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# The effects of ethanol-dissolved propolis on the storage of grapefruit cv. Star Ruby

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**Abstract:** Propolis has a strong antimicrobial effect and limits the growth of microorganisms. This study was carried out to determine the effect of propolis on the storage life of Star Ruby grapefruit. Fruits were obtained from Mustafa Kemal University, Faculty of Agriculture, Dörtyol Experimental Research Station, Dörtyol, Hatay, Turkey. Fruits were dipped in ethanol-extracted propolis (EEP) in various concentrations (1%, 5%, and 10%) immediately after harvest and then stored at 8 °C and 90% relative humidity for 6 months. The effects of propolis on the incidence of physiological disorders and fungal decay, and some fruit quality characteristics (weight loss, fruit juice content, total soluble solids, titratable acidity, juice pH, percent of fruits with green calyx, and skin color) were assessed at monthly intervals during the storage period. Treatment with 5% EEP was effective in preventing fungal decay. The percentage of weight loss was significantly higher in the control fruits (6.36%-7.83%) than in those treated with 5% EEP (5.71%) and 10% EEP (4.95%) at the end of the storage period. Star Ruby grapefruit treated with 5% EEP was successfully stored at 8 °C for 5 months.

**Key words:** Dipping, grapefruit, postharvest quality, propolis, Star Ruby

## Star Ruby altıntoplarının muhafazası üzerine etanolde eritilmiş propolisin etkisi

**Özet:** Propolis kuvvetli bir antimikrobiyal ve mikroorganizmaların çoğalmasını engelleyici etkiye sahiptir. Bu araştırma Star Ruby altıntoplarının muhafazasına propolisin etkisinin belirlenmesi amacıyla yapılmıştır. Bu araştırmada Hatay ili Dörtyol ilçesi Mustafa Kemal Üniversitesi Ziraat Fakültesi Dörtyol Araştırma ve Uygulama Bahçesinde yetiştiriciliği yapılan Star Ruby altıntopları kullanılmıştır. Derilen Star Ruby altıntopları, propolisin alkolde eritilerek hazırlanan solüsyonunun değişik dozlarına (%1, %5 ve %10) daldırılarak, 8 °C'de %90 oransal nemde 6 ay süreyle muhafaza edilmiştir. Muhafaza sırasında her ay alınan meyve örneklerinde fizyolojik ve mantarsal bozulma oranları ve ağırlık kayıpları, usare oranı, suda çözünebilir kuru madde, asitlik, pH, yeşil kapsüllü meyve ve meyve kabuk rengi gibi bazı meyve kalite özellikleri üzerine propolisin etkisi saptanmıştır. Propolisin alkolde eritilerek hazırlanan %5'lik solüsyonu mantarsal bozulmaların önlenmesinde etkili olmuştur. Ağırlık kayıpları en fazla tanık meyvelerinde (%6.36-7.83) olurken, en az %10 propolis (%4.95) ve %5 propolis (%5.71) meyvelerinde olmuştur. %5 propolis uygulanan Star Ruby meyveleri 8 °C'de 5 ay başarıyla depolanmıştır.

**Anahtar sözcükler:** Altıntop, daldırma, derim sonrası kalite, propolis, Star Ruby

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## Introduction

Most postharvest losses of citrus fruit are due to decay from green (*Penicillium digitatum* Sacc.) and blue (*Penicillium italicum* Wehmer) molds. Currently, both diseases are controlled with chemical fungicides, such as thiabendazole and imazalil (Eckert 1989). However, alternative methods are needed because of the development of pathogen strains that are resistant to these fungicides and increasing public concern regarding pesticide-contaminated food (Palou et al. 2001).

Cold injury, weight loss, and fungal decay are the dominant factors limiting the storage life of grapefruit. In postharvest handling of fruits for market, high concentrations of chemicals are used, especially for the prevention of fungal decay. The result of uncontrolled and excessive use of chemicals negatively affects human health and the environment. In addition, chemical residues on fruit can cause serious problems for export (Özdemir et al. 2005). There is a clear need for alternative natural materials for postharvest disease control that reduce fungal decay and carry lower risks for consumers. Biological control with yeast antagonists (Dündar and Göçer 2001), hot water and hot air treatments (Wild 1990; Schirra and D'Hallewin 1997; Özdemir and Dündar 2001), modified atmosphere packaging (Özdemir and Kahraman 2004), sodium bicarbonate (Smilanick et al. 2005), and chitosan treatment (Chien et al. 2007) are natural alternatives to synthetic chemical postharvest treatments for disease control in citrus.

