aceite esencial de orégano como el compuesto activo más efectivo frente al crecimiento de *E. coli* O157:H7 (Hammer et al. 1999; Dorman y Deans, 2000; Friedman, et al. 2000, 2002). Contrario a lo observado con el aceite de orégano, el aceite de canela a una concentración cinco veces mayor que la de orégano (0,5% p/v) fue sólo activo después de 30 y 60 min de exposición, mostrando valores de BA_{50} de 0,12% y 0,094%, respectivamente. El aceite de canela fue menos efectivo probablemente debida a la diferencia de reactividad de los compuestos fenólicos contenidos en ambos antimicrobianos, los cuales actúan de manera distinta frente a un determinado microorganismo. Aunque algunas investigaciones han señalado que son necesarias concentraciones similares de aceite de canela o de orégano para inhibir el crecimiento de *E. coli* O157:H7 es conocido que el cinamaldehído (compuesto activo del aceite de canela) no causa una desintegración completa de la membrana externa, mientras que carvacrol y timol (compuestos activos del aceite de orégano) producen un daño más importante en la membrana celular que conlleva a la liberación de muchos de sus componentes, siendo más efectivo frente al microorganismo (Sikkema et al., 1995; Helander et al., 1998). Con respecto a la efectividad del aceite de hierba de limón (0,5% p/v) frente al crecimiento de *E. coli* O157:H7 se observó que su efecto fue muy similar al mostrado por el aceite de canela, donde para inhibir el 50% del microorganismo fue necesario 0,059% del aceite y un tiempo de exposición de 60 min. Friedman et al, (2004), observaron valores muy similares de BA_{50} frente a *E. coli* O157:H7 presente en zumo de manzana clarificado y tratado con aceite de orégano, canela y hierba de limón por un tiempo de 60 min a 37°C.

Actividad antimicrobiana de los AEs en las PC

La efectividad de los AEs incorporados en las PC se evaluó midiendo la zona libre de crecimiento alrededor de un disco de PC a la que se le denominó, zona de inhibición del compuesto. Tal como se observó en las SFP, el aceite de orégano fue el más efectivo frente al crecimiento de *E. coli* O157:H7 en

las PC elaboradas a partir de puré de manzana. De hecho, la zona de inhibición de la PC conteniendo aceite de orégano fue mayor a medida que aumentaba la concentración de este compuesto en la formulación, observándose una destrucción total del microorganismo con una concentración de 0,1% p/v. Sin embargo, fueron necesarias concentraciones de 0,5% de aceites de canela o hierba de limón para inhibir el crecimiento microbiano alrededor de los discos de PC, necesitándose una concentración cinco veces mayor de ambos aceites para obtener una actividad antimicrobiana similar a la obtenida con aceite de orégano. Nuevamente, la diferente efectividad observada entre los aceites esenciales de canela y hierba de limón incorporados en una película de puré de manzana comparada con la de aceite de orégano puede explicarse por el tipo de compuestos activos que los conforman, así como el diferente grado de difusividad de los mismos a través de la PC. Varias investigaciones han señalado que la actividad de los aceites esenciales está relacionada con la configuración química de sus componentes, con la proporción en la que estos se encuentren presentes y su grado de interacciones (Dorman y Deans, 2000; Mariano et al. 2001; Delaquis et al. 2002). Además, Cagri et al. (2001) sugirió que la difusión de agentes antimicrobianos a través de un disco de PC depende del tamaño, forma y polaridad de las moléculas que difunden, de la estructura química de la película, así como del grado de entrecruzamiento molecular de sus componentes.

Propiedades de barrera

Permeabilidad al vapor de agua (PVA)

Se observó que la PVA de todas las PC disminuyó a medida que aumentaba la cantidad de AEs en su composición, siendo este efecto más acentuado cuando se empleó aceite de orégano (0,1%). Hernández (1994), señaló que la transferencia de vapor de agua en las PC ocurre a través de su parte hidrofílica y por lo tanto depende de la proporción hidrofóbica/hidrofílica de los constituyentes de la película. Debido a que cada AE presenta sus propias características físico-químicas, el comportamiento observado en las diferentes PC pudo deberse al tipo de AE empleado en su composición y a la proporción de compuestos hidrofílicos en la misma. La incorporación de aceite de orégano en la formulación impartió a las películas excelentes propiedades antimicrobianas, además de mejorar sus propiedades de barrera al vapor de agua.

