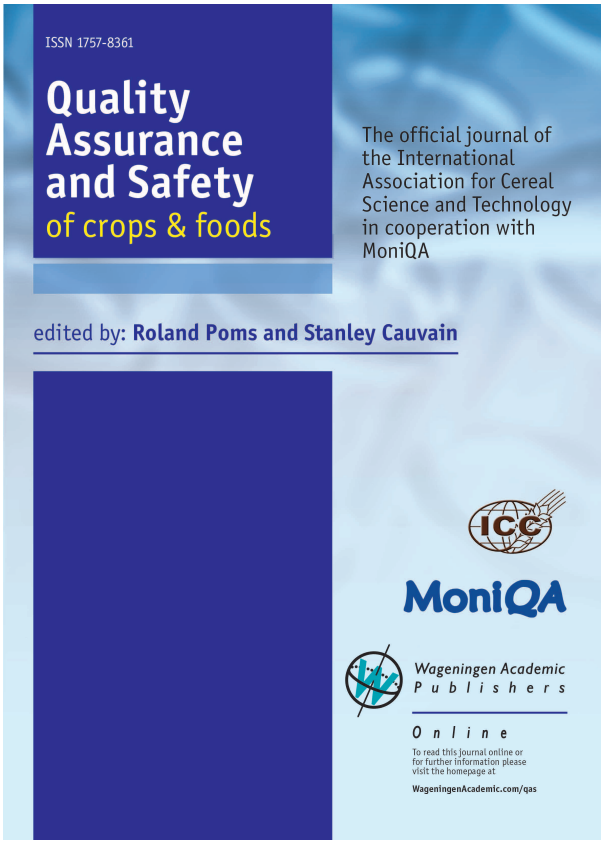

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Effect of essential oils incorporated into an alginate-based edible coating on fresh-cut apple quality during storage

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RESEARCH ARTICLE

Abstract

Edible films, as carriers of antimicrobial compounds, constitute an approach for incorporating plant essential oils (EOs) into fresh-cut fruit surfaces. Biodegradable alginate-based coatings with and without EOs were applied to fresh-cut apple, cv. Golden Delicious, in order to find healthy treatments to better preserve fresh fruit quality and safety during postharvest cold storage. Physicochemical properties (°Brix, colour and texture), polyphenoloxidase (PPO) and peroxidase activity and browning potential were determined throughout cold storage. Alginate coatings containing cinnamon oil were more effective than rosemary at inhibiting respiration rates. The addition of EOs and antioxidant was more effective than alginate alone in reducing weight loss and preserving the original colour and lightness. Moreover EOs reduce the PPO and peroxidase activity, in particular in the firsts days after processing. These results show that EOs can be used to prepare edible films for fresh-cut fruit applications.

Keywords: cinnamon oil, rosemary oil, ready-to-eat apple, shelf-life

1. Introduction

Minimal processing has been defined as a combination of procedures, such as washing, sorting, trimming, peeling, and slicing or chopping, that do not affect the fresh-like quality of the food. This type of product does not contain preservatives or antimicrobial substances and rarely undergoes any heat process before consumption. Ready-to-eat fruits and vegetable market have rapidly grown in recent years due to the health benefits associated with these foods, together with the busy lifestyles, increasing purchasing power and health-conscious consumers. Nevertheless, since the tissular integrity of fruits is more easily altered during processing, ready-to-use commodities are more perishable than the original materials (Oms-Oliu *et al.*, 2010). The main factors that affect loss of consumer's acceptability are discoloration, enzymatic browning, dryness and texture loss (Pérez-Gago *et al.*, 2010). These parameters determine the visual appearance (Pace *et al.*, 2011). For this reason the fruit processing industry requires the development of techniques capable of maintaining safety and shelf-life and preserving the original visual and organoleptic fresh-like characteristics of fresh-cut produces.

