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REDUCTION IN FRUIT ROT AND ENHANCEMENT IN FRUIT QUALITY OF KINNOW MANDARIN BY CALCIUM CHLORIDE APPLICATION

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This experiment was started at the Institute of Horticultural Sciences, University of Agriculture, Faisalabad during 2014-15 to investigate the response of calcium chloride to reduce rotting and to maintain the quality related parameters during the cold storage of Kinnow mandarin fruit. The experiment was laid out in completely randomized design (CRD) with factorial arrangement. Fruits were harvested from Sq. No. 9 and brought into the Lab., UAF. Fruits were dipped 10 mints in 0, 2, 3 and 4% $CaCl_2$ solution and stored at 5°C at 85 to 90% relative humidity for 90 days. The data was recorded every 15 days' interval during the storage. It was observed after 90 days storage that $CaCl_2$ @ 4% reduced fruit rot percentage from 11.68 to 1.33% and all treated fruit maintained their firmness better than untreated. Lower loss in fruit weight (7.39%) was also found in fruits of same treatment as compared to untreated fruit where it was 14.59% after 90 days' storage. Maximum TSS (9.41°brix), ascorbic acid contents (50.95mg$100g^{-1}$), total sugar contents (14.90%), reducing sugars (8.32%) were also found from the juice of fruit those were treated with 4% $CaCl_2$. However, TPC (218.44mg GAE $100g^{-1}$), TAC (64.29% inhibition), CAT (25.66mg/protein), POD (0.55U mg/ protein) and SOD (128.69U mg/ protein) were more in 3% $CaCl_2$ after 90 days' storage as compared to the fruits those were treated with 4% $CaCl_2$ where these were (217.89mg GAE $100g^{-1}$), (61.83% inhibition), (24.17U mg^{-1} protein), (0.53U mg^{-1} protein), (126.13U mg^{-1} protein). It was observed that per-storage treatment of 4% $CaCl_2$ for Kinnow mandarin is most effective but it reduced the contents of TPC, TAC, CAT, POD and SOD. It is concluded that 3-4% calcium chloride reduced the rotting percentage and maintained the quality of Kinnow mandarin for 90 days cold storage.

Keywords: Kinnow, antioxidants, calcium chloride, enzymatic activity, phenolics, fruit rot.

INTRODUCTION

Kinnow is our most important exportable product in all citrus fruits and hence give good return in terms of foreign exchange (Jaskani *et al*., 2005). Kinnow is stored at 4-5°C and 85-90% RH for approximately 8-12 weeks without chilling damage. Kinnow mandarin is a delicate in nature because 20-30% postharvest losses happened during the storage due to bacterial and fungal contamination on the fruit, mismanagement of diseases, low quality fruit, inappropriate weather condition, delay in harvesting, lack of proper roads and improper cold storage facilities, surplus supply in the market (Singh *et al*., 2004). While exporting high quality citrus fruits, some postharvest applications need to meet the requirements of the importing countries to allow fresh citrus from competing regions to undergo cold treatment. Cold treatment involves storing fruits at low temperatures for a period of time to ensure they are free from insects, usually fruit flies. Prolonged cooling will increase the peel problem of sensitive citrus varieties; however, mandarins, lemons, oranges, tangelos and Ellendale mandarins are all effectively treated with the lowest quality (Anonymous, 2001).

Hossain *et al*. (2005), Abdi *et al*. (2006), Misra and Gupta (2006), Singh *et al*. (2006), Hosseini and Thengane (2007) and Naeem *et al*. (2009) observed that calcium chloride maintains the cell firmness and reduce the rotting of fruit crops during storage. The role of calcium in stabilizing cell membrane and slowing senescence of horticultural and agronomic crops were widely recognized. The use of pre- and postharvest calcium application may additionally slowdown senescence in fruits without harmful impact on consumer attractiveness (Lester and Grusak, 1999). Luna-Guzman and Barrett (2000) reported that dipping treatment with calcium may enhance calcium contents notable in comparison to foliar application and does not cause fruit damage, relying on type of salt and calcium concentration. Picchioni *et al*. (1998) reported that postharvest calcium treatment keeps tissue firmness, cell expansion and membrane integrity. Exogenously applied calcium stabilizes plant cell walls and prevents cell walls from deteriorating enzymes (White and Broadley, 2003). Therefore, the aim of this study was to reduce the fruit decay and extend storage life of 'Kinnow' mandarin.

MATERIALS AND METHOD

The study was initiated in 2014-2015. Six fruit per sample was considered as experimental units. The fruits were randomly divided into four treatments, each treatment contain 144 fruits with four replicates was dipped in (2, 3 and 4%) calcium chloride solution. Fruits were replaced from the solution and shade dried. After 23-24 hours the fruits are placed in cold storage with maintained temperature 5°C with 85-90% RH for 90 days. Sampling (6 fruits from each replicate) was carried out at 6 times every 15 days. After 15, 30, 45, 60, 75 and 90 days of storage, fruit rot, weight loss, total soluble solids, titratable acidity, ascorbic acid contents, total phenolic contents, total antioxidants and enzyme activity (catalase, peroxidase and superoxide dismutase) were measured for fruit quality assessment.

