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Active Coated Films to Decrease Browning of Minimally Processed Banana Fruit

Annalisa Apicella, Elisa Carlucci, Charlène Leneveu-Jenvrin, Fabienne Remize, and Paola Scarfato*

Minimally processed fruit presents a short shelf-life because of cellular endogenous metabolism or microbial development. Fruit browning mostly results from phenolic compound oxidation. Banana (*Musa acuminata* Cavendish) cuts are particularly prone to enzymatic browning, resulting in unattractive color and modifications of aroma. In this study, the effect of two different eco-sustainable packaging films on minimally processed banana is investigated. The films differ by the substrate, either polyethylene terephthalate (PET) or a fully biodegradable mix of polylactic acid (PLA) and polybutylene adipate terephthalate (PBAT). They are coated with an amorphous PLA layer containing as antioxidant an active agent deriving from olive processing wastes. Minimally processed banana slices are prepared, packed, and stored at 4°C. Microbiological quality, color, pH, firmness, and polyphenol oxidase activity are evaluated during storage. The PET films show a better efficacy to limit browning, mainly due to the higher oxygen barrier properties compare to the PLA/PBAT. Best results are obtained with PET films with 5% of active phase.

the choice of F&V purchasing. Beside appearance and convenience, packaging characteristics appear to be strong determinants for minimally processed F&V purchase decision. The shelf life of these foods is short, typically 3–7 days, and they must be stored under refrigeration. Maintaining freshness and obtaining a long shelf-life are difficult to conciliate, especially with those packaging materials showing poor barrier performance, such as biodegradable materials.^[3] Indeed, fresh-cut F&V are rapidly spoiled because of endogenous metabolism: respiration, oxidative reactions, and activity of cell wall enzymes modify aroma, taste, and texture. In addition, the development of bacteria, yeasts, and molds can impair the safety and the microbiological quality of F&V.^[4] Bioactive phytochemicals are also catalyzed during shelf-life.

The most used technologies adopted to increase minimally processed F&V

shelf-life include a tight control of storage temperatures, sanitation of F&V during washing, and application of modified atmospheres.^[5–7] The development of active packaging is another way for that purpose.

Active films are innovative packages deliberately incorporated with components able to actively interact with the surrounding environment to improve properties of both film and foodstuff. Antioxidants, in particular, can interfere with oxidation mechanisms of many sensitive foods, thus limiting adverse effects such as changes in texture, color, nutritional profile, formation of off-flavors, and promotion of microbial growth.^[8] Many phenolic compounds such as carvacrol, thymol, or sesamol, as well as hydroxytyrosol, highly abundant in olives wastes and byproducts, showed high antioxidant potential and health benefits.^[9] Thus, a biodegradable active packaging could provide a synergic effect of improved barrier properties, prolonged shelf life, and positive environmental impact.^[10]

Banana (fruit of *Musa acuminata* Cavendish) was used to examine the effect of anti-browning packaging. Banana slices undergo the release of cell fluids, containing phenolic compounds, on the cut surface. These compounds react with oxygen to form oxidized brown compounds. This reaction is mediated by a group of enzymes, called polyphenol oxidases (PPO). In this study, active coated biodegradable and recyclable films were designed to limit browning reactions and were tested for their efficacy in increasing the shelf-life of fresh-cut banana.

1. Introduction

Minimally processed fruit and vegetables (F&V) correspond to ingredients freshly prepared by washing, peeling and cutting, which are ready-to-use by the consumer. The market for these foods is continuously increasing thanks to their image of healthiness and convenience of use.^[1,2] Sensorial appeal, social interactions, costs, time constraints, and advertising influence

