Research Article

Comparative Nutritional and Storage Study of two Mandarin Varieties by Application of Various Salts Incorporated in Wax

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Abstract | Citrus crop postharvest losses can be minimized by investigating different innovations for extension in storage period and shelf life. Various fungicides and salts are incorporated in wax to limit postharvest fruit decay and fungal attack. In the present study two salts were incorporated in commercial wax to control postharvest decay in Willow leaf mandarin (*Citrus reticulata Blanco*) and Kinnow mandarin (*Citrus reticulata Blanco*). Sodium bicarbonate and Potassium sorbate incorporated (6% w/v concentration) in wax were used to compare with commercially used fungicide (Thiabendazole). Study was conducted at 4±1 C° with 90±05% relative humidity for 60 days followed by one week of storage at ambient conditions. Least decay incidence 1.2% and 1.8% respectively in Kinnow and Willow leaf was observed in fruit treated with fungicide in wax followed by fruit treated with Potassium sorbate in wax (2.8% and 3.6%). TSS and acidity showed conversely increasing and decreasing trends respectively in both type of fruits with extension of storage period. Willow leaf proved as a close second with respect to various aspects of quality and nutritional value during the fresh fruit analysis undertook prior to storage. Kinnow and Willow leaf didn't differ significantly in juice percentage (46.33 and 43.16), total soluble solids (TSS) (11.20and 10.23), percent acid (0.69 and 0.73), total sugars percent (10.75 and 9.83), crude protein, crude fat and in DPPH activity respectively. The study depict that incorporation of salts to wax in substitution to the fungicide is an effective application to control postharvest citrus fruit decay with perks of safety and convenience.

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1. Introduction

Willow leaf is known under various regional names but most commonly called as Mediterranean mandarin is a commercial variety of Italy and Spain although cultivated in various other regions like Egypt, Brazil, Algeria and Argentina. Willow leaf is the pollen parent of three important hybrids Encore, Wilking and Kinnow along with King Mandarin as a seed parent (Reuther et al., 1967). Mediterranean mandarin Willow leaf has distinctive characteristics of mild and pleasantly aromatic flavor of juice while Kinnow, a King-Willow leaf mandarin hybrid is the major citrus fruit grown in Punjab Pakistan (Citrus Pages, 2011).

Although both Mandarin varieties Kinnow and Willow leaf have good bearing potential but there is a problem of marked alternate bearing tendency which need to be carefully managed. In addition Willow leaf has highly delicate rind leading towards excessive decay losses during on-tree storage or either during post-harvest cold storage. Oil from rind and aromatic leaves of the Willow leaf are widely used in perfumes, flavoring, confectioneries and beverages (Hodgson, 1967).

Pakistan produces mandarins dominated by Kinnow in large quantities exporting to different countries of the world fetching more than $200 Million revenue annually (TDAP, 2018) Citrus industry of Pakistan faces huge pre-harvest and post-harvest losses due to improper pre-harvest practices and postharvest management. Pre-harvest losses, low quality and poor yields at Pakistan citrus industry are mainly associated with low fruit set, malnutrition, water stress, insect-pest complexes etc. situation is further aggravated by huge post-harvest losses (Nawaz et al., 2008).

One third citrus crop is wasted due to heavy post-harvest losses ranging from 35 to 40 % occurring at Pakistan citrus industry (PHDEB, 2014) are mainly associated to loss of quality linked with physiological disorders, pathological disorders leading to weight loss and microbial attack to the produce (Iqbal, 1996).

Qualitative losses are more difficult to measure than quantitative losses of fresh fruit. Post-harvest fruit losses in mandarin crop occur mainly due to improper methods of harvesting, handling, packing and transportation (Kader, 2005) .

Literature (Palou et al., 2002; Gamagae et al., 2004; Gregori et al., 2008; Venditti et al., 2005; Mehyar et al., 2011) indicates that application of various salts mixed with wax is effective in reducing the post-harvest fruit decay and weight loss.

Mixing of various salts in varying ratios with different waxes at the time of packing have been reported to clearly improve the post-harvest storage stability of citrus fruit thus reducing the decay and weight loss (El-Mougy et al., 2008; Larrigaudiere et al., 2002; Palou et al., 2008; Valencia-Chamorro et al., 2009; Montero et al., 2010).

The present fresh fruit and storage study was conducted to compare nutritional value and bring improvement in shelf life and keeping quality of two mandarins i.e. Kinnow and Willow Leaf. Two salts in commercial wax were incorporated to enhance storage stability thus controlling postharvest decay and weight loss. Overall objective of the experiment was to improve the quality and storage stability of mandarin fruit.

