

## **Effect of Chitosan and Salicylic Acid Along with Mallow and Aloe Vera Extracts on Qualitative and Quantitative Traits of mandarin (*Citrus reticulata*) “Unshiu”**

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### **ABSTRACT**

*A factorial experiment was conducted to investigate the effect of chitosan, salicylic acid, aloe Vera gel, and mallow mucilage on the qualitative traits of mandarin “Unshiu” and determine their best concentration in a completely randomized design at the Islamic Azad University, Science and Research Branch. Experimental treatments included chitosan (0.5, 1 and 1.5%), salicylic acid (1, 1.5 and 2 mM), aloe vera gel (15, 30 and 45%) and mallow extract (15, 30 and 45%) were immersed and then stored for 45 days (storage period with 4 levels 0, 15, 30 and 45 days). Fruits juice pH, titratable acid, total soluble solids, fruit weight loss, carotenoids, ascorbic acid content, and antioxidant capacity were measured. The*

results showed that the pH, total soluble solids, weight loss, and carotenoids content increased during storage, and the amount of titratable acid, ascorbic acid, and antioxidant capacity decreased. The most effective treatment in maintaining pH, titratable acid, soluble solids, carotenoid content, ascorbic acid, and antioxidant capacity was salicylic acid 2mM, which is recommended for use after harvesting..

**Keywords:** Mandarin "Unshiu," Postharvest, Citrus, Biodegradable, Antioxidant Capacity.

## **1. Introduction**

Citrus are one of the subtropical fruits of the world with high economic value in Brazil, America, and China. Today, citrus industry is known in the world. In Iran, Citrus unshiu Marc. has a good market in terms of taste and introduction to the market. This cultivar is suitable for cool subtropical regions and extends to temperate regions of Japan, China, Spain, and countries with favorable climatic conditions (Qazvini and Moghaddam, 2016). Most fresh fruits and vegetables are known for their therapeutic value and health-promoting activities. Therefore, products produced without the use of artificial chemicals are considered worldwide due to the presence of active compounds such as phenolic compounds and their antioxidant properties (Suleria et al., 2015). In this regard, the application of natural and biodegradable compounds improves the shelf-life of garden products and increases the health indices of the population. Environmental and human-friendly compounds are one of the best methods to control better postharvest diseases (Asghari and Riaei, 2012). Chitosan is a non-toxic, biodegradable, pragmatic, and biologically efficient compound with strong antimicrobial and antifungal activity and can effectively control fruit caries. It can also easily have a coating on fruits and vegetables and reduce the respiration rate of fruits and vegetables by controlling carbon dioxide and oxygen permeability. The antimicrobial properties of chitosan are caused by amino groups with a positive charge (Hosseini et al., 2009). Aloe Vera gel is transparent, odorless, and Cohesionless and has a high absorption power, a suitable alternative for chemical coatings in post-harvesting of agricultural products. Aloe Vera gel has numerous most important compositions: vitamins, amino acids, enzymes, salicylic acid, anthracionins, and saponin.

Meanwhile, the salicylic acid and saponins have characteristics of fungicide and result in preventing the growth and proliferation of fungi, thereby causing their deaths (Choi et al., 2001). The mallow has long been used in medicinal and food items. Its most important components are mucilage, flavonoid, tannins, phenolic compounds, and anthocyanin (malvin, delphinidin, and malvidin) (Dehkordi, 2003). External use of salicylic acid protects plants from direct oxidative damage (Horvath et al., 2007). It has been shown that the treatment with salicylic acid effectively reduced the respiration of the harvested fruits and is also completely dependent on concentration (Mo et al., 2008; Srivastava and Dwivedi, 2000). In addition, salicylic acid in high concentrations affects the pore width of the aperture, leading to the closing of the aperture. The fresh weight and respiration speed directly correlate with the pore width of the aperture (Manthe et al., 1992). Gheisar Beigi et al. (2015) examined the effect of chitosan coating on lemon fruit and reported that chitosan treatment increased the firmness of the skin and fruit meat, vitamin C content, and titratable acid against the control fruits. Also, in the experiment, two varieties of pulm (Stanley and Giant) were treated with 1% chitosan. The results showed that chitosan coating has been effective on weight loss, reducing respiration, and reducing decay rates of fruit. Also, chitosan with reduced respiration prevented fruit weight and titratable acid loss, pH, and fruit firmness in two cultivars (Bal, 2018). Another study reported the effects of salicylic acid and aloe Vera gel in Thomson navel oranges. The fruits treated showed less weight and higher firmness, soluble solids content, titratable acid, vitamin C, and total phenol content (Rasouli et al., 2019). According to the results of this study on the effect of Mallow mucilage and S. thyme essential oil on the storage of pear cultivars in Isfahan, Mallow mucilage controls fruit juice more and fruit weight, and this increases fruit firmness and decrease of soluble solids compared to control group (De Oliveira et al., 2020). Due to the high importance of citrus fruits storage and improving exports status of these fruits from the north of Iran, this study aimed to improve the storage of Citrus unshiu based on biodegradable, compatible with human health.

