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ORIGINAL PAPER

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POSTHARVEST HYDROTHERMAL TREATMENTS TO MAINTAIN QUALITY OF 'NEWHALL' NAVEL ORANGE

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ABSTRACT

Hydrothermal treatments are long known curing methods for the improvement of fruits resistance against storage conditions and for controlling fungal pathogens. The optimal conditions of the hydrothermal treatments significantly vary among species and varieties/cultivars. Present research was conducted to measure the storability effect of hot water dipping (HWD). First of all, three different hydrothermal temperatures (45, 50 and 55°C) tested for 3 different HWD durations (3, 4 and 5 min). Hereafter, main studies were performed with 50°C for 5 min HWD treatment which provided highest performance in preliminary experiments. In this main studies, physical, bio-chemical, physiological and enzymatic characteristics of the fruits were also tested with 20 days interval for 120 days of storage. Results suggested that the HWD treatment reduce respiration rate and enhance the activity of some enzymes, mainly polyphenol oxidase (PPO), peroxidase (POD) and superoxide dismutase (SOD), and helps to preserve physical and bio-chemical quality of 'Newhall' navel oranges.

Key words: biochemical quality, enzymatic activity, fruit curing, hot water treatments, physical quality preservation, postharvest diseases

INTRODUCTION

Fruits are living organisms and continue to respire after harvest. Respiration, together with transpiration cause physical, bio-chemical and physiological changes and deterioration at the stored fruits. There are several measures, including cold storage, waxing, edible coatings and modified atmosphere packaging, which can be used to improve storability of fresh fruits [Kahramanoğlu 2017, Chen et al. 2019, Kahramanoğlu and Wan 2020]. Besides to that, hydrothermal treatments are also known to be beneficial in preserving fruit's storage quality [Alvindia 2015]. The hydrothermal applications had been noted to induce the activity of some enzymes, i.e. calatase (CAT), superoxide dismutase (SOD), peroxidase (POD) and/or polyphenol oxidase (PPO) which help to prevent lipid peroxidation and/or enhance fruits' resistance against postharvest pathogens [Ballester 2006, Gao 2018].

Orange fruits are among the most produced, exported/imported and consumed fruit crops around the world. Oranges are the third most produced fruit crops in the world with 78.7 M tonnes after bananas (116.7 M tonnes) and apples (87.2 M tonnes) [FAO

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2021]. Navel oranges (*Citrus sinensis* L., Osbeck) are among the most preferred oranges by consumers because of their unique flavor and high nutritional contents (especially vitamin C and other phytochemical compounds) [Albertini et al. 2006, Okatan 2020, Sekerli and Tuzcu 2020]. As for other fruits and vegetables, oranges are also very sensitive to storage conditions due to physical, bio-chemical and physiological deterioration; and fungal pathogens (mainly *Penicillium digitatum* and *P. italicum*) [Zeng et al. 2010, Wan et al. 2020]. Although fungicides were effective in preventing postharvest losses caused by fungal pathogens, development of resistance in funguses against fungicides and the increase in the consumer awareness against agro-chemicals caused a shift in the postharvest handling practices through non--chemical ways [Sharma et al. 2009, Mayachiew et al. 2010]. In this case, hydrothermal applications is one of these non-chemical measures in postharvest practices. However, the optimal temperature and curing duration were reported to be significantly vary among species and cultivars [Kahramanoğlu et al. 2020a]. At the same time, the real mechanism behind the efficacy of hydrothermal applications is not well understood. Therefore, present research was carried out to i) find out the optimal temperature for improving storability of 'Newhall' navel orange and ii) evaluate the changes in phytochemical compounds and enzymatic activity of the fruits to enable the determination of exact mechanism behind this efficacy.

