DOI: 10.21608/alexja.2023.222162.1039

Post-Harvest Application of Some Calcium Forms to Improve Quality and Shelf Life of Mandarin Fruits During Storage

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ARTICLE INFO
Article History
Receive Date: 25/7/2023
Revise Date: 4/8/2023
Accept Date: 20/8/2023

Key words:
mandarin, calcium, salts, enzyme

ABSTRACT

An experiment was conducted during 2020 and 2021 seasons to evaluate the influence of postharvest calcium salts to enhance quality and prolonging shelf life of Balady mandarin fruits. The fruits were harvested at yellow or greenish-yellow, transported to laboratory, and prepared to immersing in solutions of calcium acetate 1%, calcium citrate 0.5%, calcium nitrate 1% and calcium chloride 2% in addition to control treatment (distilled water) for 5 min. The fruit dipped in different calcium solutions were stored at 4±1°C with 85-90% Humidity for 45 days. Physical and chemical fruit characteristics were recorded every 15 days during storage. Results showed that postharvest applications of different calcium sources were significantly decreased fruit weight loss and decay, also, it seems to be successful for maintaining fruit firmness, acidity, vitamin C, SSC and SSC/acid ratio in Balady mandarin fruits compared to control treatment. Also, lower activity of polygalacturonase, pectin methylesterase, and β-galactosidase enzymes were notice in fruit dipped in calcium sources than that on control. In addition, calcium nitrate 1% and $CaCl_2$ 2% applications found to be the best for reduces weight loss, decay and keeping overall fruit quality during storage. So, it is concluded that dipping mandarin fruit in calcium nitrate 1% or calcium chloride 2% led to extend shelf life and keeping fruit quality.

INTRODUCTION

Mandarin (*Citrus reticulate* Blanco) is one of the most important fruits within the citrus fruit species grown in Egypt. Mandarin fruit is easy peeling, acceptable juice quality and consumed freshly. Moreover, mandarin fruit is a good source for vitamin C and A, in addition it contains a little amounts of vitamin B, carbohydrates and proteins (Putnik *et al.,* 2017). Although, it's nutritional benefits for human health and commercial value; mandarin fruits may have some postharvest physiological problems and during handling. Softening and fungi attack led to fruit rot, which are considered the major problems of mandarin fruits which not only cause a negative influence on fruit shelf life and marketability but also causing significant economic losses for growers and retail business (Palou, 2009; Kassim *et al*., 2020). In this respect, calcium salts have a great role in increasing shelf-life and enhancing fruit quality under storage conditions. Calcium plays a vital role in improving the quality and shelf-life of fruits due to, its participation in many physiological processes such as maintaining the structure and function of the cell wall and membranes as it interacts with pectic acid in the cell wall to form calcium pectate, thus having a direct effect on fruit stability and enzymatic activity (Ochoa-Villarreal *et al.,* 2012). Calcium also has a beneficial effect on marketing and storage by inhibiting senescence and reducing respiration rate, protein disintegration, weight loss and rotting. In this sense, Tsantili *et al.,* (2002) noted that fruits with high calcium content have lower respiration rates and a longer shelf life compared to low fruits. Rokaya *et al.,* (2016) found that using 1% calcium chloride as postharvest treatment led to delays the senescence of mandarin fruits. Also, Kaur and Kumar (2014) observed that immersing Kinnow mandarin fruit in $Ca(NO_3)_2$ at 0.5 to 1 % and $CaCl_2$ at 1 to 2% resulted in fruit retention for up to 45 days stored in cold room while preserving fruit properties such as juice content, TSS, acidity and ascorbic acid. In addition, Riaz *et al.,* (2020) reported that calcium chloride and calcium lactate at 2% treatments significantly reduced weight loss, decay and total sugar, and increased sweet orange fruit firmness and vitamin C. Rabiei *et al.,* (2011) revealed that apple fruits soaked in $Ca(NO_3)_2$ at 0.5 to 1% and calcium acetate at 0.37 and 0.7% retain maximum firmness, acidity, catalase enzyme activity, with reduced TSS, weight losses, TSS/acid ratio and peroxidase enzyme activity during cold storage at 0±2°C for 150 days. Moradinezhad *et al.,* (2019) revealed that immersing Jujube fruit in 0.5 and 1% $CaCl_2$, $Ca(NO_3)_2$ and $CaSO_4$ led to a delay in fruit ripening and preserved the firmness of the fruits, and reduce loss in weight. It was found that the treatment with calcium is beneficial in terms of the integrity and functions of the membrane, preserving phospholipids and proteins from losses, and reducing ion leakage (Wang *et al.,* 2014).

Therefore, the objective of this research is to investigate the effect of different post-harvest treatments of calcium salts on prolonging shelf life and maintaining overall quality of mandarin fruits during storage.

