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RESEARCH ARTICLE

Exogenous calcium nitrate application delays senescence, enhance nutraceutical properties and antioxidant defense system during storage

Muhammad Azam[1]*, Riadh Ilahy[2], Jiyuan Shen[3], Rashad Qadri[1], Muhammad Azhar Iqbal[4], Zarina Yasmin[5], Sakeen-Tul-Ain Haider[6], Muhammad Arslan Khan[1], Muhammad Jafar Jaskani[1], Aman Ullaha Malik[1], Sajjad Hussain[6], Muhammad Awais Ghani[1], Aqeel Feroz[4]

[1]Institute of Horticultural Sciences, University of Agriculture, Faisalabad, 38040, Pakistan, [2]Laboratory of Horticulture, National Agricultural Research Institute of Tunisia (INRAT), University of Carthage, Tunis, Tunisia, [3]College of Horticulture, South China Agricultural University, 510642, Guangzhou, [4]Barani Agriculture Research Institute, Chakwal, Pakistan, [5]Postharvest Research Institute, Ayub Agriculture Research Institute, Faisalabad, [6]Department of Horticulture, Bahauddin Zakariya University Multan, 60800, Pakistan

ABSTRACT

In recent year, the demand for guava fruit has been increasing in domestic and international markets. However, guava fruit is extremely perishable due to the short shelf life and rapid over-ripening of the fruits suggesting the need for practical tools to extend their shelf-life. In the present study, the efficiency of calcium nitrate treatment was investigated on Golla guava fruit. Fruits were treated with different concentrations (0, 1%, 2%, 3% and 4%) for 12 days storage at ambient condition. Among the dipping treatments, 4% calcium nitrate significantly reduced fruit weight loss (FWL), decay percentage, and suppressed the increase of soluble solids content (SSC). Moreover, 4% calcium-treated fruits had higher total titratable acidity (TA), and lower ripening index (SSC: TA ratio) and pH during the storage period. Higher ascorbic acid, total phenolic content (TPC) and higher reducing, non-reducing and total sugars along with retained higher activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) enzymes were observed in (4%) calcium nitrate treated guava fruits. Calcium nitrate (4%) also exhibited higher aroma, taste and flavour among the other treatments. Generally, the pre-storage calcium nitrate dipping treatment of guava fruit-maintained fruit quality for up to 12 days at ambient storage condition and could be suggested as practical solution at commercial levels in extending the shelf life of fresh guava fruits.

Keywords: Calcium; Fruit decay; Postharvest life; SOD; POD; CAT *Psidium guajava* L

INTRODUCTION

Guava belongs to family *Psidium guajava* L. and is highly palatable, delicious, and imperative fruit crop distributed throughout world mainly in tropical and sub-tropical regions (Watson & Dallwitz 1991). The guava is commonly cultivated in many countries around the world including Pakistan, Brazil, India, China, Egypt and South Africa. Nutritionally, guava fruit contains various bioactive compounds such as dietary fiber, pectins, mineral contents, carotenoids, and antioxidant. Guava fruit have higher ascorbic acid content which is three to six times higher than citrus fruit (Singh, 2010). Various physico-chemical changes occur in appearance, texture, flavour and color of guava after harvesting which influence fruit quality and acceptability (Rana et al., 2015).

Guava is a climacteric fruit characterized by higher ethylene and respiration during ripening and after harvesting which limits the storability and hampers the world trade of these fruits (Eliane et al., 2005) although their high content in nutritional and bioactive compounds (Gill et al., 2014). Guava is perishable fruit in nature, and its texture and fruit quality characteristics rapidly deteriorate during postharvest storage, therefore needs quick marketing after harvesting (Hong et al., 2012; Goutam et al., 2010). Guava fruit become over ripe and mealy under ambient conditions within a week. Postharvest losses in guava has been revealed approximately 30-50% (Lashley (1984), and even more higher than pre-harvest losses in guava (Salunkhe et al., 1991). During postharvest handling, marketable value is decreased due to rapid softening of guava fruits (de Aquino

***Corresponding author:**
Muhammad Azam, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, 38040, Pakistan. **Tel:** (+92-335-6644339).
E-mail: muhammad.azam@uaf.edu.pk

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et al., 2015). Postharvest treatments reduce the moisture loss from the surface of fruits by lowering the metabolic activities, evaporation, transpiration, and respiration within the fruits especially due to decay causing microorganism (Golding et al., 2005).

In the last decade, various treatments and preservation methods has been tested and applied to extend guava fruit quality during storage such as edible coatings, gamma-irradiations, growth retardant, calcium chloride, calcium lactate, ascorbic acid, preharvest calcium sprays, cling and shrink packaging films, control atmosphere storage and cold storage behavior (Rana et al., 2015; Gill et al., 2014; Hong et al., 2012; Goutam et al., 2010; Anjum et al., 2020; Vishwasrao & Ananthanarayan, 2016; Pandey & Joshua, 2010; Pereira et al., 2010; Singh & Pal, 2008; Teixeira et al., 2018; Javed et al., 2018). Calcium salts has been used particularly to slow down the ripening, delay aging process and control of diseases onset in fresh horticultural produces (Cheour et al.,1991; Nadia et al., 2007). Singh & Chauhan (1982) revealed that pre- and postharvest applications maintained physiological, biochemical changes and marketing by increasing shelf-life of climacteric fruits. Previous postharvest methods were developed for extension of guava shelf life which do not use commercially either due to highly costly in nature or unavailability of materials or owing to high risk of human health. In this milieu, it is of concern to evaluate the use of calcium nitrate application to increase the quality of guava fruits during ambient temperature storage conditions.