Permeabilidad al oxígeno (PO$_2$)

Las películas comestibles elaboradas a partir de puré de manzana mostraron buenas propiedades de barrera al oxígeno, exhibiendo valores de permeabilidad de 22,64±1,28 cm^{3}µm/m^2-d-kPa. Los compuestos no polares como los lípidos, actúan como excelentes barreras al vapor de agua, pero son menos efectivos como barrera a los gases. De hecho, la incorporación de AEs en la formulación causó un incremento en los valores de PO$_2$, alcanzando valores de 38,12±0,80 cm^{3}µm/m^2-d-kPa cuando se incorporó aceite de orégano al 0,1% p/v en la formulación.

Propiedades mecánicas

Fueron tres las propiedades mecánicas estudiadas en las PC, fuerza de tensión, elongación y elasticidad. Con respecto a las propiedades de tensión, se observaron comportamientos diferentes dependiendo del AE contenido en la formulación. Un incremento en la concentración de aceite de hierba de limón de

0,05% a 0,1% p/v causó un significativo descenso en la fuerza de tensión de la PC. Por el contrario, la adición de aceite de canela produjo un incremento en la fuerza de tensión ejercida por la PC. Las diferencias observadas en ambas PC fueron atribuidas a las distintas polaridades de los AEs incorporados. La presencia de AEs, generalmente no produce un cambio significativo en las propiedades de elongación de las PC. De hecho, el tipo y concentración de AEs añadido a las películas de puré de manzana no modificaron sus propiedades de elongación. De igual forma, la incorporación de AEs en la formulación no causó ningún efecto significativo en las propiedades elásticas de las películas. Sin embargo, la presencia de 0,075% de aceite de canela causó un incremento en la elasticidad de las mismas (7,60±0,59 MPa) comparado con aquellas que no contenían ningún tipo de AE (5,06±0,54 MPa). Resultados similares fueron reportados por Zivanovic et al, (2005) quienes observaron un descenso en la fuerza de tensión y un incremento en la elongación de películas de quitosano enriquecidas con AEs.

En general se observó que las películas elaboradas a partir de puré de manzana poseían una alta permeabilidad al vapor de agua y baja al oxígeno sin detectarse un cambio sustancial de los otros parámetros por la adición de AEs. Sin embargo se confirmó que la incorporación de aceite de orégano confiere una mayor actividad antimicrobiana frente a *E. coli* O157:H7, tanto en la SFP como en las PC, comparado con los otros AEs estudiados.

C.3.-Película comestible a base de alginato y puré de manzana. Incorporación de aceites esenciales o sus correspondientes compuestos activos.

Una vez establecidas las características de los PC basadas en puré de manzana y agentes antimicrobianos, se decidió por un lado, incorporar una de las matrices polisacáridas estudiadas en la primera parte del trabajo, alginato, con el fin de mejorar las propiedades de barrera y mecánicas de esta última, y por otro lado, se decidió estudiar el efecto de la incorporación de los compuestos activos (carvacrol, citral y cinamaldehído) correspondientes a los AEs estudiados anteriormente.

Efecto en las propiedades de barrera y mecánicas

Permeabilidad al vapor de agua (PVA)

Debido a la naturaleza hidrofílica de las PC basadas en polisacáridos, éstas tienden a ser barreras pobres a la humedad (Kester y Fennema, 1986; García et al., 1998), por lo que la incorporación de lípidos dentro de su formulación mejora notablemente sus propiedades de barrera al vapor de agua (García et al., 2000; Yang y Paulson., 2000). Sin embargo, nosotros observamos que la incorporación de AE o de sus correspondientes compuestos activos no mejoraron las propiedades de PVA de las PC, ya que mostraron valores similares a los observados en el control, aprox. 4,95 g-mm / kPa-h-m^2. Aunque, se observó un ligero descenso de la PVA después de la incorporación de 0,5% p/p de cinamaldehído. Este comportamiento es presumiblemente debido a que los AE están constituidos básicamente por terpenos y no por lípidos.

Adicionalmente, si se comparan los resultados de PVA obtenidos en PC de alginato-puré de manzana sin adición de AE (4,95 g-mm / kPa-h-m^2) con las PC de alginato estudiadas en el apartado C.1 (1,08 g-mm / kPa-h-m^2), se puede observar un importante incremento de la PVA debido a la incorporación de puré de manzana, el cual al ser de naturaleza hidrofílica causó un incremento en dicho parámetro.