MATERIALS AND METHODS

An experiment was conducted on fruits harvested from Balady mandarin (*Citrus reticulate* Blanco) orchard situated in Desouk distract, Kafr El-Sheikh governorate, Egypt. Trees have 16 years old, budded on sour orange (*Citrus aurantium* L.), and planted at 5×5 m apart, soil classified as clay and irrigation was flood. Fruits were harvested at 15th and 21th of December during 2020 and 2021 seasons, respectively when fruit at yellow or greenish yellow. Fruits were selected regular in size, and free from insect attacks and physical damages. As soon as fruit samples transported to laboratory of Sakha horticulture station, it was cleaned, sterilize with sodium hypochlorite solution (0.02%) for 2 minutes, washed with clean water and allowed to air-dry. The samples of fruit were splitted into six groups; group one was used to determining fruit quality characteristics at harvest date. The other groups were soaked all each alone for 5 minutes in one application of Control (water); calcium acetate $[Ca(C_2H_3O_2)_2]$ at 1%, calcium citrate $[Ca_3(C_6H_5O_7)_2]$ at 0.5%, calcium nitrate $[Ca(NO_3)_2]$ at 1% and calcium chloride $[CaCl_2]$ at 2%. After that, the fruits treated with different sources of calcium salts in addition to control were placed in carton boxes each having 3Kg of Balady mandarin fruits. The treatments were arranged in a randomized complete block design with three replicates; each replicate having 9Kg of mandarin fruits. The fruit was stored at 4±1°C with 85 – 90 % RH for 45 day. The following data was recorded every 15 days during the storage period.

1. Weight loss %:

Balady mandarin fruits were weighed on the date of harvest and in each storage period, the weight loss was calculated as mentioned in the equation: Fruit weight loss % = (Weight before storage – Weight after storage / Weight before storage) × 100.

2. Fruit decay %:

The percentage of fruit decay was determined by counted the number of fruit decayed at the exit date of sample and calculated by using the equation: fruit decay % = {number of decayed fruits/total number of stored fruits} x 100.

3. Fruit firmness (Newton):

Fruit firmness was measured using an Effegi penetrometer and the values are expressed as newton**.**

4. Fruit shelf life (days):

After each exit date of samples from refrigerator in order to estimate fruit quality, the remaining fruit are left at room temperature (22±1°C with 60 – 70 % RH) to determine shelf life in days. The fruits were examined every four days until fruit reach to 51% of senescence stage.

5.Soluble solids%, Titratable acidity%, SSC/acid ratio and vitamin C:

Juice was extracted from fruit samples for determining juice quality as: Soluble solid content was measured by hand refractometer, titratable acidity as citric acid was determined by titration with 0.1M sodium hydroxide using phenolphthalein indicator according to (A. O. A. C 1967), ascorbic acid as mg/100 ml juice by using 2, 6 dichlorophenol indophenol according to Rangana, (1977), and SSC/acid ratio was estimated.

6. Cell wall enzyme activities:

6.1. Polygalacturonase (PG)

Polygalacturonase enzyme activity was determined by using 50 μL of extract in a 1 mL solution containing polygalacturonic acid (25 g L^{-1}) and 40mM sodium acetate for 1h at 30°C. Then, 1.5 mL of the sample was boiled for 10 min, and left to cool in room temperature, and then the absorbance at 410 nm was measured by spectrophotometer according to Chen *et al.*, (2017).

6.2. Pectin methylesterase (PME)

Pectin methylesterase (PME) activity was determined by using the solution consist of 400 μL sample enzyme solution, 2 mL pectin solution 0.5%, 0.15 mL bromothymol blue 0.01%, 0.85 mL water. Absorption was measured at 620 nm by spectrophotometer according to Santos *et al.*, (2020).

6.3. β-galactosidase (β-Gal)

According to Chen *et al.*, (2017) the determination of β-galactosidase activity was done by using 0.5 ml polygalacturonic acid (10 g L-1), 0.5 mL enzyme solution and 1.5 mL salicylic acid. The mixture was heated for 5 min in boiling water, then allowed to cool to room temperature and completed to 25 mL with distilled water. absorbance at 540 nm by spectrophotometer.

7. Statistical analysis:

Statistical analysis was done by using SAS software, and the significant differences among means values show by using L.S.D. at 5% level according to Snedecor and Cochran (1990).

RESULTS AND DISCUSSION

1. Fruit weight loss (%):

The data in Table 1 show the changes in % weight loss of Balady mandarin fruits immersed in calcium acetate 1%, calcium citrate 0.5%, calcium nitrate 1% and calcium chloride 2% plus control (distilled water), and stored at 4±1°C with RH 85 – 90 % for 45 days. It's obvious from data in Table 1 that the percentage of weight loss was increased with the progress of storage period during the two seasons. Data also indicated that, Balady mandarin fruits dipped in different calcium forms had significantly lower percentage weight loss than those found in control ones. Moreover, mandarin fruits dipped in calcium nitrate 1% and calcium chloride 2% produced the lowest weight loss values during storage. In this respect, calcium nitrate 1% showed to be the best treatment for reducing fruit weight loss during storage. Similar results were obtained by Kaur and Kumar (2014) who found that Kinnow mandarin fruits dipped in calcium chloride 2% and calcium nitrate 1% had been significantly reduced fruit weight loss compared to fruits dipped in distill water (control). Also, Riaz *et al.,* (2020) revealed that application of postharvest calcium lactate and calcium chloride at concentration of 1, 1.5 and 2% led to reduces weight losses incidence and decay in the time storage of sweet orange, in addition, they found that $CaCl_2$ salt is the best than calcium lactate in reduce weight loss. Joshi *et al.,* (2020) revealed that mandarin fruits dipped in calcium chloride at 1% maintaining fruit firmness and reduced weight loss compared to those immersions in distill water. Calcium postharvest application found to reduce fruit weight loss during cold storage, this effect can be attributed to maintain cell membrane integrity and high fruit firmness and low moisture (Aghdam *et al.,* 2012, Zakaria *et al.,* 2018 and Acevedo-Barrera *et al.,* 2019). Additionally, calcium decreases fruit respiration, transpiration and damages in cell wall (Mohamed *et al.,* 2017).

