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Salicylic Acid and Putrescine to Reduce Post-Harvest Storage Problems and Maintain Quality of Murcott Mandarin Fruit

Hassan A. Ennab [1], Mervat A. El-Shemy [2] and Shamel M. Alam-Eldein [3,*]

1 Citrus Research Department, Horticulture Research Institute, Agricultural Research Center (ARC), Giza 12619, Egypt; hassan.ennab@yahoo.com
2 Fruit Handling Research Department, Horticulture Research Institute, Agricultural Research Center (ARC), Giza 12619, Egypt; mervatabd22@yahoo.com
3 Department of Horticulture, Faculty of Agriculture, Tanta University, Tanta 31527, Egypt
* Correspondence: shamel@ufl.edu or shamel.alameldein@agr.tanta.edu.eg; Tel.: +2-040-345-5584

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Abstract: Natural products have been used to improve citrus fruit quality and storability, which increase the fruit marketing period and exportation to distant markets. This study was conducted to evaluate the role of salicylic acid and putrescine on reducing post-harvest loss and maintaining the quality of stored Murcott mandarin. Fruit were harvested at 297–300 days from full bloom, and five 5-min dipping treatments were applied; salicylic acid (200 and 400 ppm), putrescine (50 and 100 ppm), and the control. One group of fruit was stored at 5 ± 1 °C and 90–95% relative humidity (RH), and the other group was stored at 23 ± 1 °C and 60–70% RH for 45 days. Quality attributes were recorded at harvest, 15, 30, and 45 days of storage. Weight loss and decay were significantly decreased with the application of salicylic acid and putrescine. Both materials were also effective maintaining fruit firmness, total soluble solids (TSS), acidity, TSS: acid ratio, and ascorbic acid during storage. Salicylic acid, especially at 400 ppm was more effective to reduce weight loss and decay, and to maintain fruit quality in comparison to putrescine treatments. It could be concluded that salicylic acid and putrescine are effectively delaying post-harvest deterioration rate and extending the storage period of Murcott mandarin fruit with acceptable quality.

Keywords: mandarins; dipping; salicylic acid; putrescine; quality; storage; weight loss; decay

1. Introduction

The oldest known budded Murcott mandarin tree was found in Florida, USA, in 1922, and named after Charles Murcott Smith [1]. It is also known as Smith Tangerine, Honey Murcott [2], or Honey Tangerine [3]. It is a hybrid of *Citrus reticulata* Blanco × *Citrus sinensis* (L.) Osbeck, Rutaceae family [4]. Trees are moderate in size and vigor with alternate bearing often occurring. Fruit is medium in size (2.5–3 inches in diameter) with reddish orange, fairly thin, and smooth peel. Flesh color is dark orange, and juice content is high with excellent TSS: acid ratio for fresh fruit market, but low storability [3]. The Mediterranean climate of Egypt is suited for citrus production. Mandarins, tangerines, clementines, and satsumas account for about one-fourth of the total citrus production in Egypt. Total cultivated area is about 46,036 ha with a total annual production of 1,038,753 t; average yield is 22.6 t/ha [5]. Murcott mandarin is a favorable fruit to the consumer, and the cultivated area is increasing rapidly in Egypt. Fruit mature from January to the end of March; making them the latest maturing mandarins type fruit with almost no competition with other fruit types and high remunerative value [6]. However, post-harvest physiological disorders, decay, and weight loss negatively affect fruit shelf life and marketability [7], especially with late-season harvested fruit in March. Burns and Baldwin [8] reported that the pattern of peel growth is different from that of the pulp. While late-season harvest refers to more pulp maturity and higher TSS: acid ratio, peel maturity refers to its susceptibility to injury, which ultimately reflected on fruit storability and marketability [9]. Therefore, proper storage is necessary to extend the marketing period and reduce market pressure at the peak harvest season [10]. The application of some natural compounds and cold storage can reduce post-harvest loss, maintain fruit quality, and extend shelf life [11].

Salicylic acid (2-hydroxybenzoic acid, $C_7H_6O_3$), a phenolic compound, natural growth regulator, and antioxidant in vascular plants [12] stimulates many physiological processes that control plant growth and development such as nutrients uptake, membrane permeability, enzymes activity, and disease-resistance mechanisms [13]. Salicylic acid improves fruit quality and storability, and has a toxicity effect on fungi during storage [14]. In addition, it can delay fruit ripening, probably through inhibition of ethylene biosynthesis [15]. It also enhances fruit firmness, reduces chilling injury, delays membrane lipid peroxidation of fruit, and maintains fruit quality during cold storage [16,17]. Post-harvest treatment with 2 mM salicylic acid significantly reduced fruit rot and maintained peel turgidity with no effect on the inner quality of stored satsuma mandarins. This effect might be attributed to the accumulation of H_2O_2 and defense-related metabolites, such as ornithine, threonine, and polymethoxylated flavones [18]. Pre-harvest spray of 8 mM salicylic acid effectively reduced fruit decay and chilling injury, slightly improved fruit firmness, and maintained TSS and acids content of cold stored ‘Lane late’ sweet orange, suggesting that salicylic acid has an anti-senescent effect [19].