2. Material and Methods

2.1 Plant material, treatments and storage conditions

This study was conducted at post-harvest division of Citrus Research Institute, Sargodha Pakistan during the citrus crop season year 2016-17. Mandarin varieties Kinnow and Willow leaf grafted on rough lemon root stock (*Citrus jambhiri Lush*).having planting age of seven years were selected for this study. Plants were spaced about 20 × 20 foot and managed under same inputs, cultural practices and agro-climatic conditions during the course of study. The study was carried out in a CRD design with three replications comprising of 20 fruits each separately for fresh fruit analysis and storage study Uniform mature fruits were selected for further physical, biochemical analysis and storage study. Mature fruit were harvested from the orchard during the month of January, washed, dried and divided into two lots. One lot was subjected to fresh fruit analysis while the second lot was waxed with prepared solutions in commercial wax according to the plan of storage study.

2.2 Storage studies

Fruit from both selected varieties Kinnow and Willow leaf were washed, waxed and dried according to the below mentioned treatment plan and stored for 60 days at constant climate chamber at 4±1 °C and 90 ±05 % RH followed by one week of storage at ambient conditions. Fruit was analyzed for physiological loss in weight, decay percentage, TSS, acidity, loss in juice percentage at intervals of 0, 30, 60 days and last week of storage at ambient conditions. TSS and acidity was estimated as mentioned below (in fresh fruit analysis section) while juice and weight loss was calculated in percentage at given intervals on the basis of initial weight of juice and fresh fruit weight recorded at the time of storage. Fruit decay percentage was determined and calculated on the basis of percentage of decayed fruits on the given intervals as described by Rokaya et al., 2016 and Miri et al., 2018.

Different salts like sodium bicarbonate and potassium sorbate were incorporated in wax at 6% (w/v) concentration to compare with commercially used fungicide Thiabendazole (0.2%) in wax along with a control treatment having only (Fomesa- shellac based) wax.

Treatment plan observed for both mandarins separately was as following;

T1= Fungicide Thiabendazole (0.2%) added in wax; T2= Sodium Bicarbonate (6%) added in wax; T3= Potassium Sorbate (6%) added in wax; T4= Wax only.

2.3 Physico-chemical characteristics

A representative sample of 20 mature fruits from each variety was selected, washed and dried to carry out the lab analysis. Fruit diameter (size) was measured by the use of digital caliper while fruit weight was measured by the use of digital electronic balance and average results were recorded in millimeter and gram respectively. Fruit firmness was measured by the usage of digital penetrometer in kilogram. Fruit juice was squeezed through electronic squeezer to calculate the average juice percentage per fruit. Peel thickness was measured in millimeters through digital caliper after removing the whiter albedo layer from the peel. All data measured was used to calculate the means of the measurements from the sample under study.

Acidity and Ascorbic acid (vitamin C) were determined and calculated as outlined by (AOAC, 2005). Total soluble solids (Brix) of juice were determined by the use of digital ATC Refractometer (automatic temperature compensation, corrected at 20 °C) (make HANNA Japan) while pH was also recorded by the use of calibrated pH meter (make OHAUS corp. USA). Titratable acid (TA) percentage (citric acid g per 100 ml) was measured by titrating 10 ml of juice against 0.1 N NaOH solutions to the persistent pink color end point achieved at pH 8.1. All measurements were made in triplicate to record the averages as previously adopted by various researchers like Nawaz et al., 2019 and Miri et al., 2018. Ascorbic acid (Vitamin C mg/100 ml) was determined by diluting one ml of juice in oxalic acid solution (0.4%), then volume was made to 10 ml by distilled water and titrated against standard dye solution to persistent pink color end point.

2.4 Total, reducing and non-reducing sugars (%)

Total, reducing and non-reducing sugar percentage was determined as described in AOAC, 1995.

The juice sample (10ml) was filtered and added in 250ml volumetric flask, 10 ml potassium oxalate (7%) and 25 ml lead acetate (2%) was also added to the solution. Volume was made with distilled water. Prepared sample was titrated against Fehling solution till red precipitates appeared. The percentage of reducing sugars was calculated as following:

$$\text{Reducing sugars (g/100ml, \%)} = 6.25\,(X)/Y$$

Where; X= ml of standard sugar solution used and Y= ml of sample aliquot used against Fehling solution.

