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Effect of Natural Waxes on Quality and Storability of Sweet Oranges (*Citrus sinensis* L.)

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Received: May 2021 **Accepted: August: 2021**

Abstract

Sweet orange fruits loss moisture and shrivel rapidly and lose consumer appeal during marketing and storage. Waxing was reported to reduce water loss, delay fruit ripening and senescence, maintain quality and extend shelf-life in orange. The effect of three natural waxes (bee, carnauba and jojoba) on quality and storability of ‘Sinnari’ and ‘Nori 16’ orange cultivars was evaluated. Parameters studied were determined using standard methods. Analysis of variance with a significance level of P≤0.05 were performed on the data using the Statistic Software Version 8.0. The three natural waxes significantly delayed senescence, reduced losses, maintained quality and extended shelf-life of orange fruits during storage for 18 weeks at 4±1 ˚C and 85- 90 % relative humidity. The results indicated that the wax treatments significantly decreased respiration rate (30.3, 21.3 and 12.3 %), reduced water loss (32.5, 12.7 and 7.8 %), decreased peel color development (31.7, 23.3 and 13.3 %), fruit softening (16.5, 12.4 and 6.8 %), and total soluble solids accumulation (10.4, 7.1 & 4.1 %), and increased the retained ascorbic acid content (45.4, 31.6 and 20.5 %) in fruits treated with bee wax, jojoba wax and carnauba wax, respectively, compared with the unwaxed (control) fruits. Bee wax was the most effective, followed by jojoba wax and least was the carnauba wax. It was recommended that natural waxes, especially bee and jojoba waxes can effectively be used to delay fruit senescence, reduce weight loss, maintain quality and extend shelf-life of orange fruits.

Keywords: Orange fruits; natural waxes; bee wax; carnauba wax; Jojoba wax; fruit quality; shelf-life

Introduction

The sweet orange (*Citrus sinensis* L.) is the most important citrus fruit. It is widely grown in all regions of the world adapted to citrus. World production of sweet oranges increased from 39.7 million metric tons (MMT) in the 1990s to 69.9 MMT in 2014 (FAO, 2016). In Sudan, oranges are grown in Kassala, River Nile, Northern, Sinnar, South Kordofan, South and West

Darfur States, with total annual production of 153.3 thousand tons (HSA, 2017).

Citrus fruits are popular, due to their characteristic favor, taste, aroma and multiple health benefits. Citrus fruits are very good source of vitamin C, ranking first in their contribution of vitamin C in human nutrition and provide significant amounts of antioxidants, such as beta-carotene and flavonoid compounds (Kader, 2002).

Sweet orange fruits loss moisture and shrivel rapidly and lose consumer appeal during marketing and storage. Waxing was reported to reduce water loss, delay fruit ripening and senescence, maintain quality and extend shelf-life in orange (Shahid, 2007), grapefruit (Abu-Goukh and Elshiekh, 2008), lime (Abdallah and Abu-Goukh, 2010), mango (Mohamed and Abu-Goukh, 2003) and tomato (Mohammed *et al*., 2018). Many countries allow food-grade waxes to be applied to fruits and vegetables, such as apples and oranges, to reduce water loss and increase consumer appeal. The rate of water loss can be reduced by 30-50 % under commercial conditions (Wills and Golding, 2016).

Most waxes in commercial use are proprietary formulations which may contain a mixture of waxes derived from plant and petroleum sources (Wills and Golding, 2016). Different waxes have different characteristics and functions. Many of these formulations have been based on a combination of paraffin wax, which gives good control of water loss, but a poorer luster to the produce, and carnauba wax, which imparts an attractive luster to the produce, but provides poorer control of water loss (Wills and Golding, 2016).

This study was carried out to evaluate the effect of three natural waxes (bee, carnauba and jojoba) on quality and storability of ‘Sinnari’ and ‘Nori-16’ sweet orange cultivars.

Materials and Methods

Experimental material

Orange fruits of ‘Sinnari’ and ‘Nori-16’ cultivars were harvested at color break from hard-green to light-green stage from Abdul-Gader Elburhani orchard at Shendi, River Nile State (18º.17` N, 24º.23` E). Fruits were selected for uniformity of size, color and freedom from blemishes and defects. Fruit were transported in field boxes to the laboratory of the Department of Horticulture, Faculty of Agriculture, University of Khartoum. The fruits were washed with tap water to remove dust treated with 0.1 % sodium hypochlorite (Clorox, 5 %) as a disinfectant then air dried.

Preparation of waxes

Jojoba oil was extracted from seeds collected from Arkaweit area, Red Sea State according to the method of Eltinay (1988). Transformation of jojoba oil to trans (Isomerizes) oil (TJO) was carried out by Arnon method (Arnon, 1981). Fifty grams from each of the 3 waxes were dissolved in 50 ml of olive oil.

