Pak. J. Bot., 53(6): 2041-2046, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-6(22)

THE SYNERGISTIC EFFECT OF *SCOLYMUS MACULATUS* L. PLANT EXTRACTS AND HOT WATER DIPPING ON THE POSTHARVEST STORABILITY OF VALENCIA ORANGES

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Abstract

Present research was carried out with an objective to test the synergism effect of *Scolymus maculatus* L. plant extracts and hot water dipping (HWD) on the storability of Valencia oranges. Experiments were conducted with four different treatments and five replications in each. Each of these replications consisted of five fruits and studies were scheduled to keep on for 150 days. Quality parameters were measured with 30-days interval. The treatments of current study are: a) immersing in pure water at 25°C for 5 min. (control); b) HWD at 50°C for 5 min.; c) immersing in pure water at 25°C for 5 min. with 1.0% *S. maculatus* L. plant extracts (Sm extract); and d) immersing in HWD at 50°C for 5 min. with 1.0% *S. maculatus* L. plant extracts (HWD + Sm extract). Results made it possible to understand that the HWD treatment is successful in slowing respiration rate and prevention of weight loss, controlling postharvest pathogens and preserving visual quality of the orange fruits. Additional to these expected results, findings of current work made it possible to conclude that the *S. maculatus* L. plant extracts have also significant influence on these quality parameters and the combination of both treatments provides highest efficacy for increasing the storability of orange fruits. Postharvest application of hot water dipping with 1.0% *S. maculatus* plant extracts to the Valencia oranges was found to maintain acceptability of the fruits for 150 days of storage at 6.0±1.0°C and 90-95% relative humidity.

Key words: Bio-materials, Combined effect, Pathogen control, Physical methods and weight loss prevention.

Introduction

Citrus fruits are very important and nutritive for human consumption throughout the world. Total citrus production was about 138.5 million tonnes in 2018, where nearly 54.5% of it was consisted of oranges. Brazil, China and India, the top 3 countries of orange production cover approximately 45.5% of the total production (Anon., 2020). Valencia oranges (*Citrus sinensis* L. Osbeck) are unique, because of their high and diverse contents of bioactive phytochemicals and are highly preferred by consumers (Albertini *et al.*, 2006). However, they are known to be not tolerant to the postharvest storage mainly because of pathogens; and their marketability is significantly reduced (Alhassan *et al.*, 2020). *Penicillium digitatum* and *P. italicum* are among the most significant citrus pathogens causing postharvest decay. Storing in cold rooms at low temperature had been known to mostly control citrus pathogens, but induce the production of reactive oxygen species (ROS) and damage fruits cell wall if the duration is extended at low temperatures (< 5-8°C) (Wan *et al.*, 2020). Fungicides are among the most preferred and easily applied management measures against postharvest citrus pathogens (Sharma *et al.*, 2009). On the other hand, consumers' acceptability of the agrochemicals in postharvest handling practices have been decreasing due to the scientifically confirmed hazards on human health and development of resistance in postharvest pathogens. Therefore, scientific world has turned its face to the physical and biological methods in postharvest handling practices (Simsek *et al.*, 2010; Silvestre *et al.*, 2011; Koch *et al.*, 2017; Gundesli *et al*., 2019).

Recent scientific researches have shown that the fruit coating with edible bio-degradable materials reduce transpiration and respiration, by lowering the exchange of water vapour and atmospheric gaseous with surrounding atmosphere (Kahramanoğlu, 2017; Riva *et al.*, 2020). Coatings with bio-degradable materials may additionally prevent the development of pathogens by inhibition of pathogens or by improving products tolerance (Ncama *et al.*, 2019). Numerous studies by researchers from all over the world (Hassanein *et al.*, 2018; Chen *et al.*, 2019; Kahramanoğlu, 2019; Kahramanoğlu *et al*., 2019; Riva *et al.*, 2020) have suggested that the different biomaterials and plant extracts improve postharvest quality of fruits, where several other studies (Chen *et al.*, 2019; Moosa *et al.*, 2019) recommended success for orange fruits. Moreover, light and 1-methylcyclopropene were also reported to significantly improve storability of fresh products (Simsek, 2011; Kahramanoğlu *et al.*, 2020b). Additional to these treatments, hot treatments prior to postharvest storage have also been reported by numerous studies (Chang *et al.*, 2020; Kahramanoğlu *et al.*, 2020a; Wan *et al.*, 2020) to significantly improve the storability of fresh fruits. However, the combination of the hot water dipping (HWD) with edible coatings and/or plant extracts have not been highly tested. In one of the combination studies, it was noted that the tea tree oil synergism into the heat treatment improves the efficacy and storability of strawberry products (Wei *et al.*, 2018). It was previously reported that the extracts of *Scolymus maculatus* L. plants consist important compounds (i.e. palmitamide, oleamide, lupeol) which may be used to treat some human diseases and may have antimicrobial activities (Abu-Lafi *et al.,* 2019). The existing knowledge of the authors' has no information about the usability of *S. maculatus* plant extracts in postharvest handling practices. Therefore, present research was designed with an aim to test the synergism effect of *S. maculatus* plant extracts and HWD on the postharvest storability of Valencia oranges.