2. Fruit decay (%):

It's clear from data in Table 2 that, Balady mandarin fruits treated with $Ca(C_2H_3O_2)_2$ 1%, $Ca_3(C_6H_5O_7)_2$ 0.5%, $Ca(NO_3)_2$ 1% and $CaCl_2$ 2% showed no decayed till 30 days from fruit stored in both seasons. At 30 days of cold storage, fruit decay started only in control treatment then increased with storage time. In this respect, at 45 days of cold storage decayed fruit was observed in fruit treated with calcium acetate 1% and calcium citrate 0.5%, but not in fruit dipped in calcium nitrate 1% and calcium chloride 2% (Table 2). Anyhow, using different calcium forms to dipped mandarin fruits caused a significant reduces in decayed fruits compared to control treatment through storage period (45 days). In this respect, control treatment exhibited the highest decayed fruits during storage stage in both seasons. Also, use calcium nitrate 1% and calcium chloride 2% did not give any decayed fruits through storage stage as compared with calcium acetate 1% and calcium citrate 0.5% in both seasons, on the other words calcium nitrate 1% and calcium chloride 2% showed to be the best treatments for reducing fruit decay percentage in both seasons. It was reported by many authors indicated that effectiveness of postharvest calcium treatments control decay and gave the lowest fruit decay percentages during storage (Zakaria *et al.,* (2018; Riaz *et al.,* (2020; Moradinezhad *et al.,* 2019). Abd El-Aziz *et al*., (2021) cleared that peach trees sprayed with chelated calcium three times, the fruits taken from this tree and stored at 0±1°C for 28 days recorded the lowest decay percentages during storage. Lower fruit decay observed in calcium treated may be due to the ability of calcium to maintain cell membrane and wall integrity, structure related pectin polymers and to maintain cell cohesion (Aghdam *et al.,* 2012; Roberto *et al.,* 2019).

3. Fruit firmness (Newton):

Data in Table 3 illustrated that, fruit firmness was significantly improved using calcium acetate 1%, calcium citrate 0.5%, calcium nitrate 1% and calcium chloride 2% compared to control. Also, it is obvious from data in Table 3 that, fruit firmness was reducing by progressive in the storage period for all treatments. In addition, Balady mandarin fruit dipped in calcium nitrate 1% were firmer followed by those dipped in calcium chloride 2% as compared with calcium acetate 1%, calcium citrate 0.5% during storage. Moreover, the highest values of fruit firmness were observed in fruit dipped in calcium nitrate 1%, in contrast the lowest values were detected in control fruit. These results agree with the findings of Srinu *et al.,* (2017), Ranjbar *et al.,* (2018) and Zakaria *et al.,* (2018) they concluded that postharvest application of different calcium salts increased fruit firmness in different fruit crops.

Table 1: The effect of postharvest treatments with different calcium sources on weight loss % of Balady mandarin fruits during cold storage in 2020 and 2021.

Treatments	2020 season				2021 season			
	Storage period in days at 4 ± 1°C with 85 – 90 % RH							
	0	**15**	**30**	**45**	**0**	**15**	**30**	**45**
Control	0	4.71	9.37	12.51	0	4.55	9.58	12.53
Calcium acetate 1%	0	3.82	6.82	8.38	0	3.52	7.30	8.61
Calcium citrate 0.5%	0	3.77	6.80	7.82	0	3.35	7.26	8.11
Calcium nitrate 1%	0	3.29	5.36	6.92	0	3.12	5.44	6.94
Calcium chloride 2%	0	3.45	5.69	7.12	0	3.34	5.50	7.00
L.S.D. ($p \leq 5\%$)	Ns	0.35	0.39	0.19	Ns	0.11	0.08	0.11

Ns = Not significant

Table 2: The effect of postharvest treatments with different calcium sources on decay % of Balady mandarin fruits during cold storage in 2020 and 2021.

Treatments	2020 season				2021 season			
	Storage period in days at 4 ± 1°C with 85 – 90 % RH							
	0	**15**	**30**	**45**	**0**	**15**	**30**	**45**
Control	0	0	1.15	1.58	0	0	0.74	1.28
Calcium acetate 1%	0	0	0	0.88	0	0	0	1.00
Calcium citrate 0.5%	0	0	0	0.50	0	0	0	0.70
Calcium nitrate 1%	0	0	0	0	0	0	0	0
Calcium chloride 2%	0	0	0	0	0	0	0	0
L.S.D. ($p \leq 5\%$)	Ns	Ns	0.02	0.11	ns	Ns	0.03	0.04

Ns = Not significant

Table 3: The effect of postharvest treatments with different calcium sources on firmness (Newton) of Balady mandarin fruits during cold storage in 2020 and 2021.

