

# Quality and Biochemical Changes of 'Sukkari' Bananas during Shelf Life as Affected by Postharvest Dipping in Ethanolic Extract of Propolis

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**The effect of postharvest ethanolic extract of propolis (EEP) dipping at 2.5%, 3.5% or 4.5% on quality and biochemical changes in 'Sukkari' bananas during 13 d of shelf life at 20 ± 2 °C and 60–70% RH were evaluated. EEP treatments, especially at 4.5%, lowered weight loss, total soluble solids (TSS) concentration and pH, and retained higher peel green color, membrane stability index (MSI), firmness and vitamin C concentration during shelf life compared with the control treatment. EEP treatments maintained higher titratable acidity (TA) and total phenol concentrations than the control only after 6 d of shelf life. Free radical scavenging capacity (FRSC) increased (lower DPPH IC50 values) during shelf life and was not affected by the treatments. The relations of such biochemical changes with α-amylase, xylanase, polygalacturonase, peroxidase and polyphenoloxidase activities were discussed. In conclusion, EEP treatments at 4.5% retained quality of 'Sukkari' bananas during shelf life and are suggested as a natural alternative to synthetic chemicals.**

Key Words: antioxidants, banana, enzymes, propolis, quality, shelf life, storage

Abbreviations: DPPH – 1,1-diphenyl-2-picrylhydrazyl, EEP – ethanolic extract of propolis, FRSC – free radical scavenging capacity, MSI – membrane stability index, PG – polygalacturonase, POD – peroxidase, TA – titratable acidity, TSS – total soluble solids

## INTRODUCTION

Bananas (*Musa* spp.) are among the few fresh fruit available to consumers worldwide throughout the year. They are considered as functional fruit due to their high nutritional and healthiness properties (Pereira and Maraschin 2015). Bananas of most cultivars such as 'Sukkari' are harvested at the full mature-hard green stage and thereafter ripening is triggered by exposure to a certain dose of ethylene for 24 h at about 18°C and 85% RH. However, the storage life of post-climacteric bananas is relatively short and the fruit rapidly softens and deteriorates (Liu 1976; Suseno et al. 2014; Awad et al. 2017). Thus, storing ethylene pre-treated bananas at 14°C with 1% oxygen retained their green color and firmness for 28 d (Liu 1976). However, following storage, these fruit ripened rapidly at 21°C in air conditions without additional ethylene treatment. In addition, being tropical fruit, bananas are highly sensitive to chilling injuries when exposed to temperatures below 13°C (Wang et al. 2014). Numerous physiological and biochemical changes

occur during shelf life including increase in weight loss, chlorophyll and polyphenolic degradation, peel browning, conversion of starch to sucrose and the decrease of pulp firmness, titratable acidity (TA) and vitamin C in several banana cultivars (Maqbool et al. 2011; Fernando et al. 2014; Wang et al. 2014; Al-Qurashi et al. 2017). Application of synthetic chemical preservatives is restricted due to rising consumer concerns regarding both human health and the environment. Accordingly, natural and safer alternative tools to modulate banana ripening and maintain quality during shelf life are critically required. The use of natural edible coatings such as chitosan, carboxymethyl cellulose, candelilla wax, gum Arabic and propolis is considered a novel approach to delay ripening, reduce water loss and decay, and extend storage and shelf life of various fruit including banana (Bautista-Banos et al. 2006; Romanazzi et al. 2013; Shi et al. 2013; Passos et al. 2016; Al-Qurashi et al. 2017).