New alternatives, such as the use of edible coatings are being investigated and applied in order to preserve fruit quality (Asgar *et al.*, 2011; Chiabrando and Giacalone, 2012; Duan *et al.*, 2011; Serrano *et al.*, 2006). Coatings on minimally processed products create a barrier semipermeable to external elements that can reduce moisture loss, solutes migration, respiration and oxidative reaction rates (Vargas *et al.*, 2008). In general, polysaccharide-based coatings have been used to extend the shelf-life of fruit by reducing respiration and gas exchange (Nísperos-Carriedo, 1994; Nussinovitch, 1997). Edible coating with pectin or gellan-lipid can significantly reduce respiration and ethylene production of fresh-cut apple during storage (Wong *et al.*, 1994). Alginate and gellan-based edible coatings have been shown to effectively prolong the shelf-life of 'Fuji' apple compared to uncoated slices (Rojas-Graü *et al.*, 2008a).

Different substances such as antibrowning agents, colorants, flavours, nutrients, spices and antimicrobial compounds

have been incorporated into edible coatings for ensuring the quality and safety of fresh-cut fruits (Eswaranandam *et al.*, 2006; Han *et al.*, 2004; Olivas *et al.*, 2003; Oms-Oliu *et al.*, 2008). Chitosan and oil coating has become a promising alternative treatment to maintain quality of food products because the antimicrobial and anti-oxidative effect of chitosan was greatly enhanced by the addition of essential oils (EOs) (Kanatt *et al.*, 2008).

EOs are aromatic oil liquids obtained from plant organs: flower, bud, seed, leaf, twig, bark, herb, wood, fruit and root. EOs are volatile, natural, complex compounds characterised by a strong odour and are formed by aromatic plants as secondary metabolites (Bakkali *et al.*, 2008). Recently, EOs and their pure components are gaining increasing interest from the point of view of their antimicrobial and anti-oxidant properties (Appendini and Hotchkiss, 2002; Burt, 2004; Capecka *et al.*, 2005; Ruberto and Baratta, 2000). The use of antioxidants from natural sources has become more popular as a mean of increasing the shelf-life of food products, preventing loss of sensory and nutritional quality (Hemeda and Klein, 1990). Furthermore, it has been demonstrated that EOs can be added to edible coatings in order to lengthen shelf-life, to prevent microorganism growth and to preserve nutritional values of foods (Du Plooy *et al.*, 2009; Salmieri and Lacroix, 2006). The antimicrobial effect of EOs incorporated into edible coatings can prolong shelf-life of different fresh-cut fruits (Rojas-Graü *et al.*, 2009). The immobilisation of the active compounds in polymer can maintain high concentrations of the active compounds on the surface of foods in order to achieve a longer storage time (Ouattara *et al.*, 2001).

Therefore, the objectives of this study was to investigate the effects of an alginate coating enriched with EOs on quality attributes and enzymes activities of apple slices during storage at 0 °C for 10 days.

2. Materials and methods

Fruit

Apples (cultivar Golden Delicious) at commercial ripeness stage (14.89°Brix, 41.61 meq/l, pH 4.65) were purchased in a local supermarket and stored at 4 °C before processing. Total acidity (Compact Titrator, Crison, Italy), pH and percentage of soluble solids (RX-1000 refractometer, Atago Company Ltd., Tokyo, Japan) were measured according to official methods (AOAC, 1995).

Edible coating solutions

Four different solutions were considered: (1) coating (C); (2) coating + antibrowning (CA); (3) coating + antibrowning + cinnamon essential oil (CAC); and (4) coating + antibrowning + rosemary essential oil (CAR). Edible coatings were elaborated from a base solution of 2% (w/v) alginate (Sigma-Aldrich Co., Steinheim, Germany) and 1.5% (v/v) glycerol (Sigma-Aldrich Co.), which were added to 70 °C sterile distilled water and stirred until total dissolution of the components (Raybaudi-Massilia *et al.*, 2008).

EOs from cinnamon bark (*Cinnamomum zeylanicum*) and rosemary (*Rosmarinus officinalis*) (Kerry, Italy) were added to the edible coating solution at 0.3% (v/v) and emulsified using a rotor-stator homogeniser (DI25 Yellow Line; IKA®-Werke, Staufen, Germany) at 20,000 rpm for 4 min (Raybaudi-Massilia *et al.*, 2008). Edible coatings without EOs were evaluated as controls. Calcium ascorbate at 1% (w/v) (Sigma-Aldrich Co.) was used as antibrowning agent (Aguayo *et al.*, 2010; Giacalone and Chiabrando, 2013).