Calcium is major component of cell wall and plays major role in improving plant health and strengthening the cell wall structure (Fry, 2004). Pre-harvest calcium sprays play crucial role in reducing the fungicides spray and improve the production, fruit quality attributes and disease resistance in various horticultural fresh products such as guava, apple, peach and kiwifruit (Chandra et al., 1999; Gerasopoulos et al., 1996; Gupta et al., 2011; Conway et al., 1994). Preharvest calcium nitrate-treated guava fruits at the breaker stage showed reduced spoilage higher firmness and remain quite acceptable up to 40 days under cold storage (Goutam et al., 2010). Chaplin & Scott (1980) found that post-harvest calcium application delays membrane lipid catabolism and maintains cell and membrane compartmentalization therefore as a result lengthening storage life of fresh fruits. Azam et al., (2021) demonstrated that ascorbic acid treatment reduced physiological weight loss, delayed decay, and improved the fruit quality by maintaining the higher ROS (reactive oxygen species) scavenging enzymes activities during ambient storage. Previously, it has been reported that different calcium salts (such as calcium chloride and calcium nitrate) are frequently applied to reduce incidence of numerous postharvest disorders in horticulture produce (Kader, 2002). Madani et al., (2016) reproted that preharvest application of calcium nitrate retained good fruit quality and increase the storability of papaya fruits. Preharvest calcium application maintained physicochemical properties, higher fruit firmness, and increased shelf life of apple and papaya fruit during different storage durations (Wójcik & Borowik, 2013; Ramakrishna et al., 2001).

Preharvest calcium nitrate application delayed ripening and retained fresh-like quality of guava cv. Sardar during storage period (Chandra et al., 1999). However, there are no appropriate studies on the postharvest application of calcium nitrate for extension of guava fruit shelf-life under storage. Therefore, the objective of the present study was to investigate the effect of different concentrations of calcium nitrate dipping to delay the postharvest senescence and maintain the quality of guava fruit as well as sensory attributes during ambient storage.

MATERIALS AND METHODS

Fruit sampling

The fresh guava fruits (cv. Golla) were picked at light green to yellow color (stage-II) early in morning during the month of November form Horticulture Research Farm, University of Agriculture Faisalabad, and immediately shifted to the Pomology laboratory for analysis. Uniform, healthy, disease- decay- and injury-free guava fruits were selected at maturity stage. Fruits were washed with tap water to remove the dust, then dried at room temperature, the fruits were dipped subsequently in different concentrations of calcium nitrate. Calcium nitrate concentrations (control, 1%, 2%, 3% & 4%) were freshly prepared by dissolving calcium nitrate (99%, Sigma-Aldrich, USA) in deionized water. The selected fruits were dipped in respective calcium nitrate solutions for five minutes, dried at ambient conditions. Finally, calcium-treated guava fruits were kept in carboard corrugated boxes at 25 ± 3 °C and stored for 12 days. The fruit samples for analyses were collected after every 3-days interval. Each treatment was comprised of three replicates and contained 40 fruits per replication.

Determination of fruit weight loss (FWL) and fruit decay (FD)

Fruit weight loss (FWL) was measured according to protocol of (Waskar et al., 1999). Briefly, ten fruits per treatment were weighted using a digital balance (PTL, RX 5000, Japan) before and after storage. The result of FWL was expressed in percentage.

$$\text{Fruit weight loss}\,(\%) = \frac{\text{Initial fruit weight} - \text{Fianl fruit weight}}{\text{Initial fruit weight}} \times 100$$

Likewise, fruit decay percentage was assessed by taking the ratio between number of decayed fruit and total number of fruit (Anjum et al.,2020).

Determination of SSC, TA, SSC: TA ratio and pH

The juice was extracted and homogenized in a beaker, then used to measure to soluble solids content with digital refractometer (ATAGO, RS-5000, Atago, Japan), and values were expressed as percent. Titratable acidity was recorded as reported by (Hortwitz, 1960). To calculate the SSC: TA ratio the values of SSC were divided with the values of the TA. The digital pH meter was used to determine the pH (HI 98107, Hanna Instruments, Mauritius).

Sugars contents

Reducing, non-reducing and total sugars were determined according to the method proposed by (Horwitz, 1960).

Determination of ascorbic acid content and total phenolic content (TPC)

Ascorbic acid content was determined from juice as previously reported by (Ruck, 1963). Total phenolic contents were assayed with the protocol of Ainsworth and Gillespie (2007) and TPC was expressed in terms of gallic acid as mg kg-^{1}.

Determination of SOD, POD and CAT enzymes activities

The pulp of guava fruit (10 g) was blended in phosphate extraction buffer having pH 7.2 and centrifuged at 12,000 g for 10 min, and the resultant supernatant was collected for enzyme assay. The activity of SOD enzyme (EC1.15.1.1) was assays as reported by (Liu et al., 2014) with minor modification. Briefly, a reaction mixture contained sodium phosphate buffer (65 mM) with having pH 7.8 [200 µL methionine (13 mM), 75 µM nitro blue tetrazolium (NBT), 10 µM EDTA, 2 µM riboflavin (0.6 µM, distilled water (800 µL)], and reacted with 100 µL of enzyme extract. After assay mixtures were illuminated to UV- light (15 min) and absorbance was noted at 560 nm. Identical solution held in the dark served as blank. Finally, SOD activity was expressed µmol s^{-1} kg^{-1}.

POD enzyme (EC1.11.1.7) was determined as described by Ali et al., (2016) with minor modification. Briefly, 100 µL crude extract were mixed with 100 µL reaction mixture (800 µL sodium phosphate buffer (100 mM with pH 6.4), 100 µL guaiacol (8 mM), 100 µL H_2O_2 (40 mM), and absorbance were noted at 460 nm, and POD activity expressed as µmol s^{-1} kg^{-1}.

