

Effect of cold storage conditions on fruit quality of different citrus cultivars of Sargodha division

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Abstract

Citrus is a major fruit of Pakistan, which is grown all over the world. Fruit quality is a great concern for growers, consumers, and processors. Different techniques are implemented to improve the shelf life of fruit and vegetables. In the current study, low-temperature techniques are used to improve the postharvest life of different citrus cultivars under cold storage conditions. This study was conducted on different cultivars of citrus, i.e., sweet orange (*Citrus sinensis* L. *Osbeck*) cv. Musambi and Malta as well as mandarin (*Citrus reticulata* L. Blanco) cv. Kinnow and Feutrell's early. All these cultivars were stored at 4 °C for 45 and 90 days. Regardless of treatments applied, the highest chilling injury was observed in sweet orange cv. Musambi (16.67%) and Malta (11%) followed by Mandarin cv. Kinnow (1.83%) and disease incidence were higher in Musambi (13.6%) followed by Feutrell's early (13%). The maximum fruit diameter was measured in Kinnow (72.58 mm) followed by Feutrell's early (64.40 mm), Malta (63.90 mm), and Musambi (61.28 mm). However, on an overall basis, higher fruit juice content percentage was measured in Feutrell's early (31%) followed by Kinnow (27.89%), Musambi (14.25%), and Malta (11.50%). The highest total soluble solids (TSS) contents were measured in Malta (12.53%), while the lowest TSS contents were recorded in Feutrell's early (11.05%) after storage of 90 days at 4°C. In conclusion, the performance of Kinnow in terms of quality parameters was better compared to Musambi, Malta, and Feutrell's early when stored at 4°C for 45 and 90 days.

Keywords: Feutrell's early, Fruit quality, Kinnow, Low temperature, Malta, Musambi

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Introduction

The citrus industry ranked 2nd largest fruit industry after grapes in the world. Citrus is grown on a local and commercial scale in about 135 countries around the globe (Naqvi, 2004). China is the leading producer of citrus, while Pakistan is ranked at 16th position in the world with production of 2.28 million metric tons annually (FAO, 2019). Pakistan is the 13th greatest producer of citrus to Brazil as the greatest producer of citrus in the world. While citrus is at the leading position in terms of cultivated area and production among fruits in Pakistan, it is cultivated on about 0.18 million hectares with 2.35 million tonnes of production and 0.28 million tonnes of export in 2017-18 (AIMS, 2018). Citrus is presumed to be a shrub or evergreen tree. The edible cultivars among from the species of Eucitrus, mandarin, sweet orange, grapefruit, sweet lime, and lemon, which are considered the best citrus fruit examples (Ghafoor et al., 2008). The major portion of this crop is cultivated in subtropical regions of Punjab

province, which contributes about 90% of total production, and the most dominant cultivar of this region is Kinnow mandarin (Citrus nobilis X Citrus deliciosa) (Memon, 2014). Citrus is generally consumed as fresh, among the fruits most popular around the world. It is nutritionally important as almost all its species are rich sources of Vitamin C, minerals, phytochemicals, and dietary fiber. In addition, vitamins A, B1, B6, calcium, folic acid, iron, magnesium, and potassium can also be supplemented by citrus (Altaf et al., 2008). The application of micronutrients enhances the quality of Kinnow mandarin (Hussain et al., 2022). However, it is also a good source of bioactive phenolic compounds and flavonones having strong antioxidant properties. Some epidemiological studies also revealed that a higher intake of citrus reduces the risk of certain cardiovascular diseases and some cancers (Mditshwa et al., 2017). Orange fruit of average size have 60 calories of energy and suffer from physiological disorders called creasing (Hayat et al., 2022., Sajid et al., 2020). Apart from functional ingredients, they are also a good source of β carotene and bioflavonoids, essential to healthy diets (Din et al., 2014).

