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Effect of Beeswax Coating with Cinnamon Oil on Quality of Sweet Peppers

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ABSTRACT

The most important postharvest problem of sweet peppers is their excessive softening that may cause flaccidity, shriveling and wilting, drying, decay and a very perishable vegetable with a short shelf life and high susceptibility to fungal and bacterial disease, in particular anthracnose and bacteria soft rot disorders that reduce the quality, marketability and consumer acceptance. The objective was to study the effect of antimicrobial compounds added to beeswax coatings to inhibit microbial contamination and maintain the quality of sweet peppers during storage. The results showed that cinnamon oil had the strongest inhibitory activity against *Colletotrichum gloeosporioides*, *Colletotrichum capsici* and *Erwinia carotovora*, compared to clove oil and thiobutacin. Coated sweet peppers stored at ambient (27 ± 2°C) or low temperature (10 ± 2°C) were firmer than uncoated peppers, lost less weight and had lower total plate counts compared to uncoated peppers. Beeswax coatings incorporated with 1.4% cinnamon oil could hold the quality and extend the shelf life of sweet peppers stored for 12 and 30 d at ambient and low temperature storage, respectively.

Keyword: sweet pepper, beeswax, cinnamon oil, antimicrobial, edible coating

INTRODUCTION

Sweet peppers (*Capsicum annuum* L.) are non-climacteric vegetables (Salveit, 1977; Lurie *et al.*, 1986; Lurie and Klein 1989; Biles *et al.*, 1993). The major postharvest problem with this crop is its excessive softening that may cause flaccidity, shriveling, wilting, drying, decay and pathological disorders that reduce its quality, marketability and consumer acceptance (Sethu *et al.*, 1996). Sweet pepper is easily perishable and has high susceptibility to fungal and bacterial disease, in particular, anthracnose is caused by the fungi *Colletotrichum gloeosporioides* and *C. capsici*, while the most common postharvest disease (bacterial soft rot) is caused by the subspecies *Erwinia carotovora* (Hardenburg *et al.*, 1990).

Beeswax, a natural wax produced in the beehives of honey bees by worker bees, is secreted from eight wax-producing mirror glands on the inner sides of the sternites on the worker bee abdomen. Beeswax contains a high proportion of various wax esters (C_{40} to C_{46} molecular species) based on 16:0 and 18:0 fatty acids, some with hydroxyl groups in the omega-2 and omega-3 positions (Kameda, 2005). It is mainly made up of ester fatty acids, various long chain alcohols and its hydrophobic (water repellant) properties. Edible films and waxes have been used for decades on fresh produce to create a semi

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permeable membrane on the surface to suppress respiration, control moisture loss, add gloss, and more recently, to provide a delivery mechanism for additional functional components (Min and Krochta, 2005). Spices such as Chinese star anise, citronella, cinnamon, cloves, thyme and sage have been investigated for their antimicrobial activity. Raybaudi-Massilia *et al.* (2008) found that cinnamon and clove essential oils at 0.3% (volume per volume) in an alginate-based edible coating best maintained the physicochemical characteristics of apple pieces while extending the shelf life. Du W-X *et al.* (2009) showed that apple-based films with cinnamon and clove oils were active against *Escherichia coli, Salmonella enterica* and *Listeria monocytogenes* by both direct contact with the bacteria and indirectly by vapors emanating from the films. Mc Hugh *et al.* (2009) found that antimicrobial substances incorporated into edible films could control the microbial contamination of fruits and vegetables by reducing the growth rate of target microorganisms or by inactivating microorganisms by direct contact, in order to inhibit the spoilage flora and to decrease the risk of pathogens.

The current study: assessed the potential of coating using beeswax as a hydrophobic phase and containing cinnamon oil to extend the postharvest life and to maintain the quality of sweet peppers against microbial contamination and the plant pathogens *Colletotrichum gloeosporioide*, *C. capsici* and *Erwinia carotovora* and examined the change in weight loss, firmness and the total plate count of coated and uncoated sweet peppers with storage time.