For determination of total sugars 25 ml sample aliquot was taken in 100ml volumetric flask in which 20 ml distilled water and 5 ml concentrated HCl was added and kept overnight to convert non-reducing sugars into reducing sugars. Concentrated NaOH (50%) was then added to neutralize the solution and distilled water was added to make the volume. The solution was added to a graduated burette and titration against 10 ml Fehling's solution was conducted by adding phenolphthalein indicator to the brick red end point. Total sugars percentage was calculated as;

$$\text{Total sugars (g/100ml, \%)} = 25\,(X)/Z$$

Where; X= ml of standard sugar solution used and Z= ml of sample aliquot used against Fehling solution.

$$\text{Non-reducing sugars (g/100ml, \%)} = \text{Total sugars \%} - \text{Reducing sugars \%} \times 0.95$$

2.5 Proximate analysis

Proximate analysis of juice for determination of crude protein, crude fat, crude fiber and total ash was carried out as described in AOAC, 2012. Crude protein was calculated through nitrogen percentage (Nitrogen % X 6.25) determined by Kjeldahl's method. Nitrogen percentage from prepared sample was determined through titration against 0.1 N H_2SO_4 till golden yellow end point. Crude fat was determined by Soxhlet's apparatus in which dried sample was extracted in petroleum ether which was dried and weighed in a petri dish. Total Ash from sample was determined by weighing the dry mineral residues heated in muffle furnace maintained at 550C°. Crude fiber was obtained by digesting sample with H_2SO_4 and NaOH and heating the residues in a muffle furnace at elevated temperature of 550°C.

2.6 Ascorbic acid, β-carotene and Antioxidant Activity (DPPH)

Vitamin C (Ascorbic acid) was determined as described by AOAC, 1995 in which diluted sample was titrated against standard dye solution. For determination of beta carotene, fruit sample was soaked in methanol under dark conditions to completely extract the carotene contents. Carotene layer separated with hexane and Sodium Sulphonate was passed through separating funnel and absorbance was measured at 436 nm as described by Ranganna (1999).

Fruit samples were analyzed for antioxidant activity (DPPH) percentage by using published procedure as by Thaipong et al. (2006). Pure ethanol based plant extract with DPPH working solution was kept in laminar chamber and absorbance was checked at 515 nm on spectrometer. Prepared stock solution was also checked at wavelength 517 nm and blank sample without plant extract prepared with ethanol and working solutions was checked at 515 nm wavelength and calculated as following;

Antioxidant activity % (DPHH) = (A blank – A sample) / A blank

2.7 Statistical analysis

Fresh fruit analysis and storage study was carried out in a completely randomized design (CRD) with three replicates The Analysis of Variance was conducted using statistix 8.1 and means were compared by LSD test at $P \leq 0.05$.

3. Results and Discussion

3.1 Fresh fruit comparison

3.1.1 Physico-chemical characteristics

The research study was carried out to explore the quality and nutritional comparison of the two promising mandarin varieties i.e. Kinnow and Willow leaf. A Commercial mandarin variety, Willow leaf of leading producers like Italy, Spain; a pollen parent of Kinnow was compared with Kinnow (predominant of local citrus industry) for nutritional value, quality and storage stability. Results of the fresh fruit analysis and storage study showed Willow leaf as a new promising substitute of Kinnow.

Although with respect of average fruit weight Kinnow has attained a bigger size (162.96 g) as compared to fruit of Willow leaf (122.58 g) but there was no significant difference found in the juice percentage attained from the two contestant varieties. Average fruit diameters of the two mandarins also did not differ significantly (Table 1). In case of fruit firmness Willow leaf was not found at par with Kinnow as Willow leaf has found a thin peel (2.12mm) as compared to that of Kinnow (2.82 mm).

Fruit weight and size are important physicochemical characteristics of fruit which mainly define the juice recovery from the fruit which is ultimately desired for fresh fruit consumer and processor. According to Fonfría et al., 1996 various factors define fruit size in which fruit position on stem, competitiveness among growth organs and genotype are important. Kinnow mandarin had significantly better weight of fruit but both mandarins did not differ in juice recovery in which peel weight from Kinnow fruit might be greater than that of Willow leaf which possess lesser peel thickness (Table 1). Results of various physicochemical parameters for Kinnow and Willow leaf were found in accordance with the results found by Neves et al., 2018; Ahmad et al., 2013 and Khan et al., 2008. Various pre-harvest factors like climatic and soil conditions and availability of various inputs on different crop maturity stages affect the fruit quality parameters. Koshita, 2015 stated that low temperatures intensify coloration of skin and availability of irrigational water helps to enhance juice recovery at maturity phase in citrus crop.