Fruits treatment

The fruits from each cultivar (880 fruits) were distributed among the four treatments in a completely randomized design with four replications. The treatments were: (1) Unwaxed (control), (2) Bee wax, (3) Carnauba wax and (4) Jojoba wax (Trans Jojoba oil). The wax treatments were applied by brushing in a thin layer over the surface of the fruits. The fruits were stored in carton boxes for 18 weeks at 4±1 ºC and 85-90 % relative humidity at Wafra For Modern Storage Co. Ltd., Khartoum North.

Parameters studied

Respiration rate, peel color, weight loss were determined on 15 fruits from each replicate every two weeks during the storage period. Respiration rate was determined using the total absorption method of Charlimers (1956) as modified by Mohamed-Nour and Abu-Goukh (2010), and was expressed in mg CO_2/kg-hr. Peel color was determined using color score; light-green (=0), trace yellow, (1), 20 % yellow (=2), 40 % yellow (=3), 60 % yellow (=4), 80 % yellow (=5), 100 % yellow (=6). Weight loss (%) was calculated according to the formula: $W_1= [(W_0-W_t)/W_0] \times 100$; where ($W_1$) is the percentage weight loss as the designed time, (W_0) is the initial weight of fruits, and (W_t) is the weight of fruits at the designated time.

Fruit flesh firmness, total soluble solids (TSS), titratable acidity, and ascorbic acid content were determined on three fruits picked randomly every two weeks during the storage period. Fruit firmness was measured by Dial Fruit Sclerometer (AGY). Two reading were taken from opposite sides of each fruit after the peel was removed, and was expressed in kilograms per square centimeter. TSS was determined directly on fruit juice extracted by pressing the fruit pulp in a garlic press, using a Kruss hand refractometer (Model HRN-32). Two readings were taken from each fruit and the mean values were calculated and corrected according to the refractometer chart.

Thirty grams of fruit pulp from the 3 fruits used for determination of flesh firmness and TSS were homogenized in 100 ml. of distilled water for one minute in a Panasonic Solid State Blender (Model MX-GX1571) and centrifuged at 10,000 rpm for 10 minutes using a Gallenkamp Protable Centrifuge (CF-400). The volume of the supernatant, which constitutes the pulp extract, was determined. Titratable acidity was determined by the method described by Ranganna (1979) and expressed as percent citric acid. Ascorbic acid content in the pulp extracts was determined using the 2,6-dichlorophenol-indophenol titration method of Ruck (1963). Thirty grams of fruit pulp were homogenized in100 ml of oxalic acid in Panasonic Solid State Blender (Model MX. GX 1571) and centrifuged at 10,000 rpm for 10 minutes using a Gallenkamp portable centrifuge (CF-400). The volume of the supernatant was topped to 250 ml of oxalic acid. Ascorbic acid was expressed in milligrams per 100 grams fresh weight.

At the end of storage period, the fruits were evaluated for general quality. Percentage of fruits in each quality grade; very good, good, fair, and poor, for each treatment was determined.

Statistical analysis

Analysis of variance and Fishers protected LSD test with a significance level of $P \leq 0.05$ were performed on the data using the Statistic Software Version 8.0.

Results and Discussion

The three natural waxes significantly delayed senescence, maintained quality and extended shelf-life of ‘Sinnari’ and ‘Nori-16’ orange cultivars during storage for 18 weeks at 4± 1 ºC and 85-90 % relative humidity. Similar results were reported for orange (Ahmed *et al*., 2007), grapefruit (Abu-Goukh and Elshiekh, 2008), lime (Abdallah and Abu-Goukh, 2010), mango (Mohamed and Abu-Goukh, 2003), and tomato (Mohammed *et al*., 2018). These effects of waxing on quality and extension of shelf-life of oranges were reflected in changes in respiration rate, peel color, water loss, firmness, total soluble solids (TSS), titratable acidity and ascorbic acid content.