CAT enzyme (EC 1.11.1.6) activity was measured as method proposed recently by Liu, et al., (2014) with slight changes. The reaction mixture was prepared comprising of 100 µL crude extract, 200 µL sodium phosphate buffer (50 mM) with pH 7, 100 µL H_2O_2 (40 mM). The absorbance was recorded at 240 nm and CAT enzyme activity was expressed as µmol s^{-1} kg^{-1}.

Sensory panel analysis

Sensory characteristics such as aroma, taste and flavour were conducted with trained panel as reported by (Shah et al., 2017). The hedonic scale consisted of 9= extremely like to 1= extremely dislike.

Statistical analysis

The experiment was designed according to completely randomized design (CRD) under two factors factorial arrangements including calcium nitrate concentrations and storage periods (Statistix -8.1® software, Tallahassee, USA). Least significant differences (LSD) among the treatments were measured following the F-test at P ≤0.05.

RESULTS

Fruit weight loss (FWL) and fruit decay (FD)

The Effect of calcium nitrate treatments, storage periods and their interaction on FWL was found significant (P ≤ 0.05). FWL increased throughout the storage period regardless of treatments (Fig. 1A). However, the lowest FWL (11.19%) was found in 3% calcium nitrate-treated fruit followed by 2% calcium nitrate-treated fruits whereas the highest FWL was observed in control fruits (20.50%) after day-12 of storage period. Fruit decay (FD) increased throughout the storage period regardless of treatments up to day-12 of storage duration. The impact of calcium nitrate treatments, storage period and their interaction on fruit decay was found significant (P ≤ 0.05). FD was found lower at day-3 then increased up to day-12 of storage (Fig. 1B). However, lower fruit decay percentage was recorded in 4% calcium nitrate (1.8-fold) and 3% calcium nitrate-treated guava fruit (1.61-fold) than control treatment after day-12 of storage. Whereas, FD was higher in untreated control (30%) and 1% calcium nitrate-treated fruits (27.40%) after day-12 of storage periods (Fig. 1B).

Soluble solids content (SSC), titratable acidity (TA), SSC: TA ratio and pH

The impact of calcium treatments, storage periods and their interaction on SSC was also significant (P ≤ 0.05). The results revealed that SSC contents increased in all the treatments up to day-9 then decreased at day-12 of storage (Table 1). Calcium nitrate treatments exhibited the reduction in increase of SSC content during storage period. SSC content was found higher in untreated control fruits (10.25%) up to day-12 of storage. However,

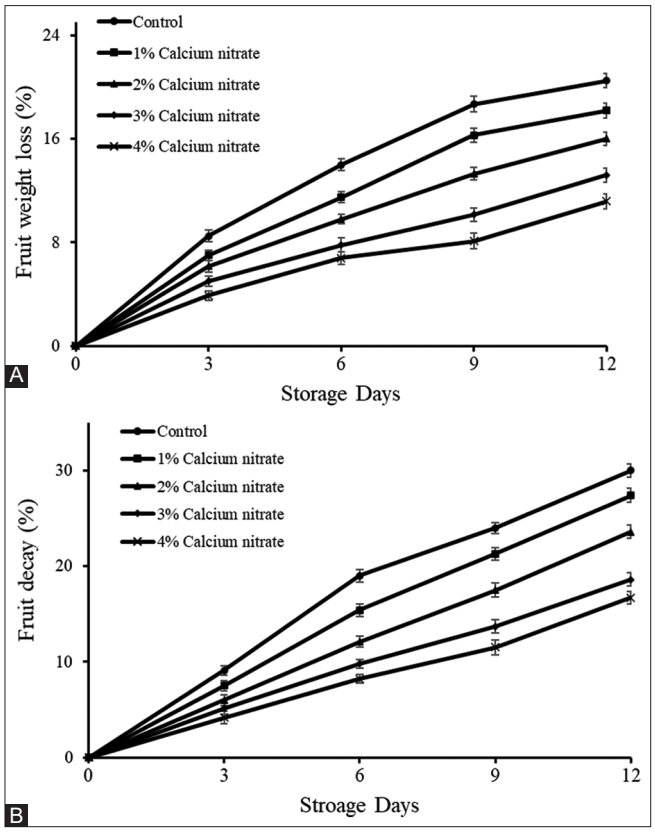

Fig 1. The effect of calcium nitrate dipping on fruit weight loss (A) and fruit decay (B) in Cv. Golla guava fruit at 0, 3, 6, 9 and 12 day of ambient storage. Values represent the means and bar indicates the standard error of three replicates.

the lowest SSC content was recorded in 3 % calcium nitrate-treated fruits (8.01%) at day-12 of storage period (Table 1).

Effect of calcium nitrate treatments, storage periods and their interaction were observed significant ($P \leq 0.05$). TA content gradually declined regardless of the treatments during storage period. However, the highest TA content was observed in 4% calcium nitrate (0.41%) followed by 3% calcium nitrate (0.39%) after day-12 of storage, respectively. Whereas, the lower TA content was showed in untreated control (0.24%) guava fruits up to day-12 of storage duration (Table 1).

The effect of calcium nitrate treatments, storage periods and their interactions on SSC: TA was significant ($P \leq 0.05$). From the table 1, the data reveled that calcium nitrate treatments were significantly affected the SSC: TA ratio during storage periods. The SSC: TA ratio gradually increased in all the treatments up to day-12 of storage. However, SSC: TA ratio was higher in untreated control fruits (42.71%) after day-12 of storage period (Table 1). The lowest SSC: TA ratio was found in 4% calcium nitrate (19.01%) and 3% calcium nitrate (21.02%) at day-12 of storage, respectively (Table 1).

The changes in pH values in calcium nitrate treated- and control fruits during12 of storage are shown in table 1.