***Weight loss (WL) and fruit rot percentage*:** The WL of Kinnow fruit samples were measured before and after every 15 days interval till the end of storage. The difference between initial weight and final weight of the fruits divided by their initial fruit weight. Fruit rot percentage was measured by their visual examination and calculated as the number of spoiled fruits divided by the total fruits multiplies by 100.

***Titratable acidity (TA) and total soluble solid (TSS)*:** The titratable acidity (TA) of the juice was calculated by the method given by Hortwitz (1960). TA was calculated by titrating 5-ml of juice with 0.1 N sodium hydroxide using phenolphthalein as an indicator. The TSS content of fruit was calculated by using a refractrometer (RX 5000, Atago, Japan).

***Ascorbic acid (mg 100 g^{-1})*:** The ascorbic acid content of the juice was determined as described by AOAC (1990). 10 ml juice was taken in a 100 ml volumetric flask and volume was prepared by adding 0.4% oxalic acid solution. Apart from this, 5 ml aliquot of filtration was taken and titrated against 2, 6- dicholorophenolindophenol dye, to the pale pink end point (lasting at least 15 seconds). Calculated ascorbic acid by using the following formula

$$\text{Ascorbic acid (mg100 ml}^{-1}) = \frac{1\times R1\times V\times 100}{R\times W\times V1}$$

***Sugar contents*:** Sugars in juices were estimated as estimated by Hortwitz (1960). In a 250 ml volumetric flask, 10 ml juice was taken and 100 ml of distilled water, 25 ml of lead acetate solution (25%) and 10 ml of potassium oxalate (20%) were added. Then make the volume with distilled water and filter. The filtrate was used to estimate the different forms of sugar and the values were estimated by using the formula.

Total sugars (%) =25×(X/Z)

Reducing sugars % = 6.25 × (X/Y)

Non-reducing sugars (%) = 0.95× (total sugars% - reducing sugars %)

***Total phenolics and total antioxidants*:** Total Phenolics (mg GAE $100g^{-1}$) the results were recorded using spectrophotometer at wavelength of 765 nm and 517 nm (Ainsworth and Gillespie, 2007).

***Enzymes assay*:** Frozen juice was used to estimate the enzymatic activities of POD, CAT and SOD after homogenization using phosphate buffer. The enzyme extracts were prepared and readings were recorded at spectrophotometer at specific wavelengths. The enzyme activity was calculated in Unit (mg^{-1} protein) (Liu *et al*., 2011).

***Statistical analysis*:** The study was laid out according to Completely Randomized Design (CRD) with factorial arrangements. The data were studied using Analysis of Variance technique using statistics 8.1 and least significant difference (LSD) was used to compare the treatment means (Steel *et al*., 1997).

RESULTS

***Fruit rot (%)*:** Maximum fruit rot (11.68%) was observed in control fruit whereas minimum fruit rot (1.33%) was record in fruits treated with 4% $CaCl_2$ followed by the fruits of other treatments 2% (2.44%) and 3% $CaCl_2$ (2.07%) respectively after 90 days storage. It was remarkable to note that fruits treated with 4% CaCl2 exhibited lower fruit rot (3.32%) during 90 days while the untreated fruits had high fruit rot (21.64%) during the period (Table 1). Treatments and storage interaction showed that fruit rot (%) increased with increased storage duration.

***Weight Loss (%)*:** Mean values of treatments showed that high weight loss (14.59%) was noted in untreated fruits while lower weight loss (7.39%) were observed in fruits

Table 1. Effects of $CaCl_2$ concentration on fruit rot (%) of Kinnow mandarin during storage.

Treatments	0 DAS	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	Mean
Control	0.00 r	7.315 f	9.050 e	12.025 d	14.660 c	17.110 b	21.64 a	11.68 A
2%	0.00 r	0.661 p	1.315 o	2.317 m	3.107 k	4.085 i	5.625 g	2.44 B
3%	0.00 r	0.331 q	1.302 o	2.317 m	2.640 l	3.287 j	4.642 h	2.07 C
4%	0.00 r	0.000 r	0.332 q	1.317 o	2.045 n	2.342 m	3.315 j	1.33 D
Mean	0.00 G	2.076 F	2.99 E	4.49 D	5.61 C	6.71 B	8.80 A	

Note: LSD (*P*= 0.05); treatments = 0.50; LSD storage = 0.66; LSD interaction = 1.01; DAS= Days After Storage

those were treated with 4% $CaCl_2$ followed by treatments 2% (8.17%) and 3% (8.12%) $CaCl_2$ respectively. Maximum weight loss (17.60%) was observed in fruits after 90 days. Minimum weight loss (3.98%) was observed in fruits when analyzed after 15days storage (Table 2). Interaction showed that directly proportion relationship between storage period and weight loss. As storage period increased then weight loss also increased. The similar trend was found in all other treatments but maximum weight loss was found in untreated fruits due to higher metabolic activities. Minimum in treated fruit during the storage which showed the superiority of $CaCl_2$ treatments over non-treated fruit.

