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Effects of Propolis on Storage of Sweet Cherry Cultivar Aksehir Napolyon

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Sweet cherries cultivar Aksehir Napolyon were dipped in ethanol extracted propolis (EEP) and water extracted propolis (WEP) in various concentrations immediately after harvest and then kept at 0 ºC, 85-90 % relative humidity for 4 weeks. The following treatments were applied to the fruits: dipping in water, dipping in ethanol (70 %), dipping in 1, 5 and 10 % concentrations of ethanol extracted propolis and dipping in 1, 5 and 10 % concentrations of water extracted propolis. The effects of propolis on incidence of fungal decay and fruit quality (weight loss, total soluble solids, titratable acidity, skin colour, stem browning, surface pitting, appearance and taste) were assessed at weekly intervals during storage. Ethanol extracted propolis treatments were effective in preventing fungal decay in cherries for 4 weeks, but adversely affected sensory quality and stem colour of cherries.

Key Words: Sweet cherry, Aksehir Napolyon, Postharvest quality, Propolis, Dipping.

INTRODUCTION

The postharvest life of sweet cherries is limited by weight loss, colour changes, softening, surface pitting, stem browning and loss of acidity and fungal decay[1]. Sweet cherries are susceptible to decay caused by several pathogens, including *Penicillium expansum* Link, *Botrytis cinerea* Pers.:Fr., *Monilinia fructicola* G. Wint. Honey, *Alternaria* spp. and *Rhizopus stolonifer* (Ehrenberg:Fries) Vuillemin)[2]. Iprodione was effective in controlling fungal decay and used extensively as a postharvest treatment of cherry until its postharvest use was cancelled[3]. The use of synthetic fungicides to control postharvest diseases of sweet cherries is not allowed in several export markets including European Union countries due to fungicide regulatory issues. Therefore, there is a need for alternative postharvest disease control method

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to reduce fungal decay with no risks for consumers. Alternative to synthetic chemicals that are of potential use in disease control on sweet cherries include biological control with yeast antagonists[3-5], hot water and ethanol treatments[6], modified atmosphere packaging[3-5] sodium bicarbonate[5], chitosan and short hypobaric treatments[7].

Propolis is a natural brownish-green resinous product that honeybees collect from different plants exudates. It possesses many biological activities such as antibacterial, antiviral, antifungal and pharmacological activitities[8-11]. Although the antimicrobial activity of propolis has been demonstrated against human pathogenic fungi, bacteria and virus[9,12]; very few *in vitro* studies have been conducted against plant pathogenic microorganism[13-17]. Ethanol extracted propolis inhibited *P. digitatum* growth *in vitro*[18] and limited the growth of *B. cinerea* on strawberry[14]. There are limited studies have been conducted *in vivo* effects of propolis against plant pathogen. The 5 and 10 % of ethanol extracted propolis extended storage life of Fremont mandarins compared to control fruits[19].

The present study was undertaken to evaluate the efficacy of propolis in controlling of fungal decay in sweet cherries during storage and determine the effects of propolis on postharvest quality of sweet cherries.

EXPERIMENTAL

Preparation of propolis extracts: Crude propolis from Hatay province (Eastern Mediterranean region of Turkey) were hand gathered. The propolis exudates collected by bees (*Apis mellifera anatoliaca, A. m. caucasica, A. m. syriaca* and their hybrids) in Hatay were mainly from a mixture of wild and medicinal aromatic plant species such as *Medicago* spp., *Trifolium* spp., *Lathyrus sativus, Coronilla varia, Lotus* spp., *Pisum arvense, Origanum syriacum, Lavandula stoechas, Thymbra spicata, Adonis* spp., *Anagalis arvensis, Hordeum bulbesum, Aegilops ovata, Convovulus* sp., *Anthemis* sp., *Salvia multicaulis, Ferula communis* and *Petroselinum sativum*. The hand collected propolis was kept desiccated and in the dark until further processing.

Propolis extracts were prepared as described by Krell[20]. Briefly, propolis was frozen to -20 °C, cut in small pieces and ground in a chilled mortar. A 10 % of the ethanol extracted propolis or water extracted propolis was prepared by adding 100 g of the specimens of propolis to 900 mL of 70 % ethanol or water with agitation for a week or 3 d, respectively at room temperature and filtered. The extracts were kept at 4 ºC in the dark until use. The amount of dissolved principles was assessed by weight difference. The 1 and 5 % propolis extracts were prepared by dilution of 10 % propolis extracts with either 70 % ethanol or water.