## 2- Materials and Methods

Citrus unshiu were harvested from a commercial garden in the Miandorud district of Sari in physiological maturation and after full ripening in late November 2019. After the proper layout, they were immediately transferred to the laboratory. The fruits were washed by urban water, and then the defective fruits were removed. The fruits were selected in size and color without any physical damage or signs of the disease. This experiment was conducted in a completely randomized design to investigate the effects of chitosan, salicylic acid, aloe Vera gel, and mallow extract on qualitative traits of Citrus unshiu and determine their best concentration. The experimental treatments included chitosan (0.5, 1, and 1.5%), salicylic acid (1, 1.5, and 2 mM), Aloe Vera gel (15, 30, and 45%), and mallow extract (15, 30, and 45%) and immersed for 3 minutes. The fruits were then spread out on the screen to dry. The fruits were then dried and transferred to cold storage at a 7-degree temperature and relative humidity of 90% and stored for 45 days (shelf-life with four levels of 0, 15, 30, and 45 days).

**Measured traits:** To measure pH, the pH meter was first adjusted by buffer solutions 4 and 7, and some of the filtered fruit extract was poured into a small beaker and then the electrodes of the device were placed in the beaker, and the pH of the extract was read and recorded. To measure titratable acid, 5 ml of fruit juice extract was mixed with 45 ml of distilled water and titrated with normal sodium hydroxide (0.1) until the pH reached about 8.2, and the results were as follows: The percentage of citric acid was expressed (Mostofi and Najafi, 2006).

$$\text{Percent of total acid} = \frac{\text{Profit volume in titration} \times \text{NaOH normality} \times \text{Equivalent acid weight} \times 1}{10 \times \text{Sample Weight}} \times 100$$

In order to measure the weight loss percentage of Citrus Unshiu before storage and after the storage period, the net weight of the fruits was measured with a digital scale with an accuracy of 0.001 g. Then the weight loss percentage of each repetition was calculated through the following equation:

$$100 \times [(A-B)/A] = \text{Fruit weight loss percentage}$$

In this formula, the letter A is related to the weight of the fruits before transfer to storage, and the letter B is related to the weight of the fruits after the end of the storage period (Arnal and Del Río, 2004). 0.5 g of Powdered and crushed mandarin “Unshiu” by liquid nitrogen was added to 4 ml of 80% methanol to measure antioxidant capacity. The fruit tissue was centrifuged with methanol at 9500 rpm for 20 minutes, and then 100 µl of the extract was added to 3400 µl of 2,2-diphenyl-1-picryl-hydrazyl solutions. 0.1 mM DPPH from Sigma Aldrich Co was added. According to the following equation, the mixture was kept in the dark for one hour after DPPH was added and then measured with a spectrophotometer at 517 nm.

$$\text{Antioxidant capacity percentage} = \frac{\text{Sample reading}}{\text{control reading}} \times 100$$

## 3- Statistical Analysis

Statistical analysis of the data obtained from this study and comparison of means was performed using SAS 9.1 and MSTAT-C software, and graphs were drawn by Excel software. The results were tested by analyzing the variance of GLM and comparing the means with Duncan's multiple range test.

## 4- Results and Discussion

Analysis of variance of the studied treatments on the measured traits is shown in Table 1. The results show that the shelf-life and the studied treatments had a significant effect on pH, titratable acid, fruit weight loss, ascorbic acid, and antioxidant capacity of fruit at a probability level of one percent. The interaction effect of shelf-life and treatments on pH, titratable acid, and ascorbic acid was significant at a 1% probability level but did not affect other measured traits.