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2. Experimental Section

2.1. Active Film Preparation and Properties

Active coated films were produced using as substrates a corona treated bi-oriented polyethylene terephthalate (PET) film (Nuroll, S.p.a, Italy), having 23 μm thickness, and a PLA/PBAT blown film (Euromaster, Pistoia, Italy) having 23 μm thickness. The active agent used was a phenolic extract deriving from olive milling wastewaters (OWE, Fangiano Farming Company, Nocera Terinese, CZ, Italy) identified by previous studies as the best candidate for the development of active bio-coatings, thanks to its high antioxidant activity and good compatibility with the polymer matrix.^[11] The active films were obtained by lab-scale coating deposition of a casting solution of amorphous PLA4060 (Natureworks, Minnetonka, USA), which conferred sealing capacity to the substrates,^[12] loaded with different amounts of OWE (0%, 3%, and 5% with respect to the mass of PLA). The final thickness of the deposited coating layer was $7.0 \pm 0.4 \mu\text{m}$. In the following, the original film substrates were identified as uncoated PET and uncoated PLA/PBAT, whereas the active multilayer films as PET $x\%$ and PLA/PBAT $x\%$, where x is the OWE percentage amount.

Uncoated and coated films were tested for permeability to water vapor, permeability to oxygen and mechanical tensile properties according to the ASTM standards F 1249, D 1434-82, and ASTM D 882-02, respectively.

Water vapor permeability was measured by M7002 Water Vapor Permeation Analyzer (Systech Instruments Ltd, Oxfordshire, UK) at 23°C and 50% R.H., and the results were performed in duplicate. Oxygen permeability tests were performed by means of a gas permeabilimeter (GDP-C, Brugger, Munchen Germany). The tests were carried out at 23°C and 0% R.H., with an oxygen flow rate of 80 mL min^{-1} . Average mean values were calculated from three measurements per each sample. Mechanical tensile tests were carried out using a CMT 4000 Series tensile tester (SANS, China) equipped with a 100 N load cell, on film specimens with a rectangular geometry (12.7 \times 80 mm^2). The crosshead speed of the test was kept at 5 mm min^{-1} for the calculation of the elastic modulus, and at 500 mm min^{-1} for the evaluation of the properties at break.

2.2. Minimal Processing of Banana

Ripe bananas were collected on local market. They were cut into slices of ca. 8 mm thickness, mixed thoroughly to allow a random selection, and immediately packed under vacuum. Each pack, having 8 cm \times 8 cm size, was filled with six banana slices. The packages were stored in a refrigerator at 4°C up to 3 days. One pack was prepared for each day of analysis, and pictures of bags were taken each day. The experiment was repeated with five independent batches of fruit.

2.3. Shelf-Life Analysis

2.3.1. Microbiological and Physicochemical Analysis

Safety and microbiological quality were determined by plate counting. Prior to analysis, one slice of banana was collected from

each sealed bag, weighted, and mixed with the same mass of saline peptone water (SPW) in stomacher for 1 min at maximal speed. Enterobacteria were enumerated on VRBG agar after incubation for 48 h at 30°C. Psychrotrophic bacteria were enumerated on nutrient agar incubated for 3 days at 10°C. Lactic acid bacteria were counted on MRS medium incubated for 48 h at 30°C. Yeasts and molds enumeration was performed on Sabouraud glucose agar with chloramphenicol after incubation at 30°C for 5 days.

Banana slices' pH was determined using a penetration electrode (5231 Crison, and pH-meter Model GLP22, Crison Instruments S.A., Barcelona, Spain). Three measurements were carried out for each sample.

Color determination was performed with a spectrophotometer CM 3500d (Minolta, Carrières-sur-Seine, France). The results were expressed according to the CIE-Lab color coordinates L^* (darkness/lightness), a^* (greenness/redness), b^* (blueness/yellowness). Numerical values of a^* and b^* were converted into saturation variable or chroma:

$$C^* = \sqrt{(a^{*2} + b^{*2})} \quad (1)$$

and a measure of chromaticity, the hue angle:

$$h^\circ = \arctan(b^*/a^*) \quad (2)$$

Color difference was also evaluated according to the following equation:

$$\Delta E = \sqrt{(L_e^* - L_c^*)^2 + (a_e^* - a_c^*)^2 + (b_e^* - b_c^*)^2} \quad (3)$$

where L_e^* , a_e^* , and b_e^* refer to assay condition and L_c^* , a_c^* , and b_c^* to initial color as control.