Propolis is a natural resin produced by honeybees and is composed of several compounds such as polyphenolics (flavonoids and cinnamic acid derivatives),

**Table 1. Fruit weight loss, peel color and pulp firmness of 'Sukkari' bananas during shelf life (SL) as affected by post-harvest ethanolic extract of propolis (EEP) dipping.**

	Weight Loss (%)	Peel Color (Index)	Firmness (N)
Initial	0.0	2.0	38.6
Treatments (T)			
Control	4.9a	5.8a	6.5b
EEP (%)			
2.5	3.5b	4.5b	6.9b
3.5	3.6b	4.4b	6.7b
4.5	3.2b	4.0b	8.0a
F-test	**	**	**
SL (days)			
6	2.9b	5.9b	8.2a
13	9.5a	6.8a	6.1b
F-test	***	***	***
T x SL			
F-test	NS	NS	NS

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ . \*\* and \*\*\*, significant at  $P \leq 0.01$  and  $0.001$ , respectively  
 NS, not significant; -, not calculated

waxes, vitamins and essential oils that reflect its antioxidant, anti-inflammatory and antimicrobial properties (Burdock 1998; Sforcin and Bankova 2011; Pastor et al. 2011). Additionally, propolis extracts contain hydrophobic composites capable of forming a biodegradable film on treated fruit surface that might enhance permeability barriers to gases in various fruit (Pastor et al. 2011; Zahid et al. 2013; Ali et al. 2014 and 2015; Passos et al. 2016). However, the chemical compositions and biological action of propolis vary with geographic zone, collection time and plant sources (Kujumgiev et al. 1999; Passos et al. 2016). Postharvest application of ethanolic extract of propolis (EEP) alone or combined with other natural edible coatings maintained quality and extended storage and shelf life of different fruit such as mandarin (Ozdemir et al. 2005), cherry (Çandir et al. 2009), apple (Yan et al. 2010), grapefruit (Ozdemir et al. 2010), grape (Pastor et al. 2011), orange (El-Badawy et al. 2012), dragon fruit (Zahid et al. 2013), mango (Mattiuz et al. 2015) and pomegranate (Kamel et al. 2015). Passos et al. (2016) reported that postharvest dipping of 'Prata' bananas in ethanolic or aqueous extract of propolis from four different botanical sources in Brazil at 2.5% (w/v) reduced weight loss only after 9 and 12 d of

shelf life at ambient conditions compared with the control. However, firmness, total soluble solids (TSS), TA, pH and sensorial attributes of fruit were not affected. To the best of our knowledge, there is little available published work on the response of banana fruit to postharvest treatment with EEP. Therefore, this study aimed to evaluate the response of 'Sukkari' bananas to postharvest dipping in EEP at different concentrations in an attempt to delay ripening and maintain quality during shelf life.

## MATERIALS AND METHODS

### Preparation of Ethanolic Extracted Propolis (EEP)

Crude propolis was collected from beehives in an apiary located in the experimental station of the Faculty of Meteorology, Environment and Arid Land Agriculture at Hada Al-Sham (110 km northeast of Jeddah 21° 48' 3" N, 39° 43' 25" E), Saudi Arabia and were then kept and air dried in the dark until extraction. The propolis exudates collected by a hybrid bee strain (*Apis mellifera lamarkii* x *A. m. carnica*) were primarily from a mixture of plant species

**Table 2. Total soluble solids (TSS), TSS/acid ratio and vitamin C concentration in pulp, and free radical scavenging capacity (FRSC) in peel of 'Sukkari' bananas during shelf life (SL) as affected by postharvest ethanolic extract of propolis (EEP) dipping.**

	TSS (%)	TSS/Acid (Ratio)	Vitamin C (g kg <sup>-1</sup> )	FRSC (DPPH IC <sub>50</sub> Values)
Initial	3.7	7.7	0.88	2.64
Treatments (T)				
Control	16.2a	33.4a	0.055c	3.6
EEP (%)				
2.5	14.8b	26.3b	0.063bc	3.4
3.5	15.1b	27.2b	0.067ab	2.9
4.5	13.8c	26.0b	0.072a	3.5
F-test	***	*	**	NS
SL (days)				
6	13.9b	12.2b	0.073a	5.3a
13	16.0a	44.2a	0.057b	1.2b
F-test	***	***	***	***
T x SL				
F-test	NS	NS	NS	NS