During the last few decades, different studies revealed that about 30-40% of fruit and vegetable production is lost before consumption in developing countries (Salami et al., 2010) mainly due to high rates of water loss and further decaying in postharvest handling (Ray & Ravi, 2005). Citrus is categorized as non-climacteric because of the absence of ripening-related enhancement in ethylene progression and respiration during postharvest (Katz et al., 2004). As described earlier, citrus is mostly consumed as fresh, which depicts that preserving natural fresh citrus qualities is important to maintain its original flavor and taste. The most critical factors in achieving this objective are postharvest treatments, storage temperature, and storage duration. At a commercial scale in pack houses, storage is considered a moat-critical phenomenon (Obenland et al., 2009). Storage temperature is a very important factor for maintaining the quality of fresh produce like fruits, vegetables, and ornamentals. On the other hand, low-temperature storage reduces the process of deterioration in temperate fruits. Besides this, extension in storage time and regularly monitoring transpiration and respiration rates during storage can also limit postharvest losses (Bisen & Pandey, 2008). Many studies showed that even fruits of the same variety possess different behaviors when stored at the same storage conditions, probably due to different harvest times, geographical locations, and other connected reasons (Haque et al., 2020., Eman & Magda, 2006). Optimal storage temperature varies among species, varieties, and plant parts. For example, storage at 13 °C of a citrus cultivar 'Ortanique' exhibits fast deterioration, while the same fruits show chilling injury (CI) when stored at 2 °C (Cohen et al., 1990).

Kinnow, the leading citrus cultivar in Pakistan, contributes about 90% of total citrus production. In fact, the citrus industry is monopolized by this single cultivar Kinnow in Pakistan (Khan et al., 2010). In case of any haphazard condition like the spread of any epidemic disease to Kinnow, it may collapse the whole citrus industry. So, it needs time to find any alternatives to this cultivar that are available in any case of a haphazard situation. This effort is made to evaluate the best cultivar in terms of shelf life and other quality attributes under common practice cold storage conditions. The present study will focus on the role of cold storage conditions on the fruit quality of different citrus cultivars of the Sargodha division.

\ Material and Methods

Plant materials and growth conditions

The present research was carried out at Mateela Kinnow Factory located in the village Mateela, Tehsil Kotmomin, District Sargodha, Punjab, Pakistan. This packed house was managed through guidelines and regulation of different food safety standards like Global GAP with reference to integrated farm assurance crops version 5.2 Control Points and Compliance Criteria AF Version 5.2-2_Mar2020. On the basis of compliance with standards, the pack-house was qualified for Global GAP certification with registration number 4049928143624. Besides, this packed house was also certified on other standards, i.e., HACCP and ISO 22000. The selection of cultivars was based on the availability of the cultivars after Kinnow. We selected two cultivars from Sweet Orange Cv. Musambi and Malta and two cultivars from mandarin (cv. Kinnow and Feutrell's early, respectively.

Sample collection, processing, and storage

All selected fruits of each cultivar were uniform in size, shape, and color, free from injuries and blemishes. Cultural practices like irrigation, fertigation, and weed control were kept uniform in all cultivars. Samples were harvested by following different maturity indices like intensity of fruit color, sugar contents, physical ripeness, and fruit firmness. The fruits of each cultivar were picked with light-picking scissors separately around the whole plant canopy. Afterward, sample fruits were shifted to the Mateela Kinnow factory for further processing. After shifting, sample fruits were subjected to necessary washing, drying, and sorting operations by the pack house plant. After that, fruits were further exposed to a waxing process in which a food polyethylene-based food wax @ 1liter/ton mixed with the recommended fungicide Thibendazole (TBZ) @ 800ppm was applied. Then, fruits were passed through a chamber of plant having 45-50°C temperature for drying. After that, the final sorting and grading process was accomplished. Then, 30 fruits of each cultivar were packed into 10 Kg corrugated boxes. After weighing, sticking, stripping, and marking, three boxes of each cultivar were moved to a cold store with homogenous conditions of 4±6°C and 85-90% relative humidity (RH). The first data before storage was recorded by the remaining uniform fruits of each cultivar. The subsequent data were noted after 45 and 90 days of storage. After removal from the cold store, the boxes were brought to the Horticulture lab of the College of Agriculture, University of Sargodha, for further analysis.