Texture analysis was performed with a TA-TX2 texture analyzer (Stable Micro Systems Ltd, Godalming, UK) equipped with a 5 kg load cell. A 2 mm diameter rod was used to penetrate the banana slices at a test speed of 0.5 mm s^{-1} . The maximum penetration force was measured and taken as firmness (N). Three pieces of banana were randomly withdrawn from each bag to carry out repeats.

2.3.2. Polyphenol Oxidase Activity

Extraction of PPO was carried out according to the procedure described by Ndiaye et al. (2009).^[13] Briefly, a 0.5 g of sample was mixed with 5 mL of McLaine citric-phosphate buffer (pH 6.5) with vortex mixer. After centrifugation at 16 000 \times g and 4°C for 20 min, the supernatant was collected and stored in ice until analysis. PPO activity was assessed in duplicate using a spectrophotometer (Jenway, Model 6405 UV-Vis, Dunmow, Essex, UK) at 420 nm. The activity was calculated from the initial slope OD as a function of time. PPO extract (100 μL) was added to Catechol solution 1.4 mL (175 mM). Every 1 min, the OD was measured.

2.4. Statistical Analysis

All statistical tests were performed with XLSTAT (Addinsoft, Paris, France). ANOVA was applied together with REGWT test to detect significant differences, with a confident interval of 95%. If indicated, Fisher's least significant difference test was applied.

Table 1. Oxygen permeability, water vapor transmission rate, and elastic modulus values of the uncoated and coated films.

Film sample	Oxygen permeability [cm $\mu\text{m m}^{-2}$ day bar]	Water vapor permeability $\cdot 10^{12}$ [g m day $^{-1}$ m 2 Pa]	Elastic modulus [MPa]	Elongation at break [%]
Uncoated PLA/PBAT	51.5 \pm 0.4	9.19 \pm 0.4	332 \pm 3	276 \pm 22
PLA/PBAT	40.7 \pm 0.33	8.31 \pm 0.2	664 \pm 5	255 \pm 18
Uncoated PET	0.16 \pm 0.001	0.74 \pm 0.1	4194 \pm 33	36.7 \pm 9.3
PET	0.22 \pm 0.002	1.11 \pm 0.2	3896 \pm 31	38.5 \pm 12.3

PBAT, polybutylene adipate terephthalate; PET, polyethylene terephthalate; PLA, polylactic acid.