Plant material: Sweet cherry fruits (*Prunus avium* L., cv. Aksehir Napolyon) were obtained from a commercial orchard in Ermenek, Karaman. Cherries were harvested at commercial maturity, as determined by skin colour (solid bright red) and hydrocooled at a water temperature of 0 to 0.2 ºC using shower hydrocooler until flesh temperature reached to 0.5-0.8 ºC for 8-10 min. Sodium hypochlorite

was added to the water to achieve a chlorine concentration of 100 mL L^{-1}. Fruits were then transferred to cold room at 8-10 °C where were sorted and selected for uniform size (diameter of 22-24 mm), colour and absence of defects according to exporting criteria of packing houses.

Propolis treatment: Cherries were subjected the following treatments: (1) dipping in water (water control, WC), (2) dipping in 70 % of ethanol (ethanol control, EC), (3) dipping in ethanol extracted propolis (EEP) at concentrations of 1, 5 and 10 % and (4) dipping in water extracted propolis (WEP) at concentrations of 1, 5 and 10 %. Treatment 1 and 2 were used as control for treatment 4 and treatment 3, respectively. Each treatment contained three replicates of about 500 g fruit each. 500 g of cherries were packed in punnets; punnets were then placed in cardboard cherry boxes (60 cm × 40 cm × 10 cm) lined with 20 µ thick, non-perforated polyethylene bags and kept at 0 ºC (± 0.5), 85-90 % (± 5 %) relative humidity for 4 weeks.

Postharvest quality evaluation: Postharvest quality of fruits was assessed at weekly intervals during storage. From each treatment weight of punnets contained cherries were recorded at the beginning and during the storage period and differences are calculated and expressed as percentage weight loss.

Skin colour was determined with a Minolta Chroma Meter CR-300 (Osaka, Japan). Colour measurements were recorded on 20 fruits from each replicate using the CIE L*a*b* colour space. From these values, hue angle (h°) was calculated as, $h° = \tan^{-1} (b^*/a^*)$. As h° was the most important colour descriptor, cherry skin colour was expressed only as h°. The hue angle is expressed in degrees and is a measure of colour that, for example, from 0 to 90° spans from red to orange to yellow[21]. Colour values for each fruit were computed as means of two measurements taken from both cheeks of each cherry along the equatorial axis.

Total soluble solids (TSS) content and titratable acidity (TA) were assessed in juice obtained from 20 fruits from each replicate. TSS content was determined with a refractometer (Atago Model ATC-1E) and TA by titration of 5 mL of fruit juice with 0.1 N NaOH to pH 8.1 and expressed as g malic acid per 100 mL juice.

Cherry with over 30% of surface stem discolouration were scored as suffering stem browning[22]. Per cent stem browning was determined by weighing fruit with brown stem in each replicate of treatments on the day removal from storage.

Fruits were considered surface pitted if pitting damaged areas on a cherry exceeded 7 mm in diameters[23]. Per cent pitting was determined by weighing surface pitted fruit in each replicate of treatments on the day removal from storage.

Any visible fungal growth was scored as decay. Fungal decay incidence was determined by weighing decayed fruit in each replicate of treatments on the day removal from storage.

A trained panel consisting of 10 people evaluated the sensory quality of the cherries, initially and then weekly throughout the storage period. Cherry quality was evaluated on a scale of 1-5 (1 = very poor, 2 = poor, 3 = fair, 4 = high, 5 = very high). Each member of the panel was requested to evaluate overall acceptability and taste.

Statistical analysis: The data were analyzed as a factorial experiment in a completely randomized block design by ANOVA using SAS software of SAS Institute, Cary, N.C.[24]. Each treatments, consists of 500 g of cherries were replicated 3 times. Mean separation was performed by Duncan's Multiple Range test at $p < 0.05$ level using SAS's Proc GLM procedure. Data for per cent weight loss, decay, surface pitting, stem browning were arcsine transformed and analyzed by ANOVA and back transformed for reporting.