Table 1: Physico-chemical characteristics of fresh fruit of Kinnow and Willow leaf Mandarins.

Variety	Fruit weight (g)	Fruit diameter (mm)	Fruit firmness (Kg)	Peel thickness (mm)	Juice (%)
Kinnow	162.96±10.44 a	71.03±3.92 a	2.42±0.1 a	2.82±0.16 a	46.33±1.45 a
Willowleaf	122.58±7.33 b	64.33±3.37 a	1.87±0.08 b	2.12±0.012 b	43.16±0.72 a

Data are means ± SE of triplicate determinations. LSD test ≤ 0.05% used for analysis

Table 2: Physico-chemical characteristics of fresh fruit of Kinnow and Willow leaf Mandarins.

Variety	TSS(Brix)	Acidity (TA) %	TSS/Acid ratio	pH
Kinnow	11.20±0.65 a	0.69±0.05 a	16.01±1.1a	3.74±0.22 a
Willowleaf	10.23±0.54 a	0.73±0.04 a	13.73± 0.09 a	3.68±0.21 a

Data are means ± SE of triplicate determinations. LSD test ≤ 0.05% used for analysis.

Fruit from both contestant mandarin varieties did not differ significantly in parameters like total soluble solids (TSS), Titratable acidity (TA), TSS/TA ratio and pH. In Kinnow average TSS was observed 11.2% while in Willow leaf as 10.2% (Table 2) while in case of TA both varieties showed 0.69% and 0.73% for Kinnow and Willow leaf respectively.

Table 3: Physico-chemical characteristics (Sugars) of fresh fruit of Kinnow and Willow leaf Mandarins.

Variety	Total sugars (%)	Reducing sugars (%)	Non-Reducing sugars (%)
Kinnow	10.75±0.33a	4.60±0.32a	6.16±0.44a
Willowleaf	9.83±0.35a	4.68±0.33a	5.09±0.05b

Data are means ± SE of triplicate determinations. LSD test ≤ 0.05% used for analysis.

Mandarins like Kinnow and Willow leaf are cherished in global citrus industry due to their reasonable size, nutritional value, physicochemical properties, sensory attributes and antioxidant characteristics (Ahmad et al., 2013). Kinnow showed a high degree of variation in various physicochemical characteristics like fruit size, shape, pH, TSS and various other parameters but it is adopted dominant citrus in Pakistan due to its capability to tolerate heat waves sometimes touching to 45 °C; this heat resistance is inherited from its parent cultivar King (Altaf et al., 2008). Results for the various physicochemical parameters like fruit weight, size, peel thickness, TSS and acidity for Kinnow were found in accordance with that stated by Khan et al., 2008.

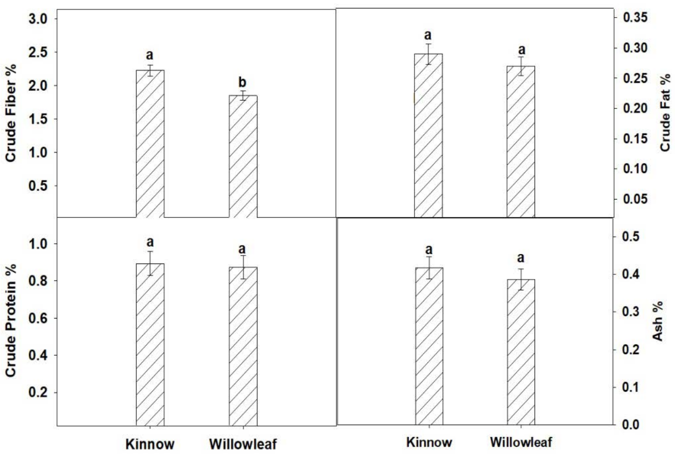
3.2 Physico-chemical characteristics (Sugars)

Both candidate mandarin varieties had no significant difference in sugar percentage except that Willow leaf had lower percentage of non-reducing sugars (5.09%) as compared to Kinnow (6.16%). Reducing sugars and total sugars percentage of both mandarins had showed no significant difference (Table 3). The sugars level increase with progression of fruit towards maturity (Hardy and Sanderson, 2010). Citrus fruit carbohydrates mainly consist of sugars; glucose, fructose (combined called reducing sugars) and sucrose (non-reducing sugar) which define the sweetness of fruit juice Hulme, 1971. The ratio of reducing to non-reducing sugars may alter with maturity, in different varieties of citrus during storage Daito and Sato, 1985. Results for sugars in both varieties were found as previous finding stated by Liu et al., 2012; Nawaz et al., 2019 and Neves et al., 2018.