MATERIAL AND METHODS

Antimicrobial activity assay

Evaluation of antimicrobial effectiveness

Three 10% (weight per volume) solutions of cinnamon oil, clove oil and thiobutacin were made by dissolving 10 g of each treatment in 95% dimethyl sulfoxide (DMSO; no effect of DMSO used as dilution solvent was observed on the microbial testing) to a final volume of 100 mL. Antimicrobial activity was estimated using a growth inhibition assay according to the method of Hamdy (2001). The fungal cells (1×10^9 cells.mL^{-1} of *Colletotrichum gloeosporioides* and *C. capsici*) were prepared and spread on a potato dextrose agar (PDA) plate; and the bacterial cells (1×10^8 cells.mL^{-1} of *Erwinia carotovora*) were spread on a nutrient agar (NA) plate following the methods outlined by Tovkach and Mukvich (2003). A No. 3 cork borer (0.6 cm in diameter) used for removing discs from the agar plates was sterilized before each separate microbial test by dipping it in ethyl alcohol (95%) and flaming it. Prior to adding the spice oil, a hole was made in the center of the agar with the cork borer. The agar core cut by the cork borer was carefully removed from the agar and each hole was filled with 40 μL of each the antimicrobial solutions. The plates were incubated in incubators at 32°C for 48 h (bacteria), and at 28°C for 48 h (mycelial fungi), and the inhibition zones produced around the antimicrobial solutions were observed. When the antimicrobial product was effective at inhibiting growth, a clear area of inhibition appeared around the antimicrobial solution. The diameters of the inhibition zones were measured in centimeters. Individual clear zone diameters were measured in triplicate and averaged to compensate for variability.

Minimal inhibitory concentrations of antimicrobial activity (cinnamon oil)

Minimal inhibitory concentrations (MICs) were determined by the twofold serial dilution method. DMSO was used as a negative control. A volume of the spice oils was added to PDA for fungal assays and NA for bacterial assays then mixed thoroughly and poured onto agar plates. Each assay in this experiment was conducted in triplicate with four quadrants on each plate, with

one for each of the microbial species and the serial dilutions. Subsequently, a micropipette was used to place a single 10 µL drop of cell solution (1×10^9 of fungal cells or 1×10^8 of bacterial cells per milliliter) into one of the quadrants. This step was repeated, placing two additional 10 µL drops of cell solution into the remaining two quadrants, with the final quadrant being used as a control. The inoculated spots were allowed to dry under a laminar flow hood before inverting the plates for incubation. The plates were incubated at 32°C for 48 h (bacteria), and at 28°C for 48 h (mycelial fungi). The lowest concentration of antimicrobial solution showing a clear zone was recorded as the minimum inhibitory concentration (MIC).

Minimal inhibitory concentrations of beeswax coating containing antimicrobial (cinnamon oil)

The MIC confirms the resistance of a microorganism to an antimicrobial agent. The MICs of the spice oils were determined using a twofold serial dilution method with DMSO as a solvent, according to the method of Andrews (2001). A beeswax emulsion was prepared by heating 18.7% beeswax in a water bath at 70°C. Then, 0.4% carboxymethylcellulose (CMC) was dissolved in 75.3% distilled water and heated on amagnetic stirrer hot plate at 70°C until the solution became clear and then 2.8% triethanolamine and 2.8% oleic acid were added as emulsifiers in the solution. The CMC solution was added to the melted beeswax and mechanically mixed using a homogenizer for 15 min at 24,000 rpm and 70°C until uniformly dispersed. The value of 512 µg/mL was chosen from the MIC results, because the highest value was used when testing for more than one plant pathogen. Nine serial dilutions were made from a stock solution of 32,768 $\mu g.mL^{-1}$ of cinnamon oil and 0.07% sucrose ester was added to the beeswax emulsion. The antimicrobial activity was estimated using a growth inhibition assay according to the method of Hamdy (2001). The MIC or zone diameter value was measured and compared against a reference standard which contains measurement ranges and their equivalent qualitative categories of susceptible, intermediately susceptible or resistant.