Permeabilidad al oxígeno (PO$_2$)

De acuerdo con lo esperable, la incorporación de alginato como matriz estructural en películas de puré de manzana, mejoró notablemente la PO$_2$, observándose valores de 10,20±0,91 cm^{3}μm/m^2-d-kPa de permeabilidad en estas nuevas PC. Si se compara con los valores de PO$_2$ obtenidos en las películas de puré de manzana del apartado anterior (22,64 ± 1,28 cm^{3}μm/m^2-d-kPa), se puede observar que la PO$_2$ de este nuevo tipo de PC es dos veces inferior. McHugh et al., (1996) sugirió que el tipo de carbohidratos empleado en la elaboración de PC puede afectar notablemente las propiedades de permeabilidad de las mismas.

Por su parte, la adición de AE no modificó significativamente la PO$_2$ de películas de alginato y puré de manzana, aunque se observó un ligero descenso de la permeabilidad en películas que contenían aceite de hierba de limón o su compuesto activo citral a una concentración de 0,5% p/p.

Propiedades mecánicas

A diferencia de lo observado en películas de puré de manzana, la incorporación de AEs y sus compuestos activos causó una importante reducción en la resistencia a la tensión de películas de alginato-puré de manzana, siendo este efecto más pronunciado en aquellas PC que contenían aceite de orégano o carvacrol en su formulación, las cuales presentaron valores de 2,47±0,37 y 2,58±0,37 MPa, respectivamente. De igual forma, los AEs causaron un efecto significativo en el porcentaje de elongación de las películas de alginato y puré de manzana, observándose un incremento de este valor en todas las películas conteniendo AEs y sus respectivos compuestos activos. Cuando carvacrol fue incorporado dentro de la formulación, se detectó el máximo porcentaje de elongación antes de romperse la película (58,33%), comparado con los 51,06% observados en películas sin antimicrobianos. Los valores de elasticidad observados en PC de alginato y puré de manzana (aprox. 7,07±1,09 MPa) fueron en general mayores que los observados en las mismas películas conteniendo agentes antimicrobianos. McHugh y Krochta, (1994), señalaron que la fuerza de tensión, elongación y modulo de elasticidad de las PC están estrechamente relacionadas con la estructura química de sus componentes.

Propiedades antimicrobianas

Al igual que en el caso de películas de puré de manzana, la efectividad de los AEs y de sus compuestos activos como agentes antimicrobianos fue estudiada tanto en la solución formadora de películas (SFP) como en las PC una vez formada. En ambos casos, la efectividad de las sustancias antimicrobianas fueron probadas frente al crecimiento del microorganismo patógeno *E. coli* O157:H7.

Actividad antimicrobiana de los AEs y sus compuestos activos en las SFPs

En general, tanto los AEs como sus compuestos activos fueron capaces de inhibir el crecimiento de *E. coli* O157:H7 en las SFPs. No se observaron diferencias significativas entre un determinado AE y su

correspondiente compuesto activo, aunque sí se observaron diferencias de efectividad dependiendo del compuesto empleado. La presencia del antioxidante N-acetilcisteína al 1% no causó ningún efecto inhibidor sobre *E. coli* O157:H7 inoculado en las SFPs sin antimicrobianos.

Entre los antimicrobianos estudiados, el aceite de orégano y su compuesto activo carvacrol mostraron tener la mayor efectividad inhibitoria frente al crecimiento de *E. coli* O157:H7, confirmándose el comportamiento antimicrobiano observado previamente en películas de puré de manzana donde éstos eran contenidos. La exposición de 0,1% de carvacrol en la SFP por un período de 3 min produjo un valor de BA_{50} de 0,020, es decir, 0,020% de carvacrol inhibió el 50% de *E. coli* O157:H7 después de 3 min de contacto entre el antimicrobiano y el microorganismo. Sin embargo, se observó el doble de actividad antimicrobiana (0,011%) cuando carvacrol estuvo en contacto por un período de tiempo más largo, 30 y 60 min. Se obtuvieron resultados muy similares mediante el uso del aceite de orégano, observándose valores de 0,025%, 0,010% y 0,012% correspondientes a períodos de contacto de 3, 30 y 60 min. El aceite de orégano contiene aproximadamente 86% de carvacrol en su composición, por lo que la similitud en sus propiedades antimicrobianas fue lo esperado. Las propiedades antimicrobianas del carvacrol están asociadas con su naturaleza lipofílica, la cual le permite acumularse en las membranas, formar parte de ellas y como consecuencia producir daños en la célula, tales como una disminución de la energía celular (Sikkema et al., 1995).