IC<sub>50</sub> value, the concentration (µg phenolic) of the test sample that decreased 50% initial radical Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ . \*, \*\* and \*\*\*, significant at  $P \leq 0.05$ ,  $0.01$  and  $0.001$ , respectively  
 NS, not significant

**Table 3. Interaction effect between treatments and shelf life (SL) on membrane stability index (MSI) of peel tissues, titratable acidity (TA) and pH in pulp of 'Sukkari' bananas as affected by postharvest ethanolic extract of propolis (EEP) dipping.**

Treatments	SL (days)					
	MSI (Index)		TA (%)		pH	
	6	13	6	13	6	13
Initial	60.3		0.48		4.9	
Control	22.1b	6.7e	0.79c	0.38d	4.4cd	5.4a
EEP (%)						
2.5	20.2b	7.6de	1.31b	0.39d	4.5c	4.9b
3.5	21.8b	11.9cd	1.50a	0.36d	4.4cd	4.9b
4.5	34.9a	20.1b	1.18b	0.36d	4.1d	5.0b

For each parameter, means within and between columns followed by the same letter are not significantly different at  $P \leq 0.05$ .

mainly *Cinnamomum camphora*, *Acacia* spp., and *Ziziphus spina-christi*. The collected propolis was frozen at  $-20^{\circ}\text{C}$  and ground into fine powder in a chilled mortar and mixed with 70% ethanol at a ratio of 1:20 with continuous shaking (Orbital shaker No. 321, Hangzhou Allsheng Instruments Co., Ltd, China) at 150 rpm for 6 d in the dark. The mixture was filtered with cheesecloth and Whatman No. 1 paper, and evaporated in a Rotary Evaporator (Julabo, Model F12, Germany). The filtrates (EEP) were frozen at  $-80^{\circ}\text{C}$  in an ultra-low temperature freezer (Sanyo, Japan) and lyophilized in a lyophilizator (Labconco, Freezone, No. 26570/D, Japan) under 5 mm-Hg pressure at  $-50^{\circ}\text{C}$ . Afterwards, different concentrations of EEP solution (2.5%, 3.5% and 4.5%) were prepared by dissolving the corresponding weight of lyophilized propolis in 25% ethanol.

### Plant Materials and Experimental Procedure

This experiment was performed on 'Sukkari' bananas imported from Yemen, and purchased in a local commercial company in Jeddah, Saudi Arabia. Fruit were harvested, packed as hands in polyethylene film in perforated cardboard (about 30 kg) and transported at  $15^{\circ}\text{C}$  from Yemen to Jeddah within 48 h from harvest. Bananas at the ripening stage 1, based on a banana ripening chart, were directly pre-treated with ethylene gas (about 0.01% by volume in air) at  $18^{\circ}\text{C}$  and 85% RH for 24 h for ripening induction at a commercial airtight ground warehouse with a great deal of bananas. Such practice is critical for ripening induction of 'Sukkari' banana. Then, uniform hands (at the ripening stage 2) were randomly selected at the warehouse and rapidly transported to the horticulture laboratory of King Abdulaziz University in Jeddah.

### Fruit Treatment

The time interval between harvest and ripening induction by ethylene treatment was 3 d. Bananas (at the color stage 2) carefully prepared in small uniform hands (about 5 fingers each, free of visual defects and with similar weight and size) were selected. A completely randomized experimental design with three replicates (six hands each) was established. Fruit of each treatment/replicate were soaked either in water (control), or 2.5%, 3.5% or 4.5% of EEP for 5 min. A surfactant (Tween 20 at 0.5 mL/L) was added to all treatments. Following air drying of about 1 h, all treatments/replicates were weighed and stored at  $20 \pm 2^{\circ}\text{C}$  and 60–70% (RH) in perforated cardboard cartons for 13 d. Before applying the treatments, additional three samples (10 fingers of each) were randomly collected for initial quality and biochemical analyses. After 6 and 13 d of shelf life, weight loss and peel color stage were recorded for each treatment/replicate. After 6 and 13 d of shelf life, samples (10 fingers of each) from each treatment/replicate were randomly collected for quality and biochemical analyses. Then, the fruit samples were peeled and the peel tissue was sliced and mixed. Random part of this peel was used for electrolyte leakage measurement and the remaining peel was kept at  $-80^{\circ}\text{C}$  for later enzyme, total flavonoids and phenols and antioxidant activity analysis. Pulp firmness was measured in each sample directly following peeling. The pulp tissue was later sliced, mixed and a random portion was used to determine TSS, TA, pH, and vitamin C.