Optimal percentage of antimicrobial (cinnamon oil) inclusion with beeswax emulsion

The percentage of the spice oils were determined using a twofold serial dilution method with DMSO as a solvent, according to the method of Andrews (2001). A beeswax emulsion was prepared using the same method described above, thereby eliminating the need for serial dilutions with cinnamon oil. Cinnamon oil was weighed into quantities from 0.8 to 1.8 g, then the oil was added to 100 mL volumetric flasks and 0.07% of sucrose ester and beeswax emulsion were added to make a final volume of 100 mL. The resulting solutions of 0.8 to 1.8% were equivalent to 8,000 to 18,000 $\mu g.mL^{-1}$. The antimicrobial activity was estimated using a growth inhibition assay.

Coating sweet peppers with beeswax emulsion containing 1.4% cinnamon oil for shelf life tests

Green and red sweet peppers (cultivars beyond genus and species were not determined) were purchased from the Wholesale Taladthai Market, Pathum Thani province, Thailand for this study. All peppers were sorted and selected according to size, color, shape, maturity stage, and absence of external defects. Each individual pepper, including the peppers that were uncoated, was washed in water and then in a 0.5% sodium hypochlorite solution prepared from 5% commercial bleach and then rinsed with water and dried gently using soft paper towels before coating.

The sample of 370 peppers was randomly distributed into two groups, of three replicates each. The beeswax emulsion was applied by hand with latex gloves by rubbing onto the pepper surface

(Chen and Nussinovitch, 2000) and then individual peppers were placed on a stainless steel screen. All peppers were allowed to air dry for 2 h at 20°C. The surface coating of the peppers was checked for uniformity. Coating was repeated on missed areas and then groups of six peppers were placed on styrofoam trays and stored in the ambient and low temperature storage. Sweet peppers were sampled for physical quality and also photographed every third and fifth day until days 18 and 30 in ambient and low temperature storage, respectively. The microbial quality of sweet peppers was measured every fifth day, for both storage conditions until days 15 and 30 in the ambient and low temperature storage, respectively.

Physical quality measurements

Weight loss

Ten peppers from the trays of each treatment were weighed on a digital balance, using the same peppers during the entire storage period. Each pepper was marked with a self-adhesive label and then weighed individually at the beginning of the experiment (Wt = 0) and during storage (Wt = t). Each pepper was returned to its original storage conditions after each measurement. The weight of each pepper was recorded every third and fifth day in ambient (27 ± 2°C and 65 ± 5% RH) and low temperature storage (10 ± 2°C and 90 ± 5% RH).

The loss in pepper weight was expressed as a normalized percentage weight loss (WL) using the following Equation 1:

$$WL\ (\%) = [(Wt=0 - Wt=t)/Wt=0] \times 100 \quad (1)$$

Puncture test

Texture analysis of sweet peppers was carried out with a Texture Analyzer (Model Lloyd TA 500, Lloyd Instruments Ltd.; Hants, UK). Whole peppers were punctured with a 4.1 mm diameter stainless steel cylindrical probe. The penetration depth was 18 mm and the crosshead speed was 100 $mm.min^{-1}$ using a load cell of 5 kg (Ozden and Bayindirli, 2002; Conforti and Zinck, 2002). Three peppers were analyzed for each treatment and each whole pepper was measured at three different locations in the middle zone of the same whole pepper. The property of 'firmness' (hardness, the maximum force applied to puncture the pepper tissue) was measured as an indicator of texture, which is very similar to that performed by the mastication that takes place during eating. Sweet peppers were discarded after the completion of the test, so new peppers were used for puncture tests at each testing.

Microbial quality measurements

Total plate count

The microbial population of the control and coated sweet peppers was measured every fifth day for both storage temperatures. The counts were carried out at day 15 and 30 of storage in the ambient and low temperature storage, respectively, using the procedure of Association of Official Analytical Chemists (1995). The total plate counts were expressed in log $CFU.g^{-1}$ (colony forming units per gram of sample) of sweet pepper sample. To enumerate the microorganisms, a sample of 10 g of each sweet pepper was removed and combined with 90 mL of sterile peptone buffered water and homogenized in a stomacher bag and pummeled with a stomacher for 2 min at medium speed. The aliquot was used for various serial dilutions. From each dilution, 1 mL aliquots were aseptically pipetted for bacteria, and for yeasts and molds. Duplicate plates were made at each dilution and incubated at 32°C for 48 h.