Apple processing and packaging

For the samples apples were peeled, cored and cut into 5-mm-thick slices using a hand-operated apple corer and slicer. Slices were immersed in the coating solutions for 1 min at 20 °C and were then allowed to drip off. For the study, 15 apple slices (total weight of 70 g) were placed in polypropylene (PP) punnets and wrapped automatically with PP film (Compac, Reggio Emilia, Italy). The PP film had an O_2 transmission rate of 750 $cm^3/m^2/24$ h at 23 °C and a vapour transmission rate of 1.1 $g/m^2/24$ h at 23 °C and 85% RH. The packages then were stored at 0 °C in darkness for 10 days.

Changes in headspace gas composition

The concentrations of oxygen and carbon dioxide inside the packages were monitored daily by sampling (0.5 ml) the headspace using a gas analyser Canal 121 (Vizag, Gas Analysis, Croissy sur Seine, France). A syringe was inserted into the package through a rubber seal placed on the film. Gases were analysed with an electrochemical sensor for O_2 level and an infrared sensor for CO_2 level. The instrument was calibrated towards air. Results were expressed as kPa of O_2 and CO_2 inside the bags.

Quality measurements

Quality measurements were determined at day 1, 7 and 10 of storage. Total acidity (meq/l), pH and percentage of soluble solids (°Brix) were measured according to official methods (AOAC, 1995). Total soluble solids content (TSS) was determined in the juice from 25 slices at 20 °C. Titratable acidity (TA) was determined by titration with 0.1 N NaOH up to pH 8.1, using 10 ml of diluted juice in distilled H_2O.

Colour measurements

Colour of coated apple slices was measured daily. 15 slices of fresh-cut apple of each edible coating condition were analysed for colour with a tri-stimulus CR-400 Chroma Meter (Konica Minolta Sensing, Sakai, Japan) with the illumining D75 and observation angle of 10° calibrated with a standard white plate (Y = 94.00, x = 0.3158, y = 0.3322).

Two readings of L* (lightness), a* (green chromaticity), and b* (yellow chromaticity) coordinates were recorded for each apple slice. Numerical values of a* and b* parameters were employed to calculate the hue angle (h°). The reported values are the mean ± standard deviation (SD) of 30 determinations.

Browning potential

Browning potential (BP) was determined according to the method of Arias *et al.* (2008) at day 1, 3, 6, 8 of storage. The extract was obtained as followed: apple slices (25 g) from each treatment were homogenised with an Ultra-Turrax T25 (IKA®-Werke), the homogenates were centrifuged (Centrifuge AVANTITM J-25, Beckman Instruments Inc., Fullerton, CA, USA) at 4,000 rpm for 10 min and the supernatant was filtered through Whatman no. 4 filter paper (Whatman Intl., Maidstone, UK). The absorbance of the clear juice was then measured spectrophotometrically (U-5100; Hitachi, Chiyoda, Japan) at 440 nm to determine BP. This measurement was replicated three times.

Determination of polyphenoloxidase activity

Polyphenoloxidase (PPO) activity was determined at day 1, 3, 6, 8 of storage.

Enzyme extraction

A portion of 50 g of apple slices was mixed with a buffer solution (1:1) at pH=6.5 containing 1M NaCl (Sigma-Aldrich) and 5% polyvinylpolypyrrolidone (Sigma-Aldrich). The mixture was blended and homogenised using an Ultra Turrax T25 (IKA®-Werke). The homogenate was centrifuged at 12,500 rpm for 30 min at 4 °C (Centrifuge AVANTITM J-25; Beckman Instruments Inc.). The supernatant was collected and filtered through Whatman no. 1 filter paper (Whatman), and the resulting solution constituted the enzymatic extract.

