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Effects of dipping time in chitosan (CS) and polyvinyl alcohol (PVA) mixture to quality of orange fruits during storage

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Abstract

Maintaining fruit freshness and quality in extended storage life represents a major obstacle in the fruit production industry. One of the easiest ways to prolong shelf life is by dipping fruits in a suitable storage solution. In this research, we assessed the effects of the dipping time of sweet orange cultivar with the mixture containing 1.15% chitosan (CS) and 0.39% polyvinyl alcohol (PVA) on the external and internal quality parameters of oranges during 12 days storage at 30 °C. The results showed that 6 min dipping time are optimum to maintain quality of tested oranges, including giving better skin color, higher total soluble solids (TSS), fruit firmness, ascorbic acid, and taste scores as well as lower weight loss, decay incidence, ethylene production, and respiration rate when compared with those of other dipping treatments. The results can be recommended to maintain the quality of postharvest orange fruits.

Keywords: dipping time; orange fruits; CS/PVA mixture; green storage; respiration.

Practical Application: Prolonging and maintaining the quality and shelf life of sweet orange fruit using a chitosan and polyvinyl alcohol mixture by dipping time.

1 Introduction

The quality of agricultural products as orange fruits are significantly affected by storage conditions and treatment methods. Orange (Rutaceae family) is classified as non-climacteric citrus fruit and is mainly growned in tropical and subtropical regions. In Vietnam, the cultivar 'sweet' orange fruit is grown predominantly in southern provinces of the country as in Mekong Delta. It is an important source of bioactive compounds, including the largest source of ascorbic acid, carotenoids, flavonoids and many other antioxidants (Couto & Canniatti-Brazaca, 2010; Davies et al., 2017; Rehman et al., 2020). It is noted that respiration rate and ethylene production are not remarkable during its ripening period. In improper storage conditions, physiological disorders and weight loss of orange are also susceptible due to dehydration of the fruit (Grierson & Miller, 2006). Besides, some pathogens on postharvest oranges, including *Lasiodiplodia theobromae*, *Phomopsis citri*, *Alternaria citri*, *Botrytis cinerea*, *Colletotrichum musae* and *Phytophthora citrophthora* have been proved to significantly affect its final quality and shelf life (Eckert & Eaks, 1989). Especially, *Penicillium digitatum* and *P. italicum* caused decay after 7-10 days of storage at 20-25 °C via wound infection, as reported in Palou (2014) finding. Therefore, many researchers have recently focused on using biological films for fruit preservation since they are safe, ecofriendly, low cost, and easy to use (Nair et al., 2020).

For preservation purposes, the biological film was mainly comprised of starch, derivative cellulose, chitosan, chitin, protein or fat. Among them, chitosan (CS) is a biological polymer with many properties as biodegradability, biocompatibility, safety, eco-friendliness, film-forming characteristics, and antibacterial ability and have been applied in food industry to extent the storage life of foods (Vasilatos & Savvaidis, 2013). PVA is a synthetic polymer having many good mechanical properties such as water solubility, tensile strength, safety, biocompatibility, biodegradability, oxygen and aroma barrier properties, flexibility, film-forming and easy preparation or uses (Silva et al., 2013) that has been applied in many fields such as drug, coating agents, adhesives, packaging, medical applications (Goodship & Jacobs, 2009). In addition, PVA is also accepted for use in American package meat and poultry product (DeMerlis & Schoneker, 2003). On the other hand, CS is known for its high degree of brittleness whereas, with less tensile strength and elasticity. As noted in many earlier researchers that blending CS with synthesis polymers as PVA could improve the mechanical properties and bioactivity of mentioned films above (Bonilla et al., 2014). A combination between CS with PVA have been intensively studied as biodegradable, biological films (Costa-Júnior et al., 2009; Tripathi et al., 2009) as well as its excellent characteristics due to hydro and ether bridge in their's structure (Chuang et al., 1999; Liang et al., 2009).

CS-PVA blend films have been studied and applied in various fields such as food packaging (Liu et al., 2017), wound dressing (Morgado et al., 2017), and air filtration (Wang et al., 2018). Our previous in-vitro study showed that CS-PVA mixture at the concentration 1.15% CS and 0.39% PVA was significantly effective in disease control of postharvest oranges (Them et al., 2021). Regardless of the favorable properties of this mixture

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in some specific fields, it has not been intensively studied to preserve agricultural product, particularly for postharvest oranges. In this study, we carried out a survey and assessment of affecting dipped time a CS and PVA mixture to quality of postharvest oranges during storage. Therefore, the objective of this study was to investigate the effective dipping time in CS and PVA mixture to maintain the quality as well as extend the shelf life of postharvest oranges during storage.

2 Materials and methods

2.1 Preparation of orange fruits

'Sweet' orange fruit (*Citrus sinensis* L. Osbeck) were harvested a mature green stage from a garden in Dong Thap province, Vietnam. Harvested fruit were placed into boxes and transported on the same day to laboratories and then recut with a portion of fruit stalk length (abount 0.5 cm) was kept on the fruit. Damaged, diseased, mechanically injured or fruit with signs of irregular ripening were excluded while only fruit with uniform size and color with an individual weight of 300 ± 2 g were chosen. Before experiments, these fruits were washed away by distilled water and then dried under a fan for 5 min.