MATERIALS AND METHODS

Materials. Navel orange (*C. sinensis* L. Osbeck cv. Newhall) fruits of present study were collected from an orchard found in Ganzhou city, China. The fruits of first year experiments (preliminary) were harvested in November 2015 and the main studies were carried out with the fruits collected in November 2016. The range of the fruit weight at the time of harvest was 240–270 g, while the color was 3.5–4.8 according to the citrus color index.

Optimal treatment of hot water dipping (HWD). To evaluate the preservation effect of HWD on postharvest loss, the preliminary experiment was performed with the following different treatments: (1) no treatment as the control, (2) HWD at 45°C for 3 min, 4 min, 5 min, respectively; (3) HWD at 50°C for 3 min, 4 min, 5 min, respectively; (4) HWD at 55°C for 3 min, 4 min, 5 min, respectively. Afterwards, the fruits from the control group and three HWD treatments were individual film-packaged and stored at room temperature (RT: 20 ±2°C, 70–85% relative humidity). The decay rate and weight loss for each group were recorded 120 d after storage.

Evaluation of decay rate and weight loss. Both the fruit weight loss and decay rate were evaluated from the 80 navel oranges per replicate of each treatment. Visual quality assessment was done for fruit decay rate, at the end of the storage (120 d). This was presented as % of rotted fruits. Weight loss was measured for 20 fruits per replicate at the initiation and end of storage (0 and 120 d) and used for the weight loss determination.

Fruit treatment and storage. These experiments were conducted with the best treatment which was determined after the above-mentioned optimum experiment studies. It was also explained in the results section that the 50°C for 5 min provided the best results, in terms of the preservation of fruit weight and prevention of decay rate. These main studies were conducted in November 2016. The selected fruits were randomly divided into two groups (HWD-treated (50°C for 5 min) and non-treated control). Then, both HWD-treated and control fruits were individually film-packaged and pre-cooled (10–12°C, 12 h). After these treatments, fruits of each group were kept at 5 ±0.5°C, and 85%–90% relative humidity (RH) for 120 days. At each sampling point (20, 40, 60, 80, 100, and 120 days), 10 oranges from each replicate were randomly picked out from both groups for analyzing bio-chemical and physiological quality parameters, as well as protective enzyme activities.

Physicochemical measurements of 'Newhall' navel orange. TSS and total sugar content in the orange juices of both groups (hydrothermal treated and control groups) were measured using a RA-250WE Brix-meter (Atago, Tokyo, Japan) and the anthrone colorimetric method, and the results were expressed as a percentage. The TA of the juices was measured according to the standard titration method with 0.1 M NaOH. Then, the VC was measured through titration with 2,6-dichlorophenol indophenol. The respiration rate was then determined based on a method described by Wan and Kahramanoğlu [2020]. It was measured by CO_2 production of the fruits by using a GHX-3051H infrared CO_2 fruit and vegetable breathing apparatus (Jingmi Scientific LLC., Shanghai, China) and expressed as mg kg^{-1} h^{-1}. The MDA content of HWD-treated and control groups was then determined according to the published methodology of Hodges et al. [1999] and expressed as mmol g^{-1} frozen weight (FW).

Protective enzymes activities. First of all, aliquots of powder (2.0 g) of fruit samples were homogenized with different ice-cold extraction buffers by following the methods as suggested by Wan et al. [2020] and the final supernatant was used in the enzyme activity assays. SOD activity was determined by assessing its ability to inhibit the photoreduction of nitroblue tetrazolium according to the method of Ballester et al. [2006] and expressed as U min^{-1} g^{-1}. Then, the POD activity was assessed based on the measurement of guaiacol oxidation at 470 nm in the presence of H_2O_2 by following the method of Kahramanoğlu et al. [2020] and expressed as U min^{-1} g^{-1}. PPO activity was finally accessed by following the catechol oxidation measurement at 420 nm by following the method of Wan et al. [2020] and expressed as U h^{-1} g^{-1}.