Polyphenoloxidase activity measurement

PPO activity was determined according to the method of Rocha and Morais (2001) and Soliva-Fortuny *et al.* (2002). Enzyme activity was assayed spectrophotometrically by adding 3 ml of 0.6 M catechol (Sigma-Aldrich) and 75 µl of extract to a 4.5 ml quartz cuvette of 1 cm path length. The changes in absorbance at 400 nm were recorded every 5 s up to 3 min from the time the enzyme extract was added using a U-5100 spectrophotometer (Hitachi). One unit of PPO activity was defined as a change in absorbance of 0.001 per min and ml of enzymatic extract immediately after extract addition. The initial reaction rate was estimated from the linear portion of the plotted curve. All determinations were performed in triplicate.

Determination of peroxidase activity

Peroxidase (POD) activity was determined at day 1, 3, 6, 8 of storage.

Enzyme extraction

Peroxidase enzyme was extracted as described by Rojas-Graü *et al.* (2008b). A portion of 50 g of apple slices was blended and mixed with 50 g of 0.2 M sodium phosphate buffer (pH=6.5). The homogenate was centrifuged at 6,000×*g* for 15 min at 4 °C with a centrifuge AVANTITM J-25 (Beckman Instruments Inc.). The resulting supernatant was collected and filtered through a Whatman no. 1 paper.

Peroxidase activity measurement

POD activity was determined spectrophotometrically at 25 °C with a U-5100 spectrometer (Hitachi) at 470 nm using guaiacol as the substrate and H_2O_2 as the hydrogen donor (Hemeda and Klein, 1990). A 0.1 ml of extract was mixed with 2.9 ml of the substrate solution (10 ml of sodium phosphate buffer 0.05 mol/l, pH 6.5; 10 ml of guayacol (1 ml/100 ml) and 1 ml of hydrogen peroxide (0.3 ml/100 ml)) (Aguero *et al.*, 2008). The POD activity unit was defined as a 0.001 change in absorbance per min and ml of enzymatic extract immediately after extract addition. The initial reaction rate was estimated from the linear portion of the plotted curve. All determinations were performed in triplicate.

Statistical analysis

The basic experimental design consisted of four different coating treatments each having three replicates. For each parameters evaluated a package contained 15 slices, was considered a replicate. Data were analysed by analysis of variance using statistical procedures of the STATISTICA ver. 6.0 (Statsoft Inc., Tulsa, OK, USA). The sources of variance being coating treatments. Tukey's test honestly significant differences was used to determine significant differences among treatment means. Means values were considered significantly different at $P \leq 0.05$. The mean values were calculated and reported as the mean ± standard deviation (n=3).

3. Results and discussion

Changes in headspace gas composition

Edible coatings are capable of reducing a modified atmosphere on coated fruit by isolating the coated product from the environment, acting as a barrier to oxygen, carbon dioxide and water vapour and decreasing the rate of respiration (Pérez-Gago *et al.*, 2010). O_2 consumption and CO_2 production in packed fresh-cut apples with or without EOs are shown in Figure 1 and 2. Significant differences were found in O_2 and CO_2 concentrations ($P \leq 0.05$) among treatments after 7 days of storage. The changes in those gases could be attributed to the effect of the EOs on fruit metabolism and to the modified atmosphere created by the edible coating, which lowered fruit tissue respiration (Rojas-Graü *et al.*, 2007). Cinnamon EO may reduce O_2 exchange since gas concentrations in the packages were slightly higher than in the other coated apple slices, reflecting the reduced ability of the fruit tissue to absorb O_2 for respiration through the cinnamon coating, leaving more O_2 in the package atmosphere (Oms-Oliu *et al.*, 2008). In contrast, an high O_2 consumption and CO_2 production was observed in the CAR apples slices. This may be a consequence of an increase of respiration due to rosemary EO.

Quality measurements

TSS is an important characteristic, giving information about the quality of fruits. TSS content increases progressively with extended storage in all the treatments investigated in this study (Table 1). EOs coatings showed significant ($P \leq 0.05$) lower values in rosemary after 1 day, and in cinnamon after 7 days of storage. At the end of storage (10 days) adding EOs to coatings did not significantly affect the TSS content.