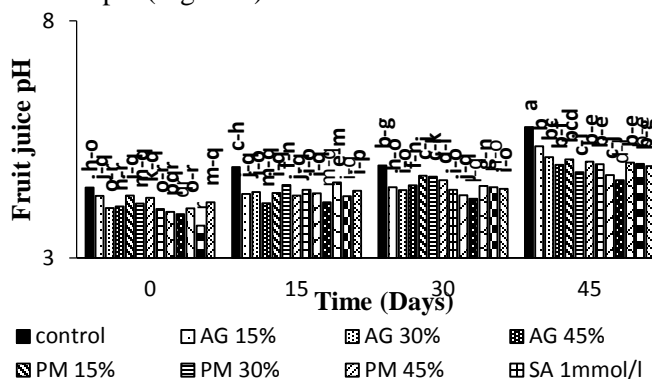
**Table 1- Variance analysis of the effect of salicylic acid (SA), Chitosan (Chi), Mallow mucilage (PM), and Aloe vera gel (AG) on some measured characteristics of Mandarin "Unshiu."**

(Mean Square)								
(Antioxidant Capacity)	(Ascorbic Acid)	(Carotenoids)	(Weight loss)	(TSS)	(TA)	pH	(Df)	(S.O.V)
7748.8**	3279.5**	106.1**	121.2**	0.025**	643.6**	4.29**	3	(Time)
26.32**	73.13**	4.25**	0.18**	0.001**	16.59**	0.265**	12	(Treatment)
11.72 <sup>ns</sup>	26.28**	1.04 <sup>ns</sup>	0.06 <sup>ns</sup>	0.0003 <sup>ns</sup>	3.47**	0.058**	36	(Time×T.)
7.81	10.04	1.12	0.042	0.031	2.31	0.10	104	(Error)
3.42	4.98	9.43	8.04	5.56	10.94	6.88	-	(CV.)

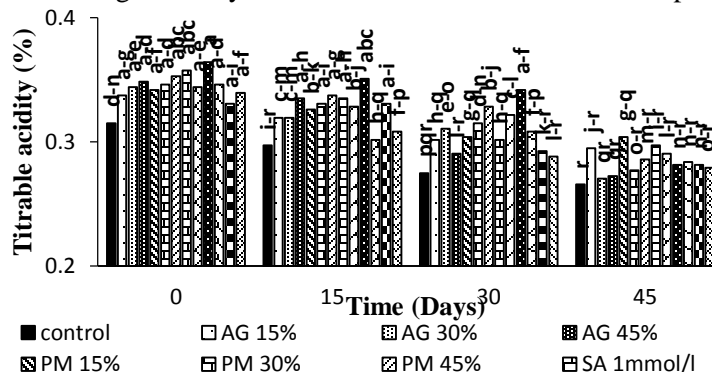
\*\* , and \* represent significance at the 0.01 and 0.05 levels, respectively, and ns represent non-significance at P < 0.05.

#### 4-1- PH and Titratable Acid

Regardless of the effect of the treatments, with increasing the storage, the pH of mandarin "Unshiu" juice was gradually increased and reached the highest level on the 45th day. The pH of fruit juice during the studies in the control treatment increased more than other treatments, and in other words, the treatments used were able to maintain the acidity of the fruit during storage. Salicylic acid with concentrations of 1 and 2 mM and then chitosan 1% had the greatest effect on preventing the increase of fruit pH. At the end of storage, salicylic acid treatment with concentrations of 1 and 2 mmol with values of 4.75 and 4.64, respectively, maintained the fruit pH (Figure 1).