Materials and Methods

Experimental research: In this study, Valencia fruits were collected from an orchard found in Northern Cyprus. Moreover, the plants of *S. maculatus* were collected within the same orchard. Both the Valencia oranges and *S. maculatus* plants were immediately transferred to the laboratory in 60 min. In the laboratory, the fruits were selected to eliminate the non-uniformity and damaged fruits. Then, the fruits were divided into 4 groups, with 125 in each group. Five fruits from each group were packed in an open box cartoons in violas to prevent mechanical damages and microbial contamination by touching each other. These boxes were named as replications. The fruits of each group were subjected to different treatments, these were: a) dipping in water at 25°C for 5 min. (control); b) hot water dipping at 50°C for 5 min. (HWD); c) dipping in water at 25°C for 5 min. with 1.0% *S. maculatus* L. plant extracts (Sm extract); and d) dipping in HWD at 50°C for 5 min. with 1.0% *S. maculatus* L. plant extracts (HWD + Sm extract). The procedure for the preparation of the Sm extract is: firstly, *S. maculatus* and water were mixed in a proportion of 10:1000 (w/v) and then warmed to 100°C, then the mixture temperature was retained at 100°C for 30 min., hereafter the mixture was filtered and cooled down to 50 or 25°C. After dipping (treating of the fruits), fruits were dried with fresh air for 30 min. and afterwards fruits of each treatment were separately stored in cold room adjusted to the temperature of 6.0±1.0°C and a relative humidity of 90-95%.

Data collection: Experimental research was planned to last in 150 days. Moreover, during this 150 day of storage, some of the fruit quality parameters were measured regularly (30-day interval) by using the below mentioned methods. At each measurement point (30, 60, 90, 120 and 150 days of storage), five boxes (with 25 fruits) from each different application (treatment) group were taken out from the cold rooms. Electronic balance (±0.01 g) was used to measure the final weight of each fruit and it was compared with the initial weight to find out the weight loss (%) of each fruit. The respiration rates (RR) of the replications were then determined by using the method and formula of Fonseca *et al.,* (2002) and calculated as CO_2 (ml CO_2 kg^{-1} h^{-1}). The 0-3 scale of Cao *et al.,* (2011) was followed for the determination of the decay incidence (DI) as percentage (%) and the method (0-5 scale from worst to best) reported by Silva *et al.,* (2015) was used for the observation of the visual quality (including pathogenic decay and fruit browning). A standard penetrometer with a 5 mm diameter probe was used to measure fruit firmness as kg cm^{-2}. Standard methods were followed to measure the soluble solids concentration (SSC), titratable acidity (TA) and ascorbic acid (AsA) contents of the fruits. SSC (%) was measured with a hand refractometer and the TA (g 100 g^{-1} citric acid) was then measured by following the standard method of titration with 0.1 N NaOH. Finally, the AsA (mg 100 g^{-1}) was determined by titrating with 2,6-dichlorophenol indophenols.

Data analysis: ANOVA (Analysis of variance) was followed to compare the raw data belonging to each quality parameters of different treatments. In case of statistical significance, Tukey's HSD test ($p<0.05$) was done to perform statistical distinction of means. All analysis was performed in SPSS 22.0 computer software and the figures were prepared with the Microsoft excel.