Organic acids are substrates for the enzymatic reactions of respiration; therefore, a reduction in the acidity is expected during storage (Yaman and Bayindirli, 2002). TA content of the apple slices decreased with increase in storage time and was significantly ($P \leq 0.05$) affected by treatment: CAR showed the highest and CAC the lowest values during storage (Table 1). Changes in weight that occurred during the postharvest period may have influenced the values for TA; it is possible that CAR samples showed the highest TA due to an higher water loss (Colelli *et al.*, 2002).

The pH values did not show significant changes during storage. Between treatments, only CAC samples showed the lower pH values at day 1.

Colour measurements

The colour parameter L* provides a measure of lightness. Results showed that the addition of antioxidant agent and EOs to the coating solution increased their L*. In particular at day 0 and 1, there were significant differences in L* of apple slices coated with EOs or without EOs, similar to the results reported by Du *et al.* (2009). A visible and significant decrease of lightness in fresh-cut apples during the storage at 0 °C was observed (Table 2). Raybaudi-Massilia *et al.* (2008) find that L* values at or below 67.2 were sensorially undesirable. In this work all the L* values were above this value. The decrease of L* was mainly indicative of sample browning and apple slices containing cinnamon (CAC) and apple slices without EO were affected to a greater ($P \leq 0.05$) decrease in comparison with the rest of the treatments (Table 2). Only the sample with rosemary and CA maintained higher L* values during storage. Cinnamon oil did not control enzymatic browning, in fact there were no differences with the coating-control. Decrease in L* of fresh-cut apples throughout the storage time also has been reported by Soliva-Fortuny *et al.* (2001) and Rojas-Graü *et al.* (2008b) in Golden Delicious and Fuji fresh-cut apples, respectively.

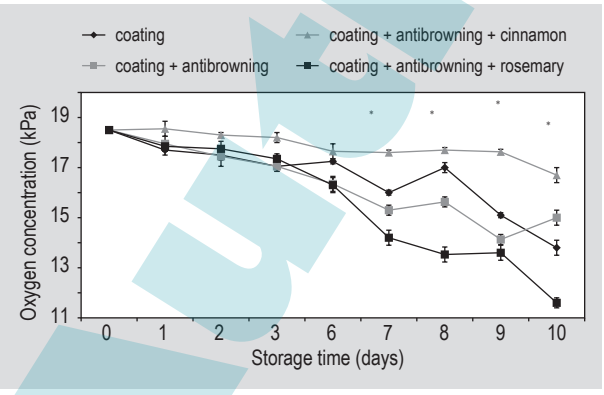

Figure 1. Evolution of oxygen concentration (KPa) of fresh-cut apples stored at 0 °C for 10 days. Data are the mean ± standard error (n=3). Asterisks indicate significant differences ($P \leq 0.05$) between treatments.

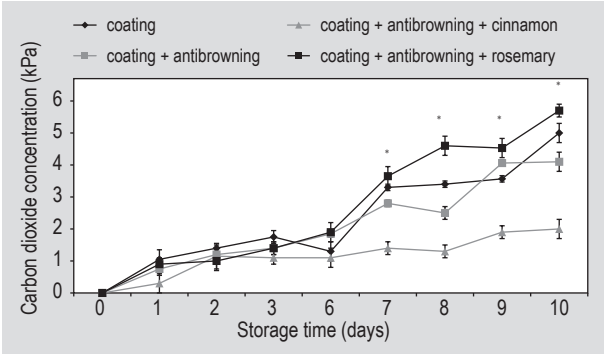

Figure 2. Evolution of carbon dioxide concentration (KPa) of fresh-cut apples stored at 0 °C for 10 days. Data are the mean ± standard error (n=3). Asterisks indicate significant differences ($P \leq 0.05$) between treatments.

Table 1. Changes in quality parameters of fresh-cut apples stored at 0 °C for 10 days. Values are the mean ± standard deviation of 3 determinations. Different letters in the same column means significantly different ($P \leq 0.05$).