Statistical analysis

Each replicate tested was subjected to a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at the $P < 0.05$ level of significance. The ANOVA was performed using the software package SPSS version 12 (SPSS (Thailand) Co., Ltd.; Bangkok, Thailand), and partial least squares using XLSTAT version 2010 (AddinSoft; New York, NY, USA).

RESULTS

Antimicrobial activity assay

The 10% cinnamon oil inhibited microbial growth more effectively than clove oil. Thiobutacin had no antimicrobial effect. The cinnamon oil was most inhibitory to the three pathogens at a concentration of 512 µg.mL^{-1}. The concentration range between 8,000 and 18,000 µg.mL^{-1} (0.8 to 1.8%) cinnamon oil was chosen for verification of the optimal percentage included with a beeswax emulsion. The results of antimicrobial activity assays showing the percentages of cinnamon oil in the beeswax emulsion are in Table 1. The concentration of 1.4% cinnamon oil in the beeswax emulsion, showed the most inhibition for *E. carotovora*, *C. capsici* and *C. gloeosporioides*.

Puncture test

At both during storage and at time zero, the firmness values of green and red peppers coated with beeswax containing 1.4% cinnamon oil demonstrated a higher firmness than the uncoated green and red peppers. The firmness values of sweet peppers decreased during storage for the coated and uncoated sweet pepper fruits as shown in Figures 1 and 2, indicating softening over time.

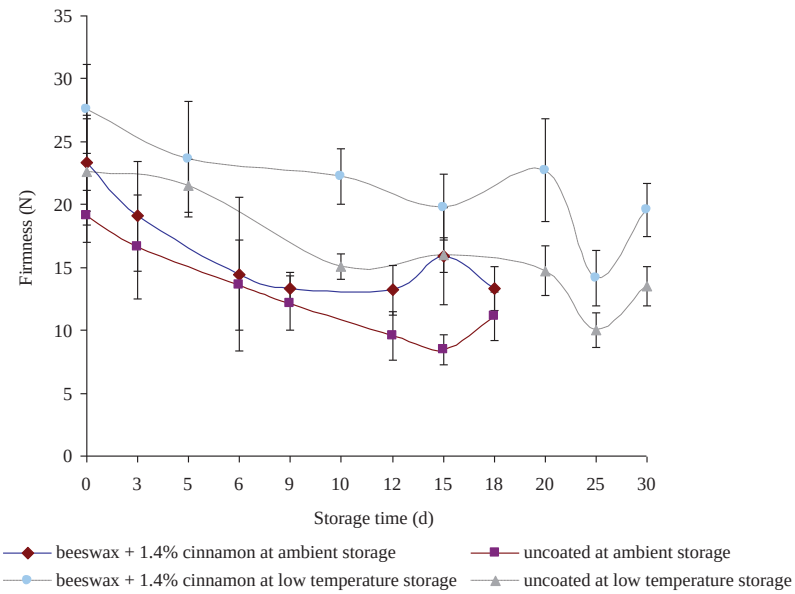

Figure 1 Firmness of green peppers in ambient and low temperature storage with and without beeswax coating. Vertical error bars show SD.

Table 1 Clear zones (mean ± SD, measured in centimeters) for different percentages of cinnamon oil in beeswax coatings.