3.3 Proximate analytical quality

Results of the proximate analysis showed that Kinnow and Willow leaf did not differ significantly in crude protein and crude fat percentage but both differ significantly for crude fiber percentage (2.23% and 1.85% respectively). There was also significant difference found among two mandarins for ash percentage; 0.41% for Kinnow and 0.38% for Willow leaf (Graph-1).

Source of dietary fiber is considered polysaccharides present in peel and pulp of citrus fruits (Spungen, 2005) in which generally insoluble portion is composed of pectin, cellulose and lignin which has ability to prevent digestive disorders (Hillemeier, 1995) with benefit of lowering cholesterol (Ting, 1980). Citrus fruits are not major source of proteins. Protein is determined by total Nitrogen content of the juice which is mostly contributed by free amino acids (Zamorani and Russo, 1974). Free amino acids present in citrus juices have only little impact on human nutrition as predominant portion is consisted on non-essential amino acids dominated by proline except lime juice (Ladanyia and Ladaniya, 2010; USDA, 2011). Fats (lipids) are mostly concentrated in the seed and rind of the citrus fruit while flesh of fruit is not a good source of fats. Outer part of the fruit surface is covered by cuticle lipids which are a natural protection to insect pest attack, chilling injury and moisture loss (Nordby and McDonald, 1991). Ash percentage indicates the dried mineral residues concentration in citrus juice. There are various mineral elements present in citrus fruits which are dominated by Potassium cations which contribute almost 40% of the total ash contents (Benk, 1965; USDA, 2011).



Graph 1: Proximate analysis of fresh fruit of Kinnow and Willow leaf Mandarins.

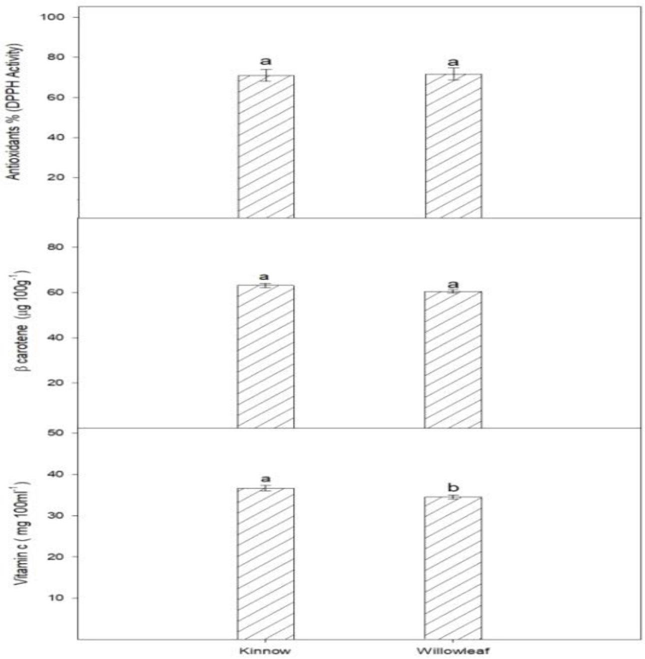
3.4 Ascorbic acid, β-carotene and antioxidant activity (DPPH)

Results for ascorbic acid (vitamin C indicates that Kinnow fruit have better concentration of vitamin C (39.21 mg/100ml) as compared to Willow leaf (31.52 mg/100ml). In case of beta carotene contents both mandarin varieties did not differ significantly having 63.12 and 60.47 µg/100g respectively for Kinnow and Willow leaf. Antioxidant activity measured as DPPH in Kinnow and Willow leaf mandarins was also remained at par showing values of 71.03 and 71.77% respectively (Graph-2).

Most important element provided by citrus fruit for human health and nutrition is vitamin C (ascorbic acid). Vitamin C is a water soluble vitamin which is found in considerable concentrations in citrus fruits. Vitamin C in citrus fruits and their products is found in variable concentrations depending upon stage of maturity, variety, handling, climate, storage and processing conditions (Liu et al., 2012). Citrus fruits providing average quantities of daily requirement are considered rich and popular source of vitamin C ranging from 23 to 83 mg/100 g of fresh edible portion (Lee and Kader, 2000). Recommended dietary allowance (RDA) by USDA for adults regarding vitamin C is 75-90 mg.