Effect on respiration rate

The respiration curves in all treatments exhibited a typical non-climacteric pattern (Fig. 1). Citrus fruits are classified as non-climacteric fruits, which exhibit most of the ripening changes, although these usually occur more slowly than those of the climacteric fruits (Kader, 2002; Wills and Golding, 2016). Similar results were reported in grapefruit (Abu-Goukh and

Elshiekh, 2008) and lime (Abdallah and Abu-Goukh, 2010). Respiration rate was significantly lower in ‘Sinnari’ cultivar than ‘Nori-16’ and at the end of the 18-weeks storage period respiration rate was 15.6 and 20.2 mg CO_2/kg-hr., in the two cultivars, respectively. These values were significantly decreased by an average of 12.3, 21.3 and 30.3 % in fruits treated with carnauba wax, jojoba wax and bee wax, respectively, compared to untreated (control) fruits (Fig. 1). Waxing was reported to reduce respiration in orange (Martinez *et al.*, 1991), grapefruit (Abu-Goukh and Elshiekh, 2008), lime (Abdallah and Abu-Goukh 2010), mango (Mohamed and Abu-Goukh 2003), and tomato (Mohammed *et al*., 2018). Waxing has been shown to influence respiration rate by decreasing oxygen (O_2) and increasing carbon (CO_2) dioxide content in the internal atmosphere of the fruits (Irving and Warren, 1960).

Effect on peel color

Peel color score progressively increased during storage of orange fruits regardless of the treatments used. At the end of the 18 weeks the storage period, the unwaxed (control) fruits reached the maximum yellow stage (color score 6) in the two cultivars, while the fruits treated with bee wax, jojoba wax and carnauba wax only reached 5.2, 4.7 and 4.0 color score in ‘Sinnari’ and 5.2, 4.5 and 4.2 color score in ‘Nori-16’, respectively (Fig. 2). The peel color development was reduced on average in the two cultivars by 31.7, 23.3 and 13.3 % in fruits treated with bee wax, jojoba wax and carnauba wax, respectively, compared to untreated (control) fruits. This is in line with earlier reports that waxing delayed chlorophyll degradation and skin color development in orange (Martinez *et al.*, 1991), grapefruit (Abu-Goukh and Elshiekh, 2008), lime (Abdallah and Abu-Goukh, 2010), mango (Mohamed and Abu-Goukh, 2003) and tomato (Mohammed *et al*., 2018). Waxing and surface coating materials significantly alter permeability of skin to gasses. The fruit, through respiration is used to reduce O_2 and increase CO_2. Under such restricted air-exchange condition, a modified atmosphere condition may be generated and some of the benefits of the modified atmosphere may be achieved (Kader, 2002). Modified atmosphere reduces the rates of respiration and ethylene production, ripening and senescence and other metabolic processes. CO_2-enriched atmosphere may also inhibit the action of ethylene, since CO_2 is a competitive inhibitor of ethylene action (Kader, 2002).

Effect on weight loss

Weight loss progressively increased during storage of orange fruits regardless of the treatment in the two cultivars. More weight loss was recorded in ‘Nori-16’ than ‘Sinnari’ cultivar in all treatments. At the end of the storage period, weight loss in the unwaxed (control) fruits was 29.8 % in ‘Sinnari’ and 34.9 % in ‘Nori-16’ orange cultivars (Fig. 3). The wax treatment significantly reduced weight loss in both cultivars. More reduction in weight loss was found in bee wax, followed by jojoba wax, and then carnauba wax in the two cultivars. At the end of storage periods, weight loss was reduced on average of the two cultivars by 32.5, 12.7 and 7.8 % in fruits treated with bee wax, jojoba wax and carnauba wax, respectively, compared to the control. Ahmed *et al*. (2007) reported that trans jojoba oil (TJO) on ‘Valencia’ orange fruits acts as barriers not only to gases migration to restrict respiration, but also to water vapor transfer, reducing transpiration and weight loss. Waxing was reported to reduce water loss in orange (Martinez *et al*,. 1991; Shahid 2007), grapefruit (Abu-Goukh and Elshiekh, 2008), lime (Abdullah and Abu-Goukh, 2010), mango (Mohamed and Abu-Goukh, 2003), and tomato (Mohammed *et al*., 2018).

Effect on fruit flesh firmness

Fruit flesh firmness progressively declined during storage of orange fruits in all treatments in both cultivars (Fig. 4). At the end of the storage period the fruit flesh firmness of the unwaxed fruits was 6.5 and 6.2 kg/cm^2 in 'Sinnari' and 'Nori-16' orange fruits, respectively. The wax treatments delayed the drop in flesh firmness during storage of orange fruits. The treated fruits were more firm than the control at any time during the storage period. At the end of the storage period, the fruit firmness was 7.1, 13.4 and 18.5 % more firm in 'Sinnari' and 6.5, 11.3 and 14.5 % in 'Nori-16' orange fruits treated with carnauba, jojoba and bee waxes, respectively, compared with the control (Fig. 4). This agrees with the findings of Martinez *et al.* (1991) and Abdallah and Abu-Goukh (2010) who observed a decrease in flesh firmness during storage of orange and lime fruits, respectively. Waxing was reported to delay fruit softening in mango (Mohamed and Abu-Goukh, 2003) and tomato (Mohammed *et al*., 2018). Fruit softening is characterized by changes in flesh firmness and has long been associated with ripening and senescence. These changes in fruit flesh firmness determine shelf-life and quality of the commodity (Wills and Golding, 2016).