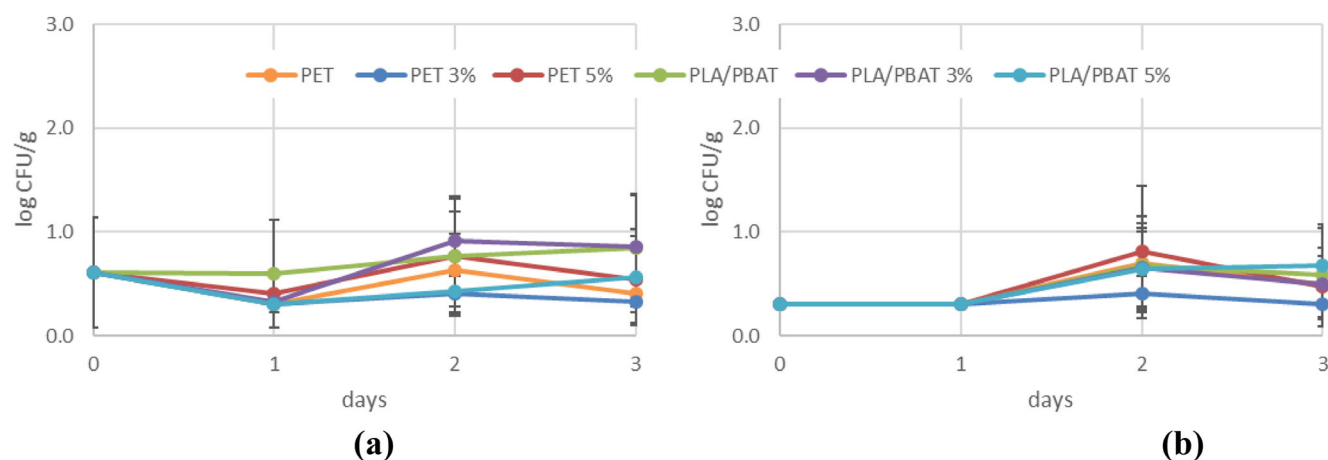


Figure 1. Enterobacteria a) and psychrotrophic bacteria b) population during storage at 4°C of banana slices, for each film. Mean values and standard deviations of the three independent experiments are reported. Three films are made of PET: control (coating without active phase), 3% (coating with 3% active phase), and 5% (coating with 5% active phase). Three films are made of PLA/PBAT: control, 3%, and 5% active phase. PBAT, polybutylene adipate terephthalate; PET, polyethylene terephthalate; PLA, polylactic acid.

3. Results and Discussion

3.1. Film Properties

Preliminary main physical–chemical characteristics of the films are summarized in **Table 1**.

As evident, the two substrates substantially differ in their oxygen and water vapor permeability values, that are up to two orders of magnitude lower for the PET compared to the PLA/PBAT. On the other hand, the PLA/PBAT substrate is a tear-resistant and flexible film, with an elongation at break value one order of magnitude higher than PET, thanks to the presence of PBAT which increases overall flexibility.

The PLA coating layer addition resulted in a slight reduction in the permeability of the PLA/PBAT substrate to both oxygen and water vapor, while the increase of the elastic modulus and the decrease in the elongation at break is a consequence of the deposition of an intrinsically rigid polymer, characterized by a high Young module. As for the observed variations among the uncoated PET and PET films, these are mainly due to the normalization over the total thicknesses, and the multilayer sample substantially maintains the functional properties of the substrate.

3.2. Evaluation of the Food Quality Evolution during Storage

Enterobacteria counts at the day of banana slice preparation (day 0) were for two batches below the detection level, at 0.3 log CFU g $^{-1}$, and for one batch at 1.2 log CFU g $^{-1}$. For psychrotrophic bacteria and yeasts and molds, the initial counts were below or at the detection limit. Eventually, for lactic acid bacteria, the counts were below the detection level for two batches and corresponded to 0.6 log CFU g $^{-1}$ for the third batch. During storage at 4°C, populations of yeasts and molds and of lactic acid bacteria remained below 0.4 log CFU g $^{-1}$.

A slight population increase was observed for enterobacteria to up to 0.9 log CFU g $^{-1}$. The same tendency was observed for psychrotrophic bacteria, reaching at the most 0.7 log CFU g $^{-1}$ (**Figure 1**). Whatever the film and the storage time, population remained low, in the range of safe and unspoiled products, and did not significantly differed between films.

The pH of fresh banana slices ranged between 4.7 and 5.3 depending on the batch, with a mean value of 5.1 \pm 0.3. They were not modified during storage, whatever the film used for packaging.

Initial firmness of banana slices had a value of 0.76 \pm 0.29 N. To get rid of the firmness variability according to sample, all