Physiological loss of weight (PLW %)

This physiological attribute was evaluated after the storage of fruits. To measure this, the following formula was used as earlier described by (Thakur et al., 2002). The difference in weight was considered as the physiological loss of weight PLW (%), and the results described as percent loss as given below:

Physiological loss in weight (%) =
$$\frac{W_2 - W_1}{W_1} \times 100$$

Where

W1= fruit weight at time of storage

W2 = weight at 45 and 90 days of storage

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Chilling injury (CI %)

Chilling or blast injury in each cultivar was calculated when samples were subjected to storage time (45 and 90 days) at a controlled temperature of $4\pm6^{\circ}$ C as prescribed by (Lafuente et al., 1997). The chilling injury (CI) was calculated by examining the fruit surface with the naked eye and counting large brown depressions along CI symptoms in affected fruits:

Chilling injury % = <u>Total no.of fruits affected by chilling injury/cultivar</u> <u>Total no.of fruits of each cultivar stored</u> × 100

Juice (%)

The juice of stored fruits in each box at each removal was extracted by a manually operated juice squeezer and weighed. The juice (%) was calculated using the formula as earlier illustrated by (Nawaz et al., 2008):

Juice (%) =
$$\frac{Average \ juice \ weight}{Average \ fruit \ weight} \times 100$$

Incidence of disease (%)

After the removal of boxes from the cold store, fruits were visualized by the naked eye. The fruits having symptoms of mold or pathogen attack were counted and separated by healthy fruits. The disease index was calculated by the following formula as mentioned by (Basit, 2019):

 $\frac{\text{Incidence of pathogens/ molds \%}}{\frac{\text{Total no of fruits affected by disease}}{\text{Total no of fruits of each cultivar}} \times 100$

Rind thickness (mm)

The rind thickness of every sample of each cultivar at 0day, 45, and 90-day intervals was measured with the help of Vernier caliper and noted in mm.

Fruit diameter (mm)

The diameter measurement of fruits after each removal from the cold store was done using a Vernier caliper around the radius of fruits, and readings indicating in mm were recorded.

Peel weight (g)

The fruits of each cultivar were peeled off manually with the help of a knife and separated peel weighted using digital electric balance and data recorded in grams.

Rag weight (g)

After removing the peel and juice extraction, the remaining rags were weighed using a digital electric balance.

Juice weight (g)

The juice of each fruit was extracted manually and sieved to get a clear juice. Juice weight was measured in grams by using a digital electric balance.

Fruit weight (g)

The fruits of each cultivar at each time before storing and during cold storage interval were weighed using a digital electric balance (A and D Limited, Tokyo, Japan), and data was recorded in grams.

Total soluble solids (TSS)

The total soluble solids (TSS) of every sample fruit were recorded using a digital refractometer (ATAGO, RX 5000). To calculate the amount of TSS, fruit juice was extracted, and 1-2 drops of juice were placed on the refractometer lens. Reading was recorded and expressed in percentage (%).

Titratable acidity (%)

The acidity of fruit juice was determined in terms of the percent anhydrous citric acid by the method of AOAC (2000).

TSS: TA

TSS/ Acid ratio was calculated as follows:

TSS: TA =
$$\frac{\text{Total soluble solids } (\%)}{\text{Titratable acidity}(\%)}$$

Juice pH

The juice of fruits was extracted by manually operating a squeezer and placed in a beaker. Before measuring, the pH meter (HANA 8520, Japan) was fixed to 7 by cleaning the tip of the pH meter with tap water.

Total sugars (%)

Firstly, we take a 100 mL volumetric flask and add the already prepared 25 mL aliquot, which was made by mixing 20 mL ddh₂O and 5 mL conc. HCl. To convert the non-reducing sugars into reducing, this solution was kept for an overnight hydrolysis stay. On the next day, this solution was neutralized by 0.1 N NaOH using phenolphthalein as an indicator, and distilled water was added to the volume. Further, this solution is taken into a burette and titrated against 10 mL Fehling solution for total sugars estimation. Total sugars calculated as:

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Total sugars (%) = $25 \times (X / Z)$

Where

X = volume (mL) of standard sugar solution titrated against 10 mL Fehling solution.

Z = volume (mL) of sample aliquot used against 10 mL Fehling solution.

Vitamin C (ascorbic acid)

For this purpose, 10 mL juice was added into a 100 mL volumetric flask, and the volume was made up to the mark by the addition of 0.4% oxalic acid; after that, 5 mL from this aliquot was taken and titrated against 2, 6-dichlorophenolindophenol dye, while the light pink color was endpoint which retained for 15 seconds.