Table 1: The effects of calcium nitrate treatments on SSC, TA, SSC: TA ratio and pH in Cv. Golla guava at 0,3,6,9, and 12 day of ambient storage temperature

Parameters	Treatment	Storage days				
		0	3	6	9	12
SSC (%)	Control		7.4 ± 0.15 m	9.5 ± 0.18 d	11.2 ± 0.16 a	10.25 ± 0.16 c
	1% Calcium nitrate		7.2 ± 0.13 o	9.01 ± 0.19 f	10.5 ± 0.18 b	9.50 ± 0.18 d
	2% Calcium nitrate	6.01± 0.13 s	7.01 ± 0.18 p	8.1 ± 0.14 j	9.5 ± 0.19 d	9.05 ± 0.19 e
	3% Calcium nitrate		6.75 ± 0.16 q	7.88 ± 0.20 k	8.80 ± 0.11 g	8.20 ± 0.21 i
	4% Calcium nitrate		6.44 ± 0.18 r	7.35 ± 0.18 n	8.40 ± 0.13 h	7.60 ± 0.23 l
TA (%)	Control		0.57 ± 0.004 b	0.39 ± 0.002 l	0.29 ± 0.003 p	0.24 ± 0.003 q
	1% Calcium nitrate		0.56 ± 0.003 c	0.40 ± 0.003 k	0.38 ± 0.004 m	0.33 ± 0.004 o
	2% Calcium nitrate	0.6± 0.002 a	0.53 ± 0.003 e	0.44 ± 0.002 h	0.42 ± 0.003 j	0.35 ± 0.003 n
	3% Calcium nitrate		0.54 ± 0.002 e	0.46 ± 0.003 g	0.44 ± 0.004 i	0.39 ± 0.002 k
	4% Calcium nitrate		0.55 ± 0.003 d	0.48 ± 0.003 f	0.46 ± 0.003 g	0.4 ± 0.004 l
SSC: TA ratio	Control		12.98 ± 0.15 q	24.35 ± 0.16 f	38.62 ± 0.17 b	42.71 ± 0.18 a
	1% Calcium nitrate		12.85 ± 0.17 r	22.52 ± 0.20 h	27.63 ± 0.16 d	28.78 ± 0.21 c
	2% Calcium nitrate	10.02± 0.21 u	13.20 ± 0.20 p	18.40 ± 0.19 l	22.61 ± 0.19 g	25.85 ± 0.22 e
	3% Calcium nitrate		12.50 ± 0.19 s	17.13 ± 0.17 n	20.01 ± 0.21 g	21.02 ± 0.19 i
	4% Calcium nitrate		11.71 ± 0.22 t	15.31 ± 0.19 o	18.26 ± 0.22 j	19.01 ± 0.24 k
pH	Control		2.44 ± 0.010 j	2.48 ± 0.031 f	2.61 ± 0.031 a	2.53 ± 0.040 c
	1% Calcium nitrate		2.42 ± 0.020 k	2.45 ± 0.025 i	2.56 ± 0.029 b	2.52 ± 0.031 d
	2% Calcium nitrate	2.2± 0.19 q	2.39 ± 0.025 n	2.41 ± 0.036 m	2.53 ± 0.041 c	2.49 ± 0.028 e
	3% Calcium nitrate		2.37 ± 0.021 o	2.39 ± 0.040 n	2.47 ± 0.025 g	2.43 ± 0.031 k
	4% Calcium nitrate		2.35 ± 0.045 p	2.35 ± 0.032 p	2.46 ± 0.039 h	2.4 ± 0.025 l

Data represent the means values with standard deviation of three replicates. Means with different letter indicate significant difference among treatments at $P \leq 0.05$ using Tukey's test. Abbreviation: SSC, soluble solids content, TA, titratable acidity, SSC: TA ratio, ripening index.

Calcium nitrate treatment showed lower pH values than control fruits. The lowest pH value was found in 4% calcium nitrate-treated fruits (2.41), whereas highest pH values were recorded in untreated control (2.53) after day-12 of storage (Table 1).

Sugars

The effect of calcium nitrate treatments, storage period and their interactions were significant ($P < 0.05$) on sugar of guava juice. The reducing sugars content increased from day-3 to day-9 of storage and then decreased at day-12 of storage regardless of the treatments (Fig. 2A). However, higher reducing sugars content were found on day-6 in 2% calcium nitrate-treated fruits, then decreased at day-12 and found higher in 3% calcium nitrate treated fruits. Moreover, 4% calcium nitrate treatment attenuated the increase in reducing sugar (5.31% to 6.02%) during at day-12 of storage period. The changes in non-reducing sugars following calcium treatments, storage days and their interaction were shown in Fig. 2B. Overall, non-reducing sugars contents were increased during storage from day-3 to day-9 and decreased at day-12 of storage. The highest increase for non-reducing sugars content were found at day-9 of storage following the application of3% and 2% calcium nitrate, with the concentration of 6.25 % and 6.08%, respectively. Similarly, lower non-reducing sugars contents were noted in 4 % calcium nitrate-treated fruit with respect to the other treatments throughout the storage period (Fig. 2C). The changes in total sugar concentrations for calcium treatments and control fruits during storage periods are shown in Fig. 2C. Regardless of the applied treatments, TS concentrations were increased up to day-9 of storage, thereafter declined at day-12 of storage (Fig. 2C). Total sugars were found higher up to day-3 in untreated control (9.3% to 11.1%), then 3% and 2% calcium nitrate treatments showed the highest TS content on day-9 with 12.8% and 12.6%, respectively. Whereas, 4% calcium treatment were recorded higher total sugars from day-3 (9.6%) to day-12 (10.2) with respect to other treatments (Fig. 2C).