Propolis is a naturally occurring brownish-green resinous product that honeybees collect from different plant exudates. It possesses many biological properties, including antibacterial, antiviral, and antifungal, and has been used for pharmacological applications (Serkedjieva et al. 1992; Siess et al. 1996; Bosio et al. 2000; Şahinler and Gül 2002; Şahinler and Kaftanoğlu 2005). Although its antimicrobial activity against human pathogenic fungi, bacteria and viruses has been demonstrated, (Burdock 1998; Kujumgiev et al. 1999), very few in vitro and in vivo studies have been conducted against plant pathogenic microorganisms (Fahny and Omar 1989; Abd al-Fattah et al. 1995; Özcan 1999; Quiroga et al. 2006). The application of 1%, 5%, and 10% concentrations of ethanol-extracted propolis (EEP) inhibited *P. digitatum* growth in vitro (Soylu et al. 2004, 2008) and limited the growth of *B. cinerea* on strawberry (La Torre et al. 1990). The application of 5% and 10% concentrations of EPP extended the storage life of Fremont mandarins, as compared to untreated control fruits (Özdemir et al. 2005). Treatment with EEP was also effective in preventing fungal decay in cherries stored for 4 weeks, but adversely affected sensory quality and stem color (Çandır et al. 2009).

The optimum storage condition for grapefruit is 8 °C and 85%-90% relative humidity (Pekmezci et al. 1984, 1995; Gürgen et al. 1984; Kaşka and Dündar 1992; Özdemir et al. 2008).

The objective of the present study was to determine the effects of environmentally friendly EEP treatment on the quality of Star Ruby grapefruit. The present study was undertaken to evaluate the efficacy of EEP in controlling fungal decay in grapefruits cv. Star Ruby during storage and to determine the effects of EEP on maintaining fruit quality during storage.

## Materials and methods

### Preparation of propolis extracts

Crude propolis was gathered by hand from Hatay province in the eastern Mediterranean region of Turkey. The propolis exudates collected by bees (*Apis mellifera anatoliaca*, *A. m. caucasica*, *A. m. syriaca*, and their hybrids) in Hatay province were primarily from a mixture of wild and medicinal aromatic plant species, including *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 stored in a desiccator and away from exposure to light until further processing.

Propolis extracts were prepared as described by Krell (1996). Propolis was frozen to −20 °C, cut in small pieces, and ground in a chilled mortar. Then, 10% EEP was prepared by adding 100 g of the collected propolis to 900 mL of 70% ethanol and agitating for 1 week. Water was then added for 3 days. The mixture was maintained at room temperature during preparation and was subsequently filtered. The

extracts were kept at 4 °C in dark storage until use. Propolis and its extracts should be stored in airtight containers in the dark. During 12 months of proper storage propolis will lose very little or none of its antibacterial activity. Alcohol extracts may be stored even longer. The amount of dissolved principles was assessed by weight difference. The 1% and 5% propolis extracts were prepared by making a dilution of the 10% propolis solution with 70% ethanol in the required proportions.

**Plant material**

Fruits of Star Ruby cultivar were obtained from 6-year-old trees grafted on *sour orange* rootstock that were planted 7 × 7 m apart at the Dörtyol (Hatay, Turkey) Research Station (36°09′E, 36°51′N, altitude 9 m) of Mustafa Kemal University, Faculty of Agriculture in 2004.

**Propolis treatment**

Grapefruits were subjected to the following treatments: (1) no treatment; (2) dipping in water (water control, WC); (3) dipping in 70% ethanol (ethanol control, EC); (4) dipping in EEP at concentrations of 1%, 5%, and 10%. Treatments 1, 2, and 3 were used as controls for the varied concentrations used in treatment 4. Each treatment contained 3 replicates of about 30 fruit. Fruits were placed in plastic boxes (60 × 40 × 30 cm), and stored at 8 ± 0.5 °C and 90 ± 5% relative humidity (RH) for 6 months.