Effect on total soluble solids

Total soluble solids (TSS %) of the two cultivars showed continuous increases during storage of orange fruits in all treatments (Fig. 5). The maximum TSS value reached at the end of storage period was 11.5 % in 'Sinnari' and 12.4 % in 'Nori-16' orange cultivars. This was in agreement with earlier reports that TSS increased during storage of grapefruit (Abu-Goukh and Elshiekh, 2008), lime (Abdallah and Abu-Goukh, 2010) and tomato (Mohammed *et al*., 2018).

The maximum TSS value reached at the end of storage period was 11.5 in 'Sinnari' and 12.4 % in 'Nori-16' orange cultivars. The accumulation in TSS was reduced on average of the two cultivars by 10.4, 7.1 and 4.1 % in fruits treated with bee wax, jojoba wax and carnauba wax, respectively, compared with unwaxed (control) fruits (Fig. 5). The increase in TSS during storage was attributed to the loss of moisture content, which led to the concentration of TSS (Abu-Goukh *et al*,. 2001; Abdallah and Abu-Goukh, 2010). Thus, the less increase in TSS during storage of orange fruit treated with waxes, compared to the control was obvious, since in both cultivars the wax treatments decreased water loss during storage (Fig. 3). A positive relationship was found between the increase in TSS and weight loss during storage of orange. This agrees with the findings of Abdallah and Abu-Goukh (2010) and Abu-Goukh *et al.* (2001), who found positive correlation between TSS and weight loss during storage of limes and onions, respectively.

Effect on titratable acidity

Titratable acidity increased during storage in all treatments in both cultivars up to 6 weeks of storage and then declined towards termination of storage period (Fig. 6). In 'Sinnari' fruits it increased from 0.45 % at harvest to 0.77 % after 6 weeks in storage and then decreased to 0.28 % at the end of storage period, while in 'Nori-16', it increased in the untreated fruits from 0.69 % to 1.1 % and then decreased to 0.29 % at the end of the storage period (Fig. 6). This is in agreement with Abu-Goukh and Elshiekh (2008) in grapefruits and Mohammed *et al*. (2018) in tomatoes, who reported that titratable acidity increased for 2-6 weeks during storage and then decreased till the end of storage period. Wax treatments decreased titratable acidity during storage of orange fruits. At the end of storage period the titratable acidity was 0.19, 0.12 and 0.01

% in 'Sinnari' and 0.18, 0.08 and 0.02 % in 'Nori-16' orange fruits treated with carnauba wax, jojoba wax and bee wax, respectively (Fig. 6). This might be explained in terms of weight loss, which was less in the waxed fruits which may lead to concentration of the acid in the fruit tissues. It was reported that waxing reduces water loss and decreased acidity during storage of orange (Martinez *et al.*, 1991; Ahmed *et al*., 2007), grapefruit (Abu-Goukh and Elshiekh, 2008), lime (Abdallah and Abu-Goukh, 2010) and tomato (Mohammed *et al*., 2018).

Effect on ascorbic acid content

Ascorbic acid content steadily declined during storage of orange fruits in all treatments in both cultivars. It decreased in the unwaxed fruits from 48.8 to 11.5 mg/100g fresh weight in 'Sinnari and from 46.9 to 15.3 in 'Nori-16' oranges (Fig. 7). This is in agreement with previous reports that showed a rapid decline in ascorbic acid content during storage of orange and pineapple (Adisa, 1968), mango (Mohamed and Abu-Goukh, 2003) and tomato (Mohammed *et al*., 2018).). The wax treatments significantly retained ascorbic acid content. At the end of the storage period, it was higher on average of the two cultivars by 45.4, 31.6 and 20.5 % in orange fruits treated with bee wax, jojoba wax and carnauba wax, respectively, compared with the unwaxed (control) fruits (Fig. 7). This is in agreement with the previous reports that waxing and surface coating retain ascorbic acid content in lime (Abdallah and Abu-Goukh, 2010) and mango (Mohamed and Abu-Goukh, 2003).