### Weight Loss Determination

The total fruit weight loss was calculated on an initial weight basis and expressed in percentage.

### Peel Color Change Estimation

Peel color change score was recorded for each sample (10 individual fingers of each) with the help of a banana ripening chart (1 to 7 scale; 1- green, 2- green with trace of yellow, 3- more green than yellow, 4- more yellow than green, 5- yellow with trace of green, 6- full yellow, and 7- yellow with brown spots).

### Firmness, TSS, TA, pH and Vitamin C Measurements in Fruit Pulp

Fruit pulp firmness was measured independently in 10 fingers (in the middle of each finger) per replicate by a

**Table 4. Interaction effect between treatments and shelf life (SL) on total phenols and flavonoids concentration in peel of 'Sukkari' bananas as affected by postharvest ethanolic extract of propolis (EEP) dipping.**

Treatments	SL (days)			
	Total Phenols (g kg <sup>-1</sup> )		Total Flavonoids (g kg <sup>-1</sup> )	
	6	13	6	13
Initial	0.29		0.21	
Control	0.44bc	0.37cde	0.20ab	0.13c
EEP (%)				
2.5	0.48ab	0.30e	0.23a	0.15c
3.5	0.40cd	0.38cde	0.15c	0.14c
4.5	0.51a	0.34de	0.17bc	0.13c

For each parameter, means within and between columns followed by the same letter are not significantly different at  $P \leq 0.05$ .

digital basic force gauge, model BFG 50N (Mecmesin, Sterling, Virginia, USA) supplemented with a probe of 11 mm diameter and the results were expressed in Newton. A homogeneous sample was prepared from these 10 fingers per replicate for measuring TSS, TA, pH and vitamin C. TSS concentration was measured as percentage in fruit pulp juice with a digital refractometer (Pocket Refractometer PAL 3, ATAGO, Japan). TA was determined in fruit juice diluted in water at a ratio of 1:2 by titrating with 0.1 N sodium hydroxide up to pH 8.2, using automatic titrator (HI 902, HANNA Instrument, USA) and the results expressed as a percentage of malic acid. Fruit juice pH was measured by a pH meter (WTW 82382, Weilheim, Germany). Vitamin C was measured by the oxidation of ascorbic acid with 2,6-dichlorophenolindophenol dye and the results expressed as g kg<sup>-1</sup> on a fresh weight (FW) basis (Ranganna 1979).

### Membrane Stability

Membrane stability was measured in peel disks according to Awad et al. (2017) and was expressed as membrane stability index percentage (MSI %). Three grams of peel disks per replicate/treatment was randomly taken and placed in 30 mL of deionized water at ambient temperature for 4 h in a shaker. Conductivity before boiling (C1) was measured with an electrical conductivity digital meter (Orion 150A+, Thermo Electron Corporation, USA). The same disks were kept in a boiling water bath (100°C) for 30 min to release all electrolytes, cooled to 22 ± 2°C with running water, and conductivity after boiling was recorded (C2). MSI was expressed in percentage using the formula:  $[1 - (C1/C2)] \times 100$ .