Ascorbic acid content and total phenolic content (TPC)

Effect of calcium treatments, storage periods ant their interaction was significant ($P \leq 0.05$) on ascorbic acid content of guava juice. The results revealed that ascorbic acid content gradually decreased in all treatment with progression of storage periods (Table 2). However, calcium nitrate treatment-treated fruits showed higher ascorbic acid values than control fruits. At day-2 of storage period, 4% calcium nitrate and 3% calcium nitrate-treated fruits showed 1.22-fold and 1.16-fold higher ascorbic acid content, as compared with control, respectively (Table 2).

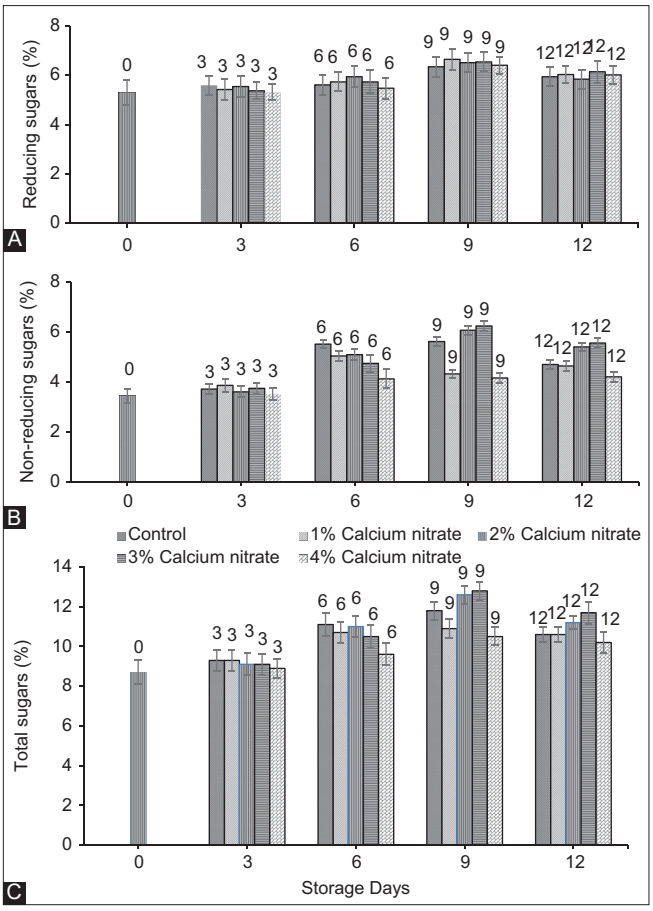

Fig 2. The effect of calcium nitrate dipping on reducing sugars (A), non-reducing sugars (B) and total sugars (C) affecting cv. Golla guava fruit at 0, 3, 6, 9 and 12 days of storage at ambient temperature. Values represent the means and bar indicates the standard error of three replicates. Different letters indicate significant difference ($P \leq 0.05$) among treatments.

The changes affecting total phenolic contents following different calcium treatments are shown in table 2. TPC were gradually decreased with advances in storage periods, regardless of treatments. However, calcium nitrate treatments showed higher TPC values than untreated control (Table 2). Overall, 4% and 3% calcium treatment-treated guava fruits showed 1.36-fold and 1.24-fold higher TPC, as compared with untreated control on day-12 of storage period (Table 2).

Enzyme activities of SOD, POD and CAT

The effect of calcium treatments, storage days and their interaction on SOD activity was found significant ($P \leq 0.05$) during the storage periods. Among all treatments, the activity of SOD significantly increased at day-3 of storage, thereafter, gradually declined up to day-12 of the storage period. However, 4% calcium treated-guava fruits showed substantially ($P \leq 0.05$) 1.51-folds higher SOD activity, than the other treatments, up to day-12 of storage period, respectively (Fig. 3A).

Table 2: The effect of calcium nitrate treatments on ascorbic acid and TPC in cv. Golla guava at 0,3,6,9, and 12 day of ambient storage

Parameters	Treatment	Storage days				
		0	3	6	9	12
Ascorbic acid (mg/100 g)	Control	247.25± 3.70 a	210.05 ± 4.01 f	181.01 ± 2.45 j	131.05 ± 3.06 q	118.05 ± 3.35 s
	1% Calcium nitrate		217.01 ± 3.51 e	187.01 ± 3.01 i	145.05 ± 3.26 n	126.04 ± 2.87 r
	2% Calcium nitrate		225.01 ± 2.78 d	195.06 ± 2.99 h	157.02 ± 2.98 m	132.31 ± 3.05 p
	3% Calcium nitrate		231.05 ± 3.64 c	201.05 ± 3.21 g	164.02 ± 3.07 l	137.05 ± 2.06 o
	4% Calcium nitrate		237.02 ± 2.67 b	210.04 ± 3.41 f	178.05 ± 3.03 k	145.05 ± 2.94 n
TPC (mg/kg)	Control	195.88± 2.50 a	171.24 ± 2.14 h	141.04 ± 3.01 k	114.47 ± 2.85 n	88.08 ± 2.87 r
	1% Calcium nitrate		175.02 ± 2.89 f	151.02 ± 3.31 j	121.21 ± 3.07 m	95.05 ± 3.87 q
	2% Calcium nitrate		180.21 ± 2.54 e	162.25 ± 3.47 i	129.05 ± 4.05 l	102.42 ± 4.25 p
	3% Calcium nitrate		184.08 ± 2.89 c	172.02 ± 4.01 g	140.52 ± 4.15 k	110.04 ± 4.70 o
	4% Calcium nitrate		189.15 ± 3.01 b	182.25 ± 3.78 d	152.02 ± 3.57 j	120.54 ± 3.08 m

The impact of calcium treatments, storage periods, and their interactions was significant ($P \leq 0.05$) on POD enzyme activity. The activity of POD steadily increased in all treatments with the advance in storage periods (Fig. 3B). However, calcium nitrate- treated fruits showed higher POD activity, as compared with control fruits. On an average, 4% calcium nitrate-treated guava fruits noticeably showed 1.48-folds higher POD activity, than other treatments, up to day-12 of storage period (Fig. 3B).