Effect on general quality

At the end of storage period, the fruits were evaluated for general quality. Table 1 showed percentages of fruits in each quality grades. In the unwaxed (control) fruits, none was found in the 'very good' quality grade in both cultivars, and only 4.3 % and 5.4 % of fruits were found in 'good' quality grade in 'Sinnari' and 'Nori-16' cultivars, respectively. Fruits treated with bee wax, jojoba wax and carnauba wax resulted on average in the two cultivars of 57.8, 52.7 and 22.1 % of fruits in 'very good' quality grades and 21.1, 19.4 and 41.9 % in 'good' quality grade, respectively. On the other hand, fruits in the 'poor' quality grade were 35.7 % and 42.1 % in the unwaxed fruits, compared to 5.8 and 3.0 % in bee wax, 4.9 and 1.2 % in jojoba wax, and 11.0 and 12.1 % in carnauba wax, respectively, in 'Sinnari' and 'Nori-16' orange fruits (Table 1). Waxing was reported to reduce water loss, increase consumer appeal, delay ripening and senescence, maintain quality and extend shelf-life in many fruits (Shahid, 2007; Abu-Goukh and Elshiekh, 2008; Abdallah and Abu-Goukh, 2010; Mohammed *et al*., 2018).

Conclusion

The wax treatments significantly decreased respiration rate, reduced water loss, decreased peel color development, fruit softening and total soluble solids accumulation and increased the retained ascorbic acid content. It is recommended that natural waxes, especially bee and jojoba waxes can effectively be used to delay fruit senescence, reduce weight loss, maintain quality and extend shelf-life and storability of orange fruits.

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تأثيـر الشـموع الطبيعية على الجـودة والعمــر التخزيني لثمــار البرتقـــال

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المستخلص

تفقد ثمار البرتقال الحلو الرطوبة وتذبل بسرعة وتفقد مظهرها الجذاب أثناء التسويق والتخزين. ثبت أن التشميع يقلل من فقد الماء، ويؤخر الإنضاج والشيخوخة، ويحافظ على الجودة، ويطيل العمر التسويقي لثمار البرتقال. أجري التحليل الإحصائي على النتائج باستخدام برنامج الحاسوب الاحصائي (نسخة 8)، **بمستوى احتمال 5 %**. قُوم تأثير ثلاثة من الشموع الطبيعية (شمع نحل العسل والكارنوبا والهوهوبا) على الجودة والعمر التخزيني لصنفي 'سناري' و 'نوري 16' من ثمار البرتقال. أن المعاملة بالشموع الطبيعية الثلاثة قد أدت معنوياً لتأخير الشيخوخة وتقليل الفاقد والمحافظة على الجودة وإطالة العمر التخزيني لثمار البرتقال أثناء التخزين لمدة 18 أسبوعا في درجة حرارة 4±1 °م و85 – 90 % رطوبة نسبية. أوضحت النتائج أن المعاملة بالتشميع قد أدت معنوياً لخفض معدل التنفس (30.3 و21.3 و12.3 %)، وقللت من فقد الماء (32.5 و 12.7 و 7.8 %)، وتلون قشرة الثمار (31.7 و 23.3 و 13.3 %)، وليونة الثمار (16.5 و12.4 و6.8 %) وتجميع المواد الصلبة الكلية الذائبة فيها (10.4 و 7.1 و 4.1 %) وزادت من المحافظة على محتوى الثمار من حامض الأسكوربيك (45.4 و31.6 و20.5 %) في الثمار المعاملة بشمع نحل العسل وشمع الهوهوبا وشمع الكارنوبا، على التوالي، مقارنة بالثمار غير المعاملة (الشاهد). وكان شمع نحل العسل أكثر فعالية، يليه شمع الهوهوبا، وآخرها شمع الكارنوبا. أوصت الدراسة بأن الشموع الطبيعية، خاصة شمع نحل العسل وشمع الهوهوبا، يمكن بفعالية أن تؤخر الشيخوخة وتقلل فقدان الوزن وتحافظ على الجودة وتطيل العمر التخزيني لثمار البرتقال.