The only fat soluble vitamin present in citrus fruits is vitamin A found in the form of provitamin A commonly called carotenoids (*α* carotene, *β* carotene and *β*-cryptoxanthin). Carotenoids are found to act as antioxidants reducing various degenerative diseases and found in variable quantities in different citrus fruits as mandarins are considered valuable dietary source followed by pink grapefruits and oranges (USDA, 2011; Holden et al., 1999). Vitamin A precursors *β*-cryptoxanthin are found in various fruits and vegetables like papaya, red chilies, pumpkins, peaches, guava but citrus fruits are considered the best rich source (Arscott et al., 2010; Burri et al., 2011). In comparison with oranges Dhuique-Mayer et al., 2005 found that Mandarins and their hybrids like clementine are best source of vitamin A.

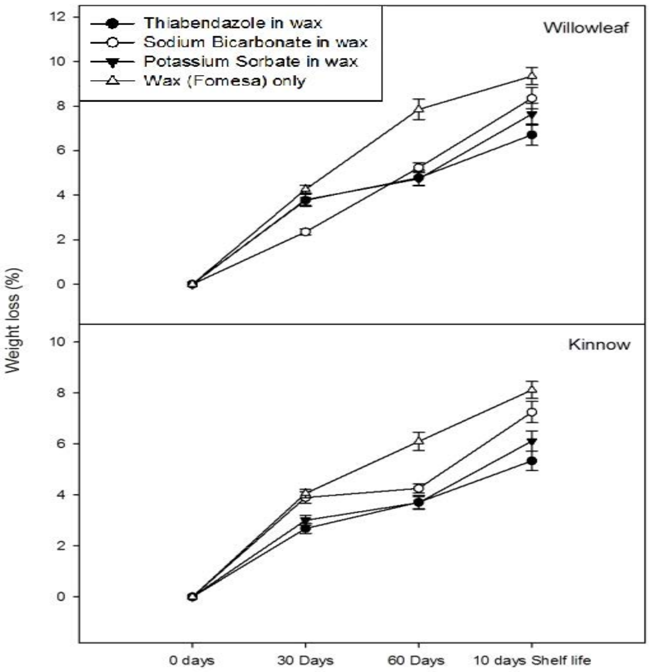
DPPH is an easy determination of antioxidant activity in the citrus juices. DPPH is a free radical which is reduced by accepting an electron from the molecules of antioxidants which are able to donate Hydrogen atom, resulting in stable molecule in the result of reduction in DPPH and yielding colorless ethanol solutions (Kour et al., 2014; Rekha et al., 2012). Antioxidants are bioactive compounds promoting various biological functions to prevent from cancer, cardiovascular disorders, diabetes and many other malfunctions in the human body (Ke et al., 2015; Rajendran et al., 2014).



Graph 2: Ascorbic acid, β-carotene and Antioxidant Activity of fresh fruit of Kinnow and Willow leaf Mandarins.