Treatments	2020 season				2021 season			
	Storage period in days at 4 ± 1°C with 85 – 90 % RH							
	0	**15**	**30**	**45**	**0**	**15**	**30**	**45**
Control	18.09	14.76	12.45	11.06	17.35	13.25	11.91	11.22
Calcium acetate 1%	18.09	15.08	12.47	11.21	17.35	14.35	12.37	11.76
Calcium citrate 0.5%	18.09	15.15	12.86	11.13	17.35	14.75	12.66	11.74
Calcium nitrate 1%	18.09	15.40	13.90	12.74	17.35	16.11	14.50	12.68
Calcium chloride 2%	18.09	15.33	13.06	12.00	17.35	15.72	14.00	12.48
L.S.D. ($p \leq 5\%$)	Ns	0.07	0.08	0.06	Ns	0.05	0.09	0.02

Ns = Not significant

In this respect, Rabiei *et al.*, (2011) found that an increase in firmness of apple fruits immersed in $Ca(NO_3)_2$ at 0.5% and $Ca(C_2H_3O_2)_2$ at 0.35% each alone during cold storage at 0±2°C for 150 days. Also, Riaz *et al.*, (2020) reported that sweet orange fruits immersed in 1, 1.5 and 2% $CaCl_2$ and $Ca(C_2H_3O_2)_2$, showed an increase in fruit firmness during storage for 35 days as compared with those immersed in distill water (control).

Higher fruit firmness observed in application of calcium could be attributed to the role of Ca in stabilizing cellular membrane (Abrol *et al.,* 2017; Abd El-Aziz *et al.,* 2021)). Also, calcium reacted with pectic acid to compose calcium pectate, led to keeping the cell wall structure and then, increasing in fruit firmness (Aghdam *et al.*, 2012; Rokaya *et al.*, 2016). Moreover, Ca regulates the activity of enzymes that cause softening (Abdelkader *et al.*, 2022). Therefore, it can be concluded that fruit rigidity showed a positive correlation with all calcium treatments, and this increase was much pronounced in treatment of calcium nitrate 1% as compared with the other treatments. This conclusion was confirmed with the findings of Abd-Rabboh (2012) and Nagy (2018).

4. Fruit shelf life (days):

Fruits from each treatment were stored in ambient conditions at 22±3°C with 60 – 70 % RH after each period of cold storage (15, 30 and 45 days) to determine shelf-life. The results in Table 4 indicated that, all calcium treatments were extended the shelf-life of Balady mandarin fruit compared to control. Moreover, fruit dipped in 1% calcium nitrate had longest shelf-life compared to other treatments. Therefore, the availability of marketing period of the mandarin fruit can be extended by postharvest dip in one of calcium sources especially calcium nitrate 1%, it seemed to be the best treatment for gave longing shelf-life of the fruits. These results are in agreement with those found by Kaur and Kumar (2014) they showed that, calcium nitrate and calcium chloride as postharvest applications increment the shelf-life of Kinnow mandarin under storage conditions. Also, Joshi *et al.,* (2020) revealed that mandarin fruit soaked in $CaCl_2$ at 1% showed longer life than those recorded in fruit dipped in distilled water. The beneficial effects of various calcium treatments on extending the shelf-life can be attributed to the vital role of calcium in decreasing weight loss, respiration rate, decay and delayed ripening, which was reflected on the extension of shelf-life of fruits (Tokala and Mahajan 2005 & Azam *et al.,* 2021).

Table 4: The effect of postharvest treatments with different calcium sources on shelf life (days) of Balady mandarin fruits after cold storage in 2020 and 2021.

Treatments	2020 season			2021 season		
	Storage period in days at 4 ± 1°C with 85 – 90 % RH					
	15	30	45	15	30	45
Control	22.16	19.66	15.83	24.71	22.65	16.78
Calcium acetate 1%	30.33	23.33	17.16	32.25	25.44	19.36
Calcium citrate 0.5%	29.83	27.33	17.33	32.42	28.14	19.42
Calcium nitrate 1%	33.16	26.83	24.00	35.33	28.28	25.32
Calcium chloride 2%	30.33	23.83	17.33	32.42	26.65	20.88
L.S.D. ($p \leq 5\%$)	1.85	1.19	1.43	1.56	1.31	1.77

5. SSC%, acidity (%), SSC/acid ratio and vitamin C:

Tables 5, 6, 7 and 8 showed the changes in soluble solids content (SSC %), titratable acidity (%), SSC/acid ratio and vitamin C of Balady mandarin fruits dipped in solutions of $Ca(C_2H_3O_2)_2$ 1%, $Ca_3(C_6H_5O_7)_2$ 0.5%, $Ca(NO_3)_2$ 1% and $CaCl_2$ 2% in addition to control (distilled water) during storage at 4±1°C with 85 – 90 % RH for 45 days. With respect to the influence of different calcium sources on SSC %, the data in Table 5 showed that, the SSC % in Balady mandarin fruit was significantly increased after 15 and 30 days during the cold storage, but decreased at the final of the storage caused of treated by various Ca salts. In addition, soluble solids content measured in fruit dipped in distilled water (control) was significantly higher than those measured in fruit soaked in various Ca treatments. The highest SSC was found in fruit soaked in distilled water (control) and the lowest was determined in fruit dipped in 1% calcium nitrate and 2% calcium chloride treatments. These findings are supported by Srinu *et al.,* (2017) who reported that 3% calcium chloride preserves TSS in comparison to control by slowing the ripening process of papaya fruits. The favorable impact of calcium salts treatments on SSC can be attributed to the go late in metabolic activity and low in respiration rate as well as its role in a vital process, this reflected on reduce degeneration of fruit and senescence which leads to decreasing the forfeit of SSC in stored room (Moradinezhad *et al.,* 2019). Also, Abrol *et al.,* (2017) show that, Ca slows the respiration, metabolism and hydrolysis of polysaccharides to monosaccharides, delaying ripening and reducing TSS of fruit during storage.

