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CALCIUM SOURCES AND CONCENTRATIONS AFFECTING TOMATO FRUIT QUALITY STORED IN DIFFERENT STORAGE CONDITIONS

B. Haleema[1*] and A. Rab[1]

[1] Department of Horticulture, The University of Agriculture Peshawar
Corresponding email: haleema_12@yahoo.co.in

ABSTRACT

Experiment was conducted in Biochemistry Laboratory of Agriculture Research Institute Tarnab (ARI), Peshawar during the year 2014 in a completely randomized design with three replications. The treatments comprised of Ca sources (calcium chloride, calcium gluconate, calcium lactate and calcium sulphate), with different Ca concentrations (0, 0.25, 0.5 and 0.75%) in two storage conditions; 32±2°C and 10± 2°C. The results revealed higher incidence of soft rot and black rot when fruits were stored at ambient temperature. Cell wall ion leakage, cell membrane ion leakage and incidence of green mold were higher with low temperature storage. Likewise, higher Ca content was recorded with calcium chloride. However, cell wall ion leakage and cell membrane ion leakage were lower in calcium chloride. The Ca content of tomato fruit was higher with 0.75% Ca concentration. The cell wall ion leakage and cell membrane ion leakage were recorded least at 0.75% concentration of calcium. The least and same incidence of black rot and were recorded at 0.5 and 0.75% Ca concentration respectively while the incidence of soft rot and green mold was found to be at minimum in 0.75% Ca concentration.

Key words: Black rot, calcium sources, calcium concentrations, ion leakage, storage temperature, soft rot, green mold.

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is widely consumed both in fresh and processed forms (Thybo *et al*., 2006). Tomato fruit is also desired for its nutritive value, as it contains important nutrients such as sugars, acids, vitamins, minerals, lycopene and other carotenoids that are essential for good health (Simonne *et al*., 2006; Toor and Savage, 2006). It is a climacteric fruit, characterized by highly perishable nature and short post harvest life, usually 2-3 weeks (Gharezi *et al.,* 2012). Due to its delicate nature, the tomato fruits are prone to postharvest losses that may range from 30 to 50 percent (Inaba and Crandall, 1986). The postharvest losses in Peshawar valley account for 20% (Iqbal, 1996). Postharvest losses may occur from harvesting to packing, storing, shipments, marketing and final delivery to the consumers (Inaba and Crandall, 1986). Thus, good postharvest management is as critical as production practices for ensuring profitable tomato crop production (Senevirathna and Daundasekera, 2010).

Temperature and relative humidity management are among the primary postharvest management practices to increase the storage life and to retain quality of tomato fruits (FAO, 2009). The rate of ripening and senescence increase with increasing temperature in tomato (Gharezi *et al*., 2012). The water loss is also directly related to temperature and relative humidity in the storage (Perez *et al.,* 2003). Storage under low temperature has been considered to be the most efficient method to maintain quality of most fruits and vegetables, because it reduces respiration rate and ethylene production, delay ripening, senescence and suppress rot development (Perez *et al.,* 2003). Tomato fruit being chilling sensitive, are damaged by prolong storage at less than 12°C (Saltveit and Morris, 1990) but store well at slightly higher (10-13°C) temperature (Roberts *et al.,* 2002).

Calcium is found to decrease fruit softening and post harvest decay (Mahmud *et al.,* 2008), delayed senescence and re- fruit quality during storage (Stanly *et al.,* 1995). Calcium application maintains cell turgor, membrane integrity, tissue firmness and delays membrane lipid catabolism thus extending storage life of fresh fruits (Picchion *et al.,* 1998). Calcium ions (Ca^{+2}) are involved in many physiological processes in vegetables and fruits. It plays a vital role in maintaining their quality. Ca^{+2} levels are inversely related to respiration and ethylene production rates in a variety of fruit crops including tomato (Garcia *et al*., 1995). The effectiveness of Ca application methods as a postharvest treatment differs among crops (Shorter and Joyce, 1998). Application of Ca has been shown to promote the firmness of strawberries and kiwifruit (Rosen and Kader, 1989; Agar *et al.,* 1999) and inhibits the ripening of tomato and pineapples (Goncalves *et al.,* 2000). Different calcium sources such as calcium chloride, calcium lactate, and calcium sulphate have been used to retain the fruit quality during storage (Morris *et al.,* 1985). The prolonged shelf life of calcium treated fruits is due to increased firmness and low ethylene production (Arthur, 2014).

Post harvest application of calcium by dipping fruits in calcium solution significantly enhance calcium

content in comparison with pre harvest sprays without causing any injury to fruits; though it depends on salt concentration and types. The concentration of solution to be used for calcium dip also depends on fruit or vegetable treated. Many studies have reported 1-2% calcium concentration as most effective concentration for diced tomatoes, cantaloupes and pears (Luna-Guzman and Barrett, 2000; Dong *et al.,* 2000).