Total soluble solids (°Brix): Means of treatment showed that maximum TSS (10.59 °Brix) was observed in control fruits while lower TSS (9.41°Brix) was found in fruits those were treated with 4% $CaCl_2$. The other treatments also showed intermediate TSS when fruits were treated with 2% (9.77 °Brix) and 3% (9.60 °Brix) $CaCl_2$ respectively. As well as the storage period was considered, as it was expected TSS was increased with the increased storage period. Maximum TSS (11.36 °Brix) was observed after 90 days. Minimum TSS (8.95 °Brix) was observed after 15 days' storage in all treatments (Table 3). The similar trend was found in all the treatments but maximum TSS was found in control fruit and minimum in treated fruit during the storage which observed the superiority of $CaCl_2$ treated fruits over control.

***Titratable acidity (%)*:** Means of treatment showed that higher TA (1.66%) was observed in fruits those were treated with 4% $CaCl_2$ whereas lower TA (1.45%) was found in untreated fruits followed by other treatments of $CaCl_2$ 2% (1.63%) and 3% (1.64%), respectively. TA was decreased with the increased storage period. Minimum TA (1.36%) was observed during long term storage. Maximum TA (1.70%) was observed after 15 days storage in all treatments (Table 4). Interaction showed that higher doses of $CaCl_2$ showed maximum TA in initial storage analysis and decrease with the delay storage. The similar trend was found in all the treatments. All levels of $CaCl_2$ treated fruits performed well than untreated fruits but 4% $CaCl_2$ performed better to keep the maximum TA for 90 days storage.

***TSS/ACID ratio*:** the results of TSS: TA ratio revealed statistically significant ($P \leq 0.05$) differences among treatments, storage interval and interaction. Means of treatments showed that maximum TSS: TA ratio (7.84) was noted in untreated fruits while minimum TSS: TA (5.65) was noted in fruits that were treated with 4% $CaCl_2$. The other $CaCl_2$ treatments also revealed in-between TSS: TA ratio when fruits were treated with 2% (6.02) and 3% (5.87) respectively (Table 5). As well as the storage period was increased TSS: TA ratio was increased. Interaction indicated that untreated fruits had a rapid increase in TSS: TA ratio

Table 2. Effects of $CaCl_2$ concentration on weight loss (%) of Kinnow mandarin during storage.

Treatments	0 DAS	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	Mean
Control	0.00 y	7.31 q	11.11 l	14.72 f	19.61 c	23.61b	25.79 a	14.59 A
2%	0.00 y	2.51 w	6.29 s	8.95 o	10.31 m	13.81 h	15.32 d	8.17 B
3%	0.00 y	3.90 v	5.95 t	7.88 p	11.59 k	13.32 j	14.18 g	8.12 C
4%	0.00 y	2.20 x	4.11 u	6.81 r	9.91 n	13.62 i	15.10 e	7.39 D
Mean	0.00 G	3.98 F	6.86 E	9.59 D	12.85 C	16.09 B	17.60 A	

Note: LSD (P= 0.05); treatments = 0.80; LSD storage = 1.07; LSD interaction = 2.08; DAS= Days After Storage

Table 3. Effects of $CaCl_2$ concentration on TSS (°Brix) of Kinnow mandarin during storage.

Treatments	0 DAS	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	Mean
Control	8.81 t	9.012 q	9.415 m	10.322 h	11.117 d	12.305 b	13.165 a	10.59 A
2%	8.81 t	8.862 s	9.217 n	9.715 k	10.192 i	10.507 f	11.165 c	9.77 B
3%	8.81 t	8.937 r	9.145 o	9.505 l	9.812 j	10.317 h	10.705 e	9.60 C
4%	8.81 t	9.020 q	9.087 p	9.212 n	9.508 l	9.818 j	10.407 g	9.41 D
Mean	8.81 G	8.95 F	9.21 E	9.69 D	10.16 C	10.74 B	11.36 A	

Note: LSD (P= 0.05); treatments = 0.19; LSD storage =0.36; LSD interaction = 0.75; DAS= Days After Storage

Table 4. Effects of $CaCl_2$ concentration on TA (%) of Kinnow mandarin during storage.

Treatments	0 DAS	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	Mean
Control	1.71 abc	1.69 cde	1.62 g	1.57 h	1.44 l	1.21 m	0.93 n	1.45 C
2%	1.71 abc	1.73 a	1.72 a	1.68 de	1.60 g	1.53 ij	1.47 k	1.63 B
3%	1.71 abc	1.71 abc	1.70 bcd	1.65 f	1.62 g	1.57 h	1.51 j	1.64 B
4%	1.71 abc	1.74 a	1.72 ab	1.69 cde	1.66 ef	1.62 g	1.56 hi	1.66 A
Mean	1.71 A	1.70 A	1.69 B	1.64 C	1.58 D	1.48 E	1.36 F	

Note: LSD (P= 0.05); treatments = 0.06; LSD storage = 0.02; LSD interaction = 0.33; DAS= Days After Storage

after 45 storage days. The fruit treated with $CaCl_2$ had maximum increase of TSS: TA ratio during 75-90 days.