**Postharvest quality evaluation**

Postharvest fruit quality was assessed at monthly intervals during the storage period. Fruit weight was recorded at the beginning and during the storage period, and is expressed as percentage of weight lost. Skin color was determined twice on the equatorial section of fruits with a Minolta CR-300 Chroma Meter (Osaka, Japan) using the CIE $L^*a^*b^*$ color space. The pH of fruit juice was measured using a digital pH meter. Total soluble solids (TSS) content was determined with a refractometer (Atago model ATC-1E). Titratable acidity (TA) was measured by titration of 5 mL of fruit juice with 0.1 N NaOH to pH 8.1 and is expressed as grams of citric acid per 100 mL of juice. Fruit juice content was calculated by dividing the weight of extracted juice by fruit weight and multiplying by 100. Moreover, the incidence of physiological disorders, fungal decay, and green calyx visually observed are expressed as a percentage of total fruits sampled. The incidence of physiological disorders was determined when injury covered more than 25% of the rind surface.

**Statistical analysis**

The data were analyzed as a factorial experiment in a completely randomized block design by ANOVA using SAS software (SAS, 1990. SAS/STAT, v.6.0, SAS Institute, Cary, NC, USA). Each treatment consisted of 30 grapefruits replicated 3 times. Mean separation was performed by Tukey's test at the $P < 0.05$ level using the SAS Proc GLM procedure. Data for percentage of weight loss, fungal decay, physiological disorders, and green calyx were arcsine-transformed and analyzed by ANOVA and back-transformed for reporting.

## Results

Weight loss increased as storage time increased (Figure 1). After 6 months weight loss reached 12.43%, without any observable shrivels. The EEP treatments reduced weight loss, as compared to the control treatments.

The incidence of fungal decay increased significantly after 4 months of storage (Figure 2). Treatment with 5% EEP produced fruits with a significantly reduced incidence of fungal decay, as compared to the control treatments. The incidence of fungal decay was highest in the 10% EEP-treated fruits (Figure 2).

Physiological disorders did not occur in any of the treated fruits, except in the case of the 10% EEP-treated fruits. Fruits treated with 10% EEP did not show evidence of physiological disorders for 3 months of storage. After 6 months of storage, however, the incidence of physiological disorders reached 1.85% in these fruits (data not shown).

Skin color L value (lightness) increased during storage (Figure 3). No differences in the skin color L value were observed between the control fruits and the EEP-treated fruits (Figure 3).

Hue values increased during storage, as compared to the time of harvest (Figure 4). The effects of EEP treatment were significant on this parameter during

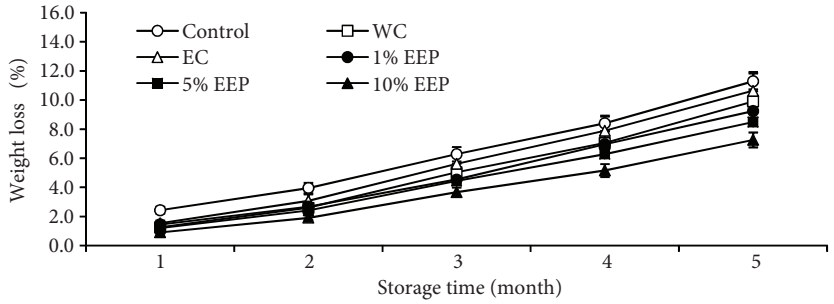


Figure 1. The effects of EEP on weight loss in grapefruit cv. Star Ruby during 6 months of storage at 8 °C.

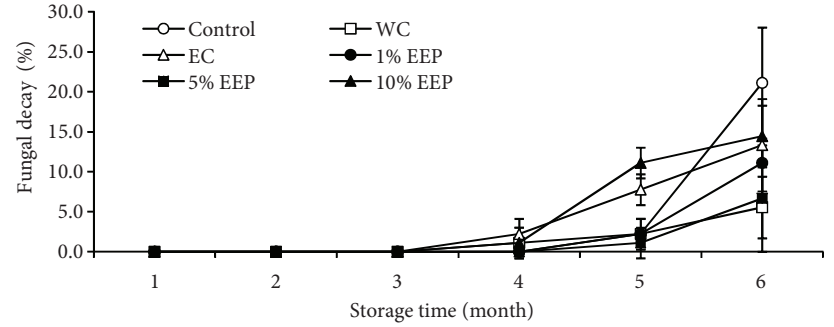


Figure 2. The effects of EEP on fungal decay in grapefruit cv. Star Ruby during 6 months of storage at 8 °C.