Data analysis. All data calculated from physical and bio-chemical experiments was expressed as the mean with standard deviation. The SPSS software was used to determine the statistical differences among the means of different treatments using the independent samples t-test at $P < 0.05$.

RESULTS AND DISCUSSIONS

Hot water dip (HWD) treatments on decay rate and weight loss of navel orange. Important results were obtained about the effects of hot water dipping on the fruits weight and decay rate. One of the most important results is that, increase in the temperature applied and increase in the duration of application caused an increase in the efficacy of treatment, until a critical temperature (55°C) and then it caused damages on the fruits' quality (Fig. 1). This might be because of the damages on cell walls. Overall, the highest weight loss and decay rate were observed from the HWD at 55°C for 5 min, and was followed by the control

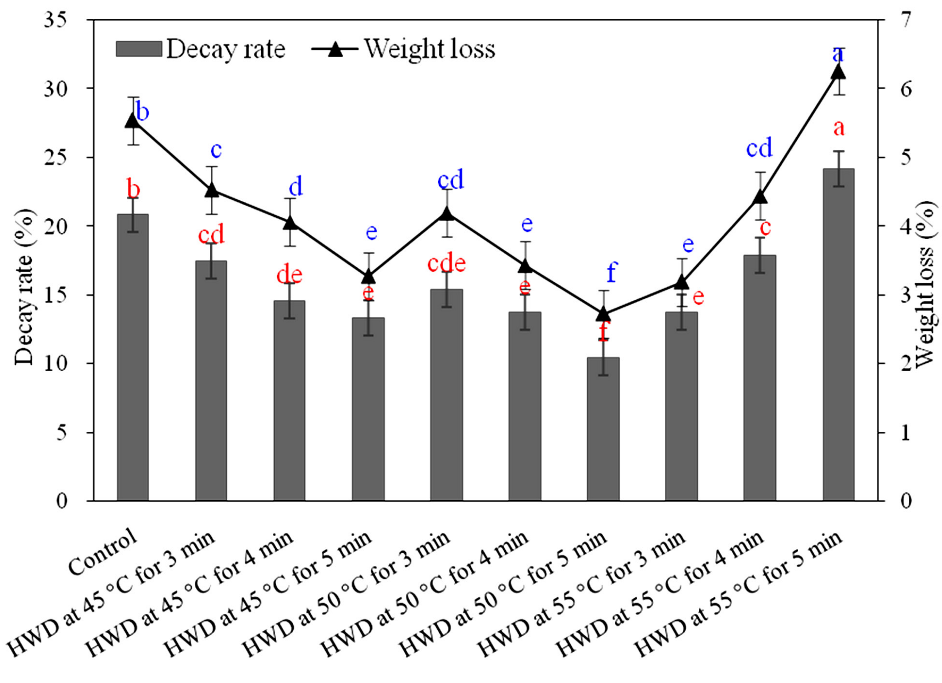

Fig. 1. Effect of different HWD conditions on decay rate and weight loss of navel orange fruit stored at 5 ±0.5°C for 120 days

treatment. According to the results obtained, the best combination was the HWD at 50°C for 5 min. The weight loss was only 2.73% at this treatment, where the decay rate was only 10%. These results are in agreement with the reports of Atrash et al. [2018] and Wan et al. [2020] who suggested that heat treatment is effective in controlling fungal decay and preserving fruit weight at citrus fruits. This success can be associated with the inducement of enzymatic activity of the fruits, as suggested by Wang et al. [2013]. This was discussed in the further sections.