Por su parte, la actividad antimicrobiana del aceite de hierba de limón y su compuesto activo citral fueron similares frente *E. coli* O157:H7, teniéndose que emplear 5 veces más concentración del compuesto (0,5%) para obtener una inhibición similar que la observada con la incorporación del aceite de orégano y carvacrol. Comparativamente, la efectividad del aceite de canela y su compuesto activo cinamaldehído fue menor, siendo efectivos frente a *E. coli* O157:H7 después de 30 y 60 min de exposición, mostrando valores de BA_{50} correspondientes a 0,11% y 0,086% para cinamaldehído y de 0,16% y 0,087% para aceite de canela. Friedman et al., (2004) indicaron que el aceite de canela posee un 85% de cinamaldehído, por lo que al igual que en el caso del carvacrol, esta similitud de efectividad entre el AE y su compuesto activo era lo esperable.

Actividad antimicrobiana de los AEs y sus compuestos activos en las PC

Aunque la efectividad de los compuestos antimicrobianos podría variar si es determinada en un medio líquido (SFP) o en un medio sólido (PC), se observó la misma tendencia de efectividad en ambos sistemas. Todas las PC que contenían sustancias antimicrobianas inhibieron significativamente el crecimiento de *E. coli* O157:H7 en las condiciones estudiadas. Coincidiendo con los resultados obtenidos en la SFP, carvacrol fue el compuesto que mostró la mayor actividad antimicrobiana, evidenciándose por una zona clara (libre de crecimiento) más grande alrededor de la película (aprox. 1,6 mm). De igual forma que lo obtenido en SFP, la efectividad observada con aceite de hierba de limón o citral y aceite de canela o cinamaldehído (0.5% p/p) frente a *E. coli* O157:H7 fue inferior que la obtenida con aceite de orégano o carvacrol.

C.4.- Aplicación de recubrimientos comestibles elaborados a partir de puré de manzana y alginato en trozos de manzana cortada. Estudio de vida útil.

Finalmente y de acuerdo con los resultados obtenidos hasta entonces, se decidió recubrir trozos de manzana Fuji en un estado de madurez intermedio con un RC elaborado con una mezcla de alginato y puré de manzana, conteniendo glicerol y N-acetilcisteína como plastificante y antioxidante, respectivamente y diferentes agentes antimicrobianos, empleándose aquellos que mostraron tener una mayor efectividad antimicrobiana (aceites de orégano y de hierba de limón) además de la incorporación de vainillina como nuevo compuesto antimicrobiano. El efecto de este recubrimiento en la vida útil de manzanas troceadas fue evaluado mediante cambios en la respiración, color, textura, características sensoriales y finalmente su estabilidad microbiológica durante un período de almacenamiento de 21 días.

Cambios de la composición gaseosa en el espacio de cabeza

El tipo y concentración de antimicrobiano incorporado dentro del RC afectó de manera diferente la evolución del O_2 y el CO_2 a través del período de almacenamiento. De hecho, cuando se incorporaron altas concentraciones de aceite de hierba de limón (1 y 1,5% p/p) o de orégano (0,5% p/p) en su formulación se pudo observar un leve descenso en las concentraciones de O_2 comparado con la brusca caída de este gas en el resto de las muestras recubiertas, las cuales alcanzaron valores tan bajos como 3,54 ppm. De igual forma, las concentraciones de CO_2 en envases que contenían trozos recubiertos con las concentraciones más altas de aceite de hierba de limón y orégano, no experimentaron un aumento sustancial de este gas durante el período de almacenamiento. Definitivamente, la incorporación de AEs en recubrimientos de alginato-puré de manzana actuó efectivamente como barrera a la difusión de los gases, tal como se había observado en los ensayos anteriores de PC. Olivas y Barbosa-Cánovas (2005), mencionaron que los RC son capaces de aislar un producto del ambiente que le rodea mediante la creación de una atmósfera modificada, la cual actúa como una barrera al oxigeno causando a su vez un descenso en la tasa respiratoria.