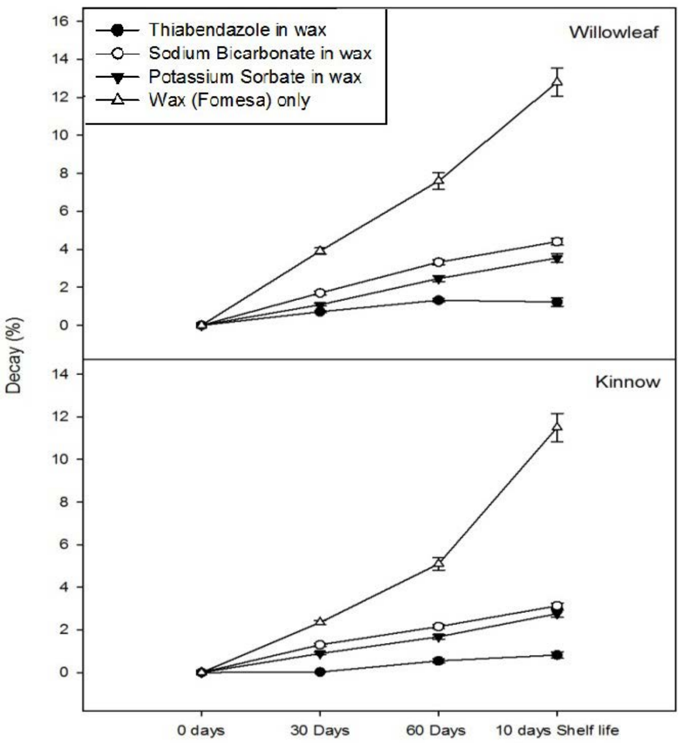
3.5 Postharvest fruit storage studies

Storage studies were carried out against parameters like physiological loss in weight, decay percentage, juice percentage, TSS and acidity. The perusal from data and graphs shown below the decay percentage (Graph 4) and weight loss percentage (Graph 3) was increased in all treatments in both mandarin varieties significantly with prolongation of storage period. Maximum decay percentage was noticed in T4 in fruits treated only with wax while minimum decay percentage was noticed in T1 in which fruit was treated with Thiabendazole fungicide in both type of mandarins. Same trend was followed by both mandarins in weight loss percentage with respect to four treatments. Graph 5 shows the juice recovery percentage which was decreased with storage period in all four treatments in both varieties but more significantly decrease was observed in case of Willow leaf mandarin. Total soluble solids increased with progression of storage period and titratable acidity (Graph 6 & Graph 7) decreased with advancement of the storage period in all treatments in both mandarin varieties.

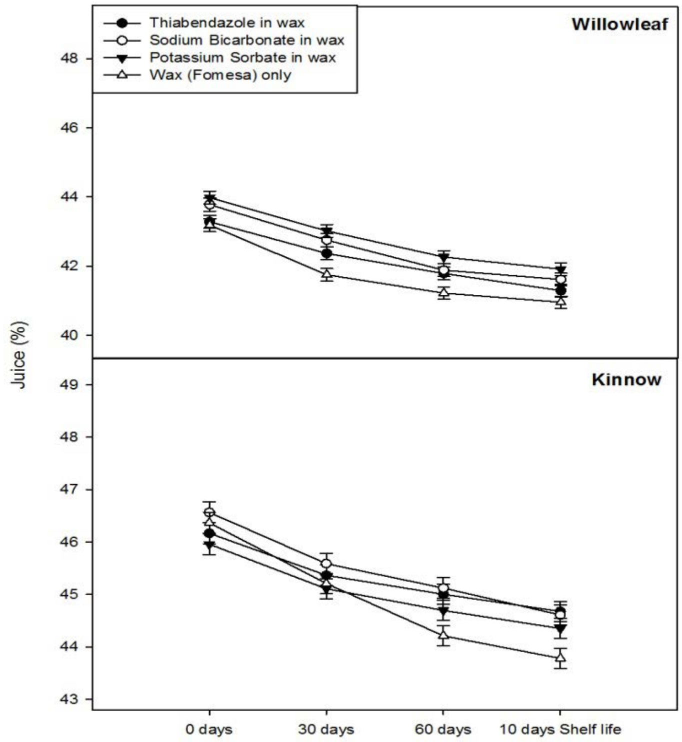


Graph 3: Effect of postharvest treatments on fruit weight loss in Kinnow and Willow leaf Mandarins.

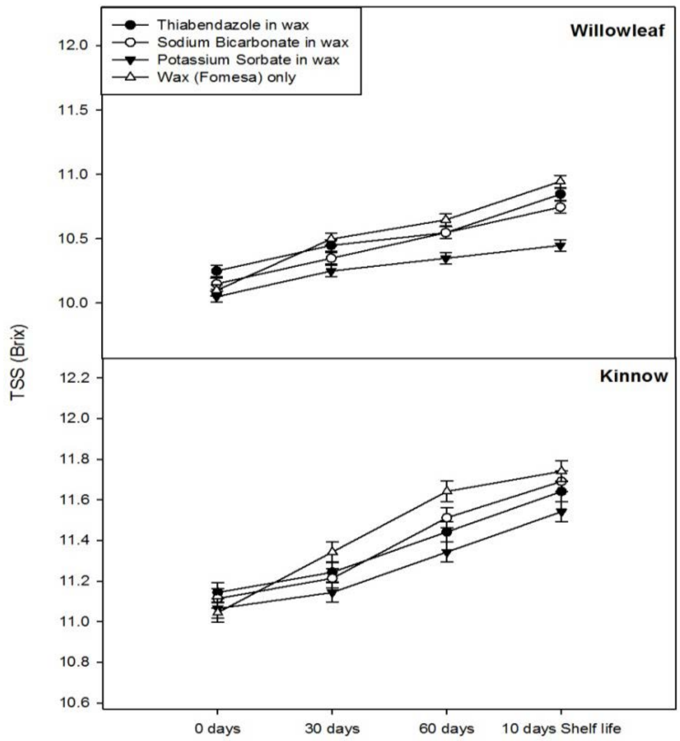
Minimum weight loss was observed in case of Kinnow treated with thiabendazole kept for one week at ambient conditions after 60 days storage at controlled conditions. In the same treatment adopted for Willow leaf weight loss percentage was significantly higher than that for Kinnow.