Keeping in view the importance of Ca role in prolonging shelf life and maintaining quality of tomato, this experiment was conducted with the objectives to evaluate the influence of different calcium sources, calcium concentrations and storage conditions on storage performance and quality of tomato fruit.

MATERIALS AND METHODS

An experiment was carried out to study effect of calcium sources and concentrations on tomato fruit quality stored in different storage conditions during the year 2014 at Agriculture Research Institute Tarnab, Peshawar. The tomato fruits were harvested at mature green stage from the field. The fruits free of mechanical injuries, diseases and disorders were selected for the study.

Four sources of calcium (calcium chloride, calcium gluconate, calcium lactate and calcium sulphate of Sigma and Ridyal company) were used. The percent calcium solutions were prepared by calculating calcium in molecular formula of the respective source. 0.25, 0.5 and 0.75% Ca solution were prepared by taking 0.92, 1.84 and 2.76g of $CaCl_2$ and dissolved in 100ml of distal water. Similar method was followed for preparing percent solutions for rest of the sources. The quantity detail of each source along with its concentration is given in Table 1.

The experiment was laid out in Completely Randomized Design (CRD) with three repeats. Tomato fruits samples of 4 kg were dipped in different calcium sources (Calcium chloride, calcium gluconate, calcium lactate and calcium sulphate) at various concentrations (0, 0.25, 0.50 and 0.75%) for 5 minutes, store at two storage conditions i.e. Ambient temperature (32±2°C and relative humidity 60±5%) and cold temperature (10± 2°C and relative humidity 60±5%) for 21 days.

Table 1: Chemical composition of different calcium sources and calculations required for preparing percent solutions.

Calcium source	Chemical Formula	Molecular weight (g)	Chemical required for 0.25% solution (g/100ml)	Chemical required for 0.5% solution (g/100ml)	Chemical required for 0.75% solution (g/100ml)
Calcium Chloride	$CaCl_2.H_2O$	147.02	0.92	1.84	2.76
Calcium Gluconate	$CH_2.(CH.OH)_4.COO)_2.Ca.H_2O$	439.78	2.75	5.50	8.25
Calcium Lactate	$(CH_3.CHOH.COO)Ca.5H_2O$	219	4.11	2.74	1.37
Calcium Sulphate	$CaSO_4.2H_2O$	172.17	1.08	2.15	3.23

Data were recorded on different quality attributes as described below:

Fruit Calcium content (mg. 100g^{-1} DW): To determine the Calcium content of the fruits, fruit were cut and locular tissue was removed and washed once again with distilled water. The fruit samples were weighed and, then, oven dried at 70ºC and weighed periodically so that weight became constant. After oven drying, the fruit samples were ground using a Tema mill which was cleaned thoroughly with a brush and acetone for each treatment and the ground fruit materials were dry-ashed. The digestion was done by the addition of 4 ml of 65% aqueous solution of nitric acid and then heating. The calcium content of fruit samples was determined by the procedure suggested by Isaac and Kerber (1971), using Atomic Absorption Spectrophotometer (GBC AA 932). The spectrophotometer was calibrated with a standard solution of 5 µg-ml^{-1}as per recommendations of the manufacture.

Ion leakage (%): Electrolyte leakage was measured for permeability assessments of cell wall and cell membrane. Pericarp disc (10 mm diameter) excised with stainless steel fruit core borer. Epidermal locular tissues were trimmed to make 4 mm thick discs. Discs were thoroughly washed with clean water for about 1 min to remove cytosol liquid and fruit juice (released after cutting). Three discs were poured in vial containing 20 ml distilled water and fixed on the stand. Stand was kept for shaking on rotatory shaker for about 30 minutes. EC of solution was recorded on electrical conductivity meter. Shaking was then repeated for 60 minutes and EC was recorded again. Three freeze and thaw cycles were then given to vial and finally total conductivity was recorded. Percent cell wall ion leakage was noted from EC readings by the formula;

$$Cell\ wall\ ion\ leakage = \frac{30}{Total\ EC}\ x\ 100$$

Whereas, percent cell membrane ion leakage was calculated from EC by the following formula;

$$Cell\ membrane\ ion\ leakage = \frac{90 - 30}{Total\ EC}\ x\ 100$$

Pathological study

Soft rot (*Erwinia carotovora*): Infected tomato fruit samples were brought to the laboratory and were surface sterilized with 0.1% $HgCl_2$ solution, fruits were then rinsed with sterile distilled water. A portion of healthy and disease fruit tissue were cut and then transferred to