Sugar contents (%): The sugar content was positively correlated with the storage period. The three CaCl2 doses (2, 3 and 4%) were aligned with each other and the juice sugar content was maintained. Mean of treatments showed that control fruits showed maximum total sugar (17.45%) while the fruits treated with 4% $CaCl_2$ exhibited minimum total sugars (14.90%). As well as the storage period was considered, as it was expected that total sugars was increased with the increased storage period. Maximum total sugars (19.36%) were observed after 90 days of storage and minimum total sugars (14.06%) were found after 15 days storage in all treatments (Table 6). Interaction showed that higher doses of $CaCl_2$ showed minimum total sugars and reducing sugars in initial storage analysis and increase with the prolong storage (Table 7). In contrast of total and reducing sugars, non-reducing sugars were decreased with the advanced storage. After 90 days, mean of treatments showed that maximum non-reducing sugars (6.25%) were recorded in those fruits treated with 4% $CaCl_2$ while minimum non-reducing sugars (6.09%) were recorded in control fruit (Table 8). Interaction between all the treatments and storage period exhibited that small variations were detected during 30 storage days. Though untreated fruits cannot keep the levels more than 45 days during storage then $CaCl_2$ treated fruits had maximum decline after 75 days of cold storage.

***Ascorbic acid contents (mg 100 g^{-1})*:** It was noted that maximum ascorbic acid contents (50.95 mg 100 g^{-1}) were observed in 4% $CaCl_2$ treated fruits whereas minimum ascorbic acid contents (36.98 mg 100 g^{-1}) were observed in untreated fruits. The other treatments also exhibited intermediate ascorbic acid contents when fruits were treated

Table 5. Effects of $CaCl_2$ concentration on TSS:TA ratio of Kinnow mandarin during storage.

Treatments	0 DAS	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	Mean
Control	5.15 mn	5.35 jkl	5.81 i	6.57 f	7.72 c	10.17 b	14.16 a	7.84 A
2%	5.15 mn	5.13 n	5.34 jkl	5.78 i	6.36 g	6.85 e	7.58 c	6.02 B
3%	5.15 mn	5.22 lmn	5.39 jk	5.76 i	6.05 h	6.56 f	7.09 d	5.87 C
4%	5.15 mn	5.19 mn	5.28 klm	5.45 j	5.70 i	6.08 h	6.67 f	5.65 D
Mean	5.15 F	5.21 F	5.45 E	5.89 D	6.45 C	7.41 B	8.88 A	

Note: LSD (*P*= 0.05); treatments = 0.49; LSD storage = 0.55; LSD interaction = 1.34; DAS= Days After Storage

Table 6. Effects of $CaCl_2$ concentration on total sugars (%) of Kinnow mandarin during storage.

Treatments	0 DAS	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	Mean
Control	13.68 x	14.46 r	16.54 i	16.46 j	17.94 d	19.39 c	23.72 a	17.45 A
2%	13.68 x	14.04 u	14.58 q	15.37 o	16.30 k	17.12 f	19.57 b	15.81 B
3%	13.68 x	13.93 v	14.41 s	14.81 p	15.61m	16.65 h	17.39 e	15.21 C
4%	13.68 x	13.84 w	14.14 t	14.44 rs	15.52 n	15.89 l	16.81 g	14.90 D
Mean	13.68 G	14.06 F	14.91 E	15.26 D	16.34 C	17.26 B	19.36 A	

Note: LSD (*P*= 0.05); treatments = 0.31; LSD storage = 0.47; LSD interaction = 1.14; DAS= Days After Storage

Table 7. Effects of $CaCl_2$ concentration on reducing sugars (%) of Kinnow mandarin during storage.

Treatments	0 DAS	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	Mean
Control	6.37 w	7.21 u	9.31 k	10.94 g	11.94 e	14.94 b	16.61 a	11.04 A
2%	6.37 w	7.34 t	8.05 p	9.18 l	10.12 i	11.04 f	14.05 c	9.45 B
3%	6.37 w	6.82 v	7.52 r	8.86 m	9.32 k	9.94 j	12.39 d	8.74 C
4%	6.37 w	7.42 s	7.89 q	8.11 o	8.73 n	9.14 l	10.62 h	8.32 D
Mean	6.37 G	7.19 F	8.19 E	9.27 D	10.02 C	11.26 B	13.41 A	

Note: LSD (*P*= 0.05); treatments = 0.30; LSD storage = 0.41; LSD interaction = 0.85; DAS= Days After Storage

Table 8. Effects of $CaCl_2$ concentration on non-reducing sugars (%) of Kinnow mandarin during storage.