Results and Discussions

Effects of HWD and *S. maculatus* plant extracts on weight loss, decay incidence, visual quality and fruit firmness: The results of current research were summarized and discussed in two main sections. In this section, the physical (external) quality parameters were presented and discussed; and in other section other physiological (internal) quality parameters were presented. The main findings of present study showed that, the weight loss of the Valencia oranges increased during the storage and reduced the marketability of the fruits. The untreated control fruits had 16.83% weight loss in 150 days of cold storage, while the weight loss at the HWD treated fruits was calculated as 13.24% (Fig. 1A). Moreover, the Sm treatment of current research was noted to have an important and statistically significant effect on the prevention of weight loss (only 12.66% loss in weight). Additional to the significant influence of these two treatments, the highest success on the prevention of the weight loss was noted from the combination of these two treatments. Therefore, the weight loss of the fruits treated with HWD + Sm treatment was found to be 10.41%. Although no significant different at 5% significance level was noted for these three treatments, the HWD + Sm treatment provided better preservation of the fruit weight. Similar results were then observed for the decay incidence (DI). Thirty day was critical in the experiments. At that time, first decay was noted from the untreated control fruits. The percentage of the DI was noted as 13.33% at that time and it was increased to 48.00% in 150 days of storage (Fig. 1B). It equals to the loss of the about half of the fruits, due to only DI. In this case, both the HWD and Sm treatment was noted to be effective in preventing DI occurrence. The DIs of the HWD and Sm treatments were calculated as 12.00% and 16.00%, respectively, at the end of the cold storage period. These values are similar to the values of DI noted in 30 day of cold storage at the untreated control fruits. Besides to these significant influences of these two treatments, the combination of both was found to provide the best performance in preventing DI. The DI of the oranges treated with HWD + Sm treatment was only 6.67% at the 150th day of cold storage.

The visual quality, is also an indication of both decay incidence and weight loss and a good indicator of the fruits' overall quality. However, the visual quality assessment also includes the browning. Therefore, as expected, the findings about the visual quality and results with weight loss were in conjunction. But rather than weight loss, visual quality is a better representative of the consumers' acceptability. In this study, both treatments were found to affect the visual quality, but this effect was non-significant (Fig. 1C). In spite of this non-significant influence, results showed that the best visual quality was obtained from the combination of the HWD and Sm treatment, as in other quality parameters. Finally for this section, the HWD and HWD + Sm treatment were found to delay the loss of fruit firmness (Fig. 1D). According to the results obtained, the fruit firmness decreased during storage. However, this decrease was rapid in the fruits treated with Sm treatment and control, and was slow in the fruits treated with HWD and HWD + Sm treatment. The initial firmness was 0.72 kg cm^{-2} and decreased to 0.55 kg cm^{-2} at the untreated control fruits in 150 days of cold storage. At the same time, the fruit firmness was 0.70 kg cm^{-2} at the oranges treated with HWD + Sm treatment. Overall, results suggested that the combination of HWD with the Sm treatment provides better performance for the preservation of the fruit firmness.

Effects of HWD and *S. maculatus* plant extracts on SSC, TA, AsA and RR: The oranges' SSC content was found to have a rising and falling trend, which increased in the first 90 days of storage and then showed a falling trend (Fig. 2A). However, the final SSC of the all treatments (12.36% - 12.60%) was found to be above the initial SSC content (12.08). The lowest SSC value was noted from control fruits, while the highest SSC value was obtained from the HWD + Sm treatment. This difference was found to be statistically in-significant. Contrary to the SSC, the TA value of the oranges decreased during the whole storage period. The initial TA value was 2.75 g 100 g^{-1} citric acid and decreased to 1.19 g 100 g^{-1} citric acid at the un-treated control fruits in 150 days of storage (Fig. 2B). At the end of the storage period, the highest TA content was noted from the fruits treated with the combination of HWD and Sm treatment. Results showed that both the HWD and Sm treatments, alone or in combination, provides favourable conditions for the delaying the loss of TA and the highest performance was obtained from the synergism effect of these treatments.