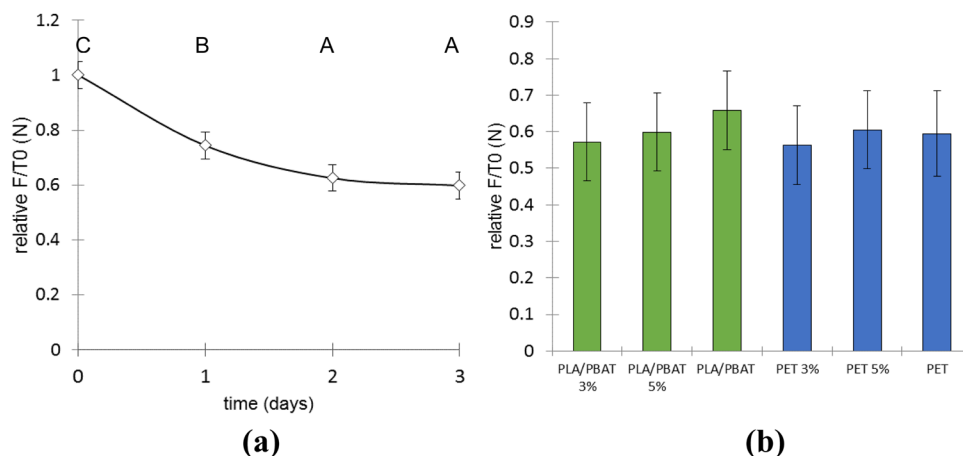


Figure 2. Changes of relative firmness to initial time (N) a) during storage up to 3 days, or b) according to film after 3 days of storage. Means and standard deviations of five independent experiments are showed. Different letters showed significant differences determined with REGWQ test.

Table 2. Change of color parameters according to time for the two control films, PET and PLA/PBAT based.

Film	PLA/PBAT (control)				PET (control)			
	0	1	2	3	0	1	2	3
L^*	67.3 ± 9.0^b	57.4 ± 8.4^{ab}	54.7 ± 9.4^a	52.7 ± 5.6^a	61.9 ± 10.5^a	53.2 ± 10.1^a	52.1 ± 14.8^a	49.1 ± 9.5^a
a^*	1.0 ± 1.1^a	2.8 ± 1.1^b	3.5 ± 1.3^b	3.7 ± 1.2^b	0.9 ± 0.6^a	2.6 ± 0.9^a	2.6 ± 1.0^a	2.8 ± 1.7^a
b^*	33.6 ± 4.0^b	30.1 ± 3.0^{ab}	28.5 ± 2.7^a	29.2 ± 2.0^a	33.9 ± 3.6^b	29.6 ± 3.1^{ab}	26.9 ± 6.4^a	25.2 ± 3.1^a
C^*	33.6 ± 4.0^b	30.2 ± 3.0^{ab}	28.7 ± 2.7^a	29.5 ± 2.0^{ab}	33.9 ± 3.6^b	29.8 ± 3.2^{ab}	27.0 ± 6.3^{ab}	25.4 ± 3.2^a
h°	1.6 ± 1.9^a	5.1 ± 1.7^b	7.0 ± 2.5^b	7.2 ± 2.2^b	1.6 ± 1.0^a	5.0 ± 1.5^b	5.9 ± 2.9^b	6.0 ± 3.2^b
ΔE	0.0 ± 0.0^a	11.1 ± 2.5^b	14.1 ± 3.8^{bc}	16.1 ± 4.3^c	0.0 ± 0.0^a	10.1 ± 2.2^b	13.1 ± 8.2^b	15.8 ± 3.9^b

Different letters in the same line for each film show significant differences determined with REGWQ test. PBAT, polybutylene adipate terephthalate; PET, polyethylene terephthalate; PLA, polylactic acid.

initial values were standardized at 1. Relative firmness showed a significant decrease according to time (Figure 2). Firmness after 2 and 3 days of storage significantly differed from initial firmness and from firmness after 1 day. However, examination of firmness after 3 days of storage did not show differences according to the film used (Figure 2).

An effect of storage on color of banana cuts was clearly established (Table 2). The values of L^* and b^* decreased during cold storage, whereas a^* values decreased. These changes correspond to a loss of brightness and yellow color component and a strengthening of red component of color. As a result, chroma value, corresponding to saturation decreased, and Hue or chromaticity increased.