For dye preparation, 42 mg NaHCO3 and 52 mg 2, 6dichlorophenol indophenol were taken together in a 200 mL volumetric flask, and the volume was made by adding distilled water.

Vitamin C was calculated by the following formula:

Ascorbic acid (mg 100 g⁻¹ juice) = $\frac{I \times R1 \times V}{R \times W \times VI} \times 100$

Where

RI = mL of dye used to titrate against VI of aliquot (sample reading)

R = mL of dye used to titrate against 2.5 mL (1 mL standard ascorbic acid +1.5 mL 0.4 % oxalic acid) of reference solution (Standard reading)

VI = mL of juice taken for titration

V = volume of the aliquot made by 0.4 % oxalic acid W = mL of the juice take

Statistical analysis

During this study, a Factorial design under Complete Randomized Design (CRD) was used in the experiment. There were four treatments; each cultivar represents a treatment, and each treatment has three replications, and every replication has 30 fruits. The data was analyzed statistically through a 2-way Analysis of Variances (ANOVA). The differences among treatment means were analyzed using the LSD test at a 5% level of significance by using the statistical software Statistix 8.1.

Results and Discussion

Physiological loss of weight (PLW %)

Physiological loss of weight (PLW) is presumed to be a major physiological attribute regarding fruit quality, evaluated as the more the physiological loss of weight will be the shelf life and vice versa. In this study, the data of PLW in Fig. 1 (A) depicts a significant difference among all the treatments at different time intervals under cold storage conditions. The mandarin cv. Kinnow exhibits (14.07%) PLW, which is lower than all remaining cultivars, while the maximum is recorded in sweet orange cv. Malta (20.79%), followed by (19.41%) and (16.95%) in Feutrell's early, Musambi, respectively, after 90 days of storage. During storage, an increase in weight loss may be due to loss in moisture, which ultimately enhances fruit softening, as earlier described by (Din et al., 2015). The loss of water ratio depends upon the water pressure gradient among fruit tissues and the surrounding atmosphere and storage temperature as well (Ghasemnezhad & Shiri, 2010). Beside, this decrease in water loss and respiration rate was due to wax layers, which also improved external appearance and led to extended shelf life, as reported by (Farooqi et al., 1975). Similarly, in 'Kinnow' mandarin was also observed by (Mahajan & Singh, 2014) wax coating reduced physiological weight loss (PWL) and improved quality as compared to nonwaxed fruits under storage at 2-3.5°C with 90-95% RH for 60 days.

Chilling injury (CI %)

Different citrus cultivars were chilling sensitive at low temperatures and expressed chilling injury symptoms when temperature decreased from an optimum limit. We also observed and noted data regarding chilling injury, which showed that sweet orange cv. Musambi, with (16.67%) chilling injury, was the more susceptible cultivar among these four cultivars under these cold storage conditions, while the lowest chilling injury was observed in mandarin cv. Kinnow about (1.83%) followed by (11%) and (9%) in Malta and Feutrell's early, respectively, after storage of 90 days under cold store. Fig 1(B) reveals data about all stored cultivars at different intervals. Mathaba et al., 2012 explicated that the application of imbalance fertilizers, high pH, and low organic matter in conventionally grown Kinnow might have developed poor fruit rind, which is highly susceptible to rind splitting and CI during storage. Khan et al. (2007) expressed that citrus cultivars were chilling sensitive at low temperatures; hence, their storage above freezing might have caused certain physiological deteriorations like chilling injury.

Incidence of disease (%)

During storage, some fungi cause many diseases in produce, which reduce the quality of produce and may rot the whole produce in case of severity. So, at each interval, data regarding disease incidence were also recorded and presented in Fig. 1(C), which reveals the disease index between day intervals and cultivars. The highest disease incidence was observed in sweet orange cv. Musambi (13.66%) and followed by mandarin cv. Feutrell's early (13%). However, the lowest disease incidence was recorded in mandarin cv. Kinnow (0%) after 45 and 90 days of cold storage. The increase in the incidence of disease with a prolonged storage period may be due to the deposition of moisture contents on the surface of fruits, which may create favorable conditions for microbial growth and ultimately cause spoilage in produce (Jaw et al., 2012).

