

Combined Effects of Hot Water and Eucalyptus Leaf Extract on the Storability of Cyprus Local Mandarin

Ibrahim Kahramanoglu

European University of Lefke

Seyda Aldag

European University of Lefke

Kenan Umar

European University of Lefke

Murat Helvaci

European University of Lefke

Turgut Alas

European University of Lefke

Serhat Usanmaz

European University of Lefke

Mehmet Askin

European University of Lefke

Volkan Okatan

Eskişehir Osmangazi University

Chunpeng Wan (✉ chunpengwan@jxau.edu.cn)

Jiangxi Agricultural University

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Abstract

Current work was conducted to evaluate the influences of hot water dipping (HWD) treatment and its combination with *Eucalyptus* leaves on the postharvest storability of Cyprus local mandarins. This experiment consisted of five different treatments, which are: 1) control (dipping fruits into tap water for 3 min); 2) hot water dipping (HWD) at 55 °C for 2 min; 3) hot water dipping (HWD) at 55 °C for 3 min; 4) hot water dipping (HWD) + *Eucalyptus* leaf (EL) at 55 °C for 2 min; and 5) hot water dipping (HWD) + *Eucalyptus* leaf (EL) at 55 °C for 3 min. According to the main findings of current work, HWD alone or in combination with the *Eucalyptus* leaf are effective for preventing the weight loss and protection of some other quality parameters. Overall all, results suggested that the incorporating *Eucalyptus* leaves into the HWD treatment improves the efficacy of the HWD, and makes it possible to reduce the dipping duration required for higher positive impact on the postharvest fruit quality.

1. Introduction

Citrus fruits are among the most important food resources of human. According to the FAO [1] total citrus production (oranges, mandarins, lemons, limes and grapefruits) was about 146.6 million tonnes in 2017. Among the all fruits and vegetables, citrus is the 13th most produced fruits and vegetables in the world and is the first among the fruit crops. Mandarins (*Citrus reticulata*) (including tangerines, clementines and satsumas) constitute about 22.8% of the total citrus production. Mandarin fruits are rich in vitamins (especially ascorbic acid), nutrients, antioxidants and other phenolic compounds [2]. Mandarin fruits are known to have short storage duration and very sensitive to storage conditions. Most important causes of postharvest losses are weight loss and fruit decay [3].

Previous studies reported that the losses arising from the postharvest decay exceed about 50% of the stored products in developing countries [4]. *Penicillium italicum* Wehmer 'blue mold' and *Penicillium digitatum* Sacc. 'green mold' are the most important reasons of the citrus pathogenic decay. Total losses caused by these two wound pathogens were noted to reach up to 80% and 30%, respectively [5, 6]. They are reproduced asexually by airborne spores and generally infect the fruits through to wounds. The spores are greatly produced by rotten fruits and easily contaminate the surrounding fruits. The severity of the pathogen is mainly depends on the amount of spores and optimal temperature which is about 20–25°C [7]. There are some important agro-chemicals have been used for the control of fungal pathogens, these are: imazalil, propiconazole and thiabendazole [8]. However, there is an increasing public concern about the use of fungicides which increased the need for controlling postharvest decay in environmentally friendly methods [4, 9].

Previous studies reported that the use of edible coatings [10–12] propolis [13], chitosan [14], plants extract or essential oils [15–19] calcium salts [20, 21] provides successful control of postharvest fruit decay and improves the storability of fruits. Apart from this, postharvest heat treatment has also reported to provide effective control of postharvest decay and prevent quality loss in storage rooms [22–26]. Previous studies show that both bio-materials and hot water treatment are effective in improving the postharvest storage quality of fruits, but the combination of these studies is very limited. According to authors' knowledge, there are very few research studied the combined efficacy of hot water treatment and bio-materials. In one of these studies Hong et al. [27] tested the combined effects of hot water and *Bacillus amyloliquefaciens* HF-01 and sodium bicarbonate for the control of mandarin fruits during storage. Therefore, current study aimed to test the combined influences of eucalyptus leaf extracts and hot water treatment on the postharvest fruit quality of local mandarins of Cyprus.

2. Results

2.1. Effects of HWD and eucalyptus leaf on the weight loss

According to the results obtained, hot water dipping (HWD) alone or in combination with the *Eucalyptus* leaf (EL) provided significant influence on the prevention of weight loss (Fig. 1). Control fruits of current work were found to have an average 5.49% weight loss in 20 days of storage, where the fruits treated with the combination of HWD and EL were found to have a weight loss from 4.04–4.58%. This result showed that the both treatments provide significant influence in reducing the weight

loss of Cyprus local mandarins. Fruit at the control treatment found to have 12.05% weight loss at the end of the experiments (60 days of storage). At that time, no significant difference was found among the other treatments. However, the least weight loss was obtained from the HWD + EL 55°C 3 min. Therefore, this result suggests that the *Eucalyptus* leaf can improve the efficiency of the hot water dipping.

2.2. Effects of HWD and eucalyptus leaf on the fruit firmness

Not surprisingly, results of current work showed that the fruit firmness had a decreasing trend during the postharvest storage of the mandarin fruits. Here, no significant difference was obtained among the control groups and other experimental treatments in 40 days of storage (Fig. 2). The extending storage showed that the control group is more sensitive to losing fruit firmness. According to the results obtained, the fruit firmness of the control group decreased from 0.56 kg cm⁻² to 0.39 kg cm⁻² in 60 days of storage. At that time, fruit firmness of all other treatments was also decreased but it was significantly higher than the control group. Those findings suggest that the HWD and EL treatments provide significant efficacy in preventing the changes in fruit firmness.