The changes over time differed for banana slices packaged in PET films: L^* and a^* values were not significantly modified, but b^* value decreased. Chroma value changed between 0 and 3 days and Hue angle between 0 and 1 day. Color difference was noticed after 1 day but did not change thereafter. The color properties of banana slices were compared between films, after 2 and 3 days of storage. The values of L^* and a^* did not exhibit differences according to the film composition. For the parameters b^* and C^* , a difference between PET control and PET with 5% active phase was detected with a Fisher test. Color difference was also different

between PET control and PET 5% films after 3 days of storage (Figure 3).

These observations were clearly supported by visual observation of browning of banana cuts (Figure 4).

After 3 days of storage, a strong browning was observed for PLA/PBAT films. Browning was less marked in PET films, especially for the PET coated with 5% active phase. This result can be related to the lower permeability to oxygen of PET films with respect to the biodegradable ones. As suggested by previous literature studies, color modifications can be related to PPO activity in banana cuts.^[14,15] However, measured PPO activity was found in the same range for all fruit samples stored in both PLA/PBAT and PET-based films, having an average value equal to $8.70 \pm 2.97 \text{ ODu min}^{-1} \text{ g}^{-1}$. Therefore, no significant differences could be detected according to time or film composition.

4. Conclusions

The effect of two antioxidant packaging films, differing by the substrate material (i.e., PET or PLA/PBAT), on the preservation of sensorial properties and microbial safety of fresh cut banana slices, was investigated. Results showed a positive effect of PET based films on color, whereas PPO activity was not affected and

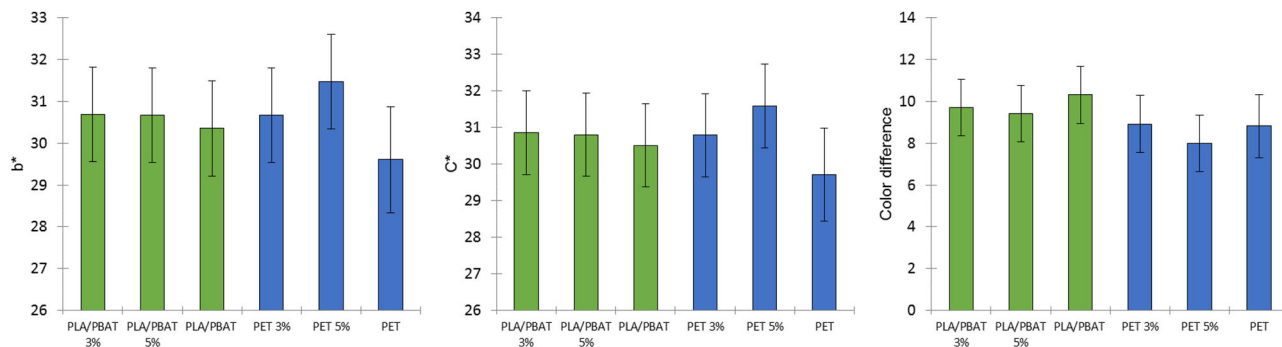


Figure 3. Color parameters according to film composition. From left to right, b^* value, chroma C^* , and color difference ΔE . Means and standard deviations of five independent experiments are showed.



Figure 4. Pictures of banana slices. Top line: PLA/PBAT-based films; bottom: PET-based films. From left to right: slices at day 0; slices at day 3 in control condition; slices at day 3 in films coated with 5% active phase. PBAT, polybutylene adipate terephthalate; PET, polyethylene terephthalate; PLA, polylactic acid.

firmness poorly preserved. PLA/PBAT based films exhibit lower barrier activity to gas, which might explain the observed differences between the effects of the two kinds of films. In addition, a slight effect of the active phase on browning limitation was observed for PET films. These outcomes pointed out the necessity of designing the food package basing on the specific shelf-life requirements of each food, in terms of respiration rates, sensitivity to environmental parameters, and shelf-life parameters. Active films with higher loads of anti-browning agents combined with high passive barrier properties limiting oxygen entrance into the bags would be a promising approach to decrease PPO activity of banana slices.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

active packaging, antioxidant, biodegradable coating, browning, PET, polylactic acid (PLA), shelf-life

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