Effects of HWD on the contents of postharvest fruit quality of navel orange. According to the results of current work, as indicated in Table 1, the TSS content of the fruits had an increasing and then decreasing trend during storage. It was also noted that the HWD treatment is effective in prevention of this change. At most of the measurement points (40, 100 and 120 days), the TSS contents of the fruits treated with HWD were measured as higher than the control group. Similar results about the slight influence on TSS was previously noted by Wan et al. [2020] and Erkan et al. [2005]. The total sugar values were similar to the TSS values of the fruits. Although the total sugar value increased during storage, at the end of the storage period, the total sugar value was found to be similar to the initial value. Again, the HWD-treated fruits had higher values than control groups. Similar results were then found for TA. The TA value of the fruits had a decreasing trend during the storage period, but this decrease delayed in HWD-treated fruits. Therefore, because of the high TA, the TSS/TA ratio of the HWD-treated fruits was lower than the control group. This is but still higher than the initial ratio.

Besides to TSS and TA, which are very important parameters for the determination of fruit flavour, The VC value is also very important for the determination of the nutritional quality of the fresh fruits. Moreover, VC values are important for postharvest handling, in which it is an indicator of the crops' resistance to oxidative stress by enhancing the scavenging of reactive oxygen species (ROS) [Sing and Sing 2013]. In this

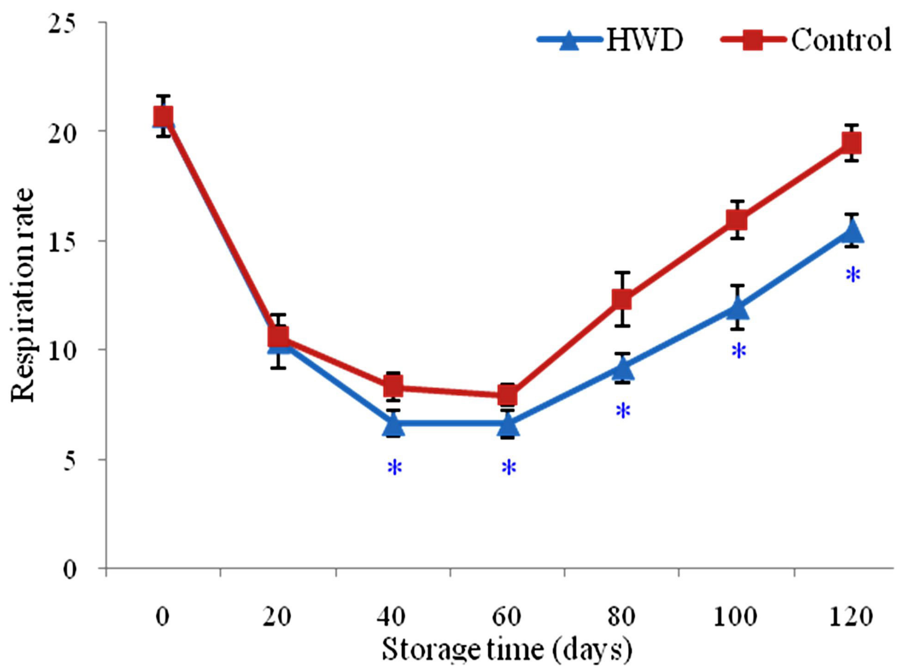

Fig. 2. Effect of HWD on respiration rate of navel orange fruits during 120 days of cold storage at 5 ±0.5°C. The symbol * (the HWD-treated fruit) represented significantly lower than the control fruit (independent samples t-test, $P < 0.05$)

Table 1. The contents of total soluble solid (TSS, %), titratable acidity (TA, %), TSS/TA ratio, vitamin C (VC, mg/100 g FW) and total sugar (%) in HWD-treated and control fruits during 120 days of storage at 5 ±0.5°C