**Table 5. Interaction effect between treatments and shelf life (SL) on antioxidant enzyme activities (U min g FW) in peel of 'Sukkari' bananas as affected by postharvest ethanolic extract of propolis (EEP) dipping.**

Treatments	SL (days)			
	Polyphenoloxidase		Peroxidase	
	6	13	6	13
Initial	402		14.6	
Control	746.0ab	436.0d	18.9d	36.2c
EEP (%)				
2.5	736.0ab	544.0c	19.8d	47.5b
3.5	690.5b	568.2c	19.7d	50.5b
4.5	813.0a	573.7c	21.0d	72.0a

For each parameter, means within and between columns followed by the same letter are not significantly different at  $P \leq 0.05$ .

### Preparation of Methanol Extract of Fruit Peel

Two grams of fruit peel (randomly collected from 10 fingers per replicate) were extracted by shaking at 150 rpm for 12 h with 20 mL methanol (80%) and filtered with Whatman No. 1 paper. The filtrate designated as methanol extract was used for estimation of total phenols, total flavonoids and antioxidant activity.

### Estimation of Total Phenols and Total Flavonoids Content

Total phenols concentration was measured according to Hoff and Singleton (1977). Fifty microliters of the methanol extract was mixed with 100 µL of Folin-Ciocalteu reagent, 850 µL of methanol and allowed to stand for 5 min at ambient temperature. 500 µL of 20% sodium carbonate was added and allowed to react for 30 min. Absorbance was measured at 750 nm. Total phenols was quantified from a calibration curve obtained by measuring the absorbance of known concentrations of gallic acid and the results expressed as g kg<sup>-1</sup> FW gallic acid equivalent. Total flavonoids concentration was determined using a modified colorimetric method described previously by Zhishen et al. (1999). Methanol extract or standard solution (250 µL) was mixed with distilled water (1.25 mL) and 5% NaNO<sub>2</sub> solution (75 µL). After standing for 6 min, the mixture was combined with 10% AlCl<sub>3</sub> solution (150 µL), 1 M NaOH (0.5 mL) and distilled water (275 µL) were added to the mixture 5 min later. The absorbance of the solutions at 510 nm was then measured. Total flavonoids was quantified from a calibration curve obtained by measuring the absorbance of known concentrations of catechin and the results



**Table 6. Hydrolytic enzyme activities (U min g FW) in peel of 'Sukkari' bananas during shelf life (SL) as affected by postharvest ethanolic extract of propolis (EEP) dipping.**

	Weight Loss (%)	Peel Color (Index)	Firmness (N)
Initial	16.0	9.2	78.4
Treatments (T)			
Control	19.8	13.8	71.2a
EEP (%)			
2.5	18.5	14.7	64.3ab
3.5	19.0	14.2	52.5c
4.5	17.2	15.8	54.3bc
F-test	NS	NS	*
SL (days)			
6	20.8a	18.0a	92.0a
13	16.5b	11.3b	28.1b
F-test	***	***	***
T x SL			
F-test	NS	NS	NS

Means within each column followed by the same letter are not significantly different at  $P \leq 0.05$ . \* and \*\*\*, significant at  $P \leq 0.05$  and  $0.0001$ , respectively  
NS, not significant

expressed as  $\text{g kg}^{-1}$  FW catechin equivalent.

### Evaluation of DPPH Radical Scavenging Assay of Fruit Peel

Free radical scavenging activity of the methanol extract of fruit peel was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method (Ao et al. 2008). A methanol extract (0.1 mL) was added to 0.9 mL of freshly prepared DPPH methanol solution (0.1 mM). An equal amount of methanol was used as control. After incubation for 30 min at room temperature in the dark, the absorbance (Abs) was measured at 517 nm using a spectrophotometer. Activity of scavenging (%) was calculated using the formula:

$$\text{DPPH radical scavenging \%} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

The inhibition concentration (IC<sub>50</sub>) was defined as  $\mu\text{g}$  phenolics of the test sample that decreases 50% of initial radical. The IC<sub>50</sub> values were calculated from the dose response curves.

### Enzyme Measurements of Fruit Peel

#### Crude Extract

One gram of fruit peel (randomly collected from 10 fingers per replicate) was homogenized with 20 mM Tris-HCl buffer, pH 7.2 using homogenizer. The homogenate was centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was designed as crude extract and stored at -20°C for peroxidase, polyphenoloxidase, polygalacturonase, xylanase and  $\alpha$ -amylase assay.