The effect of calcium nitrate treatments, storage periods and their interaction on CAT activity was found significant ($P \leq 0.05$). Among all treatments, CAT activity initially increased at day-3 of storage, then progressively decreased throughout the storage period (Fig. 3C). Nevertheless, CAT activity noticeably declined in untreated control fruits at higher levels than calcium nitrate-treated guava fruits. Moreover, CAT activity remained higher in calcium nitrate treated-fruits than untreated control fruits up to day-12 of storage. Among the calcium nitrate treatments, 4% calcium treated-fruits significantly ($P \leq 0.05$) exhibited about 1.68-folds higher CAT activity, as compared with control (Fig. 3C).

Sensory analysis

Impact of calcium treatments, storage periods and their interaction were significant ($P \leq 0.05$) on the aroma, taste and flavour of guava fruit. Overall the aroma, taste and flavor intensity decreased with storage period advance (Fig. 4A-C). However, calcium treated-fruit exhibited higher score for aroma, taste and flavour as compared to untreated control fruit. Among calcium treatments, 4% calcium nitrate-treated fruit showed significantly highest score about 1.81-folds, 1.45-folds, and 1.53-folds for aroma, taste and flavour with respect to the control fruit after day-12 of storage, respectively (Fig. 4A-C).

DISCUSSION

Weight loss is an indicator of water loss in fruits which increase with increasing respiration and transpiration rates leading momentously to boost desiccation and metabolic activities (Ali et al., 2018). The effect of calcium nitrate treatments on fruit weight loss showed significant difference than untreated control fruits during storage period. The highest FWL was found in untreated control fruit as compared with calcium nitrate treatments which might be due to an increase in respiration rate and transpiration losses. Calcium treatments may play important role in controlling the respiration rate, maintaining tissue turgidity and firmness as well as in reducing the activity of enzyme responsible for cellular structure breakdown leading to enhanced fruit shelf life (Levy & Poovaiah, 1979). Likewise, preharvest calcium nitrate application reduced the weight loss affecting guava cv. Sardar than control fruits (Goutam et al., 2010). Our results are similar with the previous finding on guava (Selvan & Bal, 2005), ber (Singh & Pal, 2008) and strawberry fruits (Martinsson et al., 2006).

Fruit decay significantly increases losses of guava fruit during storage (Akbar et al., 2020). In the present study, fruit decay in calcium nitrate-treated fruit remained lower, than untreated control fruits. Gupta et al., (2011) reported that calcium compounds application markedly reduced spoilage percentage by thickening middle lamella of fruit cells with higher accumulation of calcium pectate and thus retained cell wall structure, which hampered entering and spread of disease in fruit. Calcium nitrate has been reported to be effective in enhancing firmness, slowing-down the respiration rate, retarding ripening process and preserving the integrity of cellular organization (Faust & Shear, 1979). In the present experiment, 4% calcium nitrate-treated fruit showed less fruit decay as compared with control fruit. These results are in close agreement with previous results on guava and

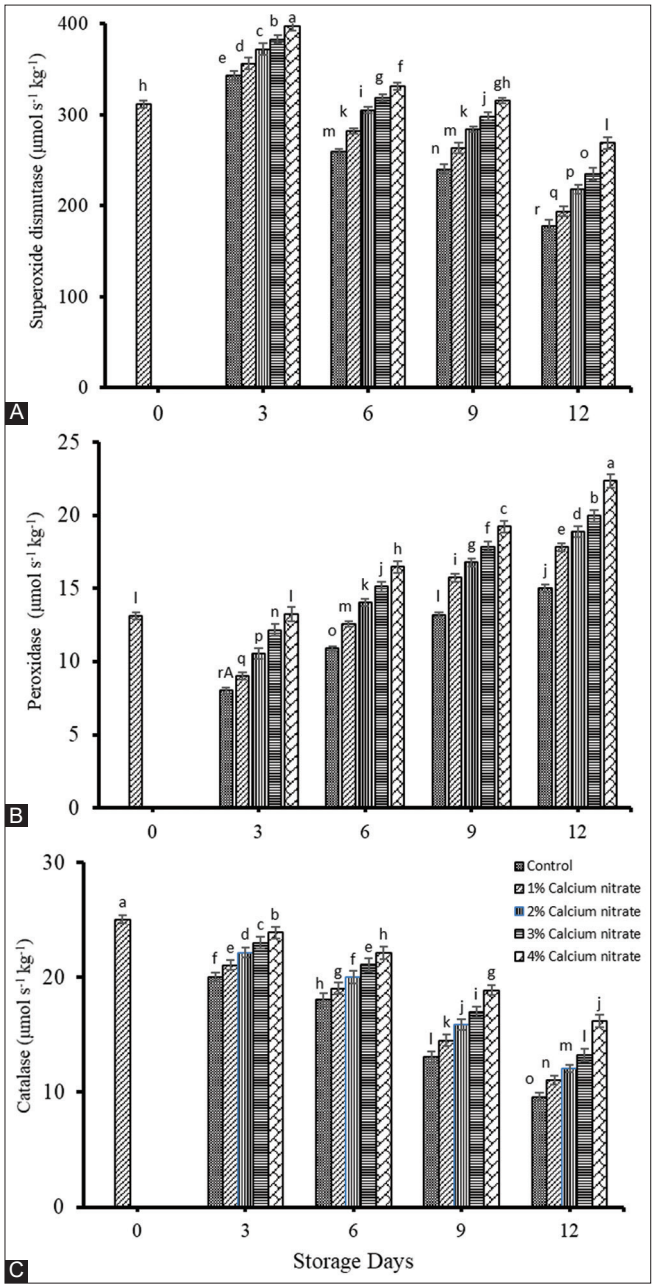

Fig 3. The effect of calcium nitrate dipping on superoxide dismutase (A), peroxidase (B) and catalase (C) in cv. Golla guava fruit at 0, 3, 6, 9 and 12 day of ambient storage. Values represent the means and bar indicate the standard error of three replicates. Different letters indicate significant difference ($P \leq 0.05$) among treatments.