2.2 Preparation of dipped solution

Chitosan (CS) has 96.6% deacetylated degree and 7.4 pH from Chitoworld Co, Vietnam. Polyvinyl alcohol (PVA) was purchased from SDFCL Sd Fine Chem Limited, India, 1700-1800 polymerized degree. The dipping mixture containing CS and PVA was prepared according to the study of Them et al. (2021), which was previously considered to have a significant influence on disease control of postharvest oranges. In brief, 1.15% (w/v) CS in 1% acetic acid (v/v) and 0.39% (w/v) PVA in boiling water at 90 °C were dissolved separately with a speed of 500 rpm at room temperature. CS and PVA solution were then combined before adding 0.1% glycerol (w/v) to the mixtures - a pliable substance for films (adjusted pH = 5.5). The final mixture needed to be stirred (200 rpm) at room temperature for 15 min to completely remove gas (Bonilla et al., 2014).

2.3 Experimatal produces

After the fruit were dried, the experiment was carried out by dipping in CS/PVA solution containing 1.15% CS, 0.39% PVA in 2, 4, 6 min, control fruit was not dipped. Dipped fruits were then dried under a fan and were dispensed into perforated cardboard boxes (10 fruit for each box) and stored at temperature (30 ± 2 °C, 80 ± 5% RH). Each treatment involved fifteen boxes of oranges treated at the same condition. The quality of oranges, including weight loss, skin color, respiration rate and ethylene production, decay incidence, firmness, ascorbic acid, total soluble solids (TSS), and sensory quality (taste) was assessed after 3, 6, 9, 12 days storage at 30 ± 2 °C. The experiment was completely randomized factorial design one factor in triplicate with 200 fruits per replication.

2.4 Fruit quality assessments

The respiration rate and ethylene production were measured by a gas analyzer (Felix-F-960, CID Bio-Science, Inc, USA). The results of respiration rate and ethylene production were expressed as $\mu mol.kg^{-1}.s^{-1}$, $nmol.kg^{-1}.s^{-1}$, respectively. At the assessment period for each of treatment, one orange fruit was put in 0.5 l plastic jar with tight-fitting lids within 120 min at 30 °C, 80 - 85% RH, and then measured with the help of septums (PBI 45 mm, grey, 1000 pcs). All experiments were performed in triplicate, five fruit for each of replicate.

Weight loss of 10 individual fruits per replicate treatment was measured by analytical balance (UX420S, 420 g ± 0.01, Japan) by the formula: Weight loss (%) = (Initial weight – weight after storage time)/initial weight × 100. Skin color (L*, a*, b*) of 10 individual fruits per replicate in treatment was measured by a chromameter (CR400, Minolta Camera Co. Ltd, Osaka, Japan). The percentage decay incidence of 10 individual fruits per replicate in treatment was calculated as the number of fruit with decay / the total fruit number × 100.

The firmness of 10 fruits in each treatment was measured as expressed in Newton force (N) by Landtek FHT-15 fruit hardress tester with 3.5 mm tipp (Guangzhou Landtek instrument Co. Ltd, China). Total soluble solids (TSS) of 10 fruits were measured in % using a digital refractometer (Atago, Tokyo, Japan) for crushed fruit flesh. Taste of 10 orange fruits in each treatment was evaluated by 10 trained panelists based on a nine-point scale (9 = excellent, 7 = good, 5 = acceptable but with limited marketability, 3 = poor and 1 = extremely poor). The fruits in each treatment were presented in separate, randomly numbered trays to panelists. Each panelist evaluated three fruit per treatment.

Ascorbic acid content was determined by the method described by Gliszczynska-Swiglo & Tyrakowska (2003). Briefly, 0.5 mL orange juice was prepared and then added 0.5 mL solution 10% metaphosphoric acid (MPA) to obtain final solution, and then homogenized sample by a vortex in 5 min, and 10.000 rpm centrifugation in 5 min. Finally, the supernatant was then injected onto the high-performance liquid chromatograph (HPLC) column to determine ascorbic acid content. Ascorbic acid was determined using Waters 600 HPLC equipments (Waters Corp., Millford, MA, USA) equipped with LiChrosorb C18 (250 × 4.0 mm, 5 mm, Merck KGaA, Germany) fitted with the same guard column. A gradient of mobile phase contained of methanol (solvent A) and 5 mmol/l KH_2PO_4, pH 2.65 (solvent B) was used according to the following program: linear increment starting with 5-22% A in 6 min and the return to the initial conditions within the next 9 min with the flow rate of 1.0 mL/min. The eluate was detected using a Waters 996 photodiode array detector set at 245 nm. Ascorbic acid content was identified based on UV spectrum and retention time with standard substrate and expressed as mg/100 g FW.

2.5 Statistical analysis

All data were analysed and presented as the mean and the standard deviations using JMP 10.0 software (SAS Institute Inc., Cary, NC, USA). The experiments were designed following a completely randomised factorial design with one factor. Significant differences between treatments were shown through the Duncan test at 95% confidence level ($p < 0.05$).