Por su parte, los niveles de etileno en manzanas cortadas y recubiertas con películas conteniendo antimicrobianos fueron bajos y se mantuvieron constantes durante los 21 días de almacenamiento, contrariamente a lo observado en la producción de O_2 y CO_2. Sin embargo, la evolución de este gas en el espacio de cabeza de los envases fue diferente en manzanas recubiertas con un RC de alginato-puré de manzana sin antimicrobianos y en aquellas muestras sin recubrir, donde la producción de etileno alcanzó niveles tan altos como 132,38 y 230,07 μl l^{-1} al final del período de almacenamiento, quedando en evidencia que el RC por si mismo produce una disminución en la producción de este gas, aunque en todos los casos inferior a la obtenida en recubrimientos con antimicrobianos. Estos resultados coinciden con los obtenidos por Wong, et al., (1994), quienes observaron una tasa de reducción en la producción de etileno del 90% en trozos de manzana recubiertos con una mezcla de polisacáridos y lípidos, atribuyendo esta reducción, tanto a la propiedades de barrera impuestas por la incorporación de lípidos, como al efecto inhibitorio causado por la solución de iones de calcio, usada como una segunda capa de recubrimiento. En nuestro caso, la incorporación de aceites antimicrobianos dentro de la formulación, así como el uso de una solución de cloruro de calcio para el entrecruzamiento de las moléculas de alginato, pudieron afectar la evolución del etileno dentro de los envases.

Alonso y Alique, (2004) indicaron que un limitado intercambio de gases entre la fruta y su entorno debido al uso de RC podría reducir drásticamente los niveles internos de oxígeno y alterar el metabolismo

respiratorio, produciendo condiciones de anaerobiosis y fermentación, y a su vez la producción de malos sabores causado por la acumulación de acetaldehído y etanol. En efecto, todas las muestras de manzana recubiertas, mostraron un ligero incremento en la producción de acetaldehído durante el almacenamiento, a excepción de aquellas recubiertas con un RC conteniendo vainilla al 0,3% y 0,6% p/p, donde los niveles de este gas alcanzaron valores tan altos como 377,47 µl l^{-1}. Estos resultados y la baja concentración de acetaldehído en los trozos de manzana sin recubrir, confirmaron la creación de una atmósfera modificada alrededor de los trozos de fruta recubiertos, la cual impidió en diferente grado el intercambio gaseoso desde el interior de la fruta hacia el espacio de cabeza del envase. Posiblemente, el hecho de que la naturaleza química de los antimicrobianos incorporados en el RC fue distinta, aceites en el caso de orégano y hierba de limón, y cristales sólidos en el caso de vainillina, hizo que la difusión del acetaldehído a través del recubrimiento fuera diferente, sugiriendo a su vez que la concentración real de este gas en el interior de la fruta fue superior al detectado en el espacio de cabeza.

Resultados muy similares fueron observados en la producción de etanol, donde se detectó la presencia de este gas después de la primera semana de almacenamiento en el espacio de cabeza de envases que contenían trozos de manzana recubiertos con vainilla 0,3 y 0,6% p/p y las concentraciones más bajas de aceite de orégano (0,1% p/p) y de aceite de hierba de limón (1% p/p). La detección de este gas en el resto de manzanas cubiertas fue más tardía, observándose los primeros indicios del gas después de 10 días de almacenamiento y no superando los 26 µl l^{-1} después de los 21 días de almacenamiento. Sin embargo, en manzanas recubiertas con un RC sin antimicrobiano, la producción de etanol fue mayor llegando a superar los 90,98 µl l^{-1} al final del período de almacenamiento.

Cambios en el color

Los menores cambios de color observados en trozos de manzana recubiertos con RC conteniendo compuestos antimicrobianos, fueron observados cuando vainilla fue incluida en la formulación en concentraciones de 0,3% y 0,6% p/p. Estos resultados coinciden con los obtenidos por Rupasinghe et al., (2006) quienes no observaron cambios significativos de color en manzanas cortadas recubiertas con una solución de carboximetilcelulosa (NatureSeal^{TM}) conteniendo vainillina en su formulación.