Plant pathogen	Cinnamon oil					
	0.8%	1.0%	1.2 %	1.4%	1.6 %	1.8%
E. carotovora	$1.53^{c} \pm 0.10$	$1.73^{b} \pm 0.12$	$1.75^{b} \pm 0.05$	$1.90^{a} \pm 0.09$	$1.98^{a} \pm 0.17$	$1.90^{a} \pm 0.13$
C. capsici	$3.47^{d} \pm 0.33$	$3.78^{cd} \pm 0.19$	$3.67^{d} \pm 0.41$	$4.08^{bc} \pm 0.33$	$4.45^{ab} \pm 0.21$	$4.63^{a} \pm 0.42$
C. gloeosporioides	$1.95^{c} \pm 0.62$	$2.73^{b} \pm 0.12$	$2.98^{ab} \pm 0.24$	$2.67^{b} \pm 0.20$	$3.45^{a} \pm 0.51$	$2.87^{b} \pm 0.71$

$^{a-d}$ = Means with the same letter are not significantly different ($P < 0.05$).

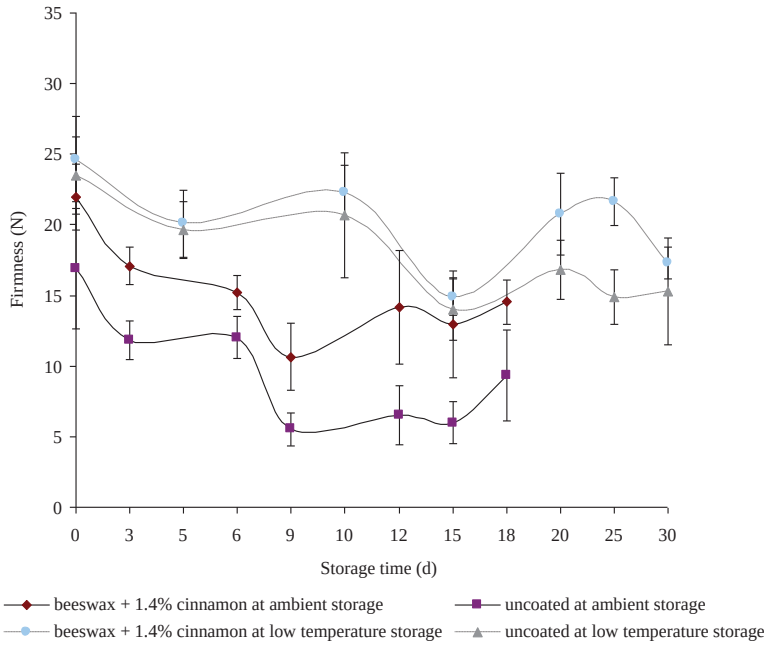

Figure 2 Firmness of red peppers in ambient and low temperature storage with and without beeswax coating. Vertical error bars show SD.

Weight loss

Statistical analysis revealed that the weight loss in the fruits coated with beeswax+1.4% cinnamon oil was significantly different from peppers without coatings. The green and red peppers coated with beeswax+1.4% cinnamon oil had less weight loss than the uncoated ones during the entire storage period. The red and green peppers stored at ambient temperature showed a greater weight loss than those kept under low temperature storage. The weight loss over 18 d for coated and uncoated peppers in ambient storage increased continuously over time. At the end of 18 d storage at ambient temperature, the weight loss of the green pepper control sample was 27%, whereas the weight loss of the sample coated with beeswax +1.4% cinnamon oil was 21%. The weight loss of the control red pepper sample was 32%, whereas the weight loss of the sample coated with beeswax +1.4% cinnamon oil was 15%. The weight loss over 30 d for coated and uncoated peppers in low temperature storage also increased continuously over time. At the end of 30 d of low temperature storage, the uncoated green peppers had lost about 11% of their original weight, whereas the weight loss of the sample coated with beeswax +1.4% cinnamon oil was about 8%. The weight loss of the control red pepper sample was approximately 11% whereas the weight loss of the sample coated with beeswax +1.4% cinnamon oil was about 7% during the storage period (Figures 3 and 4).

Microbiological analysis

The levels of mold and yeast on the green and red peppers coated with beeswax+1.4% cinnamon oil versus uncoated green and red peppers at both storage temperatures were not significantly different, as all counts for all treatments were less than 1 log CFU.g^{-1} (data not shown).