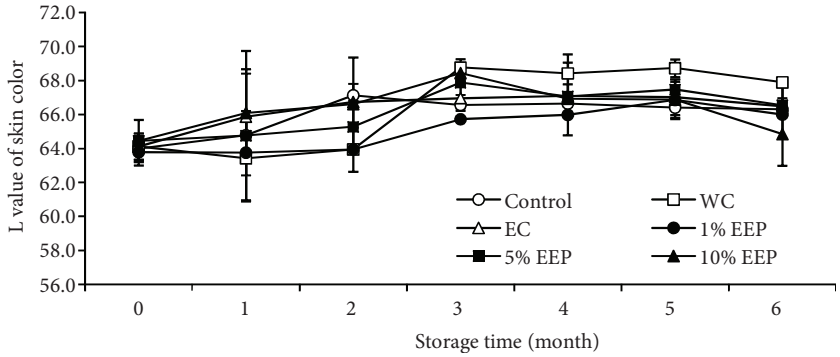


Figure 3. The effects of EEP on L value of skin color in grapefruit cv. Star Ruby during 6 months of storage at 8 °C.

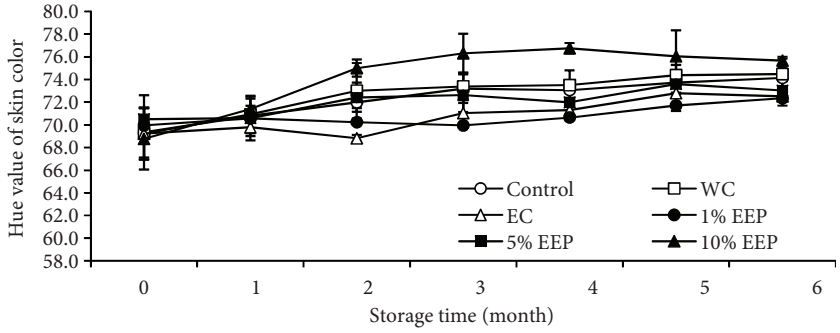


Figure 4. The effects of EEP on hue value of skin color in grapefruit cv. Star Ruby during 6 months of storage at 8 °C.

storage. Fruits treated with 10% EEP had the highest hue value (Figure 4).

Fruit juice content decreased during storage. The effects of EEP treatment on fruit juice content were not significant (Figure 5). The TSS content and the TA decreased, while the pH value increased significantly during storage (Figures 6-8); however, the effects of EEP treatment on the TSS content and the TA were not significant during 6 months of storage (Figures 6 and 7). The percentage of fruit with green calyxes was higher in the EEP-treated fruits than in the control fruits (Figure 9).

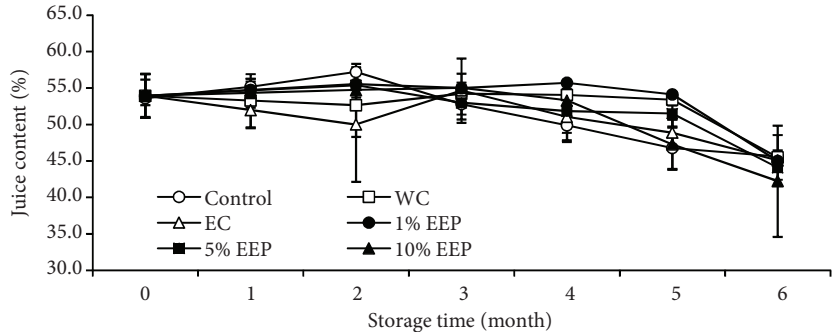


Figure 5. The effects of EEP on juice content in grapefruit cv. Star Ruby during 6 months of storage at 8 °C.

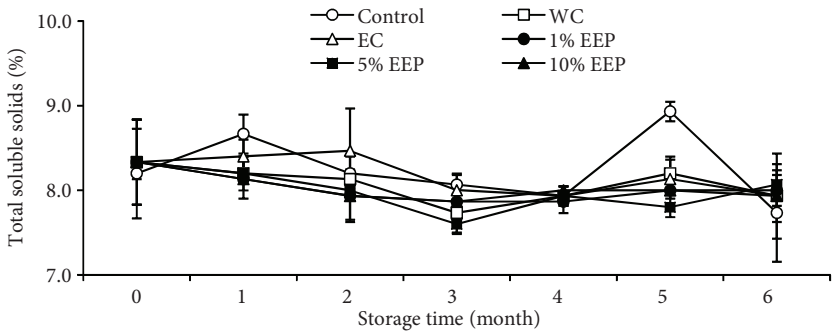


Figure 6. The effects of EEP on total soluble solids in grapefruit cv. Star Ruby during 6 months of storage at 8 °C.