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Fruit diameter (mm)

Fruit diameter was also significantly reduced during the whole storage period among all cultivars. Fig. 2 (A) data presented the fruit diameter at different storage intervals among all cultivars under cold storage conditions. The mandarin cv. Kinnow has a maximum fruit diameter (72.58 mm), while sweet orange cv. Musambi (61.28 mm) ranked last among all cultivars after 90 days of storage. The increase in storage duration causes moisture loss and ultimately reduces fruit diameter, as described by (Erkan & Wang, 2006).

Rind thickness (mm)

Rind thickness was reduced as the storage period was prolonged in all cultivars under cold storage conditions. The data in Fig. 2 (B) exhibit the data regarding the rind thickness of all cultivars at different day intervals under cold storage. The maximum rind thickness (4.45 mm) was observed in sweet orange cv. Malta while minimum recorded in mandarin cv. Kinnow was (2.90 mm) after 90 days of storage in cold storage. The decrease in rind thickness may be due to the loss of water.

Juice weight (g)

In general, a gradual decrease was observed in the juice weight of all citrus cultivars under cold storage as storage time increased. Fig. 2(C) presented data about juice weight for each cultivar at different storage intervals. The maximum juice weight was recorded in mandarin cv. Kinnow (39.8 g), minimum in sweet orange cv. Musambi (14.83 g) after storage of 90 days under cold storage. Mainly, juice weight is dependent on fruit moisture contents. Loss in moisture and a further decrease in juice content during storage among different fruits is common (Obeed & Harhash, 2006).



Fig. 1 (A) Represent physiological loss weight (%), (B) Chilling injury (%) and (C) Incidence of disease (%) as influenced by storage temperature and storage duration of different citrus cultivars

Fruit weight (g)

In this study, the storage period exceeds the fruit weight reduced in all stored cultivars. The fruit weight data for all cultivars at different time intervals were presented in Fig. 2 (D). The highest fruit weight was recorded in mandarin cv. Kinnow (158 g) while lowest in mandarin cv. Feutrell's early (95 g) after 90 days of storage. Loss in fruit weight is mostly connected with respiration through peel. The moisture loss from peel mainly depends upon the water pressure gradient between the fruit tissue and surrounding atmosphere and storage temperature (Ghasemnezhad & Shiri, 2010).

Rag weight (g)

The rag weight is another vital parameter. If the weight of the rags is higher, the juice is usually recovered in fewer citrus fruits, and the fruit with less rag weight recovers more juice. Each cultivar showed significant differences regarding rag weight. The data of each cultivar at different time intervals are presented in Fig. 2 (E). The maximum rag weight was recorded in sweet orange cv. Malta (43g) and minimum in mandarin cv. Feutrell's early (31g) after 90 days of storage. These results were also supported by the results from lemon (Sindhu & Singhrot, 1993), Nagpur mandarin (Ladaniya, 1997), and Kinnow (Alhawat et al., 1984), they reported the decline in rag weight after storage in the cold storage.

Peel weight (g)

Peel has an inverse relation according to the quality of fruit. Maximum weight of the peel means poor quality of fruit. The results regarding peel weight were found significant in this study, with a decline trend observed among all the cultivars. The peel weight was decreased as the storage period increased. The fig 2. (F) revealed data regarding peel weight for all cultivars at different day intervals. However, the highest peel weight was recorded in sweet orange cv. Malta (42.5 g) and lowest in mandarin cv. Feutrell's early (31.4g) after storage of 90 days. The reduction in peel weight might be due to continued respiration, which results in water loss and, ultimately, peel weight decrease.