Days	Treatment	TSS	TA	TSS/TA	VC	Total sugar
0	Initial	12.10 ±0. 071	0.97 ±0.016	12.41 ±0.431	45.65 ±0.394	12.35 ±0.216
20	HWD	12.68 ±0.110	0.78 ±0.016	16.34 ±0.677	52.93 ±0.438*	12.82 ±0.245
	Control	12.54 ±0.055	0.80 ±0.016	15.73 ±0.342	48.54 ±0.256	12.93 ±0.254
40	HWD	13.17 ±0.114*	0.70 ±0.008*	18.89 ±0.750	54.64 ±0.836*	13.75 ±0.275
	Control	12.82 ±0.110	0.66 ±0.014	19.51 ±0.797	50.98 ±0.857	13.61 ±0.354
60	HWD	12.97 ±0.141	0.65 ±0.011*	20.09 ±1.259*	45.99 ±0.576*	15.52 ±0.190*
	Control	12.88 ±0.110	0.56 ±0.008	23.02 ±0.991	39.12 ±0.883	14.44 ±0.201
80	HWD	12.60 ±0.089	0.57 ±0.007*	22.10 ±1.310*	39.03 ±0.458*	14.99 ±0.212*
	Control	12.47 ±0.114	0.49 ±0.013	26.60 ±0.867	34.88 ±0.217	12.94 ±0.292
100	HWD	12.42 ±0.071*	0.52 ±0.007*	23.87 ±1.040*	35.13 ±0.510*	13.79 ±0.184*
	Control	11.90 ±0.000	0.44 ±0.002	27.00 ±0.120	31.02 ±0.567	12.02 ±0.249
120	HWD	11.85 ±0.084*	0.49 ±0.015*	24.41 ±0.561*	29.54 ±0.514*	12.39 ±0.165*
	Control	11.54 ±0.089	0.37 ±0.019	30.82 ±0.473	24.04 ±0.426	11.48 ±0.179

The symbol * and * (the HWD-treated fruit) represented significantly higher and lower than the control fruit, respectively (independent samples *t*-test, $P < 0.05$)

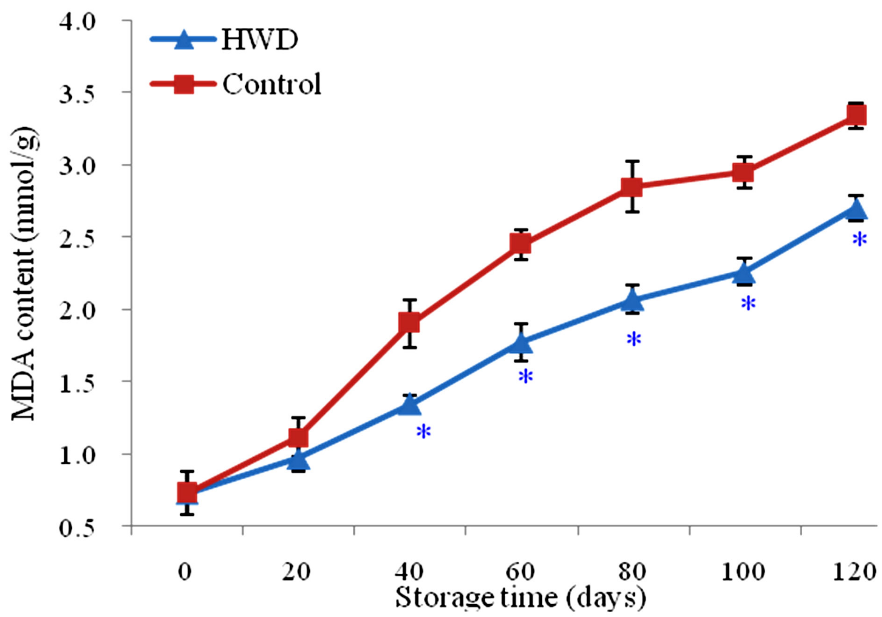

Fig. 3. Effect of HWD on MDA content of navel orange fruits during 120 days of cold storage at 5 ±0.5°C. The symbol * (the HWD-treated fruit) represented significantly lower than the control fruit (independent samples t-test, $P < 0.05$)