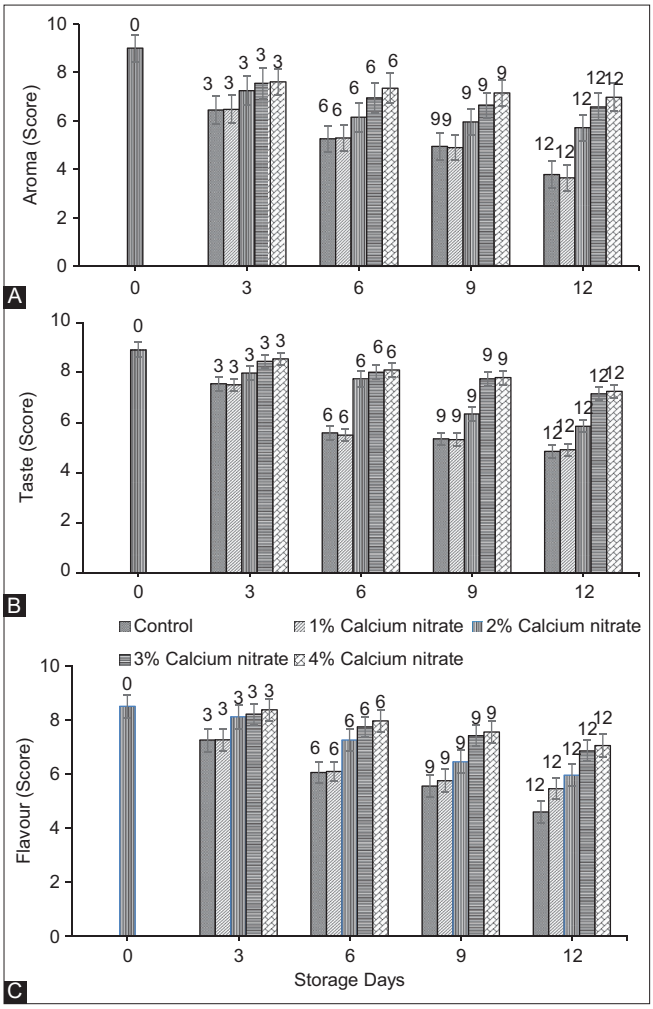

Fig 4. The effect of calcium nitrate dipping on aroma (A), taste (B) and flavour (C) score in cv. Golla guava fruit at 0, 3, 6, 9 and 12 day of ambient storage. Values represent the means and bar indicate the standard error of three replicates. Different letters indicate significant difference ($P \leq 0.05$) among treatments.

strawberry fruits (Selvan & Bal, 2005; Martinsson et al., 2006, Rehman et al., 2020).

The SSC contents were significantly affected with the calcium nitrate treatments as compared with control throughout the storage periods. The SSC concentration was increased up to day-9 of storage and then declined at day-12 of storage period in all treatments. However, calcium nitrate -treated fruits inhibited an increase in SSC concentration, as compared with control. The phenomenon of upsurge of soluble solids content during storage is conceivably due to the breakdown of starch into sugars and after complete hydrolysis no additional increase in SSC, after that deterioration occur which mainly utilized for respiration substrates (Wills et al., 1980). Gohlani & Bisen, (2020) reported that phenomenon of carbohydrates breakdown into sugar is the main responsible for lower SSC content. Goutam et al., (2010) reported that 1.5% calcium nitrate treated fruits had retained higher SSC due to the accelerated breakdown of metabolic activity during storage. Similar results have been found previously in pear (Mahajan & Dhatt, 2004) and guava fruits (Singh & Chauhan, 1982).

In our current work, the effect of calcium treatments on TA was found significant during the storage period. TA contents were gradually decreased in all treatments up to day-12 of storage period. However, calcium nitrate-treated fruits retained higher levels of TA than control fruit

throughout the storage periods. Similarly, calcium nitrate-treated fruits showed higher TA level and delay ripening process and low respiration process. Furthermore, calcium treated fruit retain higher TA during storage and shelf life which might be due to reduced respiration rate in guava fruit (Goutam et al., 2010; Killadi et al., 2007), aonla (Singh et al., 2005) and peach fruits (Navjot & Gurcharan, 2006).

In the present study, during postharvest storage periods, the changes in SSC: TA ratios were showed significant differences for calcium nitrate treatments than untreated control. In present study, SSC: TA ratio was noticed higher in control as compared with calcium nitrate-treated fruits. Calcium nitrate treatments were showed lower SSC: TA ratio which is indication of delay the ripening and senescence process however, higher SSC: TA ratio showed rapid increase in SSC and lower TA contents during storage periods. Therefore, 4% calcium treated fruit showed lower SSC: TA ratio as compared with control fruit.

The pH values showed the abundance of chemicals in fruits which lead to the breakdown of starch into sugars and subsequent hydrolysis of organic acid. The calcium nitrate-treated fruits showed lower pH values as compared with control during the progression of storage duration. The findings of the present study are therefore consistent with the previous reports that calcium application reduced the pH of strawberry fruit during storage (Kumar et al., 2012).