Polyamines are natural compounds with aliphatic nitrogen structure, found in all living organisms, and play an important role in many physiological processes related to plant growth and development, including mitosis division, embryogenesis, floral initiation and development, fruit development, maturation and ripening, plant senescence, in addition to plant response to environmental stresses [20–22]. The common polyamines found in plant cells are putrescine, spermidine and spermine [23]. Putrescine is the main product in polyamine biosynthesis, and a synthetic precursor of spermidine and spermine [24]. Spermine plays an important role in oxidative balance and amino acids biosynthesis to cope with oxidative damages associated with cell senescence [25]. Polyamines affect fruit firmness, weight loss, ethylene production, and soluble solids and acids content [26,27]. Post-harvest vacuum infiltration of putrescine notably delayed color change, increased fruit firmness, and resulted in a reduction of post-harvest mechanical damages of lemon [28] due to lower abscisic acid concentration [29]. Putrescine has also reduced weight loss and increased fruit firmness in apricot [30] and plum [31]. Plum fruit treated with 1 mM putrescine showed a delay and/or a reduction in ethylene biosynthesis, associated with higher fruit firmness, lower TSS and acids content, reduced weight loss, and delayed color change; thereby extended storage life [32]. Putrescine increased fruit quality and storage life of litchi [33], strawberry [34], pomegranate [35], mango [36], and grapes [37].

Citrus fruit in general have good keeping quality, because of the low respiration rate of the fruit. High storage temperature increases fungal decay, water loss, fruit softening, and deterioration in vitamin C [38]. Cold storage extends fruit shelf life through reducing the respiration rate and ethylene production (particularly in climacteric fruit), inhibiting the rate of biological deterioration. In addition, cold temperature reduces the incidence of fruit decay [39]. In general, the optimal storage conditions for mandarins are 5 °C and 90–95% RH for 2–6 weeks [40]. Murcott mandarins were resistant to microbial infection, but highly subjected to chilling injury and weight loss when stored at 6 °C for 2 months, followed by 14 °C for 2 weeks [41]. Murcott fruit had better flavor quality when stored for 6 weeks at 5 °C compared to those stored at 20 °C [42]. Grierson and Ben-Yehoshua [43] found that 6–7 °C extends the storage life of ‘Coorg’ mandarins to 8 weeks. However, long storage periods may cause some physiological disorders, for example storing ‘Ponkan’ mandarins at 5 °C for 3 months caused severe chilling injury followed by decay, which almost led to total fruit loss, but high TSS percentage. Fruit stored at 10 °C showed less storage period with light chilling injuries and slight off flavors. The least storage losses and best fruit quality were noticed at 15 °C, but the storage period was shorter. The shortest storage period associated with higher percentage of decay and weight loss was observed at 20 °C, but the remaining intact fruit still had acceptable quality [44]. The maintenance of an optimal temperature with high RH (90–95%) is the best scenario for citrus fruit storage. Other factors like fruit post-harvest sanitation, pre-cooling, and air velocity inside the storage room are important factors to maintain fruit shelf life [45]. Citrus species and varieties vary in their optimal storage temperature. The majority of citrus cultivars favor low temperature levels during prolonged storage, while some other cultivars like 'Fortune' and 'Nova' mandarins, 'Navelate' oranges, lemons, and grapefruits are sensitive to low temperature below 9 °C [46]. Maintaining RH at 85–95% during storage is important to avoid water loss and softening if humidity is low, and decay if humidity is high [47]. Ventilation is also important to eliminate CO_2 and other volatile components with some remarkable reduction in fruit decay [48].

To reduce post-harvest loss, this research aimed to evaluate the effect of post-harvest dipping treatments with salicylic acid and putrescine on fruit quality and storability of Murcott mandarin fruit grown under the Egyptian conditions.

2. Materials and Methods

This study was carried out on Murcott mandarin fruit taken from 10-years old trees growing in the experimental farm of the Faculty of Agriculture, Kafrelsheikh University, Kafr El-Sheikh Governorate (31°30′85″ N, 30°80′39″ E), Egypt. Trees were budded on Volkamer lemon, *Citrus volkameriana* Ten & Pasq rootstock, and planted at 4 × 4 m spacing in clay soil (59.65% clay, 18.79% silt, 21.57% sand, 1.10% organic matter, 1.85 dSm-1 electrical conductivity (EC), and pH = 7.76) with flood irrigation system. Trees were uniform in growth, vigor, size, productivity, and appeared healthy with no observed symptoms of nutrient deficiency. Trees varied in their nature of alternate bearing within the same experimental farm, therefore fruit were not collected from the same trees during both seasons; only those observing on-year and good fruit yield were chosen for fruit collection (approximately 360 kg fruit/season). All trees received the same cultural practices with no growth regulators or pesticides. Trees were subjected to the following fertilization program; 0.5 kg/tree superphosphate (37% P_2O_5) applied once a year in December, 4.5 kg/tree ammonium sulphate (20.5% N) applied three times a year in February, May and late June, 1.25 kg/tree potassium sulphate (48% K_2O) applied twice a year in March and late June.