RESULTS AND DISCUSSION

Weight loss increased as storage time extended (Table-1). After 4 weeks of storage, per cent weight loss reached to 1.62 % without any visual shrivels. Loss of weight is one of the most important causes responsible for cherry quality deterioration, which increases the fruit susceptibility to fungal decay. Visual shrivel usually appeared when water loss reaches 5 % in cherries[25]. Weight loss in cherries is higher than in other commodities due to their low skin diffusion resistance[26]. In this study, low weight loss is due to polyethylene box liner as reported previously[27-29]. The effects of propolis treatments on the weight loss were not significant during 4 weeks of storage.

Incidence of fungal decay significantly increased after 3 weeks of storage (Table-1). 1 and 5 % EEP treated fruits had significantly lower fungal decay compared to WEP treated fruits and control fruits after 3 and 4 weeks of storage. Özcan[16] showed that WEP at 4 % resulted in more than 50 % inhibition against some plant pathogens including *P. digitatum* and *B. cinerea in vitro*. In this study, WEP found to be ineffective in reducing fungal decay. The antimicrobial actions of different extracts of propolis from various geographic origins were compared[30]. For all propolis samples tested, the strength of antimicrobial activity decreased in the order of EEP, propolis volatiles and WEP. The extraction of propolis with ethanol procures all water soluble, ethanol soluble and the volatile components of propolis making EEP superior to the other two extracts qualitatively and/or quantitatively. Ethanol extracted propolis (EEP) inhibited *P. digitatum* growth *in vitro*[18] and limited the growth of *B. cinerea* on strawberry[14]. Previous studies also showed some promising results on antifungal activity of EEP[18]. Ethanol extracted propolis provided complete inhibition of naturally occurring green mold disease on wounded but uninoculated grapefruits[18]. Ethanol extracted propolis resulted in slightly lower incidence of fungal decay in Fremont mandarins compared to control fruits during the storage[19]. The data of this study indicated that EEP might provide inhibition of fungal decay in cherries for 4 weeks which is sufficient for marketing of cherries.

Surface pitting was not visible for 3 weeks of storage (Table-1). After 4 weeks of storage, incidence of surface pitting reached 11.66 %. Propolis treatments did not affected surface pitting (Table-1).

Stem browning is typically developed during sweet cherry storage has been associated with fruit ripening[22] and also due to dehydration[31]. In this study, incidence

TABLE-1
EFFECTS OF ETHANOL EXTRACTED PROPOLIS (EEP) AND WATER EXTRACTED PROPOLIS (WEP) ON WEIGHT LOSS, FUNGAL DECAY, STEM BROWNING AND SURFACE PITTING OF CHERRIES CULTIVAR AKSEHIR NAPOLYON DURING 4 WEEKS OF STORAGE AT 0 ºC

Treatments	Weight loss (%)	Fungal decay (%)	Surface pitting (%)	Stem browning (%)
After 1 weeks at 0°C				
Ethanol control	0.90a[X]	0.00a	0.00a	15.62a
% 1 EEP	0.41a	0.00a	0.00a	6.88b
% 5 EEP	0.61a	0.00a	0.00a	1.35c
% 10 EEP	0.93a	0.00a	0.00a	8.21b
Water control	0.47a	0.00a	0.00a	1.08c
% 1 WEP	0.50a	0.00a	0.00a	0.00c
% 5 WEP	0.92a	0.00a	0.00a	0.00c
% 10 WEP	0.50a	0.00a	0.00a	0.00c
Mean	0.65(d)[Y]	0.00(b)	0.00(b)	4.14(d)
After 2 weeks at 0°C				
Ethanol control	1.22a	2.30a	0.00a	13.73b
% 1 EEP	0.76a	0.00a	0.00a	19.48b
% 5 EEP	1.01a	0.00a	2.18a	12.58bc
% 10 EEP	1.11a	0.00a	0.00a	31.90a
Water control	0.80a	1.14a	0.00a	7.14cd
% 1 WEP	0.96a	0.00a	0.00a	0.00d
% 5 WEP	1.08a	0.00a	0.00a	0.00d
% 10 WEP	0.85a	0.00a	0.00a	0.00d
Mean	0.97(c)	0.43(b)	0.27(b)	10.61(c)
After 3 weeks at 0°C				
Ethanol control	1.52a	5.74b	0.00a	40.24a
% 1 EEP	1.04a	0.00d	0.00a	49.47a
% 5 EEP	1.28a	0.00d	0.00a	20.65b
% 10 EEP	1.39a	0.00d	0.00a	19.76b
Water control	0.89a	3.24c	0.00a	11.01bc
% 1 WEP	1.42a	3.35c	0.00a	4.02c
% 5 WEP	1.39a	7.28a	0.00a	15.60b
% 10 WEP	1.17a	0.00d	0.00a	3.37c
Mean	1.26(b)	2.45(a)	0.00(b)	20.52(b)
After 4 weeks at 0°C				
Ethanol control	2.33a	2.53b	14.94a	39.21b
% 1 EEP	1.58b	0.00c	15.36a	56.48a
% 5 EEP	1.43b	0.00c	12.70aa	51.35a
% 10 EEP	1.48b	2.50b	18.24a	40.18b
Water control	1.31b	3.58ab	6.86a	5.60c
% 1 WEP	1.52b	5.70a	2.43a	5.97c
% 5 WEP	1.68b	5.59a	11.40a	7.13c
% 10 WEP	1.61b	3.89ab	11.30a	7.71c
Mean	1.62(a)	2.97(a)	11.66(a)	26.70(a)