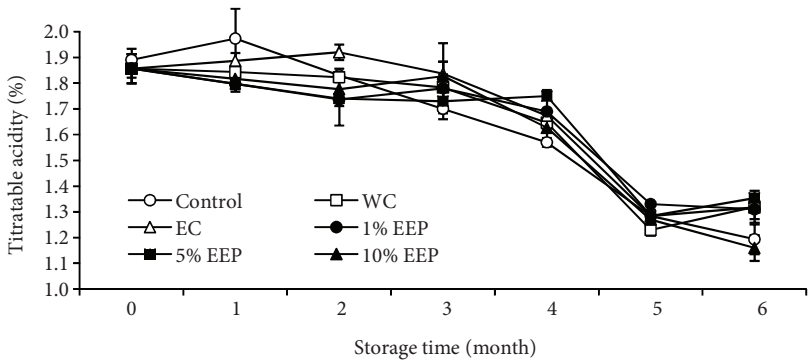


Figure 7. The effects of EEP on titratable acidity in grapefruit cv. Star Ruby during 6 months of storage at 8 °C.

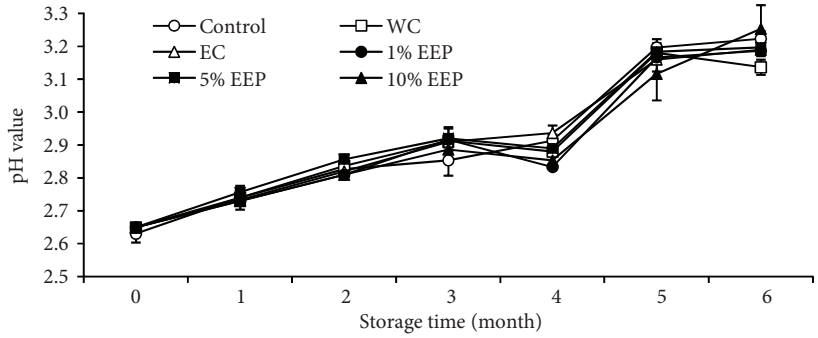


Figure 8. The effects of EEP on the pH value in grapefruit cv. Star Ruby during 6 months of storage at 8 °C.

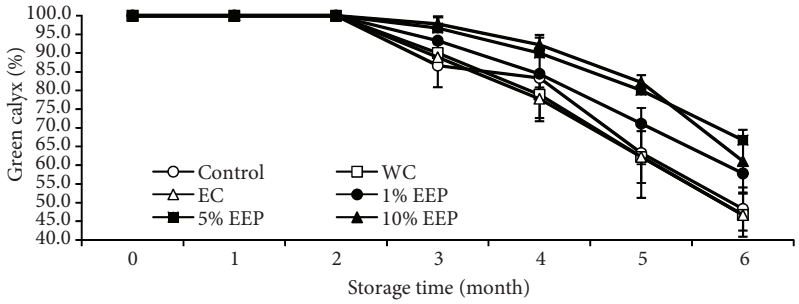


Figure 9. The effects of EEP on green calyx in grapefruit cv. Star Ruby during 6 months of storage at 8 °C.

## Discussion

Weight loss is a significant factor in citrus quality deterioration and increases susceptibility to fungal decay. In general, the fruit becomes unmarketable when weight loss exceeds 10% of the total weight of fresh produce. When propolis covered the surface of the fruit, weight loss did not exceed 10% until the sixth month of storage. In addition, it was reported that 2%-3% weight loss per month in citrus may be related to storage in conditions outside the ideal 85%-90% relative humidity and appropriate temperature (Gürgen et al. 1984; Waks et al. 1985). Water loss, which is the most important factor in the storage of fruits and vegetables, accounted for a large portion of the total weight loss observed. Water loss in the EEP-treated fruits was less than in the fruits subjected to the control treatments (Figure 1). EEP treatment covered the surface of the fruits. Similar results were obtained in other studies that tested variability in weight loss when treatments and products that covered the surface of fruits were applied (Kaşka and Dündar 1992; Pekmezci et al. 1995; Hagenmaier and Baker 1996; Özdemir and Dündar 1999, 2001). Similarly, Özdemir et al. (2005) and Çandır et al. (2009) reported that weight loss was lower in propolis-treated cherries during storage.