The total plate counts of aerobic bacteria from the green and red peppers coated with beeswax+1.4% cinnamon oil were significantly

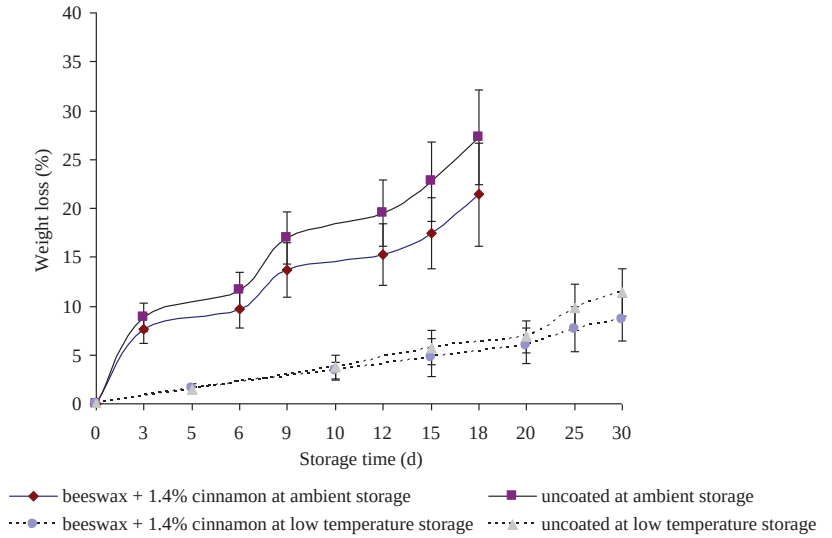

Figure 3 Weight loss of green peppers in ambient and low temperature storage with and without beeswax coating. Vertical error bars show SD.

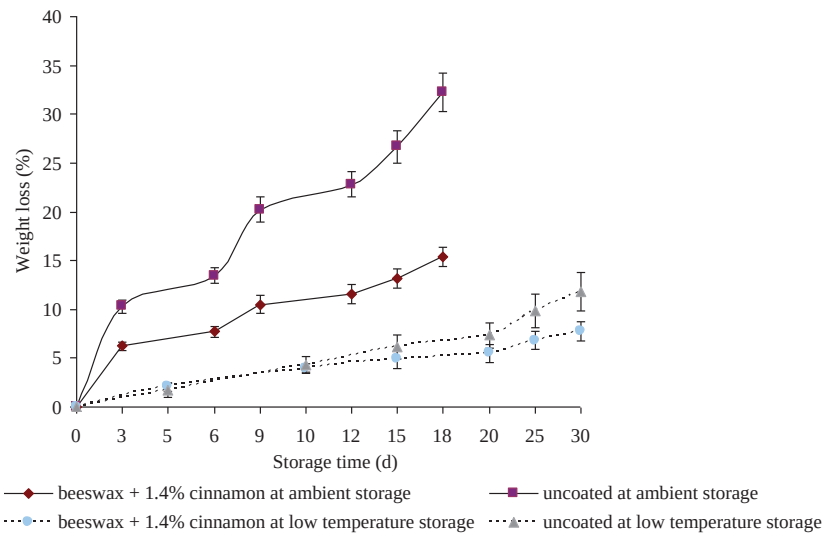

Figure 4 Weight loss of red peppers in ambient and low temperature storage with and without beeswax coating. Vertical error bars show SD.

different and lower than the total plate counts of the uncoated peppers. At 15 d, the uncoated green peppers in ambient storage had a 4% higher total plate count than the coated peppers, and the uncoated red peppers had a 12% higher total plate count than the coated peppers. After 15 d of low temperature storage, the uncoated green peppers had a 13% higher plate count than the coated peppers and uncoated red peppers had a 2% higher total plate count than the coated peppers (Table 2). This might indicate that the antimicrobial coating on the outside of sweet peppers inhibited microbial contamination.