Fruit harvest took place on 14 March 2018 and 11 March 2019 at horticultural maturity stage (i.e., full color and average TSS = 12.30 ± 0.15%), which was about 297–300 days from full bloom, according to Arras and Usai [41]. Fruit samples were randomly collected from the four directions (N, E, S, and W) and three levels (top, medium, and bottom) of the tree. Fruit were uniform in size (average diameter = 7.20 ± 0.10 cm), color, and free of physical injuries, sunburn, blemishes, bruises, and pathogen and insect attacks. Fruit were directly transported to the Post-harvest Laboratory at Sakha Horticulture Research Station, Agricultural Research Center, Kafr El-Sheikh Governorate, for cleaning and dipping treatments. Fruit were washed with tap water with no pesticides or waxing treatments. Fruit were divided into five groups, 72 kg fruit each, and one group was assigned for one of the following 5-min dipping treatments at room temperature; distilled water (control), salicylic acid (Product of Aljoumhoria Chemicals Inc., Cairo, Egypt) at 200 and 400 ppm, and putrescine (Imported from Germany by Techno Gene Inc., Doki, Giza, Egypt) at 50 and 100 ppm. Afterward, fruit were left to dry for one hour at room temperature, and then each group was divided into two equal groups of 36 kg; one group to be stored at 5 ± 1 °C and 90–95% RH, while the second group to be at room temperature (23 ± 1°C) and 60–70% RH for 45 days. Each group was divided into four subgroups of 9 kg, which were divided into three replicates (3 kg each ≈ 16 fruit) packed in one layer inside a corrugated box. One subgroup (9 kg) was used for harvest day analysis, and the remaining three subgroups (27 kg) were used for evaluation and analysis every 15 days during storage (9 kg each). Ventilation of storage room was carried out for 5 min every 15 days of storage.

Fruit weight (g) was calculated at harvest and every 15 days during storage using a bench-top digital scale Model PC-500 (Doran scales, Inc., Batavia, IL, USA). The percentage of weight loss was calculated according to the following equation [49]:

$$([\text{Fruit weight at harvest} - \text{Fruit weight during storage}] \div \text{Fruit weight at harvest}) \times 100 \quad (1)$$

The decayed fruit were weighted every 15 days of storage, and the percentage of decay was calculated according to El-Kady et al. [49], as follows:

$$(\text{Weight of decayed fruit} \div \text{Initial fruit weight}) \times 100. \quad (2)$$

Average fruit firmness (N) was measured on two sides of five fruit [49] using a hand-held Shimpo digital force gauge, Model FGV-50XY fitted with 10 mm diameter plunger tip (Shimpo company, Wilmington, NC, USA). Total soluble solids (TSS) concentration was measured with a hand-held refractometer Model RA-130 (KEM Kyoto Electronics Manufacturing Co. Ltd., Tokyo, Japan), according to Mazumdar and Majumder [50]. Total acidity was estimated as citric acid/100 mL of juice using 2,6-dichlorophenol indophenol, according to the Association of Official Analytical Chemists (AOAC) [51]. Data of TSS and total acidity was used to calculate TSS: acid ratio. Ascorbic acid content was estimated as mg/100 mL juice using 2,6-dichlorophenol indophenol, according to Rangana [52].

The experiment was arranged in complete randomized design of 5 treatments × 4 evaluation times × 3 replicates at two different temperatures. Data of each storage temperature were individually analyzed using analysis of variance (ANOVA) [53], and means were compared using Duncan's multiple range test (DMRT) at $p \leq 5\%$ [54].

3. Results and Discussion

3.1. Weight Loss (%)

Fruit weight loss is basically related to water loss [55], and this essentially due to transpiration, which accounts for 90% of total weight loss [56], and initially comes from the peel [57]. Valencia oranges stored for 2 months at 20 °C and 50–75% RH showed a reduction of 9.5% in peel weight, but 2.1% in pulp weight [56]. Water loss adversely affects the quality and limits the economic post-harvest life of fruit [58]. Oranges start showing shrinkage at weight loss of 2.5%, and become unsalable at 5% of the original weight [59]. Therefore, maintaining fruit in cold and humid conditions significantly affects stomatal behavior, and reduces the rate of water loss during storage [60]. High temperature causes peel shriveling and drying, and ultimately results in dull appearance, softening, and peel senescence [61]. Abscisic acid (ABA) level is correlated with storage temperature, for instance, fruit stored at 12 °C showed a higher rate of water loss associated with an increase in ABA, in comparison to those stored at 2–5 °C [62].

The results in Table 1 show that the percentage of weight loss generally increased with extended storage period at both cold and room temperature in 2018 and 2019 seasons. Similar results were reported on Murcott mandarins stored at 5–10 °C when compared to those stored at 22–25 °C [63]. Results also revealed that application of salicylic acid and putrescine significantly reduced weight loss in comparison to the control at both temperatures, and salicylic acid was more effective in this regard. The most pronounced effect was noticed with fruit dipped in 400 ppm salicylic acid. The application of 400 ppm salicylic acid effectively reduced weight loss of stored 'Oregon Spur' apple [64]. In this respect, 400 ppm salicylic acid-treated fruit showed an average weight loss of two seasons equal to 1.6% in comparison to 2.9% for the control at cold temperature, while these values recorded 4.1% and 7.9% at room temperature, respectively (Table 1).

Table 1. Effect of salicylic acid (SA) and putrescine (Put) on weight loss (%) of Murcott mandarin fruit at 15, 30, and 45 days of storage during 2018 and 2019 seasons.