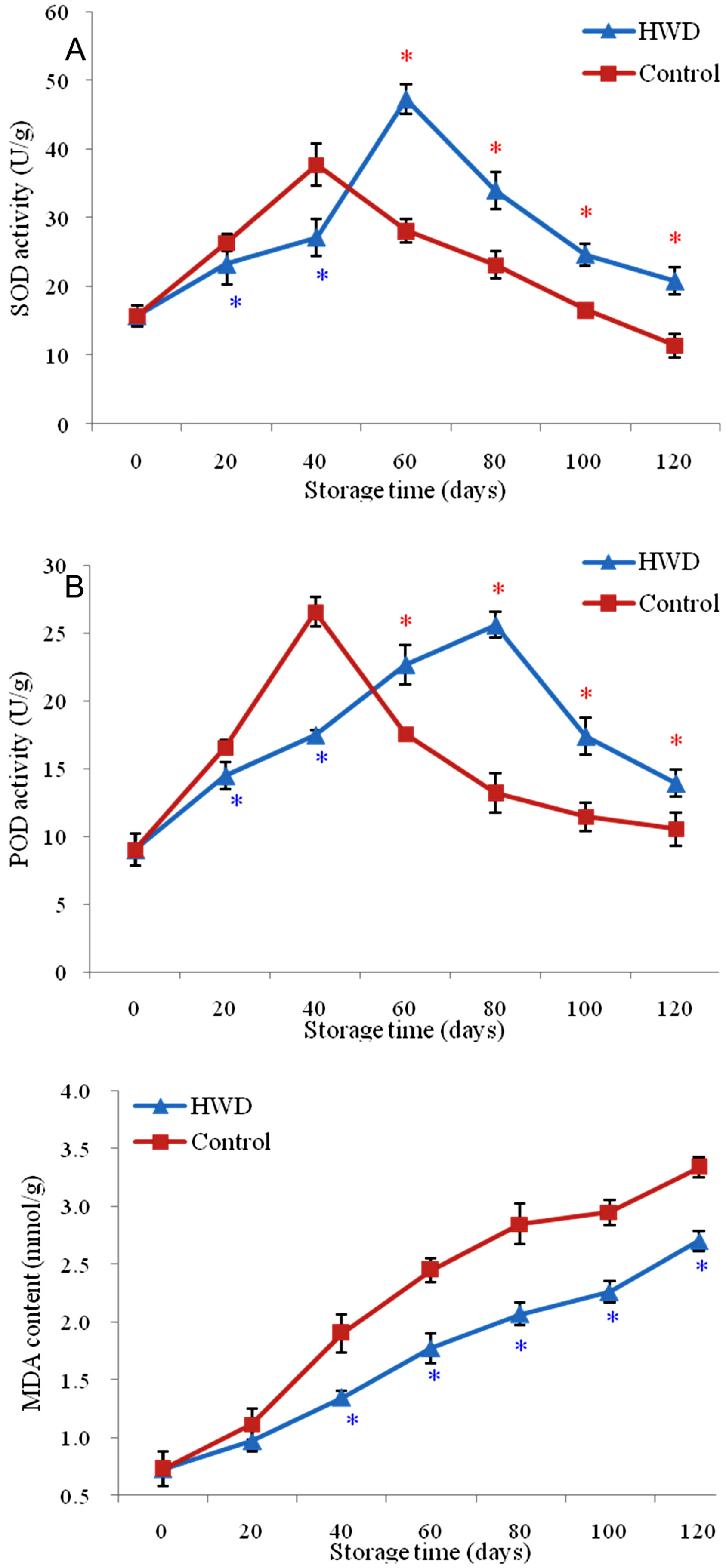

Fig. 4. Effect of HWD on enzyme activities of SOD (A), POD (B) and PPO (C) of navel orange fruits during 120 days of cold storage at 5 ±0.5°C. The symbol * and * (the HWD-treated fruit) represented significantly higher and lower than the control fruit, respectively (independent samples t-test, $P < 0.05$)

study, the VC content of the fruits of both groups increased during the first days of storage and then decreased. According to the results, the HWD-treated fruits had higher VC content than the others. As indicated above, this can be the one reason of the quality preservation of the HWD-treated fruits (Tab. 1). Similar effects of HWD were noted by several studies before [Imahori et al. 2016, Huan et al. 2017, Kahramanoğlu et al. 2020a, 2020b]. The VC was previously suggested to neutralize ROS and prevent chilling injury and quality degredation of the fruits [Naser et al. 2016]. Overall results suggest that the HWD preserves VC content of the fruits and this helps to preserve fruits' quality during storage.