2.3. Effects of HWD and eucalyptus leaf on the fruit visual quality

Visual quality is a very important parameter for the determination of the postharvest storage quality of fruits. Results of present study are meaningful in terms of the protection of the visual quality. The duration of hot water dipping and the *Eucalyptus* leaf extracts were also found to have significant influence on the visual quality. At the end of the storage duration, the best fruit quality was obtained from the HWD + EL, 55°C 2 min and HWD, 55°C 3 min (Fig. 3). No significant difference was measured for these two treatments.

2.4. Effects of HWD and eucalyptus leaf on the decay incidence

According to the results obtained, the first decay was observed 20 days after storage from the control treatment and the HWD 3 min treatment (Fig. 4.). The decay caused by pathogens found to have a continuous increase during storage and it was found to be higher than the other treatments. The HWD treatment was found to have significant influence on the prevention of the decay caused by pathogens. However, the most important result of present study is that the incorporation of the *Eucalyptus* leaf extracts into HWD, provided better control of the pathogens. According to the best of the Authors' knowledge, this is the first report of the incorporation of a leaf extract into HWD. At the end of the experimental studies, the HWD + EL / 55°C 2 min treatment was found to have the highest influence on the control of pathogens. At the 60th day of storage (the end), the decay incidence was observed as 53.33% at the control treatment while the decay incidence was only 3.33% on the HWD + EL / 55°C 2 min treatment.

2.5. Effects of HWD and eucalyptus leaf on the juice content

Results about the juice content of the mandarin fruits showed that juice reduction mainly occurred after 40 days of storage. During the first days of the storage, the juice percentage of the all fruits showed slight increase (Fig. 5.). This increase in the juice percentage is thought to be due to the high weight loss from the fruit bark. According to the results obtained, the weight loss is higher at the fruit bark. Hereafter, the juice percentages of the all treatments were found to have a decreasing trend, parallel to the high weight loss of the fruits. At the 40th day of storage, lowest juice percentage was noted from the control treatment and it was followed by the HWD 55°C 3 min. Similar trend was continued after 40th day till the end, and the highest juice percentage was noted from the HWD 55°C 2 min, HWD + EL 55°C 2 min and HWD + EL 55°C 3 min. All of these results are in conjunction with the results about weight loss and fruit decay. To sum up, it can be concluded that the *Eucalyptus* leaf extracts has high potential for the protection of fruit weight and juice content.

2.6. Effects of HWD and eucalyptus leaf on the soluble solids concentration and titratable acidity

SSC of the mandarin fruits was found to have a declining trend from 20th day to 40th day, and a slight increase then after. The main reason is the huge weight loss after 40th day of storage (Table 1.). When comparing the treatments' impact on the SSC, it was noted that the fruits treated with 3 min showed higher SSC loss as compared with the others. An important finding of

current work was found to be about the titratable acidity (TA) contents of the mandarin fruits. The initial TA was measured as 6.6 g 100 g⁻¹, and it was decreased to a range of 2.2–2.7 g 100 g⁻¹ in 20 days of storage. This was about 200% decrease as compared with the initial contents. The final TA content of the fruits was found in a range of 1.2–1.4 g 100 g⁻¹ at the 60th day of storage. At that time, the higher TA content was observed from the HWD 3 min treatment. Findings of current work showed that the HWD treatment and eucalyptus leaf extract have significant influence on the TA contents of the fruits, but this was not so important as compared with the initial values. The SSC/TA ratio is an important indicator of the fruit flavour which significantly influences fruits' acceptability and marketability. The increase in this ratio improves the fruits attractiveness for the consumers, but it is also a result in the postharvest changes. According to the results obtained, the SSC/TA ratio showed an increasing trend from the 20th day till the end. Within this increasing trend, the least increase (could be accepted as the most successful treatment) was noted from the HWD 3 min. This treatment was followed by the HWD + EL 2 min.

Table 1
Change in the mandarins' SSC and TA contents in response to different treatments

Treatments	SSC				TA				SSC/TA			
	0 d	20 d	40 d	60 d	0 d	20 d	40 d	60 d	0 d	20 d	40 d	60 d
Control	11.6a	11.4a	11.6a	11.8a	6.6a	2.5bc	1.7b	1.3b	1.76a	4.58ab	6.71c	9.07a
HWD 55°C, 2 min	11.6a	11.0a	11.0ab	10.8b	6.6a	2.4c	1.3d	1.2c	1.76a	4.64ab	8.31a	8.80a
HWD 55°C, 3 min	11.6a	11.1a	10.4bc	11.2ab	6.6a	2.7a	1.9a	1.4a	1.76a	4.20b	5.61d	7.88b
HWD + EL 55°C, 2 min	11.6a	10.8a	10.5ab	11.1ab	6.6a	2.2d	1.5c	1.3b	1.76a	4.98a	7.17bc	8.34ab
HWD + EL 55°C, 3 min	11.6a	11.1a	10.0c	11.8a	6.6a	2.6ab	1.4d	1.3b	1.76a	4.35b	7.35b	8.90a

Means at the same measurement point with same letter or letters represents no significant difference according to the Duncan's multiple range test ($p < 0.05$).