[X]Mean separation was performed by Duncan's Multiple Range Test. Treatment means (n = 3) followed by same letter within column are not significantly different at $p < 0.005$. Treatment means was compared separately for each storage time.

[Y]Letters in parenthesis indicates comparison of means of storage time. Values represents mean of all treatments for each storage time.

TABLE-2
EFFECTS OF ETHANOL EXTRACTED PROPOLIS (EEP) AND WATER EXTRACTED PROPOLIS (WEP) ON TOTAL SOLUBLE SOLIDS (TSS) CONTENT, TITRATABLE ACIDITY (TA), SKIN COLOUR CHANGES (L*, hº), APPEARANCE AND TASTE OF CHERRIES CULTIVAR AKSEHIR NAPOLYON DURING 4 WEEKS OF STORAGE AT 0 ºC

Treatments	TSS (%)	TA (%)	Skin colour		Appearance[Z]	Taste[Z]
			L*	hº		
At harvest	13.93(b)	0.74(a)	21.53(c)	12.82(b)	5.00(a)	5.00(a)
After 1 weeks at 0°C						
Ethanol control	15.13a[X]	0.69bc	24.63bc	13.82a	5.00a	4.97a
% 1 EEP	15.67a	0.70abc	23.63c	11.82a	5.00a	4.90a
% 5 EEP	15.93a	0.66c	23.67c	11.98a	5.00a	4.60b
% 10 EEP	17.10a	0.74a	26.04ab	10.57a	5.00a	4.67b
Water control	16.80a	0.74a	22.86c	10.52a	5.00a	4.97a
% 1 WEP	14.20a	0.70abc	26.98a	14.25a	5.00a	5.00a
% 5 WEP	14.13a	0.69bc	26.00ab	14.03a	5.00a	5.00a
% 10 WEP	15.53a	0.72ab	24.64bc	10.11a	5.00a	5.00a
Mean	15.60(a)[Y]	0.71(b)	24.81(b)	12.14(b)	5.00(a)	4.89(a)
After 2 weeks at 0°C						
Ethanol control	14.00c	0.63d	24.38bc	13.96a	4.17b	2.93c
% 1 EEP	15.73abc	0.65bcd	24.46bc	13.51a	4.50ab	2.80c
% 5 EEP	16.03ab	0.66bcd	24.94bc	10.57b	4.83a	2.73cd
% 10 EEP	17.13a	0.73a	23.84c	9.83b	4.00b	2.33d
Water control	15.67abc	0.71ab	24.53bc	11.97ab	5.00a	3.73b
% 1 WEP	14.33abc	0.65bcd	27.01	14.63a	5.00a	3.83ab
% 5 WEP	14.33bc	0.64cd	25.70b	13.30a	5.00a	3.90ab
% 10 WEP	16.93a	0.70abc	24.80bc	10.54b	5.00a	4.27a
Mean	15.52(a)	0.67(c)	24.96(b)	12.28(b)	4.69(b)	3.32(b)
After 3 weeks at 0°C						
Ethanol control	14.67a	0.60a	26.09a	14.74a	3.50c	1.90e
% 1 EEP	15.07a	0.67a	25.14a	13.24a	3.83bc	2.43d
% 5 EEP	15.73a	0.65a	26.84a	13.81a	3.83bc	2.70cd
% 10 EEP	15.87a	0.65a	25.95a	11.53a	4.33ab	2.53d
Water control	15.53a	0.67a	26.24a	13.23a	4.83a	3.07bc
% 1 WEP	13.93a	0.60a	27.05a	14.56a	5.00a	3.57ab
% 5 WEP	15.67a	0.66a	25.40a	12.36a	4.83a	3.80a
% 10 WEP	15.80a	0.67a	24.97a	10.83a	5.00a	3.13bc
Mean	15.28(a)	0.65(d)	25.96(a)	13.04(b)	4.40(c)	2.89(c)
After 4 weeks at 0°C						
Ethanol control	14.67a	0.59a	25.72a	14.49a	2.33c	1.67c
% 1 EEP	14.80a	0.59a	25.56a	13.89a	3.00c	2.73b
% 5 EEP	14.20a	0.60a	25.84a	15.86a	2.67c	1.93c
% 10 EEP	14.73a	0.59a	25.83a	13.64a	4.00b	2.50b
Water control	14.93a	0.67a	24.59a	14.12a	4.67ab	2.80b
% 1 WEP	15.20a	0.58a	25.60a	14.30a	5.00a	3.43a
% 5 WEP	15.13a	0.62a	25.15a	13.82a	4.67ab	3.57a
% 10 WEP	16.47a	0.62a	25.63a	15.16a	4.17ab	3.93a
Mean	15.02(a)	0.61(e)	25.49(a)	14.41(a)	3.81(d)	2.82(c)