3 Results and discussion

3.1 Respiration and ethylene production

The respiration rate and ethylene production of orange fruit during 12 days storage at 30 °C have been affected by dipping time in CS and PVA mixture as summarized in Figure 1. Ethylene and CO_2 production of fruit were progressively increased, meanwhile, O_2 was progressively decreased during storage at all the treatments. Ethylene production of postharvest oranges was decreased when increased dipping time in CS and PVA mixture. The orange fruits were treated with a dipping time of 6 min, having the lowest ethylene content (0.03, 0.046, and 0.062 nmol.kg^{-1}.s^{-1} after 6, 9, and 12 days of storage, respectively). Besides, ethylene production of control fruit was higher than that of dipped fruit as 0.092 nmol.kg^{-1}.s^{-1} after 12 days storage at 30 °C (Figure 1A). Coinciding with increasing ethylene content, the respiration processes of oranges occurred with reducing O_2 gas and enhancing CO_2 gas in measurement jar. Meanwhile, untreated fruits was seen to have stronger respiration processes than treated fruit. The CO_2 content of 6 min dipped fruit after 12 days of storage was 0.75 µmol.kg^{-1}.s^{-1}, lower than control fruit (1.09 µmol.kg^{-1}.s^{-1}) at the same time assessment (Figure 1B). Contrastly, at the same time assessmet after 12 days storage, fruit dipped in CS and PVA mixture of 6 min for O_2 content is -2.85 µmol.kg^{-1}.s^{-1}, lower than undipped fruit (-3.17 µmol.kg^{-1}.s^{-1}) (Figure 1C).

Since orange fruit is the type of fruit without the respiratory peak (non-climacteric), the respiration rate and ethylene production are not considersably increased during fruit growing and ripening (Porat, 2008). Having differences in respiration rate at all the treatments could be due to the nature of CS/PVA blending films that created a very thin film layer of coated fruit to make delaying respiration and ripe processes of fruit, resulting in reduce ethylene production. In addition, a longer dipping time might create a thicker film layer that interfered better with metabolic gas inhibiting ethylene production and slowing down physiological ripening processes of fruits. Besides, these changes were also related to biochemical changes during the ripening of fruit (Rapisarda et al., 2001)

3.2 Weight loss (%)

Dipping time of CS and PVA mixture have remarkablely affected to weight loss ratio of postharvest oranges during storage at 30 °C, and results are shown in Figure 2A. Gernerally, weight loss ratio of oranges was gradually increased following storage time at all the treatment, meanwhile, the weight loss ratio of fruit dipped in CS and PVA mixture was lower than control. The control fruit always showed weight loss ratio at the highest percentage, which was 25.28% after 12 days of stoarge, and also double than treated fruit of 4 or 6 min with CS and PVA mixture (Figure 2A)

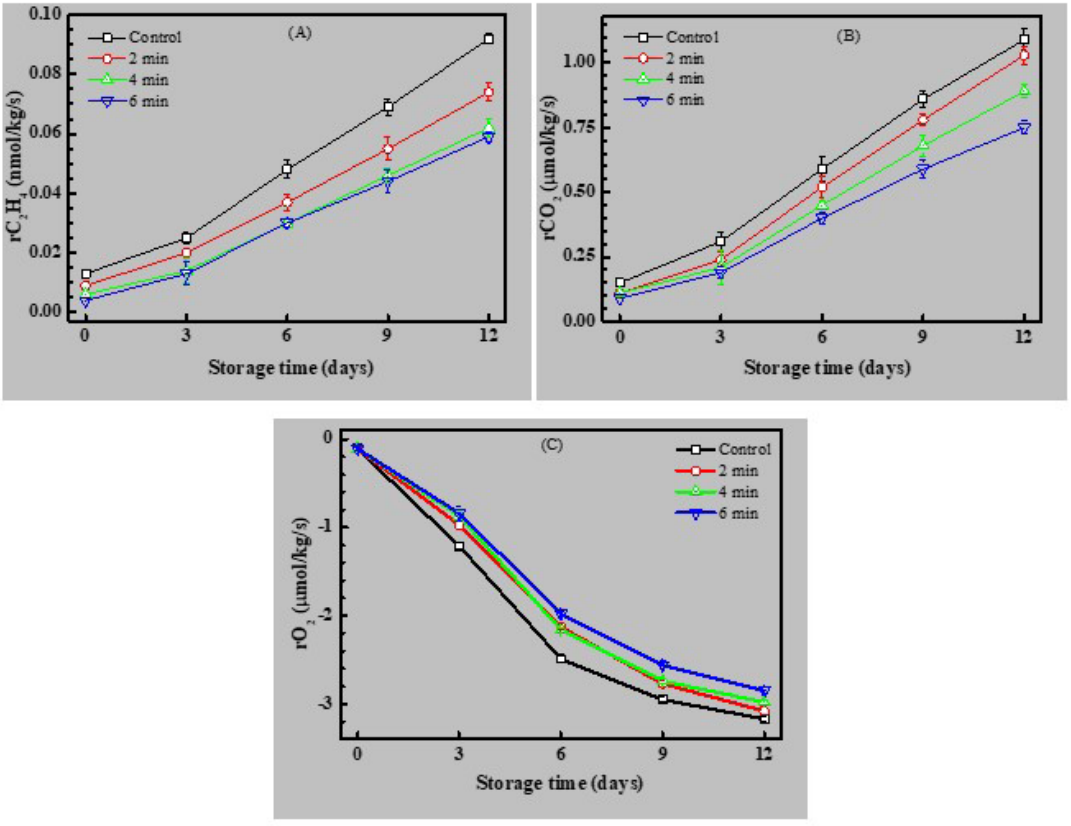

Figure 1. Effects of dipped time on changes in respiration rate and ethylene production of sweet orange fruits during storage at 30 °C: (A) C_2H_4 production; (B) CO_2 production; (C) O_2 production (data were presented as mean ± standard deviation, N= 3).