Graph 4: Effect of postharvest treatments on fruit decay in Kinnow and Willow leaf Mandarins.

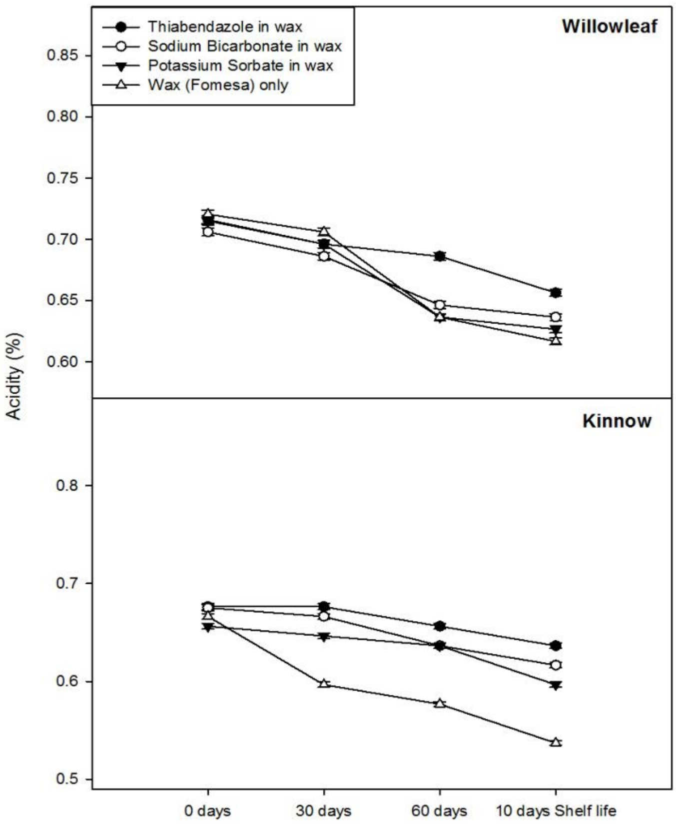


Graph 5: Effect of postharvest treatments on fruit juice recovery in Kinnow and Willow leaf Mandarins.



Graph 6: Effect of postharvest treatments on fruit juice TSS in Kinnow and Willow leaf Mandarins.

Physiological weight loss and reduction in juice recovery in all treatments was recorded minimum attributed to wax coating which retard the respiration and moisture transpiration from surface of fruit assisted by environmental temperature and relative humidity. Thus application of wax in combination with fungicide and salts was found quite helpful to retain glossiness avoiding wilting and skin shriveling as studied by Wills et al., 1999; Mahajan et al., 2004.



Graph 7: Effect of postharvest treatments on fruit juice acidity in Kinnow and Willow leaf Mandarins.

Minimum decay percentage in both fruit under test was seen in a fungicide treatment T1 with prolongation of storage period followed by T3 and T2 in which fruit was treated with Potassium Sorbate and Sodium Bicarbonate respectively. The results indicated the effective control of fruit decay achieved by the combination of two salts with wax confirming results previously obtained by El-Mougy et al., 2008 and Youssef et al., 2012.

TSS (Brix) and acidity mainly determine the fruit maturity indices and serve to determine the quality of fruit. TSS in both mandarins increased with prolongation of storage period while acidity showed a decreasing trend. Incremental trend in TSS can be attributed due to metabolic activities converting polysaccharides into sugars and reduction of moisture level due to transpiration and evaporation. The linear decline in acidity might be due to catabolism of organic acids (Mahajan et al., 2016). Overall results for TSS and acidity were found in accordance with findings of Rab et al., 2016, Sonkar et al., 2009, Youssef et al., 2012.

Conclusions and Recommendations

Postharvest losses can be minimized through best postharvest management practices and by extending storage stability of citrus fruit. Waxing with incorporated salts like Potassium Sorbate and Sodium Bicarbonate can effectively control postharvest diseases and decay in Mandarin fruits resulting in improved quality of fruit. Kinnow and Willow leaf mandarins exhibit well balanced nutritional status and can be stored up to 60 days at optimal storage conditions after proper postharvest treatment.

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Author Contribution

The authors Ehsan-Ul-Haque and Akbar Hayat designed, directed and executed the project. Muhammad Zubair, Muhammad Abdullah Jamil and Faheem Khadija performed the experiments. Muhammad Asim, Muhammad Shakeel Hanif developed the framework and wrote the article. Sajjad Hussain reviewed the whole manuscript critically.

Conflict of interest

The authors have declared no conflict of interest.

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