La luminosidad en todos las muestras de manzana recubiertas descendió a partir de la primera semana de almacenamiento, excepto en aquellas muestras cuyo recubrimiento contenía aceite de hierba de limón (1,0% p/p) o vainilla (0,6% p/p) donde los valores de L* permanecieron relativamente constantes durante todo el período de almacenamiento. Por su parte, los valores de a* se vieron modificados por la incorporación de agentes antimicrobianos en la formulación, siendo especialmente significativo cuando se empleó aceite de hierba de limón, el cual produjo tonalidades más verdes en el exterior de los trozos de manzana, pero, en contraste, produjo pigmentaciones oscuras en su interior desde los primeros días de almacenamiento. En la figura 2, se pueden observar las diferencias de color interna y externa de cilindros de manzana recubiertos con un RC conteniendo aceite de hierba de limón (1%), comparado con la aplicación de RC sin ningún tipo de antimicrobiano en su composición. Adicionalmente, se observó un pronunciado descenso del parámetro b* en aquellas muestras que contenían 1,5% de aceite de hierba de limón, fluctuando entre 28,52 y 21,77 durante el período de almacenamiento. De igual forma y debido a que el tono (h*) es calculado usando valores de los parámetros a* y b*, la tonalidad de los trozos de

manzana cuyo RC contenía aceite de hierba de limón en su composición presentó los valores más altos del parámetro h[*], contrarrestando con los resultados obtenidos en trozos de manzana sin antimicrobianos, los cuales mostraron los menores valores de h[*] durante el almacenamiento.
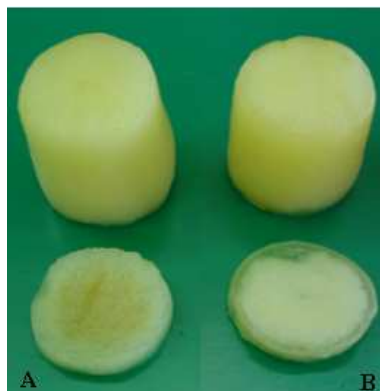

Figura 2.- Cambios en el color de cilindros de manzana Fuji cortada recubierta con un RC de alginato-puré de manzana conteniendo (A) 1% de aceite de hierba de limón (B) sin antimicrobianos, después de 7 días de almacenamiento.

Además del efecto ejercido en el color por la presencia de antimicrobianos en la formulación del RC, la presencia de N-acetilcisteína influyó significativamente en la conservación del color de los trozos de manzana, manteniéndolos libres de pardeamiento durante el almacenamiento. Por el contrario, se observaron indicios de pardeamiento desde primeras horas de almacenamiento en trozos de manzana sin recubrir, evidenciándose por altos valores de a[*] (1,34) y bajos de h[*] (87,50). En vista de estos resultados, se confirma el potencial uso de los recubrimientos comestibles como soporte y transporte de agentes antioxidantes en la superficie de manzanas cortadas, permitiendo contrarrestar los efectos del pardeamiento enzimático.

Cambios en la textura

En general, los menores cambios de textura fueron observados en piezas de manzanas recubiertas con una película de alginato-puré de manzana sin antimicrobiano y en aquellas que contenían aceite de orégano (0,1% p/p) o vainillina (0,3 y 0,6% p/p) en su formulación, siendo estas últimas las que se conservaron más firmes durante los 21 días de almacenamiento. Rupasinghe, et al., (2006), señaló que la incorporación de 12 mM de vainillina dentro de una solución de NatureSeal[TM] mantuvo los valores de firmeza de rodajas de manzana "Empire" y "Crispin" durante todo el período de almacenamiento. Sin embargo en este estudio, la firmeza inicial de manzanas sin recubrir (7,60 N) disminuyó gradualmente desde los primeros días de almacenamiento, observándose valores de 4,03 N al final de este período. La diferencia observada entre manzanas sin recubrir y aquellas recubiertas con un RC sin antimicrobianos se debieron a la presencia de cloruro de calcio dentro de la formulación. Resultados similares fueron reportados por Lee et al. (2003), quienes demostraron que la incorporación de cloruro de calcio al 1%

dentro de un RC elaborado a partir de concentrado de proteína de suero ayudó a mantener la firmeza de rodajas de manzana fresca. Olivas y Barbosa-Cánovas, (2005) indicaron que potenciadores de la textura, tales como el cloruro de calcio, pueden ser incorporados en RC para mejorar la calidad de la fruta durante el almacenamiento debido a la inhibición de la pérdida de firmeza en frutas mínimamente procesadas.