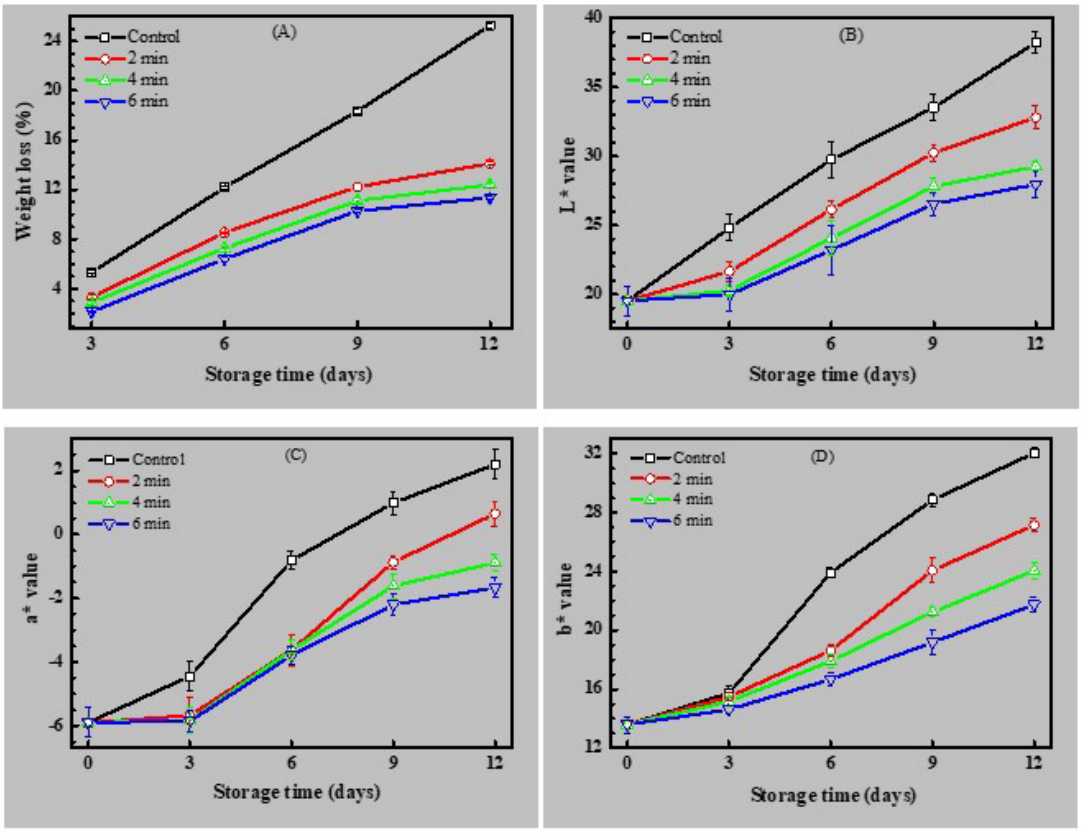

Figure 2. Effects of dipped time on changes in weight loss and skin color of sweet orange fruits during storage at 30 °C: (A) weight loss; (B) L* value; (C) a* value; (D) b* value (data were presented as mean ± standard deviation, N= 3).

CS was popularly applied in the storage of many agricultural products due to its film-forming ability, biodegradability, and bioadhesivity. Significantly, CS blending with PVA could improve mechanical properties of the film due to their's good properties such as biological dissolving ability and emulsifying capability, due to the presence of ether-bridge and hydrogen bond in their's structure (Chuang et al., 1999; Costa-Júnior et al., 2009; Liang et al., 2009; Tripathi et al., 2009; Wang et al., 2018). Causing of phenomenon natural weight loss during postharvest oranges storage at all the treatment is due to respiration activity and water vapor diffusion. Dipped fruit in CS and PVA mixture was delayed in water vapor diffusion processes due to its characteristics leading to reduce weight loss. Different dipping times would generate different thickness of the film layer resulting in variations of water vapor leakage rate and fruit respiration.

3.3 Changes in skin color

Changing the skin color of postharvest oranges during storage at 30 °C had significantly affected by the dipping time in the CS and PVA mixture, as shown in Figure 2B, C, and D. L* value at all the treatment was progressively increased during storage. Meanwhile, the L* value of the control sample obtained the highest changing, simultaneously, the skin color of 6 min dipped fruit was maintained (Figure 2B). In addition, a*, and b* value was gradually increased during storage, the green color of skin turning tendency to yellow color (Figure 2C, and D). As a result, dipping time was considered to be parellel with the color changes of fruit. Skin color changes of control fruit after 12 days of storage were the highest and significant difference compared to dipped fruit. Meanwhile, the lowest color change was belonged to dipped fruit, especially in dipped fruit with 6 min. These changes of untreated fruit were in accordance with weight loss ratio, respiration rate and ethylene production that were proved in Figure 1A, B, C and 2A.

Postharvest oranges are continuously happening ripe processes, and physiological, and biochemical conversion, inducing ethylene gas and this gas accelerated chlorophyll dissolving to reduce green color and new color synthesis as carotenoids and anthocyanin (Jobling et al., 2002). In addition, CS combining with PVA can prevent damage and delay skin color changes during storage, resulting in a significant difference among all tested treatments that we have noted. Samples with longer dipped time would create films thicker, and so O_2 gas was difficult to penetrate inside fruits retarding chlorophyll oxidation processes. So, dipped fruit in CS and PVA mixture can retard the fruit's ripe, physiological, and biochemical processes.