Treatments	0 DAS	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	Mean
Control	6.95 a	6.89 b	6.87 c	5.25 o	5.69 n	4.23 q	6.74 d	6.09 C
2%	6.95 a	6.37 g	6.21 h	5.88 l	5.87 l	5.77 m	5.24 o	6.04 D
3%	6.95 a	6.76 d	6.55 e	5.65 n	5.98 jk	6.37 g	4.75 p	6.13 B
4%	6.95 a	6.10 i	5.93 kl	6.01 j	6.45 f	6.41 fg	5.89 l	6.25 A
Mean	6.95 A	6.53 B	6.39 C	5.69 E	5.99 D	5.69 E	5.65 F	

Note: LSD (*P*= 0.05); treatments = 0.55; LSD storage = 0.70; LSD interaction =1.29; DAS= Days After Storage

with 2% (45.41 mg 100 g^{-1}) and 3% (47.95 mg 100 g^{-1}) $CaCl_2$ respectively. As well as the storage period was considered, as ascorbic acid contents were decreased with the increased storage period. Interaction showed that all $CaCl_2$ treated fruits had increase ascorbic acid contents. Untreated fruits had a small rise during 15 days followed by continuous decrease from 45-90 storage days. Higher ascorbic acid contents (58.32 mg 100 g^{-1}) were observed in 4% $CaCl_2$ during 60 days while untreated fruits showed minimum ascorbic acid contents (35.61 mg 100 g^{-1}) at the similar day during long term cold storage (Table 9). Interaction showed that higher doses of $CaCl_2$ exhibited maximum ascorbic acid contents in initial storage analysis and increase with the prolong storage.

***Total phenolic contents (mg GAE 100 g^{-1})*:** The fruits treated with 3% $CaCl_2$ showed maximum TPC (218.44 mg GAE 100g^{-1}) followed by the fruits of 2% (215.22 mg GAE 100g^{-1}) and 4% (217.89 mg GAE 100g^{-1}) $CaCl_2$ while control fruit showed minimum TPC (197.95 mg GAE 100g^{-1}) respectively. In addition to the storage period was measured, as it was expected TPC was decreased with the increased storage period. Interaction showed that TPC increase in untreated fruits during 15-30 storage days followed by decrease up to long term cold storage. The treated fruit with 3% $CaCl_2$ exhibited maximum TPC (227.59 mg GAE 100g^{-1}) during 60 days of cold storage while minimum TPC (193.73 mg GAE 100g^{-1}) were observed in control fruits at the similar day (Table 10). Results concluded that 3% $CaCl_2$ dose was effective to increase in TPC during long term storage.

***Total antioxidants (% inhibition)*:** Higher antioxidant activity (64.29% inhibition) was noted in 3% $CaCl_2$ whereas lower antioxidant activity (51.25% inhibition) was found in control fruits after 90 days storage. Moreover, antioxidant activity decreased with the increased storage duration. Like ascorbic acid and total phenolic contents, antioxidant activity of fruit was also enhanced in all the treatments from 0-60 days but reduced slowly up to 90 days (Table 11). $CaCl_2$ @ 3% also helps to maintain antioxidant activities for longest time.

***Catalase (U mg^{-1} protein)*:** CAT activity was decreased with the increased storage duration. Maximum CAT activity (25.66 U mg^{-1} protein) was observed in those fruits treated with 3% $CaCl_2$ followed by the fruits of 2% (24.75 U mg^{-1} protein), 4% (24.17 U mg^{-1} protein) $CaCl_2$ and control (15.49 U mg^{-1} protein), respectively, after 90 days storage (Table 12). Interaction showed that inverse relationship was found between CAT activity and storage period. Control treatments had showed small increase during early 15 days followed by a decrease rate in CAT activity during 75-90

Table 9. Effects of $CaCl_2$ concentration on ascorbic acid contents (mg 100 g^{-1}) of Kinnow mandarin during storage.

Treatments	0 DAS	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	Mean
Control	41.19 t	42.92 r	41.71 s	37.72 v	35.61 w	31.82 x	27.91 y	36.98 A
2%	41.19 t	43.84 p	48.16 l	50.12 g	48.83 j	45.42 o	40.31 u	45.41 B
3%	41.19 t	46.44 n	49.40 h	52.29 e	53.81 c	49.29 i	43.21 q	47.95 C
4%	41.19 t	48.76 k	51.06 f	56.55 b	58.32 a	53.71 d	47.12 m	50.95 D
Mean	41.19 F	45.49 D	47.58 C	49.17 A	49.15 B	45.06 E	30.64 G	

Note: LSD (*P*= 0.05); treatments = 0.46; LSD storage = 0.59; LSD interaction = 0.92; DAS= Days After Storage

Table 10. Effects of $CaCl_2$ concentration on total phenolic contents (mg GAE 100^{-1}) of Kinnow mandarin during storage.