Quality parameter	Treatments[1]	Storage day		
		1	7	10
TSS (°Brix)	coating	12.47±0.23 a	12.55±0.31 a	13.10±0.09 a
	coating + antibrowning	12.63±0.03 a	13.05±0.40 a	13.20±0.12 a
	coating + antibrowning + cinnamon	12.07±0.08 a	12.20±0.09 b	13.35±0.04 a
	coating + antibrowning + rosemary	11.40±0.20 b	13.25±0.04 a	13.9±0.02 a
TA (meq/l)	coating	35.43±3.96 a	32.70±0.31 b	23.03±0.95 b
	coating + antibrowning	35.22±2.24 a	35.19±0.99 a	31.06±0.43 a
	coating + antibrowning + cinnamon	34.22±2.15 b	29.47±0.18 b	25.56±1.63 b
	coating + antibrowning + rosemary	36.24±1.06 a	35.91±2.47 a	33.47±0.66 a
pH	coating	4.85±0.31 a	4.56±0.22 a	5.13±0.13 a
	coating + antibrowning	4.78±0.21 a	4.36±0.04 a	4.87±0.17 a
	coating + antibrowning + cinnamon	4.42±0.23 b	4.53±0.13 a	4.93±0.02 a
	coating + antibrowning + rosemary	4.94±0.11 a	4.59±0.24 a	4.84±0.62 a

TA = titratable acidity; TSS = total soluble solids content.

Table 2. Changes in colour parameters of fresh-cut apples stored at 0 °C for 10 days. Values are the mean ± standard deviation of 30 determinations. Different letters in the same column means significantly different ($P \leq 0.05$).

Storage day	Treatments[1]			
	coating	coating + antibrowning	coating + antibrowning + cinnamon	coating + antbrowning + rosemary
Lightness (L*)				
0	73.02±3.13 b	76.12±1.50 a	77.74±2.31 a	78.21±2.00 a
1	70.75±4.05 b	74.23±3.51 a	76.66±1.76 a	76.74±3.18 a
2	69.43 ±4.32 b	76.43±2.16 a	74.85±2.66 b	75.76±3.18 a
3	69.91±4.03 b	73.18±4.75 a	74.27±3.15 a	75.67±2.46 a
6	69.54±3.62 c	75.10±3.80 a	72.68±4.99 b	76.47±2.86 a
7	70.06±3.82 c	78.11±1.90 a	72.21±4.09 b	76.29±2.86 a
8	69.27±4.24 b	78.75±1.48 a	69.80±5.45 b	76.13±2.71 a
9	70.08±3.9 b	77.72±2.25 a	70.49±5.26 b	76.27±2.43 a
10	70.29±4.06 b	77.43±1.95 a	70.72±4.05 b	76.63±2.24 a
Hue angle (h°)				
0	96.24±3.15 b	101.72±1.65 a	103.74±2.00 a	102.10±1.36 a
1	95.54±3.01 b	100.67±3.38 a	102.41±1.67 a	100.93±4.21 a
2	93.77±3.39 b	100.26±3.04 a	100.53±3.15 a	99.15±4.00 a
3	93.45±2.95 b	100.45±3.20 a	100.42±2.90 a	100.68±3.10 a
6	93.68±2.73 b	99.24±2.76 a	98.29±4.89 a	98.83±3.18 a
7	94.47±2.91 b	101.47±2.25 a	98.37±4.05 b	99.30±3.25 a
8	92.44±2.62 b	100.82±2.27 a	95.33±5.53 b	98.98±2.66 a
9	93.30±3.03 b	99.37±2.36 a	94.33±6.29 b	97.20±2.55 a
10	93.02±3.12 b	97.86±2.09 a	93.11±5.59 b	97.66±2.97 a

The same trend was found among hue angle (h°) values of fresh-cut apples (Table 2). Hue angle decreased significantly ($P \leq 0.05$) as apples slices browned during storage. Apple slices treated with coating alone (C) and with cinnamon (CAC) affected the h° values more rapidly than the others. The browning phenomenon was greatly reduced in apple slices treated with antibrowning agent, that reacted competitively with PPO and peroxidase via the intermediate quinones to form stable colourless compounds (Richard *et al.*, 1991). This effect also has been reported in fresh-cut apples (Aguayo *et al.*, 2010; Raybaudi-Massilia *et al.*, 2007) and in fresh-cut pears (Oms-Oliu *et al.*, 2008). Nonetheless, tissue browning was notably affected by the incorporation of cinnamon EO into the coating. Raybaudi-Massilia *et al.* (2007) suggested that this is possibly as a consequence of significant damage to the cell tissue integrity, which could cause the release of enzymes and substrates that reacted to produce coloured compounds.