3.4 Changes of decay incidence, firmness, ascorbic acid, and TSS of fruit

Results of decay incidence of postharvest oranges during storage are mentioned in Figure 3A. Rot incidence of orange fruit was increased at all the treatment during storage at 30 °C.

In the first initial of 3 days of storage, oranges did not display any decay at all treatments, but after 6 days storage, oranges started to show a sign of decay. Meanwhile, control fruit having decay incidence was highest (8.2%) after 12 days of storage and significant difference than the others. However, dipped time 6 min started to show a sign of decay after 9 days of storage and reached lowest after 12 days of storage (2.14%). Our results were consistent with obtained data from above analysis as weight loss, skin color, and respiration rate changed in this present study.

Together with weight and color changes, the structure of the fruit was also altered following ripe processes. Our results showed that firmness of fruit was gradually decreased during storage at all the treatment that might be due to water loss, biochemical changes making the structure of cell becoming weak, loose, and soft fruit as shown in Figure 3B. Dipping time had an inverse ratio with decreased in firmness of the fruit. For instance, the firmness of the control fruit was fastest decreased than the others, and the lowest decrease belonged to 6 min dipped fruit.

Reducing of firmness during storage of oranges might be due to increased activity of polygalacturonase and pectinesterase enzyme from which undissolved pectin in the cell was disintegrated, weakening the binding abilities between the cell and tissues and making softer skin fruits as mentioned in the previous study (Villarreal et al., 2008; Tavarini et al., 2009). In addition, orange fruit became over-ripen until the senescence stage, including protein dissolution of the films and weak binding of the structure to reduce the hardening of oranges. So, CS and PVA mixture was used to delay biological ripening processes and reduce the softness of the fruit.

Citrus fruits and their products are becoming an essential dietary ascorbic acid source and more importantly, there is an increasing focus on studying, especially on the stability of ascorbic acid in fruit during storage (Nagy, 1980). Our results showed that ascorbic acid content in orange fruits at harvest stage was high, but continuously decreased during storage at all the treatments as noted in Figure 3C. Especially after 12 days of storage, ascorbic acid in the control fruit was significantly dropped to 31.1 (mg/kg) with the loss almost near seven times compared to freshly harvested fruit. Meanwhile, after dipping fruit for 6 min, ascorbic acid content only lost nearly a haft compared to fruit at harvest stage. Rapisarda et al. (2001) reported that ascorbic acid content changed depending on cultivars. For instance, Tarocco orange did not show any loss in ascorbic acid content during storage at 8 and 22 °C for 85 and 106 days, respectively. However, for 'Moro' oranges, ascorbic acid content was reduced in the first 50-day of storage, and similar results were also reported in blood oranges cultivars as 'Tarocco Messina', 'Tarocco Meli', and 'Moro' oranges, stored at 6 ± 1 °C for 65 days (Rapisarda et al., 2008). In addition, another study by Scandalios (1993) reported that ascorbic acid content of 'Tarocco Messina' and 'Ovale' oranges increased at first period of storage and then declined but not reduce under antioxidant protection levels of fruit at the end of storage (48.86-63.66 mg/100 mL). Besides, for 'Valencia' oranges