2.7. Effects of HWD and eucalyptus leaf on the ascorbic acid content

Similar to the results of SSC, the ascorbic acid (AsA) content of the fruits was also found to decrease in time. During the first 20 days of the storage, the AsA contents of the fruits treated with HWD or HWD + EL combination were all found to have an increasing trend, except the control treatment (Fig. 6.). The AsA content of the control fruits were all found to have continuous decreasing trend. Similar with the control treatment, other fruits were also found to have a decreasing AsA content during storage, after 20 days of storage. At the end of the experimental studies, the highest AsA content was noted from the HWD + EL treatments and no significant difference was obtained between the 2 min and 3 min treatments. The following highest AsA contents were noted from the HWD 2 min and HWD 3 min treatments. All were found to be significantly higher than the AsA content of the control treatment.

3. Discussion

Findings of current work showed that the hot water treatment is effective for the prevention of the postharvest weight loss and retention of the fruit quality. Results of present study are in agreement with the findings of Queb-Gonzalez et al. [23] who reported that the postharvest heat treatments prevent the weight loss at mandarin fruits. Hot water treatment was also recommended by several other studies for different citrus species [24, 26]. On the other hand, the combination of HWD with *Eucalyptus* leaf (EL) was recorded in current study to prevent weight loss. There are not similar studies with the use of *Eucalyptus* leaves as additive to the HWD. However, the results are in conjunction with the reports of Hong et al. [27] where the combination of hot water with *Bacillus amyloliquefaciens* HF-01 and sodium bicarbonate were reported to protect weight loss

and help to control postharvest decay at mandarin fruits. Similarly, in present study, the combination of HWD with EL was noted to have higher influence on the control of fruit decay. The positive influence on heat treatment on the control of fruit decay is a well-known and widely used method [31]. This positive influence of heat treatment on the control of fruit decay was previously attributed to be caused by the positive effect on the activities of some enzymes, i.e. SOD [32]. Heat treatment was also noted to reduce decay at the 'Clementine' mandarins [33].

The HWD treatment alone or in combination with EL in present study was also found to protect fruit SSC, TA, fruit firmness and AsA contents as compared with the control groups. The biosynthesis of AsA was previously suggested to enhance scavenging of reactive oxygen species (ROS) [26]. In some other previous studies, similar to present study, hot water treatment was reported to protect the biosynthesis of AsA [34, 35]. Similar results were reported for Mexican lime [31] and zucchini fruits [36]. Heat treatments were similarly noted by Erkan et al. [33] to delay the changes in AsA content and SSC. In a previous study, Massot et al. [37] suggested that the heat treatment activate some genes related with AsA biosynthesis and this improves the fruits resistance against fungal pathogens. The activation of the AsA, also known to neutralize free radicals and improves the fruits storability [38]. Findings of current work suggested that the incorporation of the *Eucalyptus* leaves into HWD increases the biosynthesis of AsA and this provides better performance against postharvest fungal pathogens. On the other hand, current knowledge suggests that superoxide dismutase (SOD), peroxidase (POD) and polyphenoloxidase (PPO) enzymes alleviate lipid peroxidation and enhance citrus' resistance against pathogens [22, 24, 39]. Thus, further studies are required about the effects of *Eucalyptus* leaves on the activation of enzymes, such as SOD, POD and PPO; and the relationship among these enzymes and pathogens.

4. Conclusions

Present study showed that the hot water dipping (HWD) treatment is successful for the prevention of weight loss, fruit decay, loss in textural characteristics, improving visual quality, and protection of the ascorbic acid content. According to the results obtained, the duration of HWD significantly changes the impact. Thus, an increase in dipping duration was noted to have an increase in the positive impact. An important result of present study is that the incorporating *Eucalyptus* leaves into the HWD treatment improve the efficacy of the HWD, but this positive impact could decrease as the temperature increase. Therefore, incorporating HWD with *Eucalyptus* leaves makes it possible to reduce the dipping duration and improve the effectiveness of heat treatment.

5. Materials And Methods

5.1. Materials

Cyprus local mandarins (*Citrus reticulata*) are used in the current studies. Fruits are collected at the commercial maturity when soluble solids concentration reaches to above 10% and the treatable acidity is above 5%. Fruits were hand-collected from an orchard found in the Gemikonagi city, located in the Western part of Cyprus. Harvested fruits are brought to laboratory in 1 hour and pre-selections was performed to eliminate any damaged or infected fruits. Furthermore, *Eucalyptus* leaves were also used in the present study. The leaves of the *Eucalyptus* were collected from the same village. Present experimental research on mandarin fruits, including the collection of plant material (eucalyptus leaves), comply with relevant institutional, national, and international guidelines and legislation. All materials were collected from an orchard belonging to one of the authors and there is not restriction on the collection and/or test of these materials.

5.2. Design of the experiments

This experiment consisted of five different treatments, which are: 1) control (dipping fruits into tap water for 3 min); 2) hot water dipping (HWD) at 55°C for 2 min; 3) hot water dipping (HWD) at 55°C for 3 min; 4) hot water dipping (HWD) + *Eucalyptus* leaf (EL) at 55°C for 2 min; and 5) hot water dipping (HWD) + *Eucalyptus* leaf (EL) at 55°C for 3 min. The EL doses were used as 80 g in 4 Liter of water. For the application of the EL added treatments, first of all, water was heated until 55°C, then the *Eucalyptus* leaves were added into the water and then the water was heated until 90°C. It was kept 10 min at that temperature

and then cooled until 55°C. Dipping of the fruits was performed hereafter. A total of 150 fruits were used in the experiments. Each of the treatments (#5) consisted of 30 fruits (replications). After treating, the fruits were air dried for 30 min. Before storing, the weights of fruits were measured separately and recorded for further analysis. The fruits were then put in violas orderly, and stored at $9 \pm 1^\circ\text{C}$ and 90–95% relative humidity.