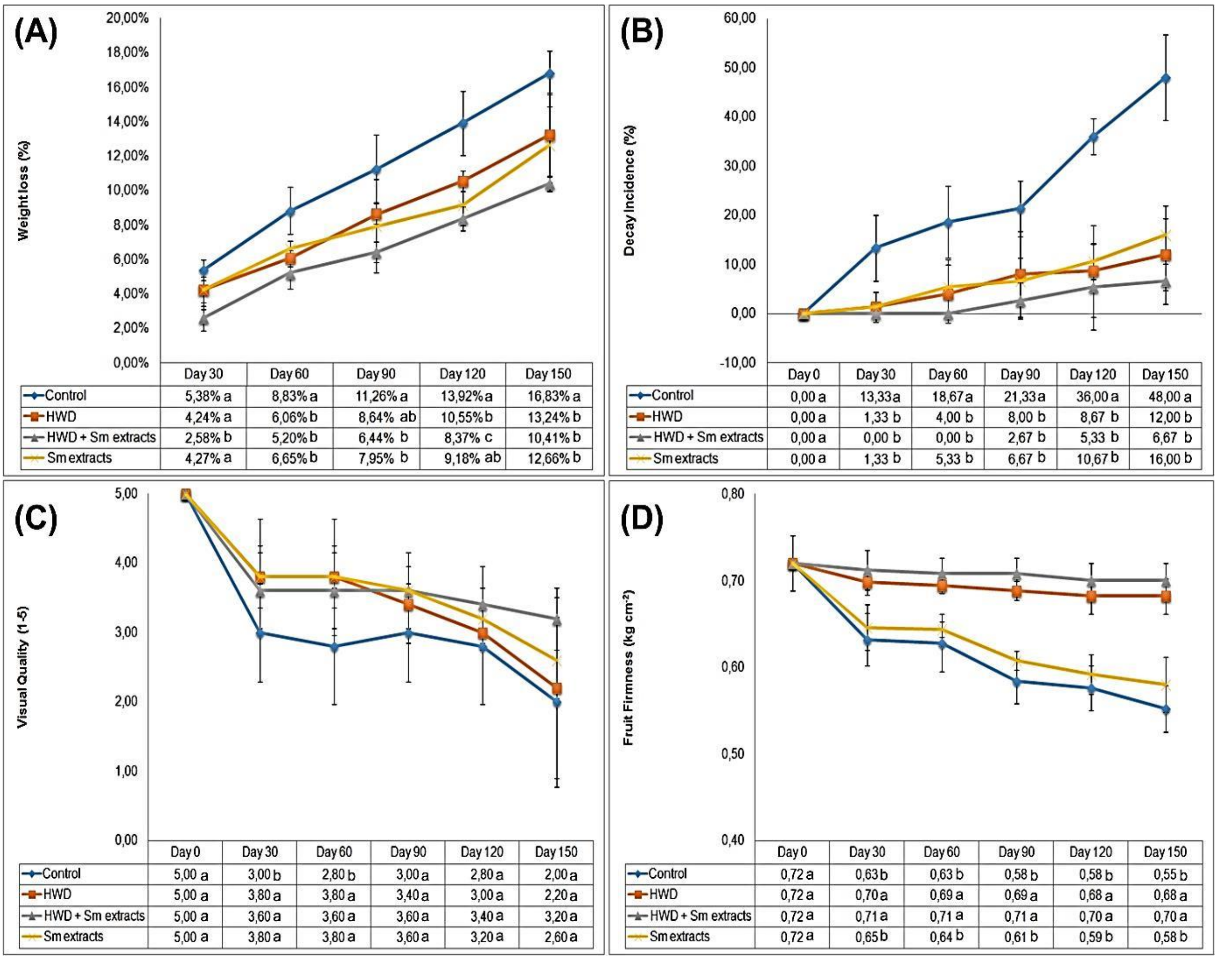

Fig. 1. Effects of HWD and *S. maculatus* plant extracts on the (A) weight loss, (B) decay incidence, (C) visual quality and (D) fruit firmness of Valencia oranges. Different letters next to the mean values at same measurement point represent significant difference at Tukey's HSD test (p≤0.05).

