Using polysaccharide-based edible coatings to maintain quality 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 cm² O₂ 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 °C. Concentration of O₂ and CO₂ 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 apple 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 apple 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.

1. 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 (Baldwin, Nisperos, Chen, & Hagenmaier, 1996; Park, 1999; Wong, Camirand, & Pavlath, 1994). 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 (former referred to as Pseudomonas elodea) are employed in the food industry as texturizing and gelling agents.
Alginates 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 & Kroetcha, 1994a, 1994b; Sothornvit & Kroetcha, 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 & Paulson, 2000).

Edible coatings may also serve as carriers of food additives such as antibrowning and antimicrobials agents, colorants, flavors, nutrients and spices (Li & Barth, 1998; Pena & Torres, 1991; Pranoto, Salokhe, & Rakshit, 2005; Wong, Gregorksi, Hudson, & Pavlath, 1996). Sulfur-containing amino acids as N-acetylcysteine, 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, and Martin-Belloso (2006).

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.

2. Materials and methods

2.1. Materials

‘Fuji’ apples (Malus domestica Borkh) stored for 3 months under controlled atmospheres (2% O₂ and 2% CO₂ at 0 °C) were provided by ACTEL, Lleida, Spain. Afterwards, apples were stored at 4±1 °C until processing. Food grade sodium alginate (Keltone® LV, ISP, San Diego, CA, USA) and gellan gum (Kelicogel®, CPKelco, Chicago, IL, USA) were used as the carbohydrate biopolymers for coating formulations. Glycerol (Merck, Whitehouse Station, NJ, USA) was added as plasticizer. Calcium chloride (Sigma-Aldrich Chemic, Steinheim, Germany) was used to induce crosslinking reaction. N-acetylcysteine (Sigma-Aldrich Chemic, Steinheim, Germany) was the added antibrowning agent. A 0.025% (w/v) of sunflower oil (La Española, Spain) with the following composition: 11 g monounsaturated, 30 g monounsaturated and polyunsaturated 57.4 g; 3.5 g omega-3 and 55–60 g omega-6, was used as the lipid source when emulsion films were prepared.

2.2. 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 °C 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, Rodriguez, Carmona, & Martin-Belloso, 2007).

2.3. 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 four 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 min. 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 cm³ (Mcp Performance Plastic LTD, Kibbutz Hamaapil, Israel) and wrap-sealed using a 64 μm thickness polypropylene film with a permeability to oxygen of 110 cm³ O₂ m⁻² bar⁻¹ day⁻¹ at 23°C and 0% RH (Tecnopack SRL, Mortara, Italy) using a MAP machine (Ilpra Foodpack Basic V/G, Ilpra, Vigenov, Italy). Trays were filled with air, heat sealed and stored in darkness at 4±1 °C. Analyses were carried out periodically during 23 days for randomly sampled pairs of trays.

2.4. 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 O₂ and CO₂ determination, respectively. The O₂ content was analyzed with a CP-Molsieve 5 Å packed column (Chrompack International, Middelburg, The Netherlands) (4 m × 0.32 mm, df = 10 mm) at 60 °C and 100 kPa. For quantification of CO₂, ethylene (C₂H₄), acetaldehyde (C₂H₄O) and ethanol (C₂H₅OH), a PorapLOT Q column (Chrompack International, Middelburg, The Netherlands) (10 m × 0.32 mm, df = 10 mm), held at 70 °C 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.

2.5. Color 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 10° 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*):

\[ h^* = \arctan \frac{b^*}{a^*}. \]

2.6. 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 center.

2.7. 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 eight 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 48 h at 30 °C to numerated mesophilic and 5 days at 5 °C 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.

2.8. 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.

3. Results and discussion

3.1. 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 O₂ and CO₂, no significant differences were observed between coated and uncoated apple wedges regarding the composition of these gases through the coatings along the evaluated period (Fig. 1, Table 1). The permeability of the plastic film used to wrap the coated apple pieces contained in the polypropylene trays to O₂ and CO₂ was moderate (110 cm³O₂ m⁻² bar⁻¹ day⁻¹ and 500 cm³CO₂ m⁻² bar⁻¹ day⁻¹) probably letting O₂ and CO₂ to pass through, preventing their accumulation in the headspace and making difficult to allow an inference on the effect of the edible coatings as selective barriers to these gases. The expected trend of O₂ and CO₂ concentration along the storage time in coated fresh-cut fruits has been reported by several authors. Thus, Wong, Tillin, Hudson, and Pavlath (1994) investigated the effect of various bilayer coatings (alginate included) on respiratory activity of coated apple pieces measuring CO₂ and ethylene production in the headspace gas composition. All the coatings studied by Wong, Tillin et al. (1994) produced a substantial rate reduction in CO₂ and ethylene, which was especially significant for the latter. The ethylene production when apple pieces were coated was 90% lower than that observed in uncoated cut apples. Fig. 2 presents the production along the time of the other gases investigated in this study. In contrast to the behavior observed for O₂ and CO₂, 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.
Fig. 2a shows the ethylene production of the coated and uncoated fresh-cut apples through storage. Ethylene levels varied from 7.55 to 28.25 μl/l in apples coated with alginate, and from 9.91 to 40.42 μl/l in apples coated with gellan, while in uncoated apple wedges the rise in ethylene production was from 19.16 to 154.35 μl/l 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 (Beaulieu & Baldwin, 2002; Kays, 1991). Wong, Tillin et al. (1994) 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. 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 apple 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 CO2 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-Gugo 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 Fig. 2b. Acetaldehyde increased during storage reaching levels as high as 141.97 μl/l in coated cut apples, while in uncoated apples the production of the gas was low (approximately 10 μl/l) and was kept constant till the end of storage. Fig. 2c shows the ethanol production in the coated fresh cut apples. The presence of this gas was detected (19.50 μl/l) after 15 days of storage in coated fruits, reaching values of 32.25 μl/l 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 O₂ and increased CO₂ 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

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).
anaerobic fermentation that is taking place. Its accumulation occurs when internal atmosphere is affected by restricting gas exchange (Park, Chimnan, & Shewfelt, 1994). From these results it can be inferred that O2 and CO2 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 O2 and under 2.5 kPa O2 + 7 kPa CO2, 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.

3.2. 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 apple wedges. Fig. 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.

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, Camirand et al., 1994; 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., 2006). These results show that alginate and gellan based coatings are good carriers for antibrowning agents since browning is prevented during all the storage period.

3.3. 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 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 could 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.

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, and 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.

3.4. 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. Fig. 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.

At the end of the 3 weeks refrigerated storage, counts of coated samples did not exceed $10^4$ and $10^5$ CFU g$^{-1}$ for mesophilic and psychrophilic respectively, with both types of coatings used, while uncoated apple wedges presented values as high as $10^7$ 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 et 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°C. 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$^{-1}$.

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.

4. 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 apple wedges coated with both alginate and gellan could have a shelf-life up to 3 weeks at 4°C; but the presence of acetaldehyde and ethanol, as a result of fermentative anaerobic processes, limit their shelf-life to 2 weeks. Results showed that the shelf life of the coated apples was extended approximately three 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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References


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).