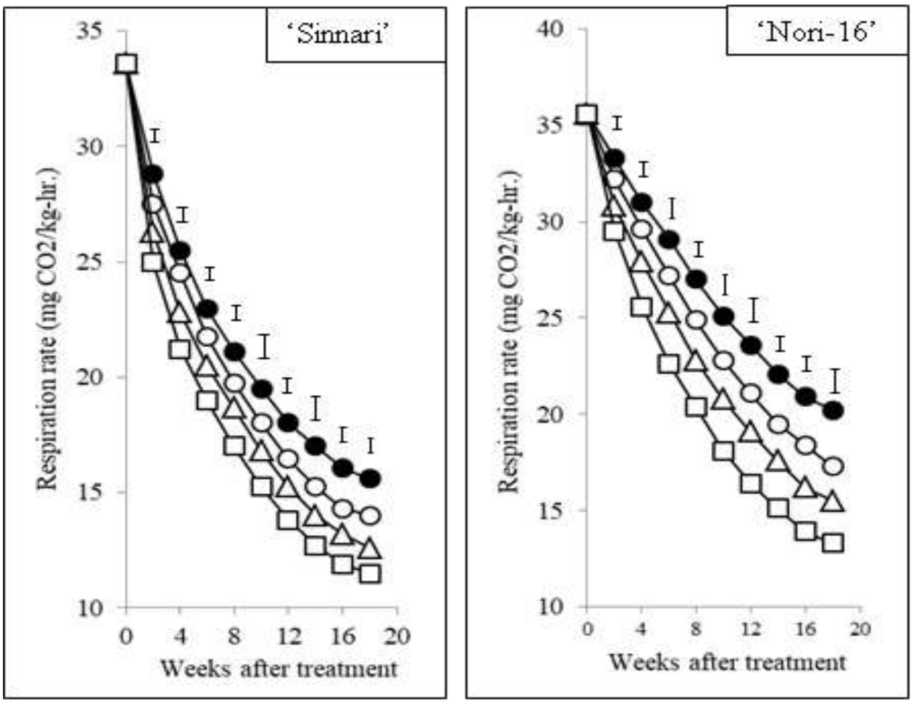

Figure 1: Changes in respiration rate during storage of 'Sinnari' and 'Nori-16' orange fruits treated with carnauba wax (○), jojoba wax (∆) and bee wax (□), compared with unwaxed fruits (control) (●) at 4±1 ºC and 85-90 % relative humidity. Vertical bars represent LSD (5 %).

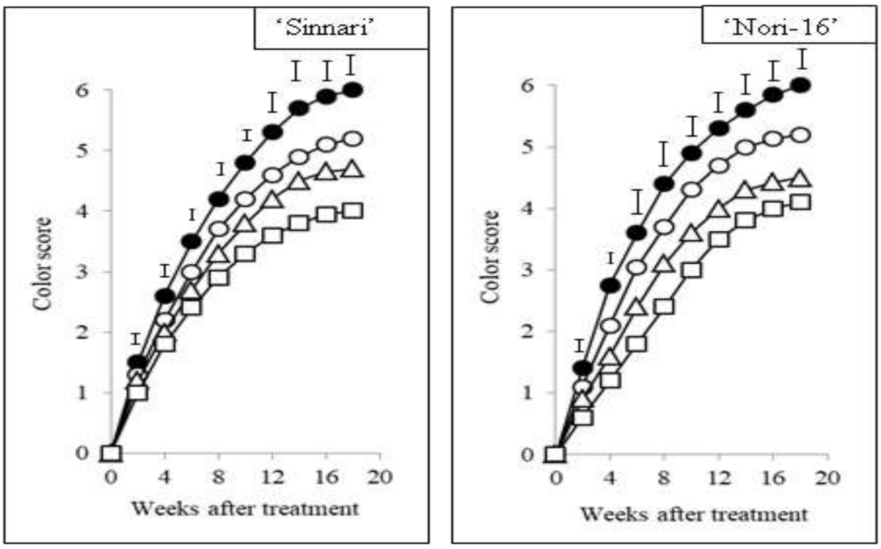

Figure 2: Changes in color score during storage of 'Sinnari' and 'Nori-16' orange fruits treated with carnauba wax (○), jojoba wax (∆) and bee wax (□), compared with unwaxed fruits (control) (●) at 4±1 ºC and 85-90 % relative humidity. Vertical bars represent LSD (5 %).

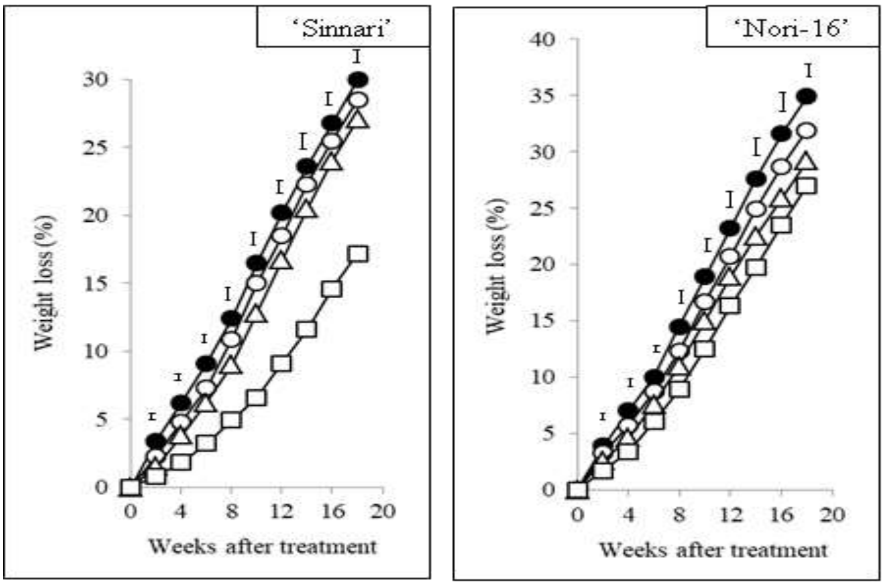

Figure 3: Changes in weight loss during storage of 'Sinnari' and 'Nori-16' orange fruits treated with carnauba wax (○), jojoba wax (Δ) and bee wax (□), compared with unwaxed fruits (control) (●) at 4±1 ºC and 85-90 % relative humidity. Vertical bars represent LSD (5 %).