Sin embargo y a pesar de las excelentes propiedades observadas por la incorporación de calcio en la formulación, trozos de manzana recubiertos con una película de alginato-puré de manzana conteniendo1% y 1,5% p/p de aceite de hierba de limón o 0,5% p/p de orégano, mostraron un abrupto descenso de la firmeza inicial desde las primeras horas de almacenamiento, alcanzando valores tan bajos como 1,9 N al final del almacenamiento en muestras recubiertas con 1,5% de aceite de hierba de limón. Posiblemente, la aplicación de este RC conteniendo 1,5% de aceite de hierba de limón afectó severamente la firmeza de los trozos de manzana debido al pH del mismo. Resultados similares han sido reportados por Lee et al., (2003) quienes observaron una intensa pérdida de firmeza en manzana Fuji fresca cortada recubierta con una solución de carragenano, la cual contenía ácido ascórbico y cítrico en su formulación, atribuyendo esta excesiva suavidad al bajo pH de la solución formadora de cobertura.

Evaluación sensorial

Un recubrimiento comestible debería poseer un sabor bastante suave o en su defecto no poseer ningún tipo de sabor, de forma que no pueda detectarse durante la consumición del alimento donde éste es aplicado (Contreras-Medellin y Labuza, 1981). Sin embargo, la incorporación de AEs dentro de cualquier formulación de RC podría causar un cambio apreciable en el sabor final del producto. De hecho, en esta investigación se observó que la incorporación de antimicrobianos de origen natural dentro de un recubrimiento de alginato y puré de manzana cambió de forma apreciable las características sensoriales de los trozos de manzana, a pesar de que sólo fueron evaluados aquellos recubrimientos que contenían las concentraciones más bajas del antimicrobiano.

La mayor puntuación (>6) de la escala hedónica utilizada para la evaluación de los atributos sensoriales fue obtenida en trozos de manzana cubiertos con una película de alginato-puré de manzana conteniendo vainillina al 0,3% p/p y en aquellas cuya composición no contenía ningún tipo de antimicrobianos. Los trozos de manzana recubiertos con un RC de alginato-puré de manzana (sin antimicrobianos), presentaron la mayor aceptación por parte de los panelistas, indicando que dicha formulación por sí misma no produce ningún cambio apreciable en las características sensoriales de la manzana fresca. Contrario a estos resultados, los trozos de manzana recubiertos con un RC conteniendo aceite de orégano en su composición fueron los que obtuvieron los valores más bajos de preferencia por parte de los panelistas, aunque mantuvieron altas valoraciones del color. El rechazo por parte de algunos consumidores de muestras conteniendo 0,1% de aceite de orégano, se debió principalmente a la detección de un sabor residual a "hierbas", el cual no fue compatible con las características sensoriales de la manzana. Sin embargo, Roller y Seedhar (2002) reportaron que la adición de 1mM de carvacrol (compuesto activo del aceite de orégano) como agente antimicrobiano, no causó reacciones adversas en la evaluación sensorial de kiwi y melón fresco cortado.

Aunque las muestras conteniendo aceite de orégano fueron las que presentaron la puntuación más baja el primer día de evaluación sensorial, se observó una caída brusca después de la primera semana de

almacenamiento en todos los parámetros evaluados en muestras conteniendo aceite de hierba de limón al 1% p/p, siendo las principales características afectadas, el color y la textura. Estos resultados coinciden con los obtenidos instrumentalmente, donde se observó una abrupta caída de la firmeza y la presencia de oscurecimiento interno en los trozos de fruta recubiertos con aceite de hierba de limón. El efecto negativo causado en ambos parámetros sensoriales, produjo el rechazo de dichas muestras por parte de los consumidores a partir de la primera semana de almacenamiento.

Son muy pocos los estudios reportados sobre el efecto del RC sobre las características sensoriales de trozos de manzana. Sin embargo, existen algunos estudios que han evaluado las propiedades sensoriales de otras matrices como el puré de frutas conteniendo algún tipo de antimicrobiano. Por ejemplo, Cerruti y Alzamora (1996) evaluaron las propiedades sensoriales de purés de frutas, incluyendo el de manzana, incorporando vainillina en su formulación, y reportaron una aceptación positiva del sabor por parte del consumidor y un mantenimiento de sabor original de la fruta fresca, similar a los resultados obtenidos por nosotros.