There was a higher rate of microbial

growth in the uncoated green and red peppers held in ambient storage. On the other hand, for the green and red peppers in low temperature storage, the rate of growth was slightly higher than for the coated peppers. As expected, the total plate counts after 15 d in ambient storage were higher for uncoated and coated red and green peppers than the plate counts for peppers held in low temperature storage. Uncoated peppers had total plate counts that were 17% (green) to 19% (red) higher in ambient storage than in low temperature storage. Total plate counts of the coated peppers were reduced 26% (green) and 10% (red) by low temperature storage (Table 2). The levels of moulds and yeasts on the green and red peppers coated with beeswax+1.4% cinnamon oil at both temperatures indicated that there were no measurable changes during storage for the coated or uncoated peppers.

DISCUSSION

Antimicrobial activity assay

Characteristically, there was a correlation between the increase in antimicrobial activity and the increase in the percentage of cinnamon oil and the increase in the diameter of the zone of inhibition. Johnson and Case (1995) found that the relative effectiveness of an antimicrobial compound was determined by comparing the diameter of the zone of inhibition with values in a standard diameter. The microorganism was considered resistant if the diameter of the zone of inhibition was less than 11 mm, intermediate if the diameter of the zone of inhibition was between 11 and 15 mm and susceptible if the diameter of the zone of inhibition was more than 15 mm. Hiran *et al.* (2010) found that 100 ppm of cinnamon oil inhibited the growth of *Aspergillus niger, A. terreus* and *Aspergillus sp.* and confirmed the conclusions of Soliman and Badeaa (2002), Matan *et al.* (2006) and Kyu Kyu Win *et al.* (2007) that cinnamon oil inhibited the growth of bacteria, yeasts and moulds. Bang *et al.* (2000) reported that cinnamon oil contained a high amount of cinnamaldehyde which inhibited the fungal cell wall synthesizing systems through reaction with the sulfhydryl groups present at the active site of the enzymes.

Puncture test

At both storage temperatures, the firmness values of green and red peppers coated with beeswax containing 1.4% cinnamon oil were higher than for uncoated peppers. The firmness values of sweet peppers decreased during storage for coated and uncoated sweet pepper fruits, indicating softening over time. These results were in agreement with those of Navarro *et al.* (2005) who found that beeswax coatings were more effective for maintaining the firmness of plums and Diaz-Perez *et al.* (2007), who found that increased water loss of sweet peppers during storage reduced fruit firmness.

Weight loss

General visible observations of peppers in ambient storage showed shriveling on the

Table 2 Total plate counts of green and red peppers that were coated and uncoated in ambient and low temperatures storage after 15 d.

Storage treatment	Total plate count (log CFU.g^{-1})					
	Green		Difference (%)	Red		Difference (%)
	Uncoated	Coated		Uncoated	Coated	
Ambient	8.38	8.08	4	9.76	8.63	12
Low temperature	6.93	6.00	13	7.95	7.80	2
Difference (%)	17	26		19	10	

CFU = Colony forming unit.

skin surface and the loss of firmness appeared to be correlated to water losses during storage. This confirmed the findings of Perez-Gago *et al.* (2003), Perez-Gago *et al*. (2005) and Perez-Gago *et al.* (2006) who used beeswax in a composite coating with whey protein isolate, whey protein concentrate and hydroxypropyl methylcellulose to preserve minimally processed intact plums, apple slices and persimmon pieces, respectively. Beeswax with the addition of 1.4% cinnamon oil and applied to coated sweet peppers was more successful in reducing water loss, maintaining firmness quality and reducing microbial contamination in low temperature storage than in ambient storage during the entire storage period as shown in Figures 5 and 6. These results corroborated the results of Avena-Bustillos and Krochta (1993) who found that emulsion coatings, including lipids, have been successful in reducing water loss.