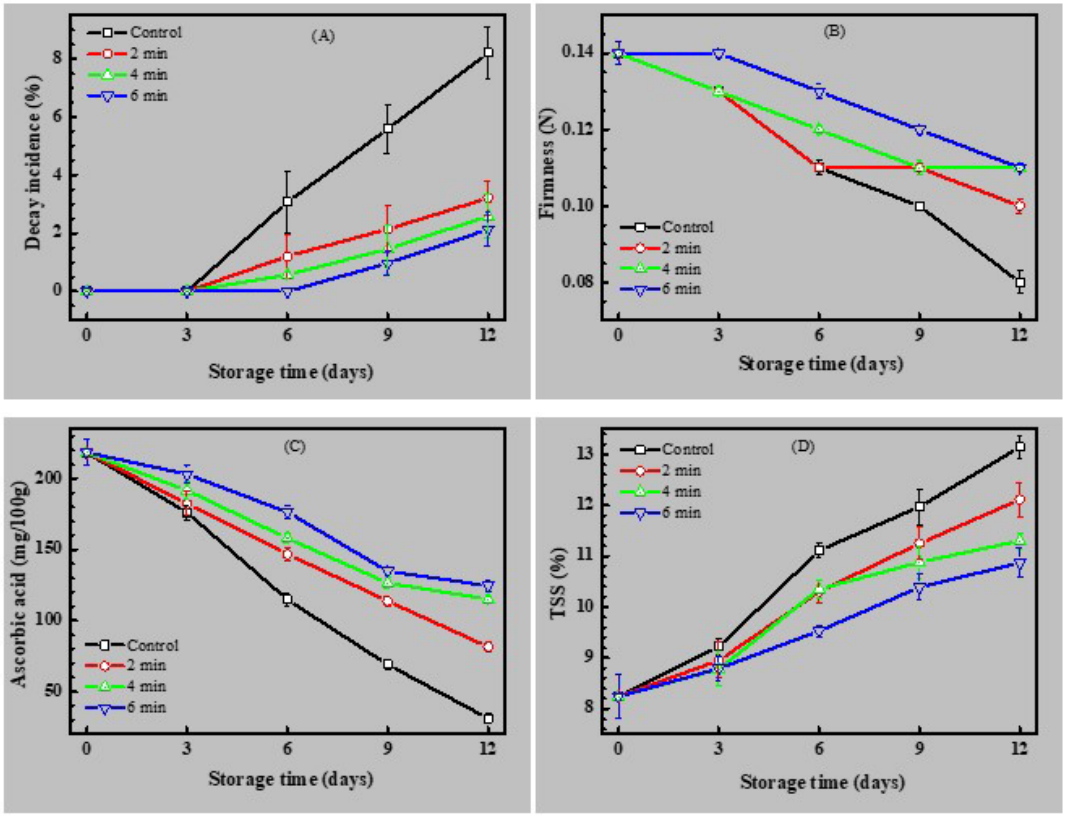

Figure 3. Effects of dipped time on changes in decay incidence, firmness, ascorbic acid and TSS of sweet orange fruits during storage at 30 °C: (A) decay incidence; (B) firmness fruit; (C) ascorbic acid content; (D) TSS value (data were presented as mean ± standard deviation, N= 3).

juice has also been stidied by Baldwin et al. (1995), it is noted that ascorbic acid content showed a decreasing trend through storage period. In other studies, the slight loss in ascorbic acid of citrus fruits during storage at different temperatures have been reported (Nagy, 1980). Reducing ascorbic acid content in 'Moro' oranges might be due to the interaction (direct condensation) with anthocyanins present in fruits at high concentrations by accelerating degradation of ascorbic acid (Poei-Langston & Wrolstad, 1981). Our results indicated that the ascorbic acid content of 'Sweet' orange was decreased during storage (Figure 3C) which was also consistent with several previous studies. For dipped oranges in CS and PVA mixture, ascorbic acid content was reduced to be less than undipped fruit that also complied with biological changes, skin color, firmness, decay incidence, respiration rate as proved above.

TSS content of oranges from all the treatments during storage at 30 °C was achieved an increasing trend with storage time as summarized in Figure 3D. The TSS value of control fruit received the highest increase from 8.24 up to 13.14% after 12 days storage. Meanwhile, 6 min dipped fruit had a lower TSS value (10.87%) after 12 days storage. Rapisarda et al. (2001) reported that TSS of 'Tarocco' oranges were increased during the storage at 8 °C for 85 days while TSS of 'Moro' oranges were slightly decreased at 106 days of storage. In addition, for 'Valencia' oranges, the TSS at the first stage increased and then reduced at the last storage period, and are also obtained by El-Zeftawi (1976), who noted TSS change during cold storage. Our results in this study were in accordance with the report for 'Tarocco' oranges by Rapisarda et al. (2001), who observed that TSS increase during storage. In another study of Baldwin et al. (1995), coated fruit with polysaccharide-based or shellac-based water wax have slightly lower TSS than uncoated fruit. Besides the presence of glycolytic enzymes in the fruit increase during storage life of 'Valencia' orange storage, a capable mechanism was explained by Echeverria & Valich (1989), in which surgar levels in harvested citrus fruit continuously increased might be due to de novo synthesis of sugars from organic acids.

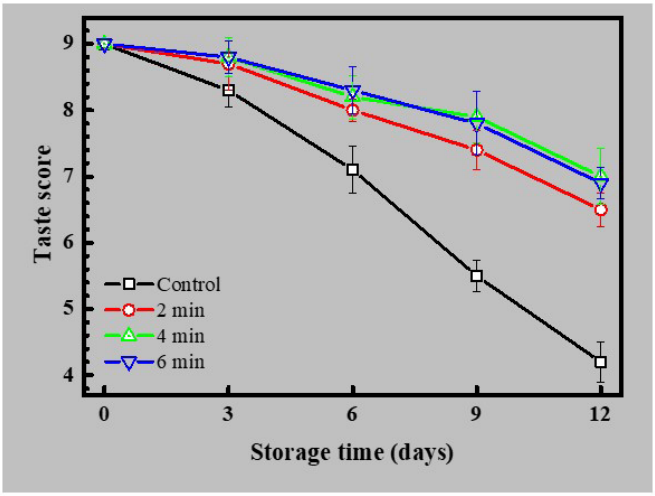

Figure 4. Effects of dipped time on changes in taste scores of sweet orange fruits during storage at 30 °C (data were presented as mean ± standard deviation, N= 3).

3.5 Sensory quality (taste) during storage

The taste of orange fruit showed a gradual decline during storage at 30 °C, as shown in Figure 4. In particular, the reduction in taste was more rapid at the end of storage life. During storage, the control fruit showed the lowest taste score (after 12 days at 30 °C with taste reach 4.2) compared to other treatments. Meanwhile, dipping fruit in 6 min showed the highest taste score at storage end (Figure 4). This phenomenon might be due to higher weight loss, respiration rate, and ethylene production, of the control treatment, resulting in higher decay incidence and changes in skin color, TSS, ascorbic acid, consequently in a lowered sensory score. Therefore, under storage period of 12 days at 30 °C, fruit dipped with 6 min exhibited better quality indicators and favorable sensorial score. Otherwise, the sensory score of the control fruit was within acceptable range but is still lower than that of 6 min dipped treatment.