Treatments	0 DAS	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	Mean
Control	208.10 qr	210.32 o	212.62 n	206.92 t	193.73	181.68 v	172.30 w	197.95 D
2%	208.10 qr	214.30 m	218.23 i	224.03 b	218.09	214.29 m	209.51 p	215.22 C
3%	208.10 qr	217.69 k	220.28 g	222.06 d	227.59	218.04 j	215.31 l	218.44 A
4%	208.10 qr	219.91 h	221.21 e	224.03 b	223.39	220.92 f	207.69 s	217.89 B
Mean	208.10 F	215.56 D	218.08 B	219.26 A	215.70 C	208.73 E	201.20 G	

Note: LSD (*P*= 0.05); treatments = 1.54; LSD storage = 1.62; LSD interaction = 2.98; AS= Days After Storage

Table 11. Effects of $CaCl_2$ concentration on total antioxidants (% inhibition) of Kinnow mandarin during storage.

Treatments	0 DAS	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	Mean
Control	60.04 j	63.55 g	57.46 m	52.50 o	45.25 p	42.37 q	37.62 r	51.25 D
2%	60.04 j	62.43 h	63.25 g	65.46 e	65.47 e	60.25 j	54.46 n	61.63 C
3%	60.04 j	66.45 d	70.54 a	69.49 b	64.53 f	60.73 i	58.26 l	64.29 A
4%	60.04 j	63.46 g	68.45 c	66.41 d	62.63 h	59.26 k	52.48 o	61.83 B
Mean	60.04 D	63.97 B	64.93 A	63.46 C	59.47 E	55.65 F	50.70 G	

Note: LSD (*P*= 0.05); treatments 1.73; LSD storage = 1.87; LSD interaction =3.34; DAS= Days After Storage

Table 12. Effects of $CaCl_2$ concentration on catalase of Kinnow mandarin (U mg^{-1} protein) during storage.

Treatments	0 DAS	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	Mean
Control	22.40 o	19.94 r	17.16 t	14.69 u	13.51 v	11.05 w	9.71 x	15.49 D
2%	22.40 o	23.58 l	26.44 f	27.42 e	29.50 b	23.73 k	20.21 q	24.75 B
3%	22.40 o	24.76 j	25.93 h	28.19 d	29.78 a	26.32 g	22.21 p	25.66 A
4%	22.40 o	23.32 m	25.62 i	29.39 c	25.91 h	23.24 n	19.34 s	24.17 C
Mean	22.40 E	22.89 D	23.77 C	24.92 A	24.67 B	21.08 F	17.86 G	

Note: LSD (P = 0.05); treatments = 0.75; LSD storage = 0.88; LSD interaction = 1.05; DAS= Days after Storage

Table 13. Effects of $CaCl_2$ concentration on peroxidase (U mg^{-1} protein) of Kinnow mandarin during storage.

Treatments	0 DAS	15 DAS	30 DAS	45DAS	60DAS	75DAS	90DAS	Mean
Control	0.531 g-k	0.541 e-h	0.570 b	0.511 l	0.511 l	0.491 m	0.471 n	0.51 C
2%	0.531 g-k	0.525 k	0.548 def	0.571 b	0.559 cd	0.530 h-k	0.505 l	0.53 B
3%	0.531 g-k	0.542 efg	0.561 bc	0.591 a	0.561 bc	0.551 cde	0.539 f-i	0.55 A
4%	0.531 g-k	0.539 f-i	0.539 f-i	0.55 def	0.531 h-k	0.509 l	0.515 l	0.53 B
Mean	0.53 C	0.54 B	0.55 A	0.55 A	0.54 B	0.52 D	0.51 E	

Note: LSD (P = 0.05); treatments = 0.02; LSD storage = 0.04; LSD interaction =0.06; DAS= Days After Storage

Table 14. Effects of $CaCl_2$ concentration on SOD (U mg^{-1} protein) of Kinnow mandarin during storage.

Treatment	0DAS	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	Mean
Control	120.30 no	123.76 l	124.20 k	125.54 i	120.58 n	117.63 p	109.27 q	120.18 D
2%	120.44 no	124.49 jk	124.80 j	127.59 g	130.47 c	126.40 h	122.67 m	125.26 C
3%	120.38 no	127.36 g	130.69 c	132.73 b	133.44 a	129.58 d	126.67 h	128.69 A
4%	120.21 no	125.36 i	127.59 g	128.57 e	129.41 d	128.19 f	123.55 l	126.13 B
Mean	120.33 F	125.24 D	126.82 B	128.61 A	128.47 A	125.45 C	120.54 E	

Note: LSD (P= 0.05); treatments = 0.47; LSD storage = 0.61; LSD interaction = 1.10; DAS= Days After Storage

storage days. But 3% $CaCl_2$ also performed better to maintain CAT activity during 90 storage days.