Effect of HWD on respiration rate and MDA content of navel orange. Results about the respiration rate of orange fruits supported the other results of present study. It was noted that respiration rate decreased up to 40th day of storage and then increased (Fig. 2). After the 40th day of storage, the RR of the HWD-treated fruits was measured as lower than the control fruits. This is the main reason behind the preservative effect of the HWD treatment. The reduction in the RR is known to reduce breakdown of carbohydrates which preserves or delays the weight loss. The reduced RR at the HWD-treated fruits is not a new phenomenon. It was previously reported for citrus fruits [Yun et al. 2013, Kahramanoğlu et al. 2020a, 2020b] and for peach fruits [Huan et al. 2017].

The end product of lipid peroxidation is named as malondialdehyde (MDA) and is also accepted as the biomarker of oxidative damage [Cai et al. 2011]. Therefore, the MDA content of the degraded fruits increase during storage and the fruits with less degradation have less MDA (Fig. 3). As a support of this general information, the MDA content of the fruits increased during storage, but the HWD-treated fruits had lower MDA content than the others. The hydrothermal treatment was previously noted by Huan et al. [2017] to reduce the MDA content in the peach fruits.

Effects of HWD on enzyme activities of SOD, POD and PPO of navel orange. The SOD and POD of fruits of present experiment were found to have a reverse relationship with RR. Both SOD and POD increased until the minimum respiration peak (40–60 days of storage); and then decreased with the increase in RR (Fig. 4). At that time, the SOD and POD values of the HWD-treated fruits were found to be higher than the control groups. This result explain the preservation ability of HWD treatment. This is because SOD and POD enzymes help to alleviate lipid peroxidation [Ballester et al. 2006, Sels et al. 2008]. The acitivity of POD enzyme was previously observed to prevent cells from by scavenging free radicals under various stress [Klessig and Malamy 1994, Sels et al. 2008]. Both the SOD and POD activities were also noted to be higher at the end of the storage period than the initial values. These results are in agreement with the reports of Yun et al. [2013]. Furthermore, as a support of present results, Wang et al. [2015] suggested that the increase in the SOD enzyme helps to reduce decay rate at sweet cherry fruit.

Additional to SOD and POD, the PPO enzyme was also noted to be higher at the HWD-treated fruits than the control group. However, the change trend of the PPO was differing than the SOD and POD. The PPO values of the hydrothermal treated fruits were always higher than the control groups. This is an important result of current work. It was previously noted that the PPO enhances disease resistance of tissues [Gao et al. 2018]. This result of current work supports the other results about the physical and bio-chemical quality preservation of the navel oranges. PPO is mainly responsible from the oxidation of phenolics into quinones, which protects the tissues from fungal pathogens [Mohammadi and Kazemi 2002].

CONCLUSIONS

Discussion of the findings of present study revealed the known benefits of hydrothermal treatment in postharvest handling practices. Present results also made it possible to determine the optimum temperature & duration combination for the hydrothermal treatment for 'Newhall' navel oranges, which was noted as HWD at 50°C for 5 min. Results also made it possible to conclude that the physical and bio-chemical quality preservation of the navel oranges is supported by the inducement of the activities of SOD, POD and PPO enzymes, together with the VC contents. The MDA content was also found to have a positive relation with the quality degradation and the results suggested that the hydrothermal treatment reduce the MDA value of the fruits.

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