As for titratable acidity, the results in table 6 revealed that, with increasing storage period titratable acidity significantly decreased in both year of study. However, fruits dipped in postharvest treatments of calcium salts had higher values of titratable acidity compared with fruit immersion in distilled water (control). In addition, postharvest application of calcium citrate 0.5% and calcium chloride 2% leads to slightly increased in titratable acidity as comparison with the other calcium salts. These findings are supported by Ranjbar *et al.,* (2018) and Moradinezhad *et al.,* (2019). Postharvest calcium treatment maintained high level of titratable acidity during cold storage may attributed to reduce fruit softening, and their effects on the glycolytic enzyme system which reflected on the preservation of acids (Abrol *et al.,* 2017 and Acevedo-Barrera *et al.,* 2019). Also, calcium reducing respiratory rate, thereby reducing the rate of changes in acidity (Tokala and Mahajan 2018 & Azam *et al.,* 2021). Similarly, Abdel-Salam *et al.,* (2016) on grapefruit, Rokaya *et al.,* (2016) on mandarin, and Joshi et al., (2020) on mandarin, they stated that the postharvest application of $CaCl_2$ led to kept acidity during storage. Besides, titratable acidity of sweet orange decreased by postharvest Ca treatments (Riaz *et al.,* 2020).

Table 5: The effect of postharvest treatments with different calcium sources on SSC % of Balady mandarin fruits during cold storage in 2020 and 2021.

Treatments	2020 season				2021 season			
	Storage period in days at 4 ± 1°C with 85 – 90 % RH							
	0	15	30	45	0	15	30	45
Control	10.40	11.45	11.60	11.35	10.80	11.95	12.15	11.95
Calcium acetate 1%	10.40	10.75	11.35	10.07	10.80	10.75	10.95	10.57
Calcium citrate 0.5%	10.40	11.10	11.15	10.85	10.80	10.65	11.00	10.15
Calcium nitrate 1%	10.40	10.35	10.90	10.95	10.80	10.65	10.70	10.30
Calcium chloride 2%	10.40	10.50	11.20	10.57	10.80	10.80	11.05	10.32
L.S.D. ($p \leq 5\%$)	Ns	0.57	0.34	0.47	Ns	0.67	0.60	0.38

Ns = Not significant

Table 6: The effect of postharvest treatments with different calcium sources on acidity % of Balady mandarin fruits during cold storage in 2020 and 2021.

Treatments	2020 season				2021 season			
	Storage period in days at 4 ± 1°C with 85 – 90 % RH							
	0	15	30	45	0	15	30	45
Control	0.86	0.84	0.77	0.74	0.88	0.77	0.73	0.69
Calcium acetate 1%	0.86	0.90	0.81	0.75	0.88	0.90	0.86	0.86
Calcium citrate 0.5%	0.86	1.06	0.91	0.75	0.88	0.99	0.86	0.84
Calcium nitrate 1%	0.86	1.02	0.86	0.75	0.88	1.03	0.87	0.71
Calcium chloride 2%	0.83	0.93	0.90	0.77	0.88	0.98	0.91	0.84
L.S.D. ($p \leq 5\%$)	Ns	0.18	Ns	Ns	Ns	0.14	0.10	0.10

Ns = Not significant

As for the influence of calcium sources after harvest on SSC/acid ratio of Balady mandarin fruit at the time of storage, it is clear from data in Table 7 that as the storage period increased, the SSC/acid ratio was significantly increased. Moreover, fruits dips in different calcium sources had lower SSC/acid ratio than untreated fruit under cold storage conditions. Since fruits soaked in Ca-citrate had the lowest SSC/acid ratio values as comparison with the other calcium forms during cold storage. Although, postharvest calcium salts application had a positive effect on SSC/acid ratio values than control, it is no regular trend for behavior of SSC/acid ratio values with calcium treatments in the two years of study. This result was confirmed with Rabiei *et al.,* (2011) who observed that, apple fruit treated with Ca after harvest had significantly lower TSS/acid ratio than that on control fruits. Also, El-Dengawy *et al.,* (2019) cleared that calcium chloride at 2% reduced TSS/acidity ratio of peach fruits during cold storage.