5.3. Fruit quality analysis

Fruits were stored for 60 days and fruit quality analysis was performed with 20 days interval (20, 40 and 60 days of storage). At the mentioned days, 10 fruits from each of the above mentioned treatment were taken out from the storage room and the following quality parameters were measured. The final weights of each fruit were measured by a digital scale (± 0.01 g) immediately after storage. Thus, the final weights and the recorded initial weights of each fruit were used to calculate the weight loss. The fruit firmness of each fruit at each measurement point was determined by a hand penetrometer as kg cm^{-2} . Visual quality analysis of each fruit was carried out by following the 0–5 scale of Silvia et al. [28]. Moreover, the Decay Incidence (DI) of the mandarin fruits, which was caused by *P. italicum* and *P. digitatum*, was calculated as %, by using the 0–3 scale of Cao et al. [29]. To determine the juice content of each fruit, the fruits were half-cut and then the juice was squeezed by an automatic machine. The seeds and pulp was filtered through 1 mm sieves to obtain clear juice. Then the juice content was calculated as %. Then, a hand refractometer was used to assess the soluble solids concentration (SSC) of fruit samples as %. Fruits' TA was then measured by using the clear juice samples through the formula of AOAC [30]. Finally, the ascorbic acid (AsA) content was determined by titration with 2,6-dichlorophenol indophenols standard method.

5.4. Statistical analysis

Data of the experiments were collected in Excel sheets to summarize (calculation of the means and standard deviation) and then subjected to the analysis of variance (ANOVA). In case there is a significant difference, separations of the means were conducted with Duncan's multiple range test ($P < 0.05$). SPSS 22.0 was used for the statistical analysis.

Declarations

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Conflicts of interest/Competing interests:

Not Applicable

Availability of data and material:

Raw data is available upon request

Code availability:

Not Applicable

Authors' contributions:

All Authors – decided and conceptualized the experiments; İ.K., S.A., K.U., M.H., T.A., S.U. and M.A.A. – collected the materials, designed the experiments and collected the data; İ.K., M.A.A., V.O. and C.W. – performed the statistical analysis; İ.K. and C.W. – write the initial draft of the paper; All Authors – edited the paper and gave the final approve.