Browning potential

Enzymatic browning reduces not only the visual quality of apple slices but also results in undesirable changes in flavour and loss of nutrients. BP of samples did not differ significantly at day 1, 3 and 6, in according with McHugh and Senesi (2000) (Figure 3). Only at the end of storage time CAC showed lower values of BP compared with other treatments.

Determination of polyphenoloxidase activity

The discoloration that occurs in cut apple is mostly due to enzymatic browning of cut apple and is catalysed by PPO (Monsalve-Gonzalez *et al.*, 1995; Sapers, 1993). In this work, PPO activity of minimally processed increased from the early days after processing (Figure 4).The treatment with EOs coating reduced PPO activity during shelf-life evaluation (Figure 4), which was associated with delayed flesh browning of apple slices. Treatment of the combination of coating and cinnamon essential oil was the most active one in inhibiting the PPO activity in apple slices, in according with Xing *et al.* (2011). PPO activity of the coating-treated slices increased and reached a peak at day 3, and then decreased (Figure 4). By contrast, CA and CAR treatments increased slowly and reached a peak at day 6, and then decreased.

Determination of peroxidase activity

POD activities of apple slices increased during storage (Figure 5). The apple slices treated with cinnamon EO had reduced activities of POD during 8 days of storage and showed that the coating inhibited the enzyme. This result was also obtained for PPO activity. Similar results were reported by Zhang and Quantick (1997). Treatments CAC and CAR showed lower POD values at day 1 and 3 but then POD activity increased, whereas application of EOs delayed the increase in the enzyme activity in the firsts days of storage (Ponce *et al.*, 2004).

4. Conclusions

Edible films, as carriers of antimicrobial compounds, constitute an approach for incorporating plant EOs into fresh-cut fruit surfaces. The results showed that alginate coatings containing cinnamon oil were more effective than rosemary at inhibiting respiration rates. The addition of the EOs and antioxidant was more effective than alginate alone in reducing weight loss and preserving the original colour and lightness. Moreover EOs, in particular cinnamon EO, reduced the browning of fruit, PPO and peroxidase activity, in the firsts days after processing. These results show that EOs can be used to prepare edible films for fresh-cut fruit applications.

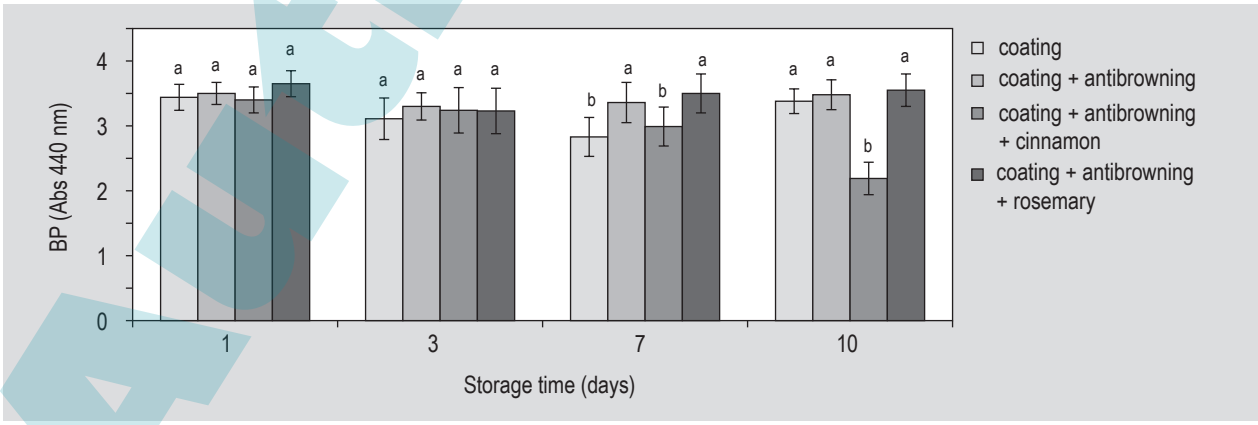

Figure 3. Browning potential (BP) of fresh-cut apples stored at 0 °C for 10 days. Data are the mean ± standard error (n=3). Mean values followed by the same letter in each storage time are not significantly different at $P \leq 0.05$.