Juice pH

In citrus, the pH of the juice shows evidence of acidity and basicity. In this study, results for juice pH were found to be significant among all cultivars. Fig. 3 (A) exhibited the results regarding juice pH for all cultivars at different time intervals during cold storage. However, the highest pH of juice was recorded in mandarin cv. Kinnow (4.16) while lowest in sweet orange cv. Malta (3.95) after storage of 90 days in a cold storage period. This could be due to the breakdown of pectin to pectenic acid. Catabolic processes

increase due to a high respiration rate that causes the breakdown of organic acids and ultimately results in high pH, as described by (Rivera- López et al., 2005). Our findings are in accordance with the results of (Tariq et al., 2001), who reported an increase in pH was observed in three citrus cultivars (sweet oranges, Satsuma, mandarins, and lemons) when kept at 5 $^{\circ}$ C with 90% RH. Similarly (Palma et al., 2003) reported that pH increased during storage of three different cultivars of mandarins ('Okistu' Satsuma; 'Nules' and 'Oroval' Clementine's), with little distinction among stored fruits at 1 and 5 $^{\circ}$ C.

Juice (%)

The results regarding juice% were significant in this study, as storage duration prolonged juice% decline among all cultivars in cold stores. The data regarding juice among all cultivars at different day intervals is presented in Fig. 3 (B). The highest juice (%) was recorded in mandarin cv. Feutrell's early (31%) and lowest in sweet orange cv. Malta (11.50%) after storage of 90 days in a cold store. The juice (%) is mainly associated with the moisture content of the fruit. During storage, the decline in juice content and moisture loss is typical in different fruits (Rab et al., 2016). In sweet oranges, moisture loss may be the main reason for reduced juice content during storage, as described earlier by (Pailly et al., 2004).

Vitamin C (Ascorbic acid)

Ascorbic acid is an influential antioxidant and is a vital part of human nutrition. It supports saving the human body from numerous diseases and scavengers, the kinds of reactive oxygen species manufactured in the body (Papadakis et al., 2003). The ascorbic acid contents were significantly affected by cold store temperature among all cultivars during storage. Fig. 3(C) showed the results regarding ascorbic acid contents for all cultivars at different time intervals during storage. However, maximum ascorbic acid contents were found in sweet orange cv. Malta (43.6 mg 100g⁻¹) while the minimum was in mandarin cv. Feutrell's early (28.16 mg 100g⁻¹) after a storage period of 90 days. Overall, a decrease in ascorbic acid contents with an increase in storage time was observed. This may be due to environmental conditions around the fruit, as earlier described by (Maftoonazad & Ramaswamy, 2019) that a higher concentration of O₂ causes an increase in ascorbic acid loss. Similarly, another study demonstrated that ascorbic acid loss was more distinct under higher temperatures than refrigerated storage conditions. In line with these results (Qiu & Wang, 2015) also reported that at 4 °C Satsuma" mandarins have higher ascorbic acid contents as compared to those stored at 20 °C. These findings also supported our results that at 4 °C all citrus cultivars show higher retention of ascorbic acid contents. The decrease in ascorbic acid content is probably due to the conversion of ascorbic acid to dehydroascorbic acid, which is heat labile and escapes out from fruit.



Fig. 2 (A) represent fruit diameter, (B) Rind thickness, (C) Juice weight, (D) Fruit weight, (E) Rag weight and (F) Peel weight as influenced by interaction of cold storage temperature and storage duration of different citrus cultivars



Fig. 3 (A) Represent Juice pH, (B) Juice % and (C) Vitamin C as influenced by interaction of cold storage temperature and storage duration of different citrus cultivars

Total soluble solids (TSS)

The TSS was significantly affected by cold storage conditions in all the Cultivars. Among the fruits kept at cold store temperature, the highest mean TSS contents were recorded in sweet orange cv. Malta (12.53%) while the lowest recorded in mandarin cv. Feutrell's early (11.06%) after a storage period of 90 days. The remaining data for all cultivars were presented in Fig. 4 (A). These findings are supported by the results of (Ahmad et al., 2013), who revealed that TSS contents were also increased as the storage period prolonged. The increase in total soluble content may be due to the loss of water during the storage or the polysaccharide dispersible in the cell wall of mature fruit (Hernández-Muñoz et al., 2006). The TSS content is enhanced due to the hydrolysis of insoluble polysaccharides into sugars at a quick rate at high temperatures (ambient) and at a slower rate at lower temperatures, i.e., in cold stores (Jawandha et al., 2012).