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Figures

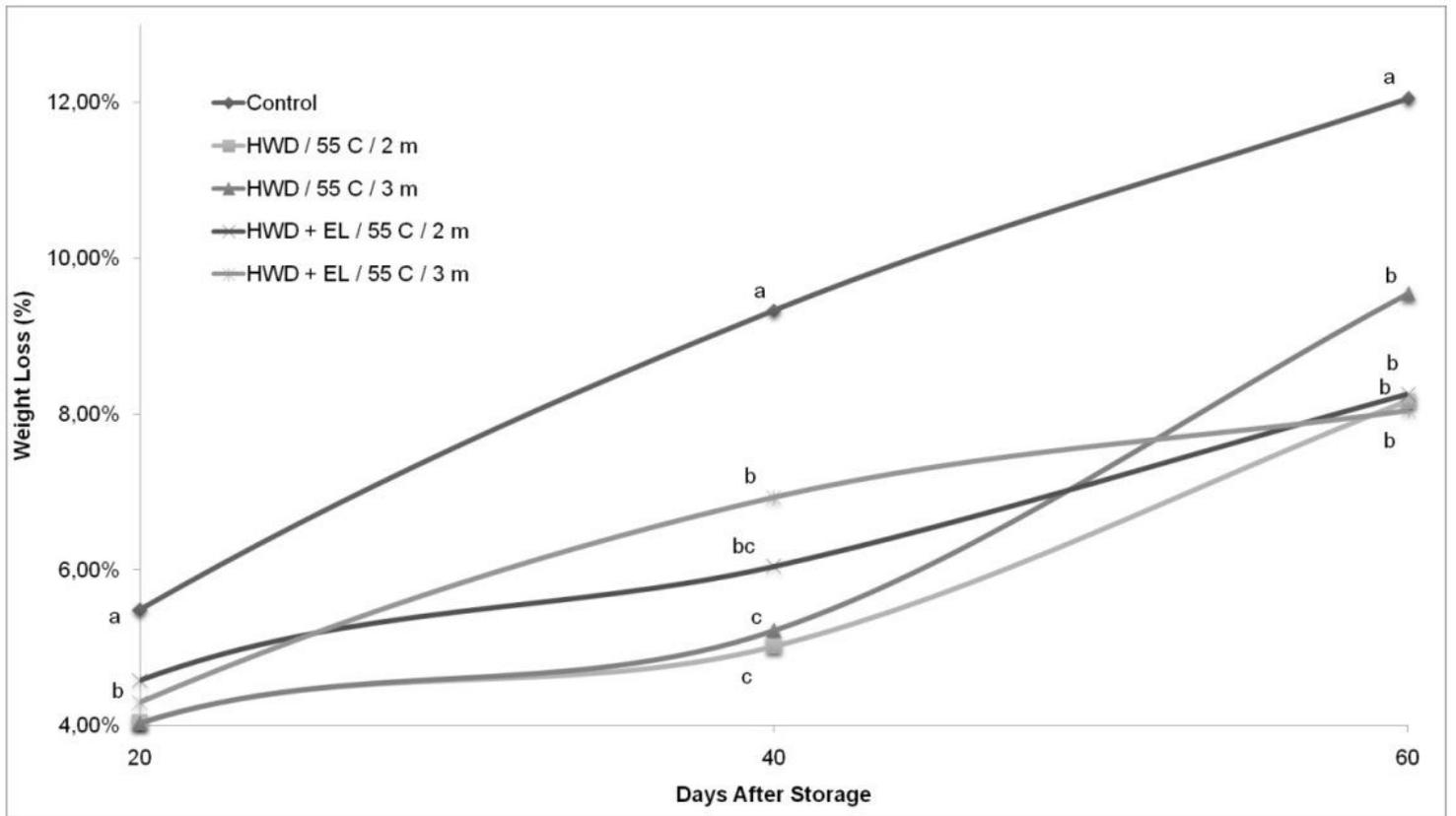


Figure 1

Change in the mandarins' weight loss in response to different treatments. Means at the same measurement point with same letter or letters represents no significant difference according to the Duncan's multiple range test ($p < 0.05$).

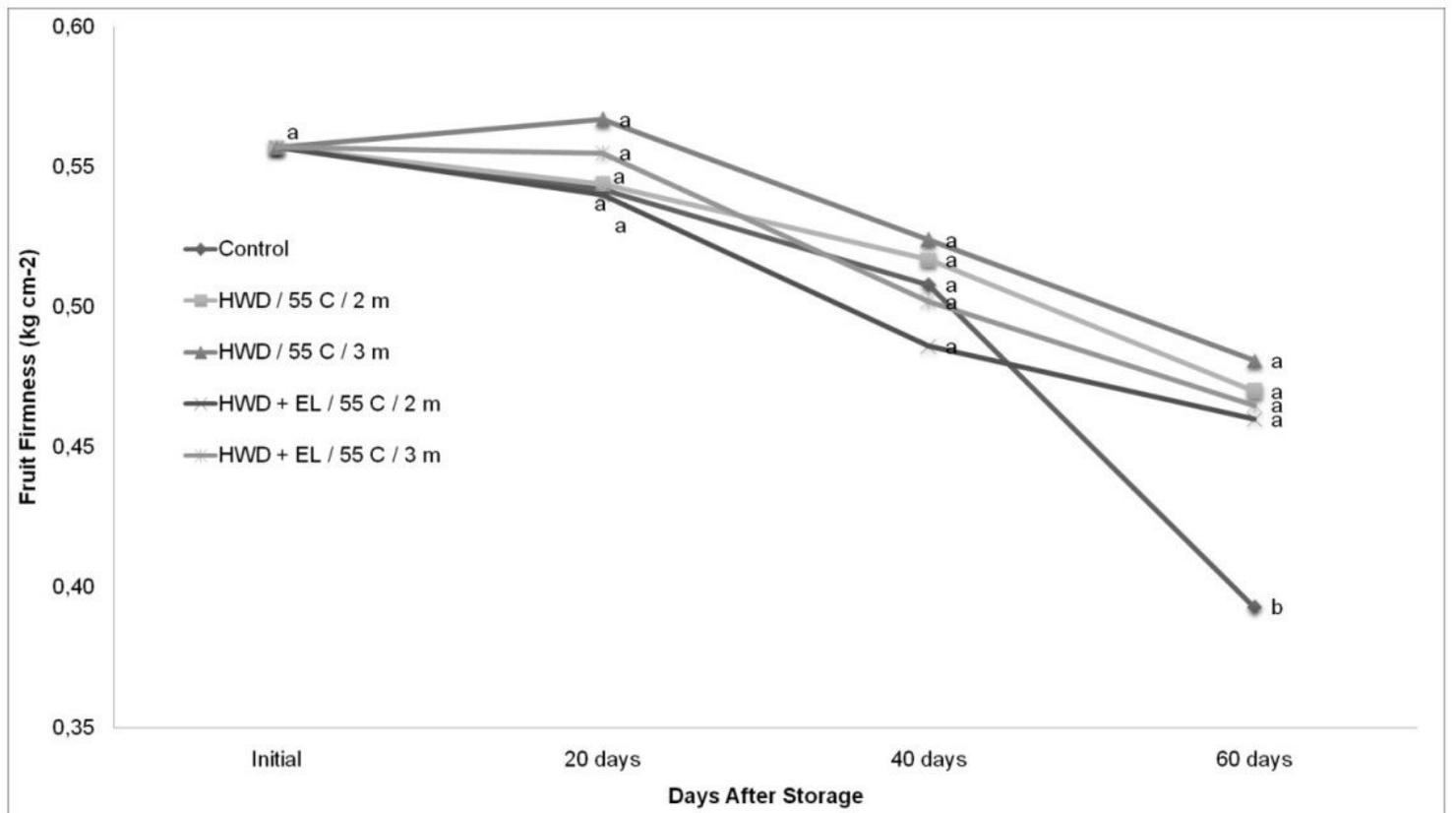


Figure 2

Change in the mandarins' firmness in response to different treatments. Means at the same measurement point with same letter or letters represents no significant difference according to the Duncan's multiple range test ($p < 0.05$).