Treatments	2018					2019				
	5 ± 1 °C and 90–95% RH									
	0	15	30	45	Mean	0	15	30	45	Mean
Control	0	3.29 a	3.85 a	5.17 a	3.08 a	0	3.39 a	3.78 a	4.12 a	2.82 a
SA 200 ppm	0	2.01 d	2.18 d	3.48 d	1.92 d	0	2.11 c	2.28 c	2.38 d	1.69 c
SA 400 ppm	0	1.83 e	1.90 e	3.32 e	1.77 e	0	1.80 e	2.02 d	2.16 e	1.50 d
Put. 50 ppm	0	2.29 b	2.87 b	4.05 b	2.30 b	0	2.43 b	2.75 b	2.98 b	2.04 b
Put. 100 ppm	0	2.08 c	2.49 c	3.68 c	2.06 c	0	1.93 d	2.28 c	2.53 c	1.69 c
Mean	0	2.30 c	2.66 b	3.94 a	–	0	2.33 c	2.62 b	2.83 a	–
	23 ± 1 °C and 60–70% RH									
Control	0	6.43 a	10.15 a	14.48 a	7.77 a	0	7.11 a	10.71 a	14.53 a	8.09 a
SA 200 ppm	0	5.17 d	6.53 d	6.65 d	4.59 d	0	5.31 d	5.45 d	5.70 d	4.11 d
SA 400 ppm	0	4.91 e	6.11 e	6.25 e	4.32 e	0	4.98 e	5.26 e	5.43 e	3.92 e
Put. 50 ppm	0	5.53 b	6.85 b	7.10 b	4.87 b	0	5.70 b	5.81 c	6.03 b	4.38 b
Put. 100 ppm	0	5.31 c	6.62 c	6.91 c	4.71 c	0	5.59 c	5.88 b	5.96 c	4.36 c
Mean	0	5.47 c	7.25 b	8.28 a	–	0	5.74 c	6.62 b	7.53 a	–

Means followed by the same letter within a column are not significantly different using Duncan's multiple range test (DMRT) at $p \leq 0.05$.

These results support the previous findings on the role of salicylic acid and cold storage reducing respiration rate and weight loss of Ponkan mandarin [27] and guava [65]. 'Ponkan' mandarins treated with 400 ppm SA, and stored at 8–10 °C and 75–90% for three months recorded 3.5% weight loss [27]. The combined application of salicylic acid and putrescine significantly decreased weight loss of stored apricot [66], plum [67], and peach fruit [68]. This might be due to a reduction in respiration rate and changes in cell wall properties related to water permeability [69]. Reduction of weight loss in putrescine-treated pear fruit [70] can be attributed to the conjugation of polyamines to phospholipids and protein components of cell membranes, resulting in stabilization and consolidation of cell integrity and membrane permeability [71].

3.2. Firmness (N)

The word "firmness" in citrus fruit is an indication of fruit turgidity and peel thickness [72]. Fruit firmness decreases with maturation, because the pulp tends to pull away from the peel late in the season, especially in mandarins, in addition to the excessive development of the albedo in late-bloom varieties [73]. The lower the temperature in winter, the thicker the peel of the fruit [74]. In climacteric fruit, the reduction in fruit firmness with maturation is mostly related to the dissolution of the pectic components of the cell wall [75], but this is not a dominant reason in citrus fruit softening. Although, citrus fruit is rich in pectin, its dissolution into soluble pectin with maturation is very slow; however, it is a rapid process in certain mandarin cultivars. In general, the concentration of cellulose, hemicellulose, and pectic substances in mature citrus fruit peel is very low [76], but the concentration of malonic acid is high [77]. In addition, the polysaccharide components of the cell walls of flavedo decrease with maturation [78]. On the other hand, the cells of albedo tissue become small with low cytoplasmic content and metabolic activity, large intercellular spaces, and weak cell wall, which break easily with maturation [79]. Citrus fruit softening after harvest is mainly related to water loss from the peel [80], which increases with maturation and senescence [58]. Fruit softening is also associated with pathogens that invade the peel and secrete cell-wall degrading enzymes [81]. Application of growth regulators and storage at low temperature and high humidity ultimately reduce water loss and retain peel turgidity [58].

Like weight loss (Table 1), the reduction in fruit firmness with prolonged storage period was more noticeable at room temperature (Table 2). Similarly, salicylic acid and putrescine effectively reduced fruit softening in comparison to the control at both temperatures in 2018 and 2019 seasons. Salicylic acid was more effective than putrescine in this respect, and the most noticeable effect was related to the application of 400 ppm salicylic acid. For instance, two-seasons average firmness of 400 ppm salicylic acid-treated fruit and control was 0.303 and 0.270 N at cold temperature, whereas these values were 0.286 and 0.240 N at room temperature, respectively (Table 2). Putrescine effectively maintained fruit firmness of lemon [29] and plum [32] during cold storage. Putrescine maintains fruit firmness through making cross-linkage with the carboxyl groups of the pectin in the cell wall leading to a strengthened cell wall. It also decreases the activity of cell wall degrading enzymes, such as pectin methyl esterase, pectin esterase, and polygalacturonase [82]. Salicylic acid was more effective than putrescine in maintaining fruit firmness during storage (Table 2), and both showed anti-senescent effect due to reduction in endogenous ethylene production [83] that protects cell wall structure [84] and maintains cell turgor pressure [37].

Table 2. Effect of salicylic acid (SA) and putrescine (Put) on firmness (N) of Murcott mandarin fruit at harvest and 15, 30, and 45 days of storage during 2018 and 2019 seasons.