With regard to vitamin C, the data in Table 8 cleared that, using all calcium sources as postharvest solution dips for Balady mandarin fruits maintained the higher vitamin C content at time of storage than fruit dipped in control treatment. This result is in harmony with those of Zakaria *et al.,* (2018) who found postharvest application of Ca-nanoparticles, bulk calcium and EDTA calcium were the best for keeping ascorbic acid content of strawberry fruit than those in control treatment under cold storage conditions. Balady mandarin fruit content of ascorbic acid decreased gradually with the progress of storage period. Moreover, fruits dipped in calcium nitrate 1% and calcium chloride 2% contained higher values of vitamin C than that immersed in the other calcium treatments during storage period. On the other hand, the lowest values of vitamin C were found in fruit soaked in distilled water (control) storage duration. So, it can be concluded that, using calcium salts as postharvest treatment are functional in preventing vitamin C content loss under storage conditions; this result was much pronounced in fruit dipped in calcium nitrate. Similar results have been obtained by Kaur and Kumar (2014) who reported that Kinnow mandarin fruit dipped in 0.5% calcium nitrate had the maximum content of ascorbic acid as compared with those dipped in 2% calcium chloride. The role of calcium for maintained vitamin C content during storage can be attributed to its effect in reducing fruit decay, dehydration and delay the decline in fruit quality. This explanation was confirmed with obtained by Azam *et al.,* (2021) who concluded that calcium treatments slowdown the oxidation reactions which reflected in reduce fruit degradation and senescence. Also, calcium application might have reduced oxidizing enzyme activities which resulting in higher ascorbic content during storage (Lo'ay and Dawood 2019).

Table 7: The effect of postharvest treatments with different calcium sources on TSS/acid ratio of Balady mandarin fruits during cold storage in 2020 and 2021.

Treatments	2020 season				2021 season			
	Storage period in days at 4 ± 1°C with 85 – 90 % RH							
	0	15	30	45	0	15	30	45
Control	12.09	14.10	15.06	15.33	12.27	16.36	15.77	17.31
Calcium acetate 1%	12.09	11.94	14.01	13.42	12.27	11.94	12.73	12.29
Calcium citrate 0.5%	12.09	10.47	12.25	14.46	12.27	10.75	12.79	12.08
Calcium nitrate 1%	12.09	10.14	12.67	14.60	12.27	10.33	12.29	12.71
Calcium chloride 2%	12.09	11.29	12.44	13.72	12.27	11.02	12.14	12.28
L.S.D. ($p \leq 5\%$)	Ns	1.28	2.39	1.21	Ns	1.65	1.53	1.02

Ns = Not significant.

Table 8: The effect of postharvest treatments with different calcium sources on vitamin C mg/100 ml juice of Balady mandarin fruits during cold storage in 2020 and 2021.

Treatments	2020 season				2021 season			
	Storage period in days at 4 ± 1°C with 85 – 90 % RH							
	0	15	30	45	0	15	30	45
Control	36.72	34.50	32.64	29.80	37.24	34.00	31.50	26.86
Calcium acetate 1%	36.72	35.00	33.24	30.10	37.24	34.30	32.30	28.60
Calcium citrate 0.5%	36.72	35.38	33.52	31.09	37.24	34.21	31.28	28.91
Calcium nitrate 1%	36.72	36.41	35.24	32.76	37.24	36.16	33.57	30.21
Calcium chloride 2%	36.72	35.60	33.70	32.03	37.24	35.00	31.77	29.17
L.S.D. ($p \leq 5\%$)	Ns	0.35	0.07	0.36	Ns	0.14	0.24	0.37

Ns = Not significant

6. Cell wall enzyme activities:

6.1. Polygalacturonase (PG)

Results in Figure 1 indicated that, the activity of polygalacturonase enzyme was significantly increased with the prolonging storage period. In addition, postharvest treatments of calcium sources significantly reduced the activity of polygalacturonase enzyme compared to control treatment. These results agree with those of Ranjbar *et al.*, (2018) they concluded that apple fruits dipped in nano-calcium and $CaCl_2$ had lower activity of polygalacturonase than control treated fruits. Moreover, polygalacturonase enzyme activity was the lowest in fruit dipped in 1% calcium nitrate followed by those dipped in 2% $CaCl_2$, whereas the highest activity of polygalacturonase enzyme in fruit soaked in distilled water. similarly, Chuni *et al.*, (2010) reported that the activity of polygalacturonase enzyme in dragon fruits dipped $CaCl_2$ was significantly reduced and the rate of reduction in polygalacturonase activity was increased with increasing $CaCl_2$ concentration from 0.0, 2.5 to 7.5g/l and dipping duration. Also, Ahlawat *et al.*, (2018) revealed that, dipping Kinnow mandarin fruits in calcium lactate at 3% was the most effective to minimize the activity of polygalacturonase enzyme and maintained fruit firmness during storage.

6.2. Pectin methylesterase (PME)

Pectin methylesterase activity in Balady mandarin fruits tended to increase with storage duration regardless the used applications. Also, Figure 2 show the fruits immersed in all calcium salts had lower values of pectin methylesterase enzyme activity than those soaked in distill water, which gave the high pectin methylesterase enzyme activity during storage period. Moreover, calcium nitrate 1% and calcium chloride 2% led to reduce the activity of pectin methylesterase enzyme compared to calcium acetate 1% and calcium citrate 0.5%. These results agree with those of Saba *et al.*, (2016) who show pectin methylesterase activity in peach fruits dipped in 1% of calcium chloride and propionate 1% during cold storage was decreased compared to untreated ones. Also, Jain *et al.*, (2019) showed that Bar fruits dipped in calcium chloride at 1 and 2% resulted in significantly lowering of activities of cellulase, polygalacturonase and pectin methylesterase enzymes during cold storage. In addition, calcium treatments delayed cell wall hydrolysis, which reflected to reduce softening, maintained firmness and extended the shelf life of the fruits (Kittemann *et al.*, 2010 and Ahlawat *et al.*, 2018).