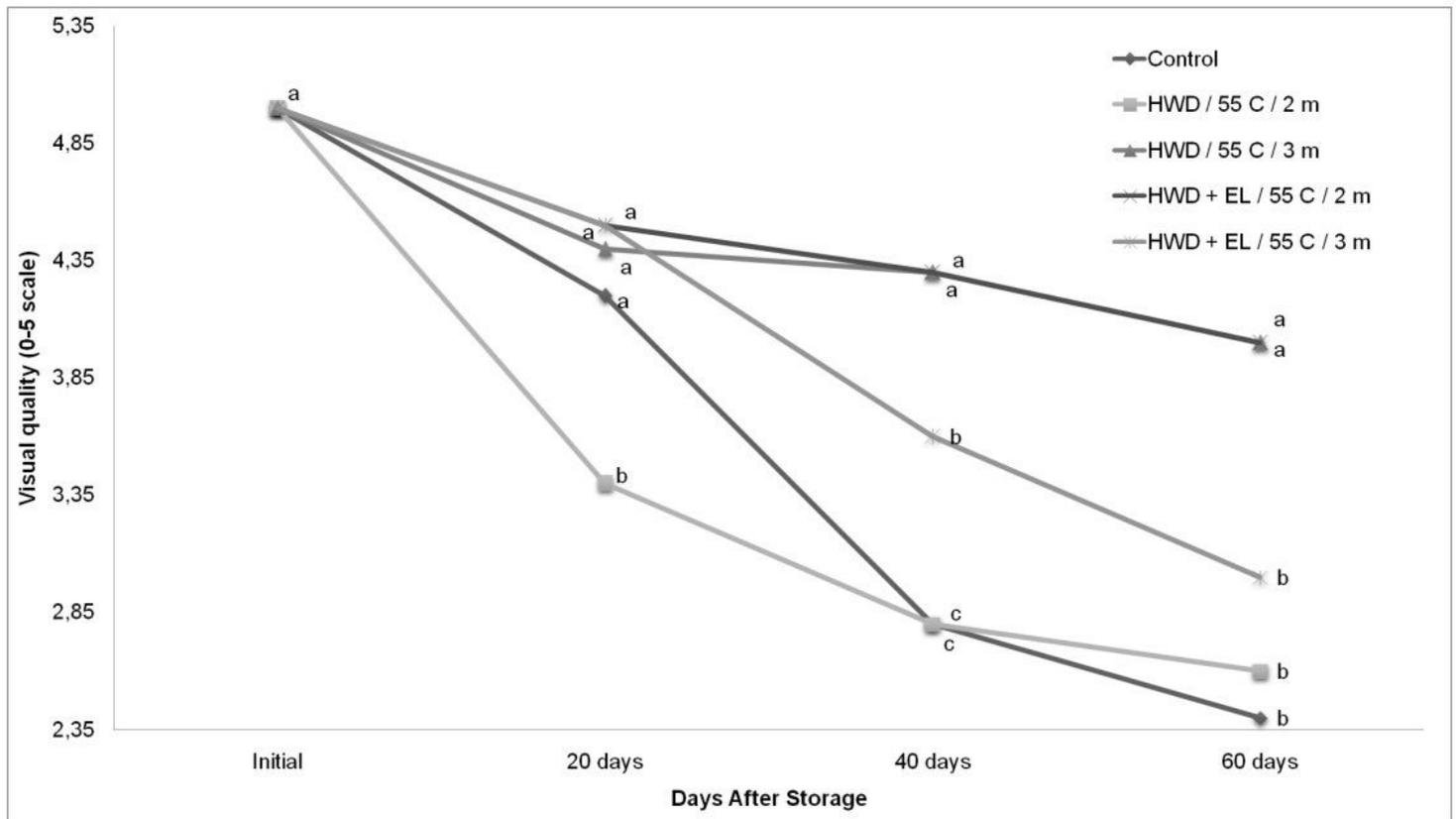


Figure 3

Change in the mandarins' visual quality in response to different treatments. Means at the same measurement point with same letter or letters represents no significant difference according to the Duncan's multiple range test ($p < 0.05$).