***Peroxidases (U mg^{-1} protein)*:** Maximum POD activity (0.55 U mg^{-1} protein) was noted in fruits treated with 3 % $CaCl_2$ while control fruit exhibited minimum POD activity (0.51 U mg^{-1} protein) after 90 days of storage. The other treatment also showed similar POD activity when fruits were treated with 2% (0.53 U mg^{-1} protein) and 4% (0.53 U mg^{-1} protein) respectively after 90 days of storage (Table 13). As well as the storage period was considered, as expected POD activity was decreased with the increased storage period. Interaction showed that maximum POD activity (0.59 U mg^{-1} protein) was observed in 3% $CaCl_2$ treated fruits during 45 storage days whereas minimum POD activity (0.51 U mg^{-1} protein) was noted in untreated fruit when evaluated at the similar time.

***Superoxide dismutase (U mg^{-1} protein)*:** It is observed that 3% dose of $CaCl_2$ showed maximum SOD activity after 90 days of storage. The similar trend was found in all the $CaCl_2$ treated fruits but minimum SOD activity in control fruits which showed the superiority of $CaCl_2$ treatments over control fruits. Maximum SOD activity (128.69 U mg^{-1} protein) was noted in treated fruits with 3% $CaCl_2$ followed by the fruits of 2% (125.26 U mg^{-1} protein), 4% $CaCl_2$ (126.13 U mg^{-1} protein) and control (120.18 U mg^{-1} protein). In addition to the storage period was measured, as expected SOD activity was decreased with the increased storage period. Maximum SOD activity (133.44 U mg^{-1} protein) was found in 3% $CaCl_2$ treated fruits after 60 days storage and minimum SOD activity (120.58 U mg^{-1} protein) was noted in control fruit at the similar day (Table 14).

DISCUSSION

Kinnow fruit have short shelf life. Therefore, sometime it cannot be reached to the consumer. In that period heavy losses occur. In general different fungicides and chemicals are used to increase the shelf life of Kinnow such as dipping in fungicide, waxing, application of growth regulators but these have some residual harmful effects on human health (Bhardwaj *et al*., 2010).

$CaCl_2$ is harmless and stated it can facilitate to increase storage life of different fruits and vegetables (Gill *et al*., 2005). In the present study minimum decay percentages was found in $CaCl_2$ treated fruits as compared to untreated fruits which showed higher values of decay. Consequently, dipping treatments with 3% $CaCl_2$ enhance the fruit quality and much less vulnerable to fruit rot during storage. The maximum fruit rot percentage was found in the control fruits was the result of reduced tissue strength and cell disruption. Similar results with decay of plum fruits at low temperature were mentioned by Mahajan *et al.* (2008). In addition to

delaying membrane lipid catabolism and increasing shelf life of fruit by $CaCl_2$ treatment which maintains membrane integrity, tissue firmness and cell turgor (Nirupama *et al*., 2010). Pre and postharvest treatment of $CaCl_2$ reduces postharvest disorders, slow down fruit ripening and reduced weight loss (Lara *et al*., 2004) and decay (Hernandez-Munoz *et al*., 2006) fruit softening and delayed total soluble solids reduction during storage (Garcia *et al*., 1996).

The lower weight reduction in $CaCl_2$ treated fruits indicated the superiority of $CaCl_2$ to reduce the weight loss during storage compared to control. The major reason for weight loss during storage is rapid respiration and transpiration rate which occur due to moisture loss and also depends on the water content of the fruit (Banarus *et al*., 1994). $CaCl_2$ application helps in decreasing the respiration rate hence reduces the ripening process and maintain the fruit firmness (White and Broadly, 2003). The outcomes of the present study are consistent with the results of Lester and Grusak (1999), who showed that $CaCl_2$ treatments can be effective in maintaining membrane function and membrane integrity because of reduced loss of phospholipids and proteins and reduced ion leakage, which may also reason for the reduced weight loss found in the treated fruits. Serrano (2004) also reported the effect of $CaCl_2$ treatment on the weight loss prevention. The lower weight reduction in $CaCl_2$ treated fruits can also be due to the effect of $CaCl_2$ in delaying the effect of natural physiological methods such as start of the climacteric, respiration, ripening development and senescence as suggested with the aid of Hussain *et al.* (2012).

The results revealed that $CaCl_2$ treated fruits had lower values of soluble solids contents as compared to control. Similar results were found that $CaCl_2$ treated fruits decrease in the TSS was possibly due to reducing of metabolic process, rate of respiration and the slower conversion from starches to sugars, hence delaying the ripening process (Rohani *et al*., 1997; Akhtar *et al*., 2010). Free sugars continuously increase with storage period and that this increase was delayed by $CaCl_2$. (Cheour *et al*., 1991). The increase in TSS due to the enzymatic conversion of the complex polysaccharides with starches and pectin's into monosaccharides during maturation (Hussain *et al*., 2008). The $CaCl_2$ dose improved the calcium content of the fruit and reduced the postharvest losses and senescence process regarding acids, sugars and antioxidant contents (Ali *et al*., 2013a).