Treatments	2018					2019				
	5 ± 1 °C and 90–95% RH									
	0	15	30	45	Mean	0	15	30	45	Mean
Control	0.311 d	0.268 d	0.247 e	0.226 d	0.263 e	0.319 c	0.289 c	0.260 c	0.240 d	0.277 c
SA 200 ppm	0.314 b	0.296 ab	0.266 c	0.249 b	0.281 b	0.338 ab	0.309 b	0.279 b	0.250 c	0.294 b
SA 400 ppm	0.316 a	0.304 a	0.284 a	0.265 a	0.292 a	0.348 a	0.328 a	0.309 a	0.270 a	0.314 a
Put. 50 ppm	0.312 cd	0.279 c	0.259 d	0.231 d	0.271 d	0.328 bc	0.299 bc	0.274 b	0.255 bc	0.289 b
Put. 100 ppm	0.313 bc	0.287 bc	0.272 b	0.237 c	0.277 c	0.319 c	0.304 b	0.279 b	0.260 b	0.290 b
Mean	0.313 a	0.287 b	2.66 c	0.242 d	–	0.330 a	0.306 b	0.280 c	0.255 d	–
	23 ± 1 °C and 60–70% RH									
Control	0.311 d	0.259 d	0.201 c	0.180 d	0.238 d	0.319 c	0.267 d	0.207 e	0.178 e	0.243 e
SA 200 ppm	0.314 b	0.290 a	0.274 a	0.196 c	0.269 b	0.338 ab	0.289 b	0.270 b	0.240 b	0.284 b
SA 400 ppm	0.316 a	0.281 b	0.280 a	0.245 a	0.279 a	0.348 a	0.298 a	0.279 a	0.250 a	0.294 a
Put. 50 ppm	0.312 cd	0.274 c	0.245 b	0.181 d	0.253 c	0.328 bc	0.269 d	0.240 d	0.221 d	0.265 d
Put.100 ppm	0.313 bc	0.287 bc	0.255 b	0.225 b	0.268 b	0.319 c	0.275 c	0.250 c	0.230 c	0.269 c
Mean	0.313 a	0.276 b	0.251 c	0.206 d	–	0.330 a	0.279 b	0.249 c	0.223 d	–

Means followed by the same letter within a column are not significantly different using DMRT at $p \leq 0.05$.

3.3. Decay (%)

Fruit susceptibility to post-harvest diseases accelerates with maturation and senescence, because peel becomes less firm; thereby less force is required to invade the peel by the pathogens [85]. Mechanical injuries at harvest and during handling are the main sites of peel invasion by the wound-invading *Penicillium* and *Geotrichum* [61]. Cold storage associated with high humidity is important for maintaining the resistance of the peel and fruit calyx against pathogens infection [81]. Moreover, low temperature obviously retards pathogens growth on infected fruit; however, disease symptoms will appear a few days after transferring the infected fruit to ambient temperature [86]. Generally, decay is the main post-harvest problem in warm and humid climates like Florida, whereas fruit shrinkage and softening are the major problems in arid climates like Egypt [38].

Fruit decay generally increases with increased temperature and duration of storage. Fruit dipping in salicylic acid and putrescine was effective in reducing fruit decay in comparison to untreated fruit (Table 3). In this regard, salicylic acid was more effective than putrescine at both storage temperatures during both seasons. At cold temperature, no decay was noticed with both salicylic acid concentrations for the entire period of storage, while decay started by the 30 days of storage with other treatments. At room temperature, the most pronounced reduction in decay was noticed with 400 ppm salicylic acid treatment; however, the presence of fruit decay started by 30 days of storage with all treatments in both seasons. It could also be noticed that average decay percentage of two seasons in salicylic acid-treated fruit was zero, whereas this percentage was 19% for the untreated fruit at cold storage. However, at room temperature, this percentage was about 23% and 69% at room temperature, respectively (Table 3). These results are consistent with previous reports on 'Ponkan' mandarins treated with 400 ppm SA, and stored at 8–10 °C and 75–90% for three months with a total decay of 2% [27].

Table 3. Effect of salicylic acid (SA) and putrescine (Put) on decay (%) of Murcott mandarin fruit at 15, 30, and 45 days of storage during 2018 and 2019 seasons.

Treatments	2018					2019				
	5 ± 1 °C and 90–95% RH									
	0	15	30	45	Mean	0	15	30	45	Mean
Control	0	0	0.35 a	0.40 a	0.19 a	0	0	0.33 a	0.42 a	0.19 a
SA 200 ppm	0	0	0.00 d	0.00 d	0.00 d	0	0	0.00 d	0.00 d	0.00 d
SA 400 ppm	0	0	0.00 d	0.00 d	0.00 d	0	0	0.00 d	0.00 d	0.00 d
Put. 50 ppm	0	0	0.21 b	0.29 b	0.13 b	0	0	0.23 b	0.31 b	0.14 b
Put. 100 ppm	0	0	0.15 c	0.20 c	0.09 c	0	0	0.18 c	0.22 c	0.10 c
Mean	0	0	0.14 b	0.18 a	–	0	0	0.15 b	0.19 a	–
	23 ± 1 °C and 60–70% RH									
Control	0	0	1.28 a	1.47 a	0.69 a	0	0	1.31 a	1.45 a	0.69 a
SA 200 ppm	0	0	0.40 c	0.65 c	0.26 c	0	0	0.35 d	0.63 d	0.25 c
SA 400 ppm	0	0	0.31 d	0.57 d	0.22 d	0	0	0.29 e	0.48 e	0.19 d
Put. 50 ppm	0	0	0.65 b	0.70 c	0.34 b	0	0	0.68 b	0.77 c	0.36 b
Put. 100 ppm	0	0	0.60 b	0.78 b	0.35 b	0	0	0.60 c	0.88 b	0.37 b
Mean	0	0	0.65 b	0.83 a	–	0	0	0.65 b	0.84 a	–

Means followed by the same letter within a column are not significantly different using DMRT at $p \leq 0.05$.