#### Peroxidase Assay

Peroxidase (EC 1.11.1.7) activity (POD) was assayed according to Miranda et al. (1995). The reaction mixture contained in 1 mL: 0.008 mL of 0.97 M H<sub>2</sub>O<sub>2</sub>, 0.08 mL of 0.5 M guaiacol, 0.25 mL of 0.2 M sodium acetate buffer, pH 5.5 and least amount of enzyme preparation. The change in absorbance at 470 nm due to guaiacol oxidation was followed for 1 min using a spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme that increases the O.D. 1.0 per min under standard assay conditions.

#### Polyphenoloxidase Assay

Polyphenoloxidase (EC 1.14.18.1) (PPO) activity was assayed with catechol as a substrate according to the spectrophotometric procedure of Jiang et al. (2002). The extract (0.2 mL) was rapidly added to 2.8 mL of 20 mM catechol solution prepared in 0.01 M sodium phosphate buffer (pH 6.8). The increase in absorbance at 400 nm was recorded for 3 min using a spectrophotometer. One unit of enzyme activity was defined as the amount of the enzyme that causes a change of 0.1 in absorbance per min.

#### Polygalacturonase, $\alpha$ -amylase and Xylanase Assays

Polygalacturonase (EC 3.2.1.15) (PG),  $\alpha$ -amylase (EC 3.2.1.1) and xylanase (EC 3.2.1.8) activities were assayed by determining the liberated reducing end products using galacturonic acid, maltose and xylose, respectively, as standards (Miller 1959). The reaction mixture (0.5 mL) contained 5 mg substrate, 0.25 mL of 0.2 M sodium acetate buffer (pH 5.5) and a suitable amount of crude extract. Assays were carried out at 37°C for 1 h. Then 0.5 mL of dinitrosalicylic acid reagent was added to each tube and heated in a boiling water bath for 10 min. After cooling to room temperature, the absorbance was measured at 560 nm. Substrates used were polygalacturonic acid, starch and xylane for polygalacturonase,  $\alpha$ -amylase and xylanase, respectively. One unit of enzyme activity was defined as the amount of enzyme which liberated 1  $\mu\text{M}$  of reducing sugar per min under standard assay conditions.

## Statistical Analysis

The obtained data were statistically analyzed as a completely randomized design with three replicates by analysis of variance (ANOVA) using the statistical package software SAS (SAS Institute Inc., 2000, Cary, NC., USA). Comparisons between means were made by least significant difference (LSD) at  $P \leq 5\%$ .

## RESULTS

Weight loss of fruit increased to 9.5% after 13 d of shelf life and was significantly lower at EEP treatments than the control (Table 1). Peel color index increased from 2 to 6.8 during shelf life and was significantly lower at EEP treatments than the control. Pulp firmness sharply decreased from 38.6 to 6.1 N during shelf life and was significantly higher at 4.5% EEP treatment (8.0 N) than the other treatments including the control (6.5 N). There were no significant interaction effects between treatments and shelf life on weight loss, peel color index and pulp firmness (Table 1). TSS content increased from 3.7% to 16.0% during shelf life and was significantly lower at all EEP treatments than the control (Table 2). TSS/acid ratio sharply increased during shelf life and was significantly lower at all EEP treatments than the control. In this respect, differences among EEP treatments were insignificant. There were no significant interaction effects between treatments and shelf life on TSS and TSS/acid ratio (Table 2). MSI sharply decreased from 60.3 to 22.1 during shelf life and was significantly higher at 4.5% EEP than the other treatments both after 6 and 13 d of shelf life (Table 3). Vitamin C concentration decreased during shelf life in all treatments. EEP at both 3.5% and 4.5% retained higher vitamin C concentration than the control (Table 2). FRSC (DPPH  $IC_{50}$  values) was significantly higher (lower DPPH  $IC_{50}$  values) after 13 d than after 6 d of shelf life. However, differences among treatments were insignificant (Table 2). There were no significant interaction effects between treatments and shelf life on vitamin C concentration and FRSC (Table 2). EEP at 3.5% retained higher MSI than the control only after 13 d of shelf life. TA concentration showed higher level after 6 d of shelf life than after 13 d. EEP treatments retained higher TA concentration than the control only after 6 d of shelf life (Table 3). However, after 13 d, differences among treatments were insignificant. pH increased from 4.9 to 5.4 during shelf life in the control treatment and was not significantly affected by the applied treatments after 6 d of shelf life. However, after 13 d of shelf life, all EEP treatments showed lower pH compared with the control (Table 3). Total phenols concentration was lower after 13 d than after 6 d of shelf life at 2.5% and 4.5% EEP treatments, while it remained relatively stable in the other