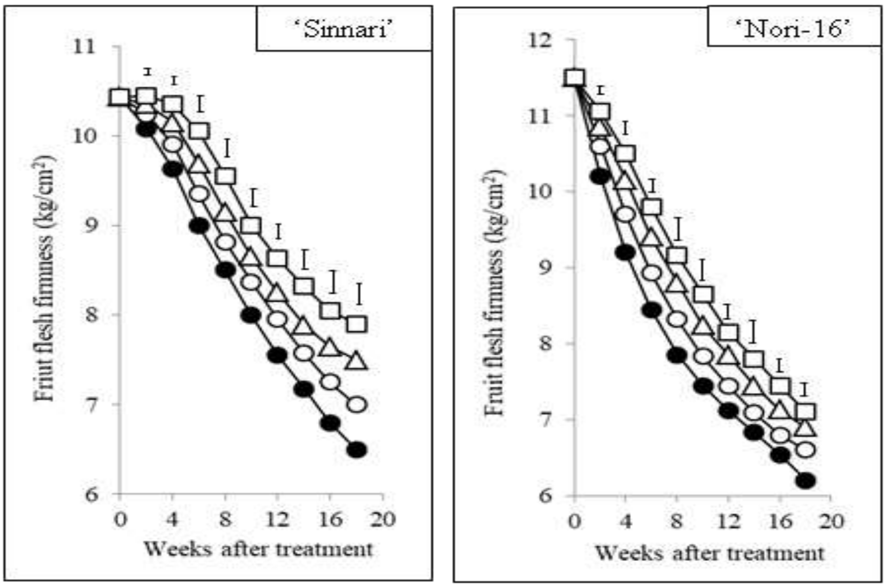

Figure 4: Changes in fruit flesh firmness during storage of 'Sinnari' and 'Nori-16' orange fruits treated with carnauba wax (○), jojoba wax (Δ) and bee wax (□), compared with unwaxed fruits (control) (●) at 4±1 ºC and 85-90 % relative humidity. Vertical bars represent LSD (5 %).

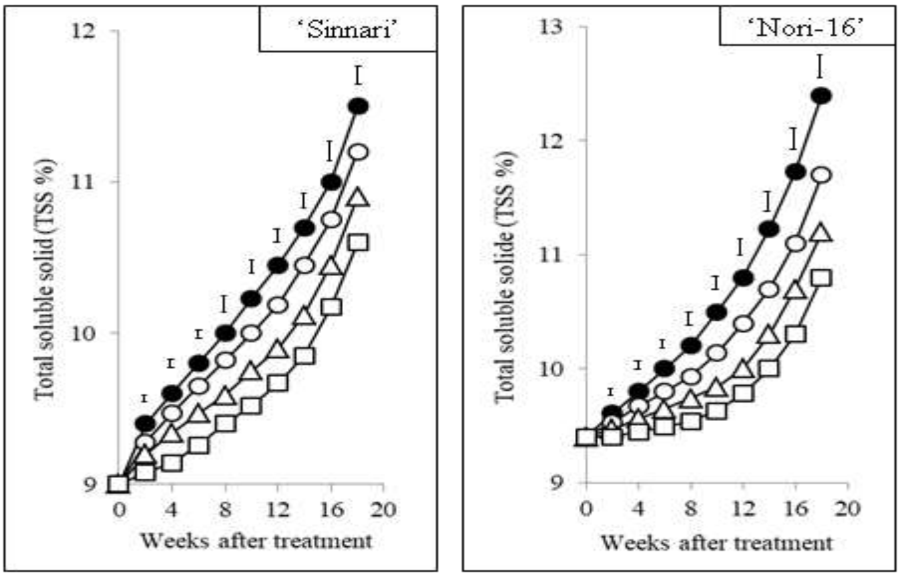

Figure 5: Changes in total soluble solids (TSS) during storage of 'Sinnari' and 'Nori-16' orange fruits treated with carnauba wax (○), jojoba wax (Δ) and bee wax (□), compared with unwaxed fruits (control) (●) at 4±1 ºC and 85-90 % relative humidity. Vertical bars represent LSD (5 %).