Estabilidad microbiológica

El crecimiento de microorganismos aerobios psicrófilos así como el de mohos y levaduras durante el almacenamiento, se vio significativamente afectado por la presencia de agentes antimicrobianos dentro de la formulación. De hecho, se observó que los recuentos de estos microorganismos en manzanas recubiertas sin antimicrobianos aumentaron aprox. 5,5 ciclos logarítmicos en el caso de psicrófilos, alcanzando valores de 10^7 UFC.g^{-1} al final de los 21 días de almacenamiento. Con respecto a la efectividad de los agentes antimicrobianos, se observó una inhibición total de microorganismos aerobios psicrófilos (con un límite de detección de 1,0 log UFC/g) y de mohos-levaduras (con un límite de detección de 2,0 log UFC/g) cuando se encontraban incorporados 1,0 y 1,5% de aceite de hierba de limón y 0,5% de aceite de orégano en el RC. Posiblemente, la efectividad de estos compuestos se vio favorecida por el bajo pH de la solución, tal como se indicó anteriormente. Burt (2004), señaló que generalmente a menor pH, mayor es la efectividad de los compuestos antimicrobianos. El recuento microbiológico de las otras muestras fue inferior en todos los casos, no excediendo de 10^4 UFC/g al final el período de almacenamiento para aerobios psicrófilos y de 3 log UFC/g para mohos y levaduras. Los recuentos de levaduras no excedieron el límite máximo establecido por la IFST para frutas procesadas por métodos no térmicos, el cual es de 6 log UFC/g (IFST, 1999). Sin embargo, los recuentos de mohos y levaduras en trozos de manzanas tratados con 0,1% de aceite de orégano alcanzaron valores de 4,1 log UFC/g al final del período de almacenamiento, siendo la efectividad de este compuesto disminuida a través del tiempo. Lanciotti et al., (1999) sugirió que la adición de aceites esenciales provenientes de cítricos en una mezcla de frutas cortadas (manzana, pera, uva, melocotón y kiwi) inhibió la proliferación de la flora natural de dichas frutas.

Adicionalmente y con la finalidad de estudiar el efecto de los agentes antimicrobianos sobre un microorganismo indicador, se evaluó la efectividad de los mismos sobre *Listeria inocua* inoculada sobre trozos de manzanas antes de ser recubiertos. No se observó incidencia de *L. inocua* en trozos de manzana no inoculados, por lo que los recuentos observados de dicho microorganismo fueron frutos únicamente de la inoculación del mismo en las muestras.

La incorporación de agentes antimicrobianos dentro de la formulación de RC inhibió significativamente el crecimiento de *L. inocua* en trozos de manzana comparado con las muestras control (sin antimicrobianos), donde se observaron recuentos de 6,2 UFC/g al final del almacenamiento, confirmándose una vez más que el RC no ejerce ningún efecto en el crecimiento de *L. inocua*. Al igual que en el caso anterior, la mayor actividad antimicrobiana fue ejercida por el aceite de hierba de limón (1,0 y 1,5% p/p) y de orégano (0,5% p/p) los cuales redujeron el número de colonias de *L. inocua* por debajo del límite de detección (2,0 log UFC/g) durante la primera semana de almacenamiento, confirmándose posteriormente la completa destrucción del microorganismo debida al efecto de los antimicrobianos.

En todos los casos se observó una reducción del recuento inicial de *L. inocua* inoculada (10^6 UFC/g) inmediatamente después de la aplicación de los RC conteniendo los agentes antimicrobianos. El recuento inicial de trozos de manzana donde se empleó vainillina disminuyó aprox. 3 ciclos logarítmicos inmediatamente a la aplicación del RC. Sin embargo, esta disminución del recuento inicial de *L. inocua* fue inferior cuando se empleó aceite de orégano (0,1% p/p) en la formulación, donde se observó únicamente una reducción de 1,6 ciclos logarítmicos. De hecho, se observó que la efectividad de este compuesto fue inferior y temporal, alcanzando recuentos muy similares a los obtenidos en el control (6 log UFC/g) después de 21 días de almacenamiento.

Como se confirmó en los trabajos anteriores, 0,1% p/p de aceite de orégano es suficiente para inhibir el crecimiento de microorganismos en películas comestibles. Sin embargo, fue necesaria una concentración superior de este compuesto (0,5% p/p) para obtener resultados inhibitorios similares a los obtenidos en PC, quedando es evidencia que su efectividad disminuye cuando es aplicado en forma de cobertura. Los resultados obtenidos están en concordancia con lo reportado por Dawson et al., (2002), quienes señalaron que las PC conteniendo antimicrobianos son más efectivas en la inhibición de un determinado microorganismo cuando éstas son aplicadas en un medio nutritivo que cuando son aplicadas en alimentos reales.

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