A decreasing trend was found regarding titratable acidity in control fruits. The decline in TA due to changes of the organic acid into sugar contents within the commodity and frequently associated with the maturity. These acids make a significant contribute to the quality and taste of the fruit. The acid content of fruits reduced with the ripening due to respiration and fermentation (Ball, 1997). During maturation, the sugar content rises, and malic acid begins to accompany citric acid degradation (Salunkhe and Desai, 1984) which may lead to a decrease in the acidity value. In our results, it was observed that $CaCl_2$ application moderately affects titratable acidity in comparison to untreated fruit. The acidity retained in 4% $CaCl_2$ can be considered as a decrease in metabolic activitity of organic acids. Ishaq *et al.* (2009) also made similar remarks where $CaCl_2$ enhances the acidity values of apricot fruit during cold storage. Dipping treatment in the $CaCl_2$ solution did not affect the titratable acidity (Manganaris *et al*., 2007).

As a result, ascorbic acid content decreased during storage; however $CaCl_2$ treated fruits exhibited higher ascorbic acid contents after 90 storage days. Ascorbic acid is an essential nutrient as compared to other nutrients and may be very sensitive to degradation during storage because of its oxidation at low temperature. Ascorbic acid is an important indicator of the quality and oxidation of citrus fruits during postharvest storage (Veltman *et al*., 2000). As the storage interval increases and exposure to excessive O_2 concentrations it will lose. However, it has been mentioned that $CaCl_2$ is powerful in delaying the maturation and oxidation of tissues that maintain ascorbic acid content. Ruoyi *et al.* (2005) reported that $CaCl_2$ retained vitamin C content during storage of peaches. The similar trend was observed by Ullah (2009) described that, respiration and transpiration is two key physiological techniques that lead to decreased level of ascorbate. The quality of fruit cannot be improved after harvest it is because all parameters relating to quality are developed while fruit is attached with plants. In addition, the harvested fruit still maintains its life processes and at the same time there is no longer the transfer of food material and water from the mother plant to the fruit. Consequently, it must rely on stored food reserves for survival. Ultimately, the reserves have been exhausted which includes ascorbic acid content for that reason the produce suffer an aging process subsequent in breakdown due to natural deterioration. However, the losses of ascorbic acid content may be due to the low temperature during storage. Especially under post-harvest storage conditions, ascorbic acid content is lost due to reduced antioxidant activity (Davey *et al*., 2000).

Our results investigated that treated fruits with 3% $CaCl_2$ showed higher phenolic contents as compared to control fruits with a small increase during early storage. In fruits and vegetables phenolic compounds are act as free radical scavengers and prevent free-radicals which cause damage to cells against oxidative stress (Wada and Ou, 2002; Chun *et al*., 2003). But, phenolic components decrease with the increase in storage period. However, fruits treated with $CaCl_2$ exhibited maximum phenolic compounds may be due to decreased rate of softening, acidity and respiration in fruits (Ali *et al*., 2013b).

Higher antioxidant activities were noted in fruits treated with 3% $CaCl_2$. Antioxidants are recognized to play important

function in obstructing oxidative injury in cells. Results confirmed that antioxidant ability reduced in untreated fruits as compared with the $CaCl_2$ treated fruits. There is an effective relationship among phenolic compound and antioxidant activity (Wang and Lin, 2000). According to our results, phenolic compound decreased with increased storage duration due to enzymatic activity and oxidation. At the end, antioxidant activity reduced in fruits (Kevers *et al*., 2007) which are similar to the results of Shirzadeh *et al*. (2011).

Antioxidative enzymes such as CAT, POD and SOD stimulate the plant defense system against different biotic and abiotic stress. Maximum enzyme activities were found in 3% $CaCl_2$ treated fruits as compared to untreated fruits. SOD is an abundant defensive enzyme that prevents superoxide damage in anaerobic organisms. POD and CAT also facilitate the removal of free radicles that can cause stress damage to cells. The study of El-hilali *et al*. (2003) exhibited that $CaCl_2$ decreases chilling injury and POD activity of fruits with prolong storage at low temperatur. Ranadive and Haard (1972) found a relationship between peroxidase activity and fruit cell wall lignification. Ca^{2+} appears to be very important for POD activity because it induces the cross-linking of polygalacturonan chains into structure that can be recognized by isoperoxidase (Penel *et al*., 1999).

The similar finding was revealed by Mortazavi *et al*. (2007) suggested that maximum CAT activity was noticed in fruits treated with 3% $CaCl_2$ while the minimal CAT activity was observed in untreated fruits. It has already been mentioned that calcium applications continued a higher CAT activity in apricot (Ali *et al.,* 2013b) and loquat (Akhtar *et al.,* 2010) during storage. Reduction of electrolyte leakage through $CaCl_2$ application increases enzyme antioxidant activity, cell wall integrity and stability. The improvement of tissue browning in the controls may be related to the increase of CAT activity. This may be due to the reason that $CaCl_2$ control higher respiration rates in treated fruits compared to the non-treated fruits.

***Conclusion*:** Pre harvest foliar spray of calcium chloride reduced the decay % and maintained the quality of Kinnow fruit during cold storage. The doses of 3 % and 4% $CaCl_2$ performed better than others to marinated the quality during 90 days cold storage,

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