Salicylic acid significantly reduced fruit decay of stored mandarins and sweet oranges due to enhancing the activity of antioxidant enzymes and improving resistance to fungal attack, the accumulation of H_2O_2 and defense-related metabolites like ornithine, threonine and polymethoxylated flavones [18], and the anti-senescent effect that maintains fruit firmness, which eventually reduced microbial attack [19]. Pre-harvest treatment of salicylic acid reduced post-harvest chilling injury and fungal diseases in melon [87], mango [88], and apple [63]. On the other hand, putrescine reduced ABA concentration and fruit senescence [29], and led to increased fruit firmness and reduced post-harvest mechanical damages and decay in lemon [28]. Putrescine has also enhanced fruit resistance to chilling injury and decay, and prolonged the storage period of peach [68] and pear [70].

3.4. TSS, Acidity, and TSS: Acid Ratio

Results in Table 4 confirm the anti-senescence role of salicylic acid [19] and putrescine [32] on stored Murcott mandarins compared to the control at both storage temperatures during both seasons. The increase in TSS throughout the storage period was less in treated fruit than the control. In this regard, salicylic acid was more effective, and the least TSS was noticed in 400 ppm salicylic acid-treated fruit. The average of two seasons showed that the increase in TSS of 400 ppm salicylic acid-treated fruit after 45 days storage at cold temperature was 12.8% compared to 12.1% at harvest, whereas those values recorded 15.1% and 12.3% for the control fruit, respectively. It is also noticeable that TSS values were higher at room temperature in both seasons.

On the other hand, Table 5 indicates that acid content was also maintained at the highest level throughout storage with 400 ppm salicylic acid-treated fruit stored at cold temperature during both seasons. However, fruit stored at room temperature maintained high acid content with all treatments with almost no significant differences among treatments throughout the storage period. These results are consistent with previous findings of salicylic acid-treated apples [84] and putrescine-treated strawberry [89] and apricots [90]. Their effect is mainly due to reduction in respiration rate and the catabolism of sugars and acids content, associated with fruit ripening process [15,91]. Sugars constitute about 75–76% of the TSS in citrus fruit [92]. Higher content of acids at harvest increased the keeping quality of lemon and mandarin fruit [93]. Salicylic acid increases polyamines level in 'Ponkan' mandarin, reduces the degradation of antioxidants system in 'Cara' orange [94], and inhibits ethylene production in pear [95]. Putrescine effectively maintains the levels of TSS and acidity due to the reduction in respiration rate [82] and ethylene synthesis [35].

Table 4. Effect of salicylic acid (SA) and putrescine (Put) on TSS (%) of Murcott mandarin fruit at harvest and 15, 30, and 45 days of storage during 2018 and 2019 seasons.

Treatments	**2018**					**2019**				
	5 ± 1 °C and 90–95% RH									
	0	**15**	**30**	**45**	**Mean**	**0**	**15**	**30**	**45**	**Mean**
Control	12.15 ab	12.68 a	13.18 a	13.48 d	12.87 b	12.48 a	13.08 a	13.45 ab	14.87 a	13.47 a
SA 200 ppm	12.13 b	12.47 c	12.97 c	13.67 c	12.81 c	12.38 b	12.8 7 c	13.37 b	14.07 d	13.17 c
SA 400 ppm	12.05 c	12.25 d	12.79 d	13.49 d	12.65 d	12.21 c	12.65 d	13.18 c	13.88 e	12.98 d
Put. 50 ppm	12.21 a	12.57 b	13.07 b	13.87 a	12.93 a	12.11 d	12.97 b	13.47 a	14.27 b	13.21 b
Put. 100 ppm	12.20 a	12.51 c	13.01 bc	13.81 b	12.88 b	12.17 cd	12.91 bc	13.41 ab	14.21 c	13.18 bc
Mean	12.14 d	12.49 c	13.00 b	13.66 a	–	12.27 d	12.89 c	13.37 b	14.26 a	–
	23 ± 1 °C and 60–70% RH									
Control	12.15 bc	12.87 a	13.57 a	15.07 b	13.42 a	12.48 a	13.48 a	14.19 a	15.68 a	13.97 a
SA 200 ppm	12.13 c	12.66 c	13.36 c	14.26 d	13.10 c	12.38 b	13.02 d	13.98 c	14.87 d	13.56 c
SA 400 ppm	12.05 d	12.44 d	13.11 d	14.08 e	12.92 d	12.21 c	12.97 d	13.73 d	14.69 e	13.40 d
Put. 50 ppm	12.21 a	12.76 b	13.46 b	14.40 c	13.21 b	12.11 d	13.37 b	14.08 b	15.07 b	13.66 b
Put. 100 ppm	12.20 ab	12.70 bc	13.40 bc	15.40 a	13.43 a	12.17 cd	13.27 c	14.02 bc	15.01 c	13.61 b
Mean	12.15 d	12.67 c	13.38 b	14.54 a	–	12.27 d	13.22 c	14.00 b	15.06 a	–

Means followed by the same letter within a column are not significantly different using DMRT at $p \leq 0.05$.

Table 5. Effect of salicylic acid (SA) and putrescine (Put) on acidity (%) of Murcott mandarin fruit at harvest and 15, 30, and 45 days of storage during 2018 and 2019 seasons.