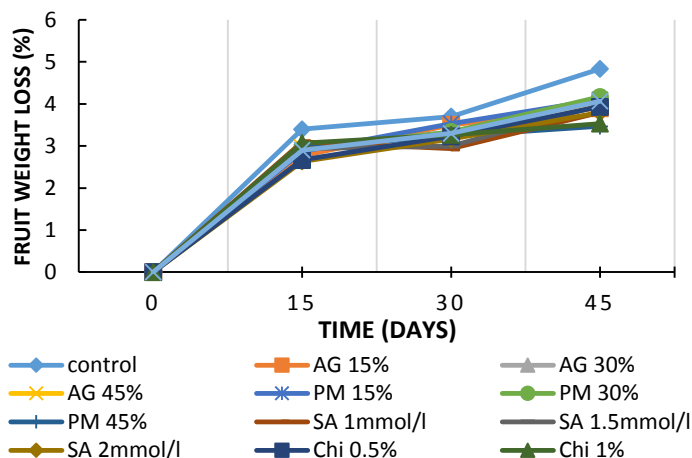


**Figure 1- Effect of salicylic acid (SA), Chitosan (Chi), Mallow mucilage (PM), and Aloe Vera gel (AG) on fruit juice pH of mandarin "Unshiu."** Columns with similar letters are not statistically significant. The trend of titratable acid changes in mandarin "Unshiu" is opposite to the pH changes, and with increasing storage, the titratable acid of the fruit gradually decreased and reached a minimum on day 45. The amount of titratable acid in the fruit during the studies in the control treatment was lower than in other treatments, indicating more organic acid decomposition during storage. In this index, the application of salicylic acid with a concentration of 2 mmol had the greatest effect on the preservation of organic fruit acids, which at the end of the storage was not significantly different from other treatments except control (Figure 2).



**Figure 2- Effect of salicylic acid (SA), Chitosan (Chi), Mallow mucilage (PM), and Aloe Vera gel (AG) on fruit TA of mandarin "Unshiu."** Columns with similar letters are not statistically significant.

### 4-2- Weight Loss of Fruit

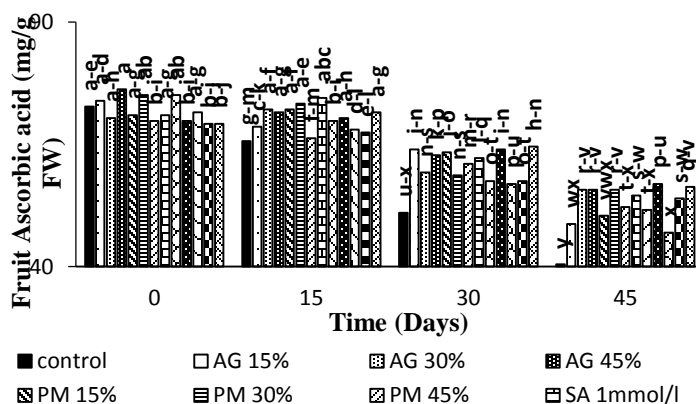


**Figure 3- Effect of salicylic acid (SA), Chitosan (Chi), Mallow mucilage (PM), and Aloe Vera gel (AG) on fruit weight loss of mandarin “Unshiu.”**

The upward trend in weight loss in fruits treated with 1% chitosan, 45% aloe Vera gel, and 45% Mallow mucilage is slower than on the 30th day, and on the 45th day, these treatments physically cover by evaporating and closing the pores of the skin surface to evaporate. They prevented water and prevented the fruits from losing more weight than before (Figure 3).

### 4-3- Ascorbic acid of the fruit

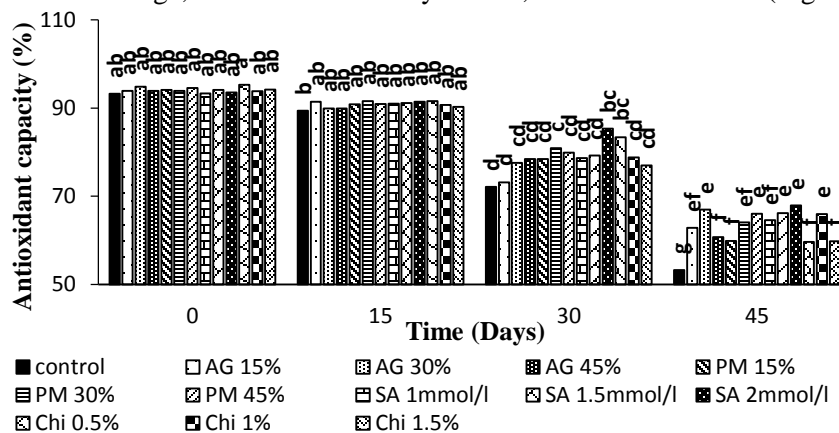
The amount of ascorbic acid in the fruit, regardless of the effect of the treatments, gradually decreased over time and finally reached its lowest level at the end of the study. The highest amount of ascorbic acid was observed at the end of storage in fruits treated with salicylic acid with a concentration of 2 mM with an average of 56.9 mg / g body weight, which with control treatment, aloe Vera gel 15%, Mallow mucilage 15% and chitosan 0.5%. There are no significant differences with 40.48, 48.69, 50.45, and 46.93 mg / g weight, respectively (Figure 4).