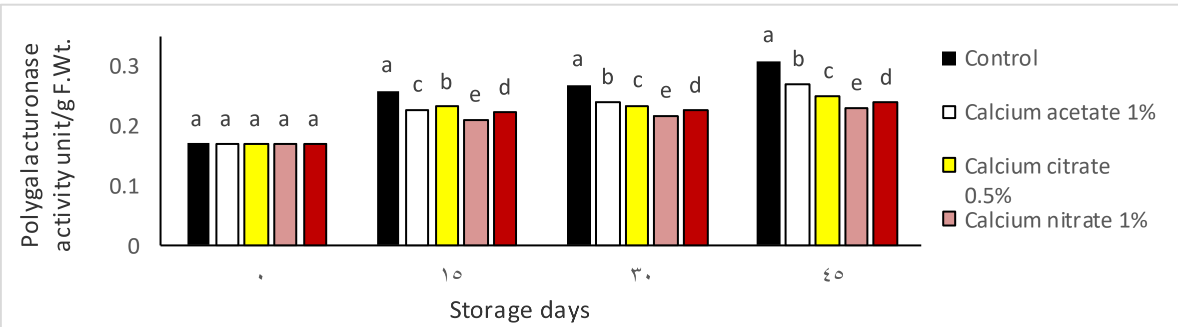

Figure 1: The effect of postharvest treatments with different calcium sources on polygalacturonase activity (unit/g F.Wt.) of Balady mandarin fruits during cold storage period

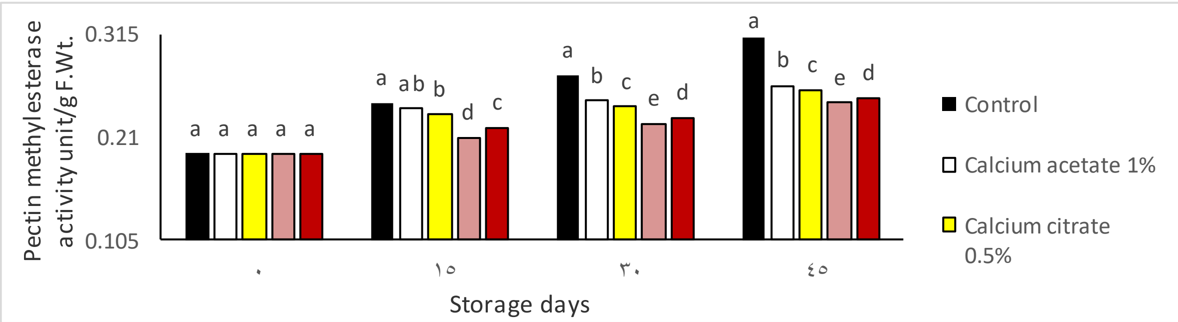

Figure 2: The effect of postharvest treatments with different calcium sources on pectin methylesterase activity (unit/g F.Wt.) of Balady mandarin fruits during cold storage period

6.3. β-galactosidase (β-Gal)

The results illustrated in Figure 3 showed that β-galactosidase activity increased with the increase in period of cold storage. Balady mandarin fruits dipped in $Ca(C_2H_3O_2)_2$ 1%, $Ca_3(C_6H_5O_7)_2$ 0.5%, Ca $(NO_3)_2$ 1% and $CaCl_2$ 2% showed a decrease in β-galactosidase activity as compared with fruits dipped in distill water (control) during storage period. Moreover, mandarin fruits dipped in calcium nitrate 1% and calcium chloride 2% have been lower β-galactosidase activity than fruit immersed in Ca acetate 1% and calcium citrate 0.5 during cold storage. The result was in harmony with Manganaris *et al.*, (2005) and Hussain *et al.*, (2012). Also, Ranjbar *et al.*, (2018) reveal postharvest calcium treatments tended to decrease the activity of β-galactosidase, and nano-calcium showed to be superior compared to calcium chloride during cold storage.

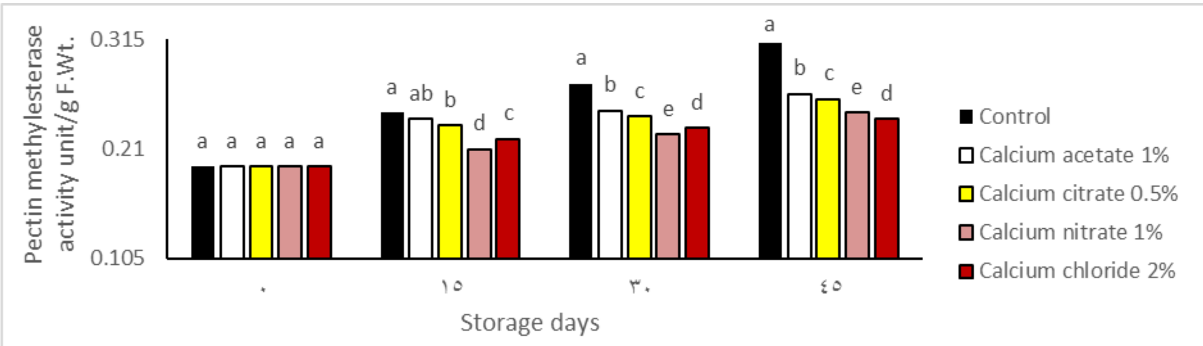

Figure 2: The effect of postharvest treatments with different calcium sources on pectin methylesterase activity (unit/g F.Wt.) of Balady mandarin fruits during cold storage period