4 Conclusions

It was clearly demonstrated that dipping time of CS and PVA mixture could significantly influence the quality of orange fruits during 12 days of storage. Among the four tested dipping time, the dipping time of 6 min in 1.15% CS + 0.39% PVA mixture showed the best preservative efficacy and maintained the best quality of sweet orange during 12 days storage at 30 °C with fewer changes in weight loss, skin color, firmness, ascorbic acid content, and TSS. In addition, this dipping time was capable of lower ethylene production (0.059 $nmol.kg^{-1}.s^{-1}$) and respiration rate (0.75 $\mu mol\ CO_2.kg^{-1}.s^{-1}$) and lower decay incidence and higher taste rating than control fruit after 12 days storage at 30 °C. Data analytics used in this study suggest that 6 min dipped time could be applied for prolonging preservation, maintaining quality, and reducing the decay of orange fruits.

Conflict of interest

None.

References

Baldwin, E. A., Nisperos-Carriedo, M., Shaw, P. E., & Burns, J. K. (1995). Effect of coatings and prolonged storage conditions on fresh orange flavor volatiles, degrees brix, and ascorbic acid levels. *Journal of Agricultural and Food Chemistry*, 43(5), 1321-1331. http://dx.doi.org/10.1021/jf00053a037.

Bonilla, J., Fortunati, E., Atarés, L., Chiralt, A., & Kenny, J. M. (2014). Physical, structural and antimicrobial properties of poly vinyl alcohol–chitosan biodegradable films. *Food Hydrocolloids*, 35, 463-470. http://dx.doi.org/10.1016/j.foodhyd.2013.07.002.

Chuang, W. Y., Young, T. H., Yao, C. H., & Chiu, W. Y. (1999). Properties of the poly (vinyl alcohol)/chitosan blend and its effect on the culture of fibroblast in vitro. *Biomaterials*, 20(16), 1479-1487. http://dx.doi.org/10.1016/S0142-9612(99)00054-X. PMid:10458561.

Costa-Júnior, E. S., Barbosa-Stancioli, E. F., Mansur, A. A., Vasconcelos, W. L., & Mansur, H. S. (2009). Preparation and characterization of chitosan/poly (vinyl alcohol) chemically crosslinked blends for biomedical applications. *Carbohydrate Polymers*, 76(3), 472-481. http://dx.doi.org/10.1016/j.carbpol.2008.11.015.

Couto, M. A. L., & Canniatti-Brazaca, S. G. (2010). Quantification of vitamin C and antioxidant capacity of citrus varieties. *Food*

Science and Technology, 30, 15-19. http://dx.doi.org/10.1590/S0101-20612010000500003.

Davies, C. V., Gerard, L. M., Ferreyra, M. M., Schvab, M. D. C., & Solda, C. A. (2017). Bioactive compounds and antioxidant activity analysis during orange vinegar production. *Food Science and Technology*, 37(3), 449-455. http://dx.doi.org/10.1590/1678-457x.20816.

DeMerlis, C. C., & Schoneker, D. R. (2003). Review of the oral toxicity of polyvinyl alcohol (PVA). *Food and Chemical Toxicology*, 41(3), 319-326. http://dx.doi.org/10.1016/S0278-6915(02)00258-2. PMid:12504164.

Echeverria, E., & Valich, J. (1989). Enzymes of sugar and acid metabolism in stored 'Valencia' oranges. *Journal of the American Society for Horticultural Science*, 114, 445-449.

Eckert, J. W., & Eaks, I. L. (1989). Postharvest disorders and diseases of citrus fruits. *The Citrus Industry*, 5, 179-260.

El-Zeftawi, B. M. (1976). Cool storage to improve the quality of Valencia oranges. *Journal of Macromolecular Science. Part A*, 44(5), 411-418.

Gliszczynska-Swiglo, A., & Tyrakowska, B. (2003). Quality of commercial apple juices evaluated on the basis of the polyphenol content and the TEAC antioxidant activity. *Journal of Food Science*, 68(5), 1844-1849. http://dx.doi.org/10.1111/j.1365-2621.2003.tb12340.x.

Goodship, V., & Jacobs, D. K. (2009). *Polyvinyl alcohol: materials, processing and applications* (Vol. 16, No. 12, 142 p.). Shrewsbury: Smithers Rapra Technology.

Grierson, W., & Miller, W. M. (2006). Storage of citrus fruit. In W. F. Wardowski, W. M. Miller, D. J. Hall & W. Grierson (Eds.), *Fresh citrus fruits* (2nd ed., pp. 547-581). Longboak Key: Florida Science Source Inc.

Jobling, J., McConchie, R., & Cannon, A. (2002). *Practical concepts in postharvest biology and technology. The AusAID CARD project at the University of Sydney and Sydney Postharvest Laboratory, funded by AusAID.* Australia: University of Sydney and Sydney Postharvest Laboratory.

Liang, S., Liu, L., Huang, Q., & Yam, K. L. (2009). Preparation of single or double-network chitosan/poly (vinyl alcohol) gel films through selectively cross-linking method. *Carbohydrate Polymers*, 77(4), 718-724. http://dx.doi.org/10.1016/j.carbpol.2009.02.007.

Liu, Y., Wang, S., Lan, W., & Qin, W. (2017). Fabrication and testing of PVA/Chitosan bilayer films for strawberry packaging. *Coatings*, 7(8), 109. http://dx.doi.org/10.3390/coatings7080109.