**Figure 4- Effect of salicylic acid (SA), Chitosan (Chi), Mallow mucilage (PM), and Aloe vera gel (AG) on fruit ascorbic acid of mandarin “Unshiu.” Columns with similar letters are not statistically significant.**

### 4-4- Antioxidant capacity

On day 45, there was a significant difference between treatments with the control treatment. There is no significant difference between 2 mM salicylic acid treated at the highest level (67.93%) and 30-45% aloe Vera, 30-45% Mallow mucilage, 1 and 1.5 mM salicylic acid, and 1.5% chitosan. (Figure 5).



**Figure 5- Effect of salicylic acid (SA), Chitosan (Chi), Mallow mucilage (PM), and Aloe vera gel (AG) on fruit Antioxidant capacity of mandarin “Unshiu” during storage at 7 °C for 45 days. Columns with similar letters are not statistically significant.**

## 5- Discussion

The trend of titratable acid changes in mandarin “Unshiu” fruit is opposite to the pH changes, and with increasing storage, the titratable acid of the fruit gradually decreased and reached a minimum on day 45. In this regard, it has been reported that changes in pH of fruit extract at the time of ripening are mostly due to leakage of organic acids from vacuoles into the cell cytoplasm. (2007)). Increased citric acid activity upon ripening or decreased acidity may be due to their conversion to sugars and their greater use in fruit metabolism (Doreyappa Gowda and Huddar, 2001). In this study, fruit weight loss gradually increased over time. The upward trend in weight loss in fruits treated with 1% chitosan, 45% aloe Vera gel, and 45% Mallow mucilage is slower than on the 30th day, and on the 45th day, these treatments physically cover by evaporating and closing the pores of the skin surface to evaporate. They prevented water and prevented the fruits from losing more weight. As the rate of aging in the fruit increases, so does the respiration rate. The rate of weight loss is mainly related to transpiration and respiration, and water loss through transpiration depends on the difference in water vapor pressure between the fruit tissue and around the fruit and the storage temperature (Hernández-Muñoz et al., 2006). This study showed that salicylic acid could not maintain the weight of mandarin “Unshiu” during storage, which is consistent with the results of the use of salicylic acid in orange fruit (Dadgar, 2014). In the present study, the amount of ascorbic acid in the fruit, regardless of the effect of the treatments, gradually decreased over time and finally reached its lowest level at the end of the study. Also, the highest amount of ascorbic acid was observed at the end of storage in fruits treated with salicylic acid with a concentration of 2 mM, which was not significantly different from the control treatment, 15% aloe Vera gel, 15% Mallow mucilage, and 0.5% chitosan. Ascorbate levels are reported to decrease during storage, but salicylic acid treatment delays the loss of vitamin C, and salicylic acid-treated fruits contain high amounts of ascorbate and dehydroascorbate (the oxidized form of ascorbic acid) (Huang et al., 2008). Aloe Vera gel coating maintains firmness and improves fruit texture, reduces wounds and other physical injuries, prevents the use of vitamin C to repair cells, and relieves stress, resulting in vitamin C accumulating in plant cells (Cordenunsi et al., 2003). Salicylic acid treatment has been reported to affect antioxidant enzymes such as superoxide dismutase, peroxidase, and catalase (Srivastava and Dwivedi, 2000). Studies have shown that aloe Vera has antimicrobial, antioxidant, antiviral, and anti-inflammatory properties (Asghari et al., 2013; Pal et al., 2013). According to the results of this study, aloe vera was able to maintain its antioxidant activity until the forty-fifth day of storage. According

to the results of this study, the treatment of grapes with aloe Vera gel prevents the reduction of antioxidant activity during storage (Zacarias et al., 2020).

## 6- Conclusion

The results of analysis of experimental data in this study showed that the best treatment to increase the storage of mandarin "Unshiu" during 45 days of storage was salicylic acid treatment with a concentration of 2 mm, which was more effective than other treatments.

## Appreciation

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