Microbiological analysis

The results of the total plate counts on peppers of different ripeness, stored at two different temperatures indicated that temperature effects were more related to the total plate count of the sweet peppers than the presence or absence of the coatings. Microorganisms grow faster under ambient compared to low temperature conditions because at low temperature the reaction rate for the individual enzymes in the organism becomes much slower and reduces the fluidity of the cytoplasmic membrane, thus interfering with transport mechanisms (Mossel *et al.*, 1995). Beeswax incorporated with 1.4% cinnamon oil coating on the outside of green and red pepper fruits inhibited microbial contamination compared to uncoated fruit at both storage temperatures perhaps as a result of the cinnamaldeyde and aldehyde groups which are chemical compounds

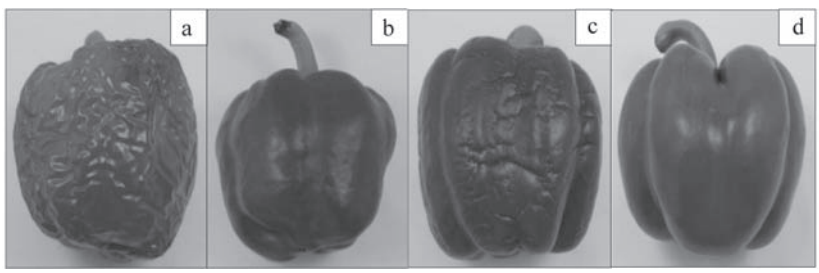

Figure 5 Green pepper samples at 15 d of storage: (a) Uncoated in ambient storage conditions; (b) Coated in ambient storage conditions; (c); Uncoated in low temperature storage conditions; and (d) Coated in low temperature storage conditions.

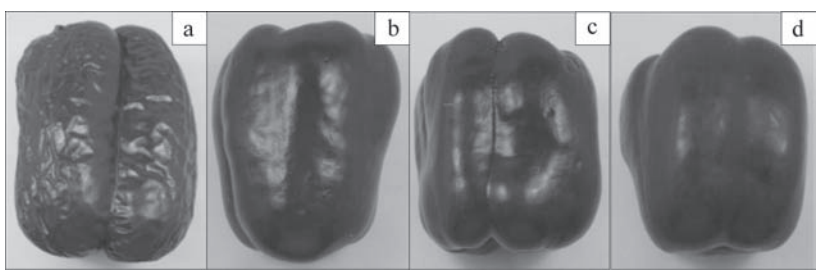

Figure 6 Red pepper samples at 15 d of storage: (a) Uncoated in ambient storage conditions; (b) Coated in ambient storage conditions; (c) Uncoated in low temperature storage conditions; and (d) Coated in low temperature storage conditions.

found in cinnamon oil which has a long CH chain and a conjugated double bond outside the ring resulting in enhanced antifungal activity (Wang *et al.,* 2005). More research on methods should be under taken to better evaluate coatings to reduce losses due to spoilage.

CONCLUSION

The beeswax coating containing 1.4% cinnamon oil was the most effective overall concentration for the inhibition of *Colletotrichum gloeosporioides*, *C. capsici* and *Erwinia carotovora*, which caused anthracnose and bacterial soft rot infection during storage.

Storage of the coated and uncoated sweet peppers with beeswax+1.4% cinnamon oil under ambient conditions resulted in significant changes in the physical and microbial quality during storage when compared with peppers held in low temperature storage. The significant physical changes comprised firmness and weight losses during storage at both temperatures. The firmness of the green and red peppers coated with beeswax containing 1.4% cinnamon oil was higher than the uncoated peppers and there were significant differences between ambient and low temperature storage after some storage times. A statistically significant weight loss of coated red and green peppers was observed under both temperature storage conditions but a greater loss resulted from ambient storage. The observed decrease in weight loss when a coating with beeswax+1.4% cinnamon oil was used prolonged the storage quality. Microbial properties were significantly ($P < 0.05$) affected in terms of the total plate count under both storage conditions. The total aerobic bacteria counts from the green and red peppers coated with beeswax+1.4% cinnamon oil were significantly different and lower than those of the uncoated peppers stored under ambient and low temperature conditions. The shelf life of the sweet peppers coated with beeswax and 1.4% cinnamon oil was extended to 12 d in ambient and 30 d in low temperature storage. Coated sweet peppers with beeswax+1.4% cinnamon oil reduced the moisture loss, maintained fruit firmness and extended the shelf life of sweet peppers.

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