Morgado, P. I., Miguel, S. P., Correia, I. J., & Aguiar-Ricardo, A. (2017). Ibuprofen loaded PVA/chitosan membranes: A highly efficient strategy towards an improved skin wound healing. *Carbohydrate Polymers*, 159, 136-145. http://dx.doi.org/10.1016/j.carbpol.2016.12.029. PMid:28038742.

Nagy, S. (1980). Vitamin C contents of citrus fruit and their products: a review. *Journal of Agricultural and Food Chemistry*, 28(1), 8-18. http://dx.doi.org/10.1021/jf60227a026. PMid:7358939.

Nair, M. S., Tomar, M., Punia, S., Kukula-Koch, W., & Kumar, M. (2020). Enhancing the functionality of chitosan-and alginate-based active edible coatings/films for the preservation of fruits and vegetables: a review. *International Journal of Biological Macromolecules*, 164, 304-320. http://dx.doi.org/10.1016/j.ijbiomac.2020.07.083. PMid:32682968.

Palou, L. (2014). *Penicillium digitatum, Penicillium italicum* (green mold, blue mold). In S. Bautista-Baños (Ed.), *Postharvest decay: control strategies* (pp. 45-102). London: Academic Press. http://dx.doi.org/10.1016/B978-0-12-411552-1.00002-8.

Poei-Langston, M. S., & Wrolstad, R. E. (1981). Color degradation in an ascorbic acid-anthocyanin-flavanol model system. *Journal of Food Science*, 46(4), 1218-1236. http://dx.doi.org/10.1111/j.1365-2621.1981.tb03026.x.

Porat, R. (2008). Degreening of citrus fruit. *Tree and Forestry Science and Biotechnology*, 2(1), 71-76.

Rapisarda, P., Bellomo, S. E., & Intelisano, S. (2001). Storage temperature effects on blood orange fruit quality. *Journal of Agricultural and Food Chemistry*, 49(7), 3230-3235. http://dx.doi.org/10.1021/jf010032l. PMid:11453756.

Rapisarda, P., Bianco, M. L., Pannuzzo, P., & Timpanaro, N. (2008). Effect of cold storage on vitamin C, phenolics and antioxidant activity of five orange genotypes [*Citrus sinensis* (L.) Osbeck]. *Postharvest Biology and Technology*, 49(3), 348-354. http://dx.doi.org/10.1016/j.postharvbio.2008.02.002.

Rehman, S. U., Abbasi, K. S., Qayyum, A., Jahangir, M., Sohail, A., Nisa, S., Tareen, M. N., Tareen, M. J., & Sopade, P. (2020). Comparative analysis of citrus fruits for nutraceutical properties. *Food Science and Technology*, 40(Suppl. 1), 153-157. http://dx.doi.org/10.1590/fst.07519.

Scandalios, J. G. (1993). Oxygen stress and superoxide dismutases. *Plant Physiology*, 101(1), 7-12. http://dx.doi.org/10.1104/pp.101.1.7. PMid:12231660.

Silva, F. E. F., Di-Medeiros, M. C. B., Batista, K. A., & Fernandes, K. F. (2013). PVA/polysaccharides blended films: mechanical properties. *Journal of Materials*, 2013, 413578. http://dx.doi.org/10.1155/2013/413578.

Tavarini, S., Degl'Innocenti, E., Remorini, D., Massai, R., & Guidi, L. (2009). Polygalacturonase and β-galactosidase activities in Hayward kiwifruit as affected by light exposure, maturity stage and storage time. *Scientia Horticulturae*, 120(3), 342-347. http://dx.doi.org/10.1016/j.scienta.2008.11.013.

Them, L. T., Dung, N. A., Dien, T. V. T., Ngoc, L. S., Van, P. T. H., & Manh, T. D. (2021). Antifungal activity of a chitosan and polyvinyl alcohol (PVA) mixture on damage-causing strains isolated from postharvest oranges grown in Vietnam. *International Food Research Journal*, 28(4), 737-744.

Tripathi, S., Mehrotra, G. K., & Dutta, P. K. (2009). Physicochemical and bioactivity of cross-linked chitosan-PVA film for food packaging applications. *International Journal of Biological Macromolecules*, 45(4), 372-376. http://dx.doi.org/10.1016/j.ijbiomac.2009.07.006. PMid:19643129.

Vasilatos, G. C., & Savvaidis, I. N. (2013). Chitosan or rosemary oil treatments, singly or combined to increase turkey meat shelf-life. *International Journal of Food Microbiology*, 166(1), 54-58. http://dx.doi.org/10.1016/j.ijfoodmicro.2013.06.018. PMid:23827808.

Villarreal, N. M., Rosli, H. G., Martínez, G. A., & Civello, P. M. (2008). Polygalacturonase activity and expression of related genes during ripening of strawberry cultivars with contrasting fruit firmness. *Postharvest Biology and Technology*, 47(2), 141-150. http://dx.doi.org/10.1016/j.postharvbio.2007.06.011.

Wang, Z., Yan, F., Pei, H., Li, J., Cui, Z., & He, B. (2018). Antibacterial and environmentally friendly chitosan/polyvinyl alcohol blend membranes for air filtration. *Carbohydrate Polymers*, 198, 241-248. http://dx.doi.org/10.1016/j.carbpol.2018.06.090. PMid:30092996.