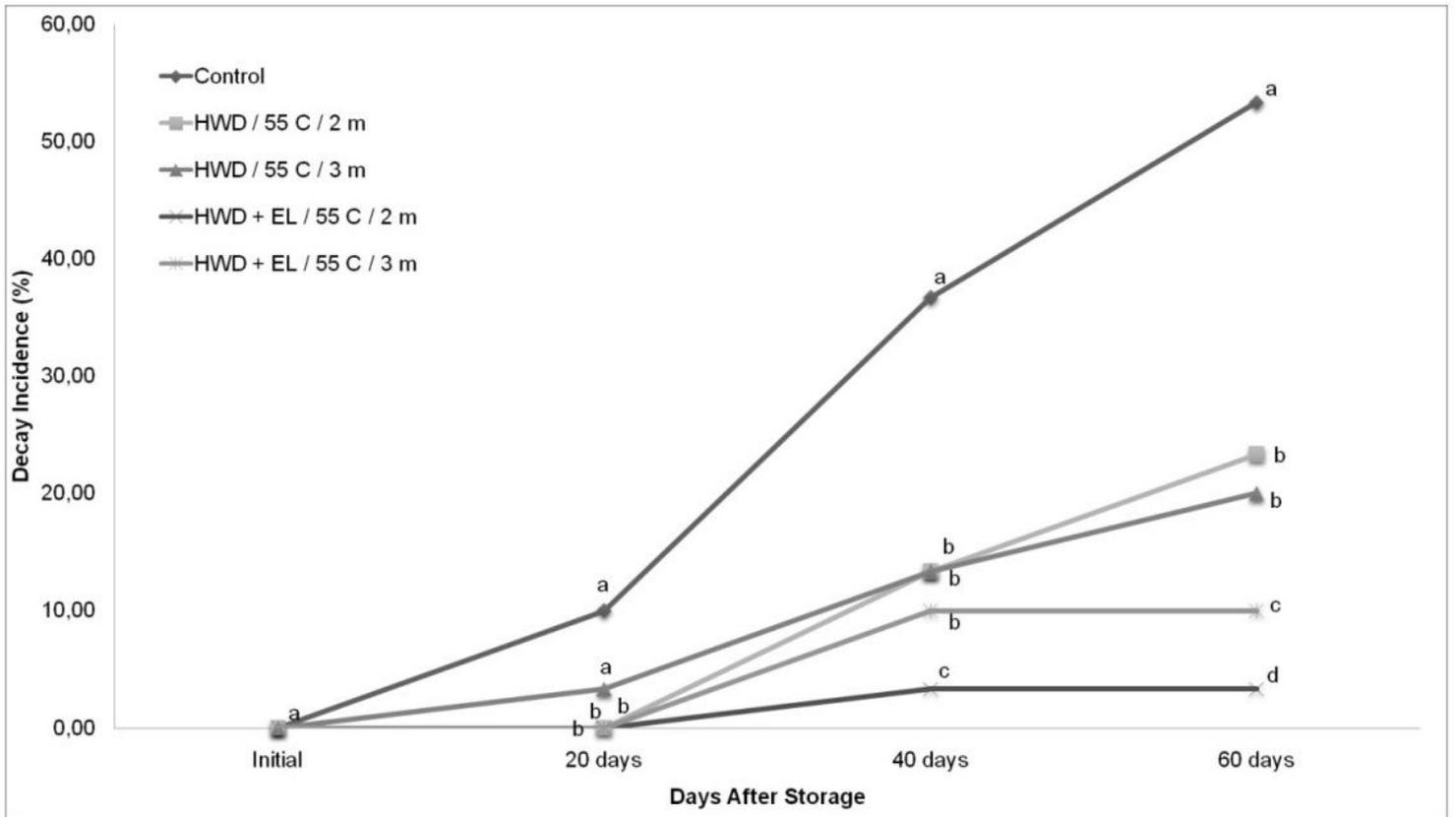


Figure 4

Change in the decay incidence of mandarins in response to different treatments. Means at the same measurement point with same letter or letters represents no significant difference according to the Duncan's multiple range test ($p < 0.05$).