Generally, EEP treatment of grapefruits can be commercially used to reduce fungal decay. In the present study the incidence of fungal decay was highest in the 10% EEP-treated fruits (Figure 2). Physiological disorders were the main cause of fungal decay in the 10% EEP-treated fruits. Özcan (1999) showed that treatment with 4% water-extracted propolis resulted in more than 50% inhibition of some plant pathogens, including *P. digitatum* and *B. cinerea*, in vitro. The antimicrobial action of different extracts of propolis from various geographic regions was also compared (Garedew et al. 2004). For all propolis samples tested the level of antimicrobial activity decreased along with the order of EEP and propolis volatiles. The extraction of propolis with ethanol procures all of the water-soluble, ethanol soluble, and volatile components of propolis, making EEP superior to the other 2 extracts, qualitatively and quantitatively.

EEP treatment inhibited *P. digitatum* growth in vitro (Soylu et al. 2004, 2008) and limited the growth of *B. cinerea* on strawberry (La Torre et al. 1990). Our previous research showed some promising results related to the antifungal activity of EEP. EEP treatment provided complete inhibition of naturally occurring green mold disease on wounded and uninoculated grapefruits (Soylu et al. 2004, 2008). EEP treatment also resulted in a slightly lower incidence of fungal decay in Fremont mandarins than in control fruits during the storage period (Özdemir et al. 2005). Data from another study indicated that EEP might provide inhibition of fungal decay in cherries for 4 weeks of storage, which is a sufficient time for marketing (Çandır et al. 2009).

Physiological disorders were defined by the presence of a localized brown-gray stain on the fruit surface. This discoloration progressed to cover the entire fruit surface and secondary pathogens developed on the surface during prolonged storage. This was not related to cold injury or other known physiological disorders. This might have been a result of the adverse effect of 10% EEP treatment. This is the main cause of fungal decay in the 10% EEP-treated fruits. The observed disorder was in fact not truly a physiological disorder, but was not fungal decay either, because in vitro we did not find any fungal pathogens on the disorder fruits. Propolis treatment did not affect physiological disorders in cherries during 4 weeks of storage (Çandır et al. 2009), but Özdemir et al. (2005) reported that propolis treatment reduced physiological disorders in mandarins during 4 months of storage.

Çandır et al. (2009) reported that propolis treatment had little or no effect on skin lightness in cherries during storage. Çandır et al. (2009), on the other hand, reported a significant effect of propolis treatment on the hue value of skin color in cherries during storage. This change is mainly due to a decrease in the $a^*$ and $b^*$ parameters of skin color (data not shown).

The effects of propolis treatment on fruit juice content were not significant during 6 months of storage (Figure 5). Similar results were obtained by Özdemir et al. (2005). Özdemir et al. (2005) also reported that propolis treatment had little effect on the TSS content and on the TA content of Fremont mandarins during 4 months of storage. Çandır et al. (2009) reported that propolis treatment had little or no effect on the TSS content and the TA content of cherries during 4 weeks of storage.

Green calyxes were protected by the EEP treatment (Figure 9). Calyx browning typically develops during citrus storage and has been associated with fruit ripening (Özdemir and Dündar 1999), but may also be due to dehydration (Özdemir et al. 2008). Similarly, in many studies on grapefruit storage it was determined that green calyxes decreased and the incidence of browning increased over time (Gürgen et al. 1984; Pekmezci et al. 1984; Özdemir et al. 2008). Çandır et al. (2009) reported that the incidence of stem browning increased significantly in cherries treated with EEP during 4 weeks of storage. Özdemir et al. (2005), on the other hand, reported no significant effect of propolis treatment on green calyxes in Fremont mandarins.

According to the data we obtained, EEP treatment was effective in preventing fungal decay in grapefruits, but adversely affected sensory quality (appearance and taste) in all of the fruits treated with 10% EEP. All of the propolis treatments had a positive effect on postharvest quality attributes, such as weight loss, skin color, and green bottom.

The 5% EEP treatment was effective in preventing fungal decay in grapefruits. The 5% EEP treated Star Ruby grapefruits were successfully protected for about 5 months at 8 °C and 85%-90% RH, without losing important qualities after the harvest. The fruits treated with propolis were brighter and flashier in other treatments. As such, the fruits treated with propolis did not require waxing, which is commonly used to increase the attractiveness for commercial sales in packinghouse operations.

The appearance of the 10% EEP-treated fruits was very poor, especially after 6 months of storage. Furthermore, the 10% EEP-treated fruits were sticky and left a stain on the fingers and hands after handling, as well as on the sides of plastic storage boxes. This is not a desired situation. Moreover, acceptance by consumers will be difficult.

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