The effects of calcium nitrate treatments and storage period on reducing, non-reducing and total sugar was found significant. Overall, sugar concentrations were rapidly increased up to 9 days of storage and then slightly decline up to day-12 of storage period (Fig. 2A-C). However, 4% calcium nitrate treatment attenuated the increase in sugars content throughout the storage conditions. Sugar levels increase with ripening and senescence of fruit during storage. Likewise, starch conversion increased the sugar concentration during storage. Calcium application helps in slowing down the ripening process, subsequent senescence and conversion of starch into sugars. Our results are in agreement with the previous findings in guava fruit during storage (Rehman et al., 2020; Chawla et al., 2018). A similar finding was reported with the $CaCl_2$ application on guava fruit during storage (Javed et al., 2018).

Ascorbic acid is an important antioxidant bioactive compounds that inhibit the deterioration of fruit during storage due to oxidation and reduce the detrimental effects of free radicals. In the present study, ascorbic acid contents decreased during storage, however 4% calcium nitrate-treated fruits showed higher ascorbic acid content as compared with untreated control fruits. It has been found that ascorbic acid concentration decreased due to oxidative breakdown with the progression of storage period. Oxidizing enzymes (such as ascorbic acid oxidase, peroxidase, catalase and polyphenol oxidase) might be responsible for decrease of ascorbic acid contents in fruits (Singh et al., 2005). Calcium treatments help in slowing-down the oxidation reactions, thereby reducing fruit degradation and senescence (Khaliq et al., 2016). Therefore, calcium nitrate application might have reduced oxidizing enzyme activities which resulting in higher ascorbic content during storage (Goutam et al., 2010) Similar findings were made in an aonla (Singh et al., 2005), peach (Navjot & Gurcharan, 2006) and guava fruits Killadi et al., 2007).

Phenolic compounds are secondary metabolites inhibiting free radical formation during oxidative stress. TPC slow down the oxidative breakdown and sustain the nutritional value of food (Shah et al., 2017). In our study, TPC contents gradually decreased following all treatments throughout the storage period. However, 4% calcium nitrate-treated fruit noticeably exhibited higher TPC contents than other treatments, up to day-12 of storage. Our results are in agreement with previous findings where *aloe vera* gel coating of guava fruit enhanced TPC during ripening and storage period probably by improved inhibition of free radical formation during oxidative stresses (Rehman et al., 2020; Ribeiro et al., 2020). Likewise, a similar trend was reported by Sliva et al., (2013) and Sun et al., (2011) in gabiroba and litchi fruit.

Calcium treated fruits retained higher activities of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in guava cv. Golla at ambient storage condition (Fig. 3A-C). These antioxidant enzymes play important role in reducing the breakdown triggered by reactive oxygen species. In the present study, the activities of these enzymes help inhibited the damages caused by free radicles naturally produced through oxidative stress during storage condition. The higher antioxidative enzyme activities are correlated with delay in repining and inhibition of senescence process (Javed et al., 2020; Rehman et al., 2020; Ribeiro et al., 2020). SOD play important role in reducing the stress conditions and extending the shelf life. The antioxidative enzymes (SOD, POD and CAT) show an imperative role in scavenging of reactive oxygen species and prevent harmful effects of H_2O_2 in tissues and membrane lipid peroxidation of litchi fruits. In our experiment, 4% calcium nitrate-treated fruits showed higher enzymatic activities than control fruits, indicating the potential of calcium treatments in the postponement of senescence in guava fruits. The application of edible coatings had higher activities of CAT and SOD enzymes than untreated control during storage of guava fruit (Rehman et al., 2020; Ribeiro et al., 2020). The activities of antioxidative enzyme reported to detoxify

different free radicals and reduce browning by alleviating oxidative damage. Therefore, keeping higher activities of these enzymes is of primary importance in reducing the occurrence of browning (Ali et al., 2018).

The results revealed that calcium treatments maintained the aroma, taste and flavor scores of guava fruit than untreated control (Fig 4. A-C). It is obvious that calcium treated fruit did not show any detrimental effects during storage periods particularly 4% calcium dose was more effective in reducing the decay and maintaining acceptable taste and aroma scores during storage periods. Calcium salts application retained higher score of palatability in guava (Singh et al., 2005), pear (Mahajan & Dhatt, 2004) plum (Mahajan et al., 2008) and peach fruits (Gupta et al., 2011) during storage conditions. So, calcium nitrate application was useful not only in reducing the physiological weight loss, decay percentage but also in maintaining the physiochemical traits, enzymatic antioxidative status and sensory attributes of cv. Golla guava fruits stored at ambient temperature.

CONCLUSIONS

Pre-storage exogenous application of calcium nitrate plays important role in retaining higher physico-chemical and sensory attributes in fresh guava fruit. The use of 4% Calcium nitrate reduced the physiological weight loss, decay percentage, and reduced the increase in SSC concentration during storage up to 12 days at ambient temperature. Fruit treated with calcium nitrate also maintained higher TA level, ascorbic acid and TPC contents as well as higher SOD, POD and CAT activities along with acceptable sensory quality. The pre-storage application of calcium nitrate at reasonable levels may improve the shelf-life of guava fruits which lead to improved marketability of this highly perishable fresh product and improve the income particularly of small holders.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

Author contributions

M.A. conceptualized the work and conceived the experimental design M.A., S.A, M.A.F., and A.K collected samples and carried out formal analyses R.Q., A.K., M.A.F., and M.D., performed physiological analysis, performed statistical analyses and helped in the interpretation of the results and in the revision of the manuscript M.A. and J.S., A.S.K., W.H.A.Q., M.J.J., and J.S., contributed to the writing and interpretation of the results M.A., W.H.A.Q., and S.H., J.S., read the paper and approved the published version of the document. All authors have read and agreed to the published version of the manuscript.

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