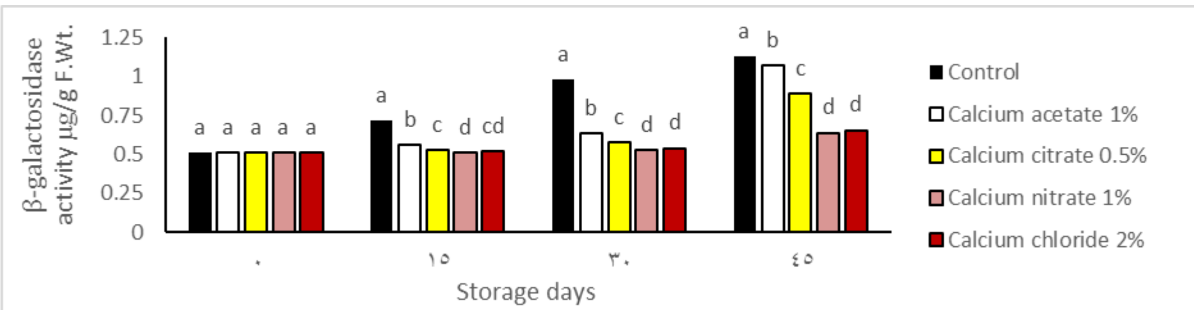

Figure 3: The effect of postharvest treatments with different calcium sources on β-galactosidase activity (µg/g F.Wt.) of Balady mandarin fruits during cold storage period

CONCLUSION

The results of this study revealed that, activities of polygalacturonase, pectin methylesterase, β-galactosidase enzymes, weight loss and decay in Balady mandarin fruits were significantly reduced by using postharvest application of calcium sources. Also, fruit immersing in different calcium sources led to increasing firmness, acidity, vitamin C, SSC and SSC/acid ratio under cold storage condition. Moreover, calcium nitrate 1% or calcium chloride 2% were the best application for reducing weight loss, decay and activities of polygalacturonase, pectin methylesterase, β-galactosidase enzymes and keeping quality of Balady mandarin fruits during cold storage period for 45 days comparing with the other calcium salts.

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الملخص العربي

تطبيق إستخدام بعض صور الكالسيوم بعد الجمع لتحسين جودة ومدة صلاحية ثمار اليوسفى أثناء التخزين

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أجريت الدراسة الحالية خلال موسمي 2020 و2021 وذلك لدراسة تأثير أملاح الكالسيوم على تقليل التدهور والحفاظ على جودة واطالة عمر ثمار اليوسفي البلدي. تم جمع ثمار اليوسفي عند اكتمال التلوين (أصفرأو أصفر مخضر)، نقلت الثمار فى الحال الى معمل محطة بحوث البساتين بسخا وتم تجهزها للغمس لمدة خمس دقائق في أحد المحاليل المائية التالية: أسيتات الكالسيوم 1٪، سترات الكالسيوم 0.5٪، نترات الكالسيوم 1٪، كلوريد الكالسيوم 2٪ وكذلك الماء المقطر (الكنترول). تم تخزين الثمار فى درجة حرارة 4 ± 1 مئوية ورطوبة نسبية 85–90٪ لمدة 45 يوم. تم تسجيل الصفات الطبيعية و الكيميائية للثمار بعد 0 و15 و30 و45 يومًا خلال فترة التخزين.

أظهرت النتائج أن فقدان الوزن وتدهور ثمار اليوسفى البلدى إنخفضت بشكل كبير عن طريق إستخدام أملاح الكالسيوم. أيضا نقع الثمار فى أحد أملاح الكالسيوم بعد الجمع أدى إلى زيادة صلابة الثمار وزيادة متحواها من الحموضة، حمض الأسكوربيك، المواد الصلبة القابلة للذوبان ونسبة المواد الصلبة القابلة للذوبان/ الحمض خلال فترة التخزين. علاوة على ذلك، لوحظ أثناء التخزين إنخفاض نشاط إنزيمات polygalacturonase و pectin methylesterase و β-galactosidase في الثمار التي غمست في محاليل الكالسيوم و ذلك عند المقارنة بالكنترول. بالإضافة إلى ذلك، وجد أن إستخدام نترات الكالسيوم 1٪ وكلوريد الكالسيوم 2٪ أكثر فاعلية في تقليل فقدان الوزن والتدهور والحفاظ على الجودة الكلية في ثمار اليوسفي البلدي.

وعليه، يمكن الاستنتاج أن المعاملة بعد الحصاد لثماراليوسفي بمحاليل نترات الكالسيوم 1٪ أو كلوريد الكالسيوم 2٪ ادت الى تحسين الجودة وإطالة عمر ثمار اليوسفي البلدي.