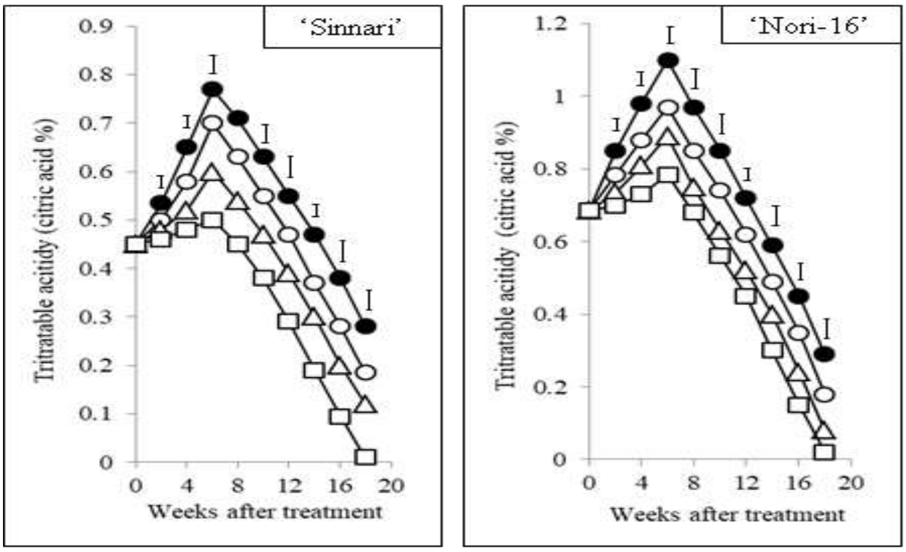

Figure 6: Changes in titratable acidity during storage of 'Sinnari' and 'Nori-16' orange fruits treated with carnauba wax (○), jojoba wax (Δ) and bee wax (□), compared with unwaxed fruits (control) (●) at 4±1 ºC and 85-90 % relative humidity. Vertical bars represent LSD (5 %).

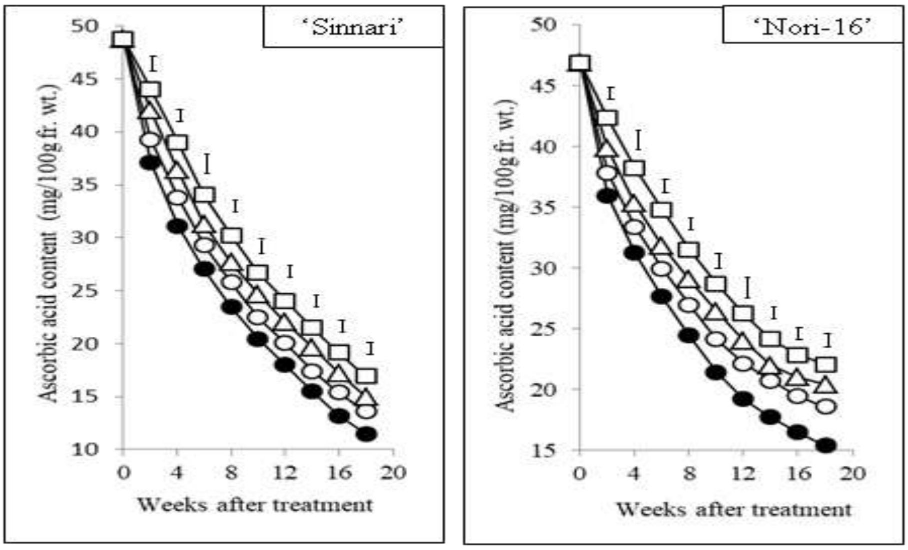

Figure 7: Changes in ascorbic acid content during storage of 'Sinnari' and 'Nori-16' orange fruits treated with carnauba wax (○), jojoba wax (Δ) and bee wax (□), compared with unwaxed fruits (control) (●) at 4±1 ºC and 85-90 % relative humidity. Vertical bars represent LSD (5 %).

Table 1: Percentage of fruits in each quality grade of 'Nori-16' and 'Sinnari' orange fruits treated with carnauba wax, jojoba wax and bee wax, compared with unwaxed (control) fruits.

Cultivar	Treatment	Quality grade (%)			
		Very good	Good	Fair	Poor
'Nori-16'	Unwaxed (control)	0	5.4	52.5	42.1
	Carnauba wax	23.5	40.4	24	12.1
	Jojoba wax	53	20.4	25.4	1.2
	Bee wax	56	21	20	3
'Sinnari'	Unwaxed (control)	0	4.3	60	35.7
	Carnauba wax	20.6	43.4	25	11
	Jojoba wax	52.4	18.4	24.3	4.9
	Bee wax	59.5	21.2	13.5	5.8