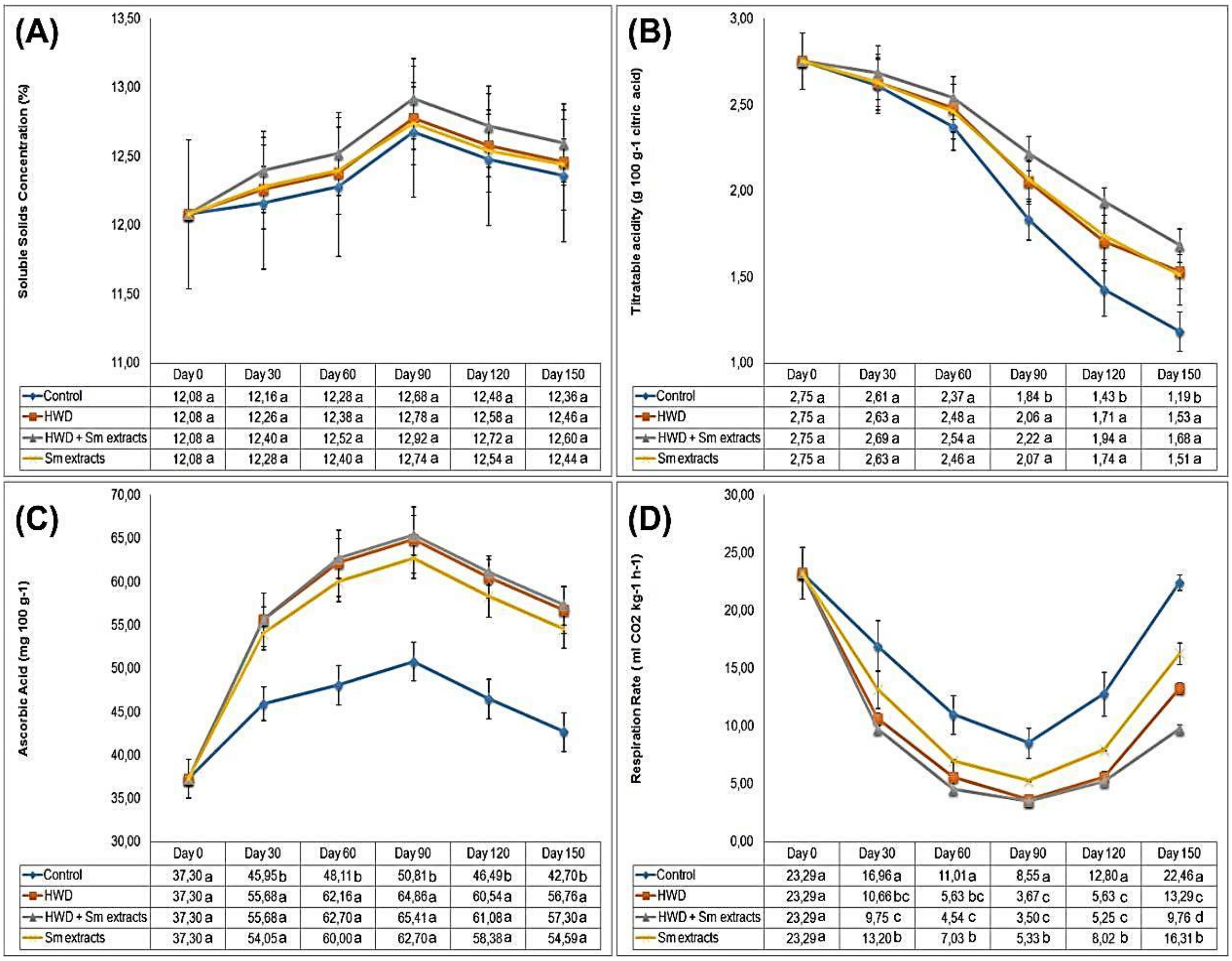

Fig. 2. Effects of HWD and *S. maculatus* plant extracts on the (A) SSC, (B) TA, (C) AsA and (D) RR of Valencia oranges. Different letters next to the mean values at same measurement point represent significant difference at Tukey's HSD test ($p \leq 0.05$).

Meaningful results were also obtained for the AsA content of the oranges. The results of current work showed that AsA has a similar trend with SSC, which is a "rising and falling". The rising continued for 90 days and the falling was observed hereafter (Fig. 2C). Similar with the other quality parameters, the treated fruits were found to have better (higher) AsA values than the un-treated fruits. The highest AsA content was observed from the fruits treated with the HWD or HWD + Sm treatment. According to the obtained results, the initial AsA was 37.3 mg 100 g^{-1} and increased to 42.70 mg 100 g^{-1} in control fruits and to 57.30 mg 100 g^{-1} in the fruits treated with HWD + Sm treatment. The other results of current research were about the respiration rate of the fruits (Fig. 2D). The RR results of oranges explain the other results of present study. The RR of the fruits was found to decrease during the first 90 days of storage and then had an upward trend. This explains the reason of 90 days to be a mid-point for SSC and AsA contents. On the other hand, the RR of the control fruits was found to be higher than the others, which explains the quality loss or reduced storability of the control oranges. In this case, the HWD + Sm treatment was found to have better performance in reducing the RR of the oranges.