Treatments	**2018**					**2019**				
	5 ± 1 °C and 90–95% RH									
	0	**15**	**30**	**45**	**Mean**	**0**	**15**	**30**	**45**	**Mean**
Control	1.22 b	0.89 e	0.77 e	0.63 c	0.88 e	1.25 a	0.92 c	0.80 e	0.66 e	0.91 d
SA 200 ppm	1.20 c	1.08 b	0.97 b	0.86 a	1.03 b	1.21 ab	1.11 a	1.00 b	0.89 b	1.05 b
SA 400 ppm	1.25 a	1.11 a	1.00 a	0.89 a	1.06 a	1.24 a	1.14 a	1.03 a	0.92 a	1.08 a
Put. 50 ppm	1.24 a	1.01 d	0.91 d	0.79 b	0.99 d	1.17 b	1.14 a	0.93 d	0.81 d	1.01 c
Put. 100 ppm	1.20 c	1.04 c	0.94 c	0.83 ab	1.00 c	1.20 ab	1.04 b	0.97 c	0.86 c	1.02 c
Mean	1.22 a	1.02 b	0.91 c	0.80 d	–	1.21 a	1.07 b	0.94 c	0.82 d	–
	23 ± 1 °C and 60–70% RH									
Control	1.22 b	0.73 b	0.62 c	0.52 b	0.77 c	1.25 a	0.70 c	0.59 b	0.48 b	0.76 c
SA 200 ppm	1.20 c	0.93 a	0.82 b	0.71 a	0.92 b	1.21 ab	0.90 b	0.79 a	0.68 a	0.90 ab
SA 400 ppm	1.25 a	0.96 a	0.85 a	0.74 a	0.95 a	1.24 a	0.93 a	0.82 a	0.71 a	0.93 a
Put. 50 ppm	1.24 a	0.96 a	0.85 a	0.74 a	0.95 a	1.17 b	0.93 a	0.82 a	0.71 a	0.91 ab
Put. 100 ppm	1.20 c	0.93 a	0.83 b	0.72 a	0.92 ab	1.20 ab	0.89 b	079 a	0.69 a	0.89 b
Mean	1.22 a	0.90 b	0.79 c	0.69 d	–	1.21 a	0.87 b	0.76 c	0.65 d	–

Means followed by the same letter within a column are not significantly different using DMRT at $p \leq 0.05$.

Citrus TSS: acid ratio increases very slightly during storage due to acids catabolism in respiration [96], and this explains results of Table 6. Fruit stored at room temperature had higher values of TSS: acid ratio than those stored at cold temperature. Salicylic acid- and putrescine-treated fruit showed lower TSS: acid ratio than the control at both temperatures in both seasons. These results were previously confirmed on putrescine-treated strawberry [90]. Data also revealed that salicylic acid was slightly more effective than putrescine at cold temperature, but no significant differences were noticed at room temperature (Table 6). The increase in TSS: acid ratio with prolonged storage period was mainly due to using more acids than sugars in respiration [65].

Table 6. Effect of salicylic acid (SA) and putrescine (Put) on TSS: acid ratio of Murcott mandarin fruit at harvest and 15, 30, and 45 days of storage during 2018 and 2019 seasons.

Treatments	2018					2019				
	5 ± 1 °C and 90–95% RH									
	0	15	30	45	Mean	0	15	30	45	Mean
Control	9.96 ab	14.24 a	17.12 a	21.40 a	15.68 a	9.98 ab	14.22 a	16.81 a	22.53 a	15.89 a
SA 200 ppm	10.10 a	11.55 c	13.37 cd	15.89 cd	12.73 d	10.23 a	11.60 c	13.37 c	15.62 d	12.71 c
SA 400 ppm	9.63 c	11.03 d	12.80 d	15.16 d	12.16 e	9.85 b	11.10 c	12.79 d	15.09 d	12.21 d
Put. 50 ppm	9.84 bc	12.45 b	14.41 b	17.57 b	13.57 b	10.35 a	11.38 c	14.49 b	17.63 b	13.46 b
Put. 100 ppm	10.17 a	12.02 b	13.84 bc	16.63 bc	13.17 c	10.14 ab	12.41 b	13.82 c	16.52 c	13.22 b
Mean	9.94 d	12.25 c	14.31 b	17.33 a	–	10.10 d	12.14 c	14.25 b	17.47 a	–
	23 ± 1 °C and 60–70% RH									
Control	9.96 ab	18.41 a	21.89 a	29.56 a	19.95 a	9.98 bc	19.26 a	24.05 a	32.73 a	21.50 a
SA 200 ppm	10.10 a	13.61 b	16.30 b	20.13 b	15.26 bc	10.23 ab	14.46 bc	17.71 b	21.91 b	16.08 b
SA 400 ppm	9.63 c	12.96 b	15.42 c	19.03 b	14.26 d	9.84 c	13.94 d	16.75 b	20.70 b	15.31 c
Put. 50 ppm	9.84 bc	13.35 b	15.83 bc	19.46 b	14.62 cd	10.35 a	14.38 cd	17.18 b	21.26 b	15.79 bc
Put. 100 ppm	10.17 a	13.65 b	16.14 b	21.04 b	15.34 b	10.14 abc	14.91 b	17.75 b	21.76 b	16.14 b
Mean	9.94 d	14.40 c	17.12 b	21.92 a	–	10.11 d	15.39 c	18.69 b	23.67 a	–

Means followed by the same letter within a column are not significantly different using DMRT at $p \leq 0.05$.