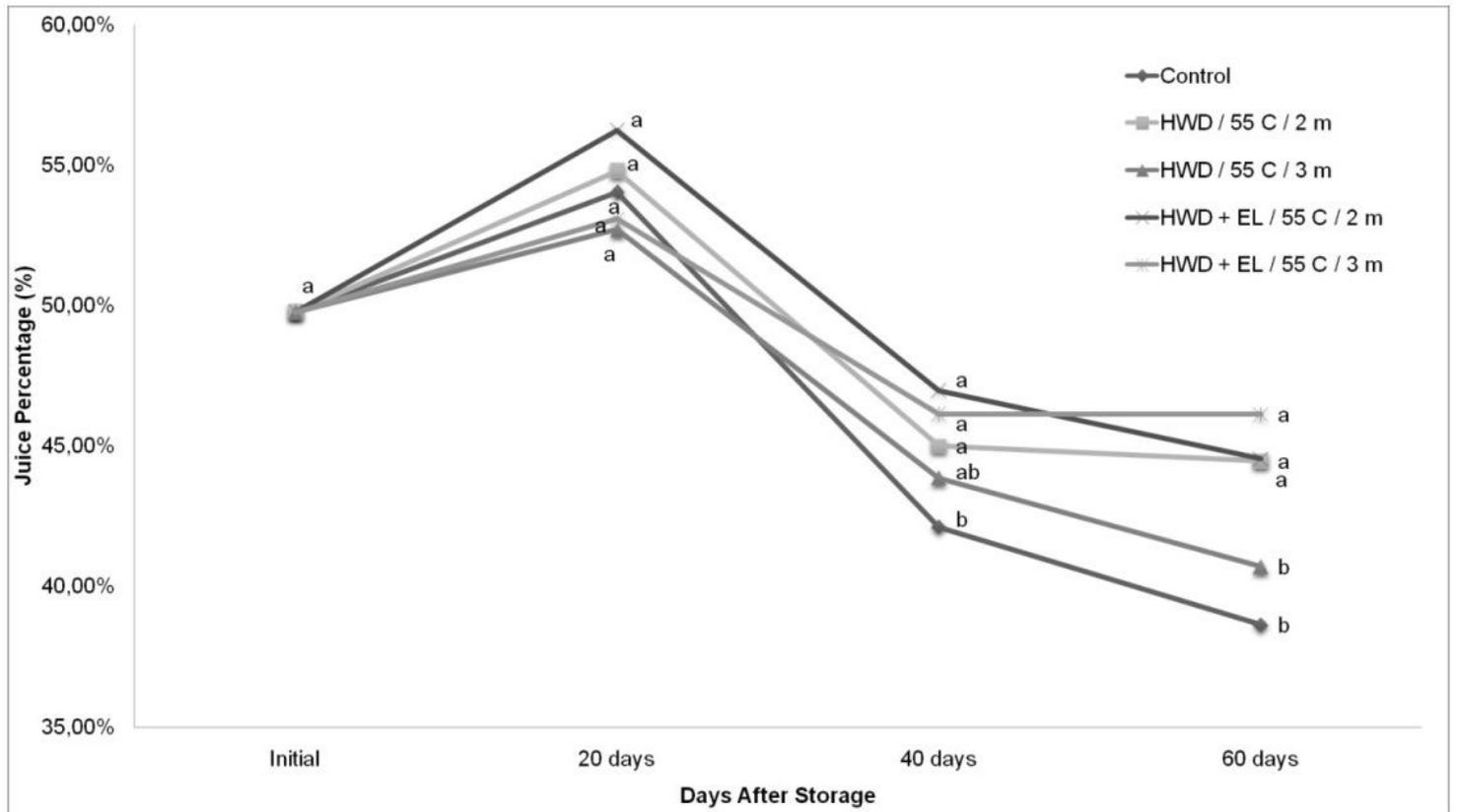


Figure 5

Change in the mandarins' juice content in response to different treatments. Means at the same measurement point with same letter or letters represents no significant difference according to the Duncan's multiple range test ($p < 0.05$).

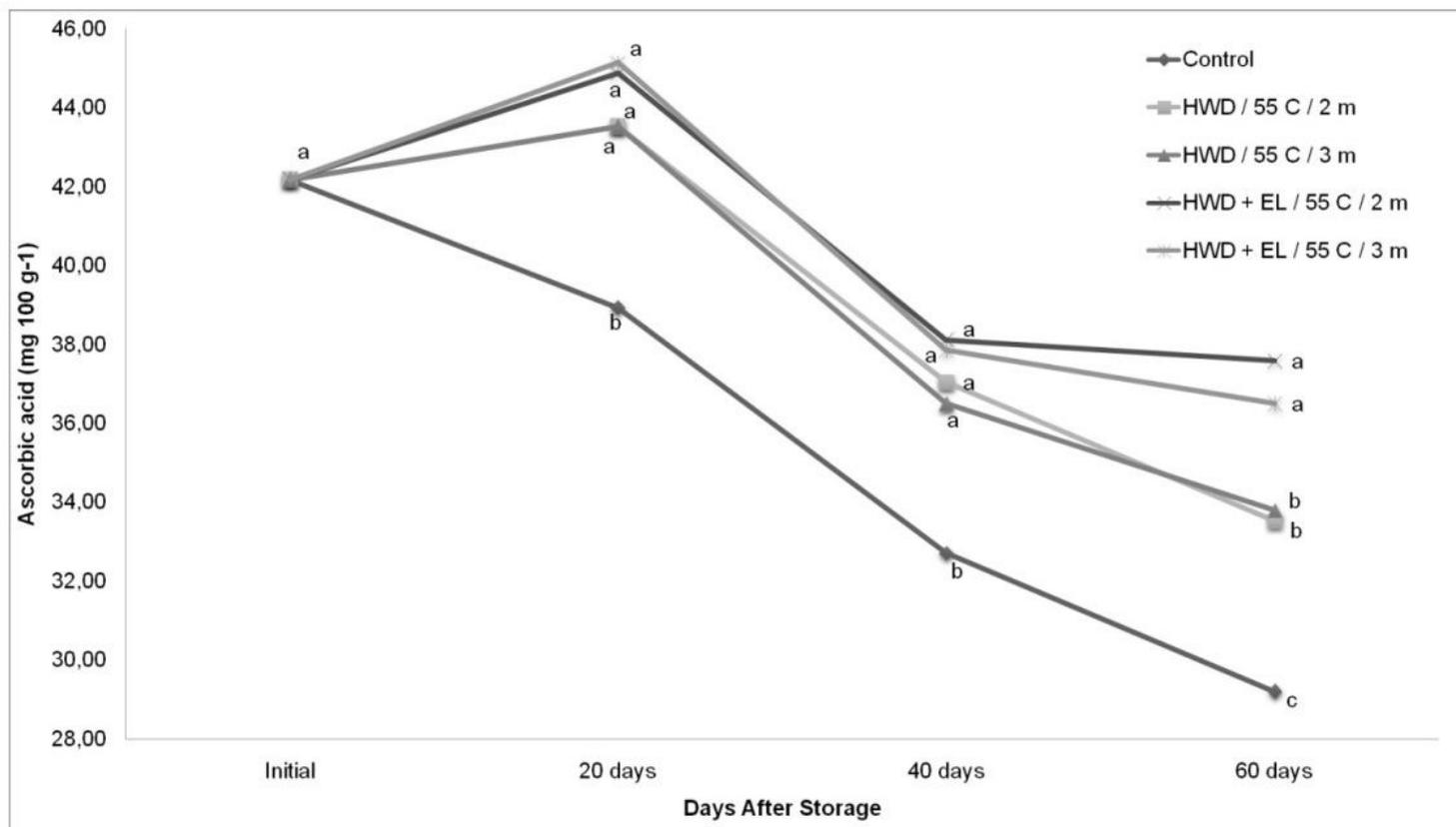


Figure 6

Change in the mandarins' ascorbic acid content in response to different treatments. Means at the same measurement point with same letter or letters represents no significant difference according to the Duncan's multiple range test ($p < 0.05$).