[X]Mean separation was performed by Duncan's Multiple Range Test. Treatment means (n = 3) followed by same letter within column are not significantly different at $p < 0.005$. Treatment means was compared separately for each storage time. [Y]Letters in parenthesis indicates comparison of means of storage time. Values represents mean of all treatments for each storage time. [Z] Appearance and Taste was evaluated on a scale of (1-5) where 1 = very poor, 2 = poor, 3 = fair, 4 = high, 5 = very high.

of stem browning was negligible in cherries treated with water or WEP, but increased significantly in cherries treated with ethanol or EEP (Table-1). No differences in incidence of stem browning were observed between WC fruits and WEP treated fruits. Therefore WEP had no effect in maintaining green stem. EC and EEP treatments resulted in higher incidence of stem browning. This might be due to adverse effect of ethanol used to extract propolis on stem colour. However, Karabulut *et al.*[6] did not observed stem browning in cherries treated with 10 to 50 % ethanol. The 70 % of ethanol used in this study appeared to be an abusively high concentration for stem colour.

Total soluble solids (TSS) content increased while titratable acidity (TA) decreased significantly during storage (Table-2). Propolis treatments had no or little effect on TSS content and TA content of cherries.

Skin colour parameters of L* value (lightness) increased during storage compared to initial value at harvest (Table-2). Propolis treatments had no or little effect on skin lightness during storage. High relative humidity inside box liner might contribute to brighter cherries during storage.

Skin colour parameters of hue angle (h°) increased during storage compared to initial value at harvest (Table-2), indicating skin colour became dark mahogany. This change during cold storage is mainly due to a decrease of a* parameter and b* parameter of skin colour (data not shown) which reflects the cherry-red colour transition to purple[32]. Similarly, Crisosto *et al.*[23] observed increased in h° of Bing cherries stored in solid box liners during the 45 day storage period. Propolis treatments did not affected h° value of skin colour during storage (Table-2).

Sensory evaluation by numbers of the panel revealed a decline in external appearance during storage (Table-2). Ethanol extracted propolis treated cherries became unacceptable after 4 weeks of storage while WEP treated cherries remained acceptable throughout the storage. Panelists also evaluated the taste of cherries. Water extracted propolis treated cherries had higher rating for taste compared to EEP treated cherries (Table-2). Taste scores of EEP treated and EC cherries dropped to below 3 after 2 weeks of storage. Water extracted propolis treated cherries was rated as 3 or higher for 4 weeks of storage. Adverse effect of EEP on sensory quality might be due to ethanol used to extract propolis since WEP cherries had acceptable appearance and taste throughout the storage.

Ethanol extracted propolis treatments were effective in preventing fungal decay in cherries, but adversely affected sensory quality (appearance and taste) and stem colour of cherries. Reverse is true for WEP treatments. Efficacy of higher concentrations of propolis extracted with either water or ethanol at reduced concentration (< 70 %) in controlling fungal needs further investigation.

None of propolis treatments had any adverse effect of postharvest quality attributes such as weight loss, pitting, skin colour, TSS content and TA.

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