3.5. Ascorbic Acid (Vitamin C)

As an organic acid, ascorbic acid followed the same trend as acidity (Table 6) declining with prolonged storage (Table 7) due to acid consumption as substrates in respiration [97]. However, this trend was slower with all dipping treatments, which maintained the level of ascorbic acid higher than that of the control throughout storage at both temperatures in both seasons. The most pronounced effect was noticed with the application of 400 ppm salicylic acid, for instance the average of both seasons indicated that fruit treated with 400 ppm salicylic acid and stored at cold temperature showed a reduction of 12.2% after 45 days of storage compared to 23.3% for the control fruit. Ishaq et al. [91] reported that ascorbic acid content is gradually decreased during storage. Salicylic acid [84] and putrescine [78] had a significant influence on maintaining the level of ascorbic acid during storage. Ascorbic acid is an important quality factor in citrus fruit, and it is very sensitive to oxidation during storage in comparison to other acids. Oxidation led to the conversion of dehydroascorbate to diketogulonate. This effect can be inhibited with putrescine, which reduces or delays the activity of ascorbate oxidase, and consequently maintains ascorbic acid [91]. Similar results have been reported in mango [36] and apricot [30]. Salicylic acid inactivates ascorbate peroxidase [98], and increases ascorbic acid in apples during storage [64]. A positive effect of salicylic acid on TSS and ascorbic acid content has been reported in strawberry fruit [99].

Table 7. Effect of salicylic acid (SA) and putrescine (Put) on Ascorbic acid (mg/100 mL juice) of Murcott mandarin fruit at harvest and 15, 30, and 45 days of storage during 2018 and 2019 seasons.

Treatments	2018 Season					2019 Season				
	5 ± 1 °C and 90–95% RH									
	0	15	30	45	Mean	0	15	30	45	Mean
Control	39.26 bc	37.11 b	34.68 d	30.45 e	35.38 c	37.44 cd	33.62 d	31.54 c	28.36 d	32.74 d
SA 200 ppm	40.67 a	38.35 a	37.57 b	35.99 b	38.14 a	36.28 d	35.21 c	34.01 b	33.17 b	34.67 bc
SA 400 ppm	40.34 ab	38.82 a	38.15 a	36.52 a	38.46 a	41.71 a	39.25 a	37.90 a	35.49 a	38.59 a
Put. 50 ppm	37.90 d	35.52 c	34.78 d	32.40 d	35.15 c	39.42 b	35.80 bc	32.27 c	30.19 c	34.42 c
Put. 100 ppm	38.77 cd	36.64 b	35.89 c	34.18 c	36.37 b	38.15 bc	36.67 b	34.31 b	31.08 c	35.05 b
Mean	39.39 a	37.28 b	36.21 c	33.90 d	–	38.60 a	36.11 b	34.00 c	31.65 d	–
	23 ± 1 °C and 60–70% RH									
Control	39.26 bc	31.64 c	28.61 c	23.57 d	30.77 d	37.44 cd	31.35 b	26.38 c	22.00 d	29.29 c
SA 200 ppm	40.67 a	34.97 b	31.88 b	29.45 b	34.24 b	36.28 d	34.02 a	30.28 ab	29.90 a	32.62 b
SA 400 ppm	40.34 ab	37.38 a	35.17 a	31.39 a	36.07 a	41.71 a	33.31 a	31.48 a	29.91 a	34.10 a
Put. 50 ppm	37.90 d	33.42 bc	31.55 b	28.77 b	32.91 c	39.42 b	32.80 a	29.93 b	26.55 c	32.17 b
Put. 100 ppm	38.77 cd	33.51 bc	29.17 c	27.33 c	32.20 c	38.15 bc	33.13 a	30.15 ab	27.82 b	32.31 b
Mean	39.39 a	34.18 b	31.28 c	28.10 d	–	38.60 a	32.92 b	29.64 c	27.24 d	–

Means followed by the same letter within a column are not significantly different using DMRT at $p \leq 0.05$.

4. Conclusions

Results indicated that fruit dipping in salicylic acid or putrescine was effective in maintaining the quality of Murcott mandarin fruit during storage, in terms of reducing weight loss and decay, and maintaining fruit firmness, TSS: acid ratio and vitamin C at acceptable levels that extend fruit storability and marketability chances. The best results were achieved with 400 ppm salicylic acid-treated fruit stored at cold temperature (5 ± 1 °C). Fruit storage at cold temperature was ceased after 45 days, because extending the storage period may result in more weight loss and decay, as well as fruit softening, which may lead to more mechanical damage during handling and marketing of the fruit [40]. Moreover, by the end of the 45-day storage period in late May, Murcott mandarin fruit become the latest maturing mandarins type fruit in Egypt with almost no competition with other fruit types, except bananas and late oranges, in addition to high remunerative value. Extending the cold storage period until late June will minimize the importance of storage due to the increase in fruit weight loss, decay, and softening, which will be reflected in actual crop loss, in addition to the higher competition with other fruit types available at market by this time of the year, such as early grapes, apricots, and watermelons. It is also suggested stopping storage at room temperature after 15 days due to the subsequent high percentage of decay (≈30%), and even the weight loss of the remaining fruit was about 5%, which makes them unsaleable [59]. The future prospective of this research may extend to cover other quality control parameters of Murcott mandarins, such as enzymes activity, phytohormones, and flavour components. It may also include research at the molecular level to differentiate between pulp and peel maturity, and identify the optimal harvest period based on peel maturity to reduce post-harvest injuries.

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