In this research, the well-known (Chang *et al.,* 2020; Wan *et al.,* 2020) effects of HWD on the preservation of fruits' storability were confirmed. Besides to that, may be the most notable finding of current research is that the effects of HWD was found to increase with the combination of the *S. maculatus* plant extracts. This result is unanimous with the report of Wei *et al.,* (2018) who reported that the combination of tea tree oil into heat treatments provides better performance for the prevention of the storability of strawberry fruits. The *S. maculatus* plant extract alone had also been noticed to have a meaningful positive effect on weight loss, decay incidence, visual quality, titratable acidity, ascorbic acid and significantly reduced the respiration rate. These results are unanimous with the several previous researches (Chen *et al.,* 2019; Kahramanoğlu 2019; Riva *et al.,* 2020; ElKhetabi *et al.,* 2020) which reported significant preservation ability for different plant extracts, essential oils, plant derived products and edible coatings. Most of those studies and the current research may suggest that the preservation ability of the materials have a significant correlation with the reduction of RR. The respiration shortly is the breakdown and loss of stored sugar which equals to the fruit deterioration (Kahramanoğlu, 2017). Therefore, it can be concluded from the current results that the higher efficacy of *S. maculatus* plant extracts in preserving postharvest quality of orange fruits is attributed to the characteristics of reducing the RR of the fruits.

Present results showed that the combination of HWD and *S. maculatus* plant extracts provided better storability for the Valencia oranges (where 90 days was recommended by Rehman *et al.*, (2018)) which can be explained with the higher efficacy in blocking the exchange of atmospheric gaseous and reducing respiration rate (Kator *et al.*, 2018). Additional to the *S. maculatus* plant extracts, the HWD is a well-known treatment which highly reported to reduce the respiration rate and improve the storability of the fruits (Chang *et al.*, 2020; Kahramanoğlu *et al.*, 2020a; Wan *et al.*, 2020).

In present study, the *S. maculatus* plant extracts and HWD, alone or their synergism, were found to preserve visual quality of fruits. Plant extracts and other biomaterials were previously suggested to preserve visual quality of different fruits in cold storage (Mohebbi *et al.*, 2014; Supapvanich *et al.*, 2016). Some of the previous studies recommended that the different biomaterials and heat treatment may prevent the synthesis of PPO and POD enzymes in the fruit cells, which results with the prevention of fruit browning and chilling injury (Sharma & Rao, 2015; Tahir *et al.*, 2018; Murmu & Mishra, 2018). Both the *S. maculatus* plant extracts and HWD were also found to reduce the decay incidence, while their combination provided best performance. No studies were performed in present research about the mechanism of the pathogen control. However, it was previously suggested by Kahramanoğlu *et al.*, (2020b) that the mechanism can be grouped in two: "1) inducement of fruits' tolerance to pathogens by the biosynthesis of some secondary metabolites and 2) direct prevention of the spore and/or pathogen development with the inducement of ROS". Some plant extracts, heat treatment and light were also reported to result in the activation of pathogenesis-related proteins (PRs) and help to provide tolerance to the fruits against some pathogens (Wan *et al.*, 2020). Additional to these results, both the *S. maculatus* plant extracts and HWD were noted to increase AsA values. AsA is a vitamin that has an important place in human nutrition and cannot be stored in the human body. For this reason, it is very for consumers and its presence in the food is always questioned. It, on the other hand, improves fruits' resistance/ tolerance to oxidative stress and improves postharvest quality of the fruits. The efficacy of HWD on the AsA is well known (Erkan *et al.*, 2005; Huan *et al.*, 2017) which can also be attributed to the induction of the genes related with the AsA biosynthesis (Massot *et al.*, 2013). Further studies are required for the determination of the mechanism behind this.

Conclusions

Valuable results of present study confirmed the high ability of HWD on the prevention of postharvest fruit quality. On the other hand, this is the first study to prove the higher ability of *S. maculatus* plant extracts for the prevention of the storage quality. The combined efficacy of HWD and *S. maculatus* plant extracts is also a novel result for the science. Current work showed that, both the incorporated or alone applications of HWD and *S. maculatus* plant extracts have important effect for the prevention of postharvest quality of Valencia oranges. Overall, results recommended that the synergism of *S. maculatus* plant extracts and HWD provides suitable conditions for the Valencia oranges and the fruits can be stored for 150 days in cold room at 6.0±1.0°C and a RH of 90-95%. Further studies are necessary for the determination of the mechanism behind this significant influence of *S. maculatus* plant extracts. Furthermore, current results also showed that the incorporation of plant extracts with heat treatment provides better performance and there are numerous plant materials in the published literature which could be tested together with heat treatment.

Acknowledgments

This work was supported by the Chongqing Postdoctoral Science Foundation (Xm2015010)，the Fundamental Research Funds for the Central Universities (XDJK2015C166, SWU114045), the Fundamental and Advanced Research Projects of Chongqing City (cstc2016jcyjA0527).

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(Received for publication 22 March 2020)