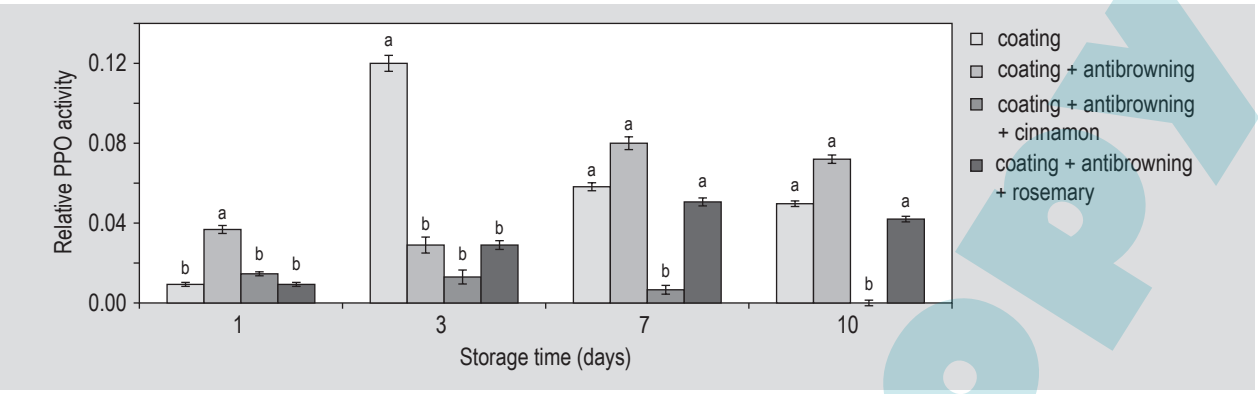

Figure 4. Relative polyphenoloxidase (PPO) activity of fresh-cut apples stored at 0 °C for 10 days. Data are the mean ± standard error (n=3). Mean values followed by the same letter in each storage time are not significantly different at $P \leq 0.05$.

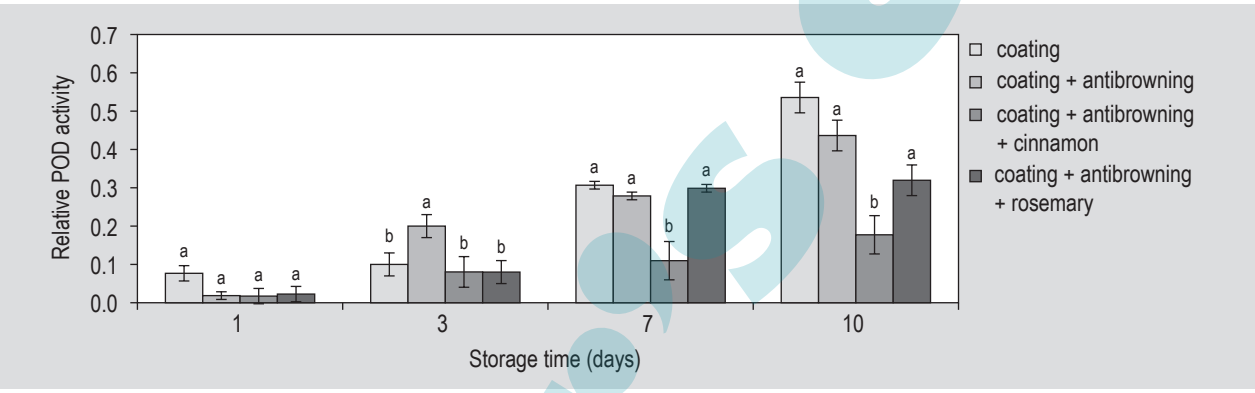

Figure 5. Relative peroxidase (POD) activity of fresh-cut apples stored at 0 °C for 10 days. Data are the mean ± standard error (n=3). Mean values followed by the same letter in each storage time are not significantly different at $P \leq 0.05$.

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