treatments (Table 4). EEP at 4.5% showed higher total phenols concentration than the control only after 6 d of shelf life (Table 4). However, after 13 d, differences among treatments were insignificant. Total flavonoids concentration was higher after 6 d than after 13 d of shelf life in the control and 2.5% EEP treatments, while it remained relatively stable in the other treatments (Table 4). In this respect, after 6 d of shelf life, EEP at 3.5% showed lower total flavonoids concentration than the control while, after 13 d, differences among treatments were insignificant (Table 4). PPO activity was significantly lower after 13 d than after 6 d of shelf life. After 6 d of shelf life, differences among EEP treatments and the control were insignificant (Table 5). However, after 13 d of shelf life, all EEP treatments showed higher PPO activity than the control. POD activity was significantly higher after 13 d than after 6 d of shelf life. POD activity was not significantly affected by treatments after 6 d, but after 13 d of shelf life, EEP treatments showed higher POD activity than the control. In this respect, EEP at 4.5% gave higher POD activity than the other EEP treatments (Table 5).  $\alpha$ -amylase and xylanase activity was significantly lower after 13 d than after 6 d of shelf life. However, differences among treatments were insignificant (Table 6). PG activity was significantly lower after 13 d than after 6 d of shelf life. In this respect, PG activity was significantly lower at 3.5% and 4.5% of EEP treatments than the control (Table 6).

## DISCUSSION

Being a climacteric fruit, banana has a relatively high metabolic activity with high respiration rate and ethylene production following harvest and, consequently, a shorter shelf life (Liu 1976; Suseno et al. 2014; Awad et al. 2017). In this regard, EEP application as a natural edible coat was evaluated for its impact on the physiology of 'Sukkari' bananas in an attempt to modulate the ripening process during shelf life at ambient conditions. Both of the active metabolic processes (transpiration and respiration) are responsible for weight loss of fruit during shelf life (Ayranci and Tunc 2003; Bhande et al. 2008; Maqbool et al. 2011). The hydrophobic composites of propolis extracts are capable of forming a biodegradable semipermeable film on fruit surface that might limit water loss and gas exchange in various fruit (Zahid et al. 2013; Ali et al. 2014 and 2015; Passos et al. 2016). Such characteristics of propolis might explain the observed lower weight loss of EEP-treated fruit than the control (Table 1). Similarly, weight loss reduction during storage by propolis treatment was also previously published on mandarin (Ozdemir et al. 2005), cherry (Çandir et al.