Titratable acidity (%)

In citrus, titratable acidity is important for evaluating the ripening process and nutritional quality (Mayuoni et al., 2011). The results regarding titratable acidity were found to be significant during this study. Overall, a decreasing trend in acidity with the passage of storage time was observed among all cultivars. Fig. 4 (B) exhibit the results of all cultivars at different day interval for titratable acidity under cold storage conditions. However, the maximum titratable acidity was found in mandarin cv. Kinnow (1.24%) and lowest in sweet orange cv. Malta (0.43%) after storage of 90 days under cold store. Our results are also in line with the results of (Thakur et al., 2002), who revealed a reduction in the acidity of Kinnow fruit juice during storage. The decline in acidity was found to be slow at cold storage temperatures. This may be because the high respiration rate makes a necessary respiratory substrate for the catabolic process in fruits. The same findings were also reported by (Panwar et al., 2017) in litchi. The reduction in percent acidity may be due to wax coatings, which fill the pores of the skin, reduce the respiration rate, and subsequently retain organic acids in fruits, as earlier reported by (Petracek et al., 1998).

TSS: TA

The results of TSS: TA for all stored cultivars at different time intervals are shown in Fig. 4 (C). The overall increasing trend

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was observed with the prolonging of the storage period. The maximum TSS: TA was found in sweet orange cv. Malta (30.58) while lowest in mandarin cv. Kinnow (9.68) after 90 days of storage. As TSS enhances and acidity decreases during storage, the TSS/Acid ratio also increases with an extended storage period (Lee & Kader, 2000). The enhancement in TSS and a simultaneous decrease in TA increased (TSS/TA), also termed the Sweetness Index (SI) (TSS/TA). An increase in SI was observed among all stored citrus cultivars. Our results indicated that sweet orange cultivars have higher SI, which is also supported by the results of (Schirra & Chessa, 1988); they explained that sweet orange varieties Hamlin and Valencia orange have higher SI after storage under cold store conditions. As SI considered the fruit quality index, a higher increase in it also leads to the development of off-flavors due to the formation of ethanol in the fruit, which is an indicator of spoilage.

Total sugars (%)

Sugars are an important parameter of quality measurement in Kinnow fruits, as they are an important source of energy when used by humans. Total sugars were significantly affected by cold storage conditions among all cultivars during this study. Generally, an increasing trend with the passage of storage time was observed during this study. Fig. 4 (D) reveals results about total sugars for all cultivars after 90 days of cold storage. The highest total sugar contents were noticed in mandarin cv. Kinnow (7.96%) while lowest in sweet orange cv. Musambi (6.71 %) after adjusted storage time. The increment in total sugars may be due to the complete oxidation of these sugars to CO_2 and water with the production of Adenosine Triphosphate during postharvest storage (Owureku-asare et al., 2014). Besides, this reduction in water during storage can also result in concentration of resultant sugars present so ultimately increase in total sugars (Rapisarda et al., 2001).

Correlation analysis

PLW (%) had a positive and significant relation with CI (%), disease incidence (%), TSS, juice pH, and TSS: TA, while negatively related with juice (%), fruit diameter, TA%, fruit weight, juice weight, rind thickness, peel weight, rag weight, vitamin C and total sugars. TSS has negative relations with fruit diameter, juice (%), TA (%), fruit weight, juice weight, rag weight, peel weight, rind thickness, vitamin C, and total sugars. However, there are significant positive relations with PLW (%), CI (%), disease incidence, juice pH, and TSS: TA (Fig. 5) and fruit storage and waxing are shown in Fig. 6).

Conclusion

In this study, different citrus cultivars were subjected to low storage temperatures for different storage durations, and their shelf life and quality. It was proved from current study that the highest chilling injury was observed in sweet orange cv. Musambi and Malta followed by Mandarin cv. Kinnow and disease incidence were higher in Musambi followed by Mandarin cv. Feutrell's early when these cultivars were stored at 4 $^{\circ}$ C for 45 and 90 days.



Fig. 4 (A) represent (TSS%), (B) (TA%), (C) TSS:TS and (D) Total sugars (%) as influenced by interaction of cold storage temperature and storage duration of different citrus cultivars



Fig. 5 Pearson Correlation Metrix (Significant level: 0.05) for different physiological and biochemical responses of different citrus cultivars under cold storage conditions



Fig. 6 Fruit waxing and storage for citrus cultivars at 4 °C

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