2009), apple (Yan et al. 2010), grapefruit (Ozdemir et al. 2010), grape (Pastor et al. 2011), orange (El-Badawy et al. 2012), dragon fruit (Zahid et al. 2013), mango (Mattiuz et al. 2015) and banana (Passos et al. 2016). EEP-treated fruit retained more green color and less yellow as estimated by the peel color index (Table 1), which reflects a delay in fruit ripening compared with the control. Such change is mainly due to chlorophyll degradation by the action of chlorophyllase, as well as the accumulation of carotene and xanthophyll during shelf life (Salvador et al. 2007; Seymour et al. 2008). Also, EEP treatments, especially at 4.5%, delayed ripening of 'Sukkari' bananas during shelf life as indicated by higher MSI, firmness and TA and lower TSS concentration and TSS/acid ratio than the control (Tables 1, 2 and 3). Moreover, EEP at 4.5% retained higher total phenol concentration after 6 d of shelf life (Table 4), and EEP treatment at both 3.5% and 4.5% retained higher vitamin C than the control during shelf life (Table 5). Such effects might be due to the general preservative effects of propolis as an antioxidant and antimicrobial agent (Burdock 1998; Sforcin and Bankova 2011; Pastor et al. 2011). Also, modification of internal atmosphere of fruit generated by propolis film on fruit surface might partially explain such positive effects (Pastor et al. 2011; Zahid et al. 2013; Ali et al. 2014 and 2015; Passos et al. 2016). The observed changes in total phenols and flavonoids concentrations during shelf life (Table 4) are in accordance with those of Kondo et al. (2005) who found that total phenols decreased in the peel of 'Namwa' bananas stored at 6°C but slightly changed in fruit stored at 12°C for 6 d. Also, Wang et al. (2014) found that total phenols in the peel of 'Brazil' banana slightly increased during the first 10 d of storage at 7°C then sharply decreased thereafter. The decrease in phenols concentration in fruit during ripening might be due to breakdown of the cell structure because of the senescence phenomena during storage (Macheix et al. 1990). Our results showed that FRSC of fruit peel increased (lower DPPH IC<sub>50</sub> values) during shelf life (Table 2), confirming those of Al-Qurashi et al. (2017) and Awad et al. (2017) on 'Sukkari' bananas. Also, total antioxidant activities (mmol TE/100g fw) measured by DPPH and ferric reducing antioxidant power (FRAP) assays of 'Hom Thong' and 'Khair' bananas increased in fruit pulp during 10 d of shelf life at 25°C, but rapidly decreased at senescence stage (Fernando et al. 2014). However, IC<sub>50</sub> values of superoxide (O<sub>2</sub><sup>-</sup>) and DPPH of the peel of 'Namwa' bananas stored at 6 or 12°C decreased during the first 2 d followed by a gradual increase during the following 8 d of storage (Kondo et al. 2005). The decrease in total phenols and flavonoid levels with the increase in FRSC during shelf life (Tables 2 and 4) suggest qualitative changes in

phenolic classes toward higher antioxidant potential. In another study, no significant correlation between total phenol concentration and antioxidant activity was detected, except for vitamin C level and FRAP values in 'Khair' banana pulp (Fernando et al. 2014). Phenolics antioxidant activity possibly has a concentration saturation limit above which the activity could not increase further with the concentration (Dani et al. 2012). In addition, other compounds such as carotenoids, vitamins and some minerals may contribute to FRSC of fruit synergistically with phenolics (Dani et al. 2012). Accordingly, several parallel assays should be used to investigate the principles of antioxidant/oxidation activity of a certain horticultural commodity. The significantly lower PG activity accompanied by higher MSI of peel of EEP-treated fruit than the control might explain the observed retention of fruit firmness by EEP treatments during shelf life (Tables 3 and 6). Both POD and PPO, as defensive enzymes against stress and pathogen attacks (Campos-Vargas and Saltveit 2002), showed much higher activity during shelf life in the EEP-treated fruit compared with the control and initial values (Table 5). Fruit ripening and senescence is considered an oxidative process in which the transition from maturation to ripening/senescence stage is accompanied by a progressive shift toward an oxidative state (Goulao and Oliveira 2008). During fruit ripening, the burst in reactive oxygen species production was accompanied by up-regulations of antioxidant enzyme activities such as POD, PPO and catalase and expression of their related genes (Ali et al. 2011; Zhang et al. 2013). In conclusion, EEP treatments especially at 4.5% retained quality of 'Sukkari' bananas during shelf life and are suggested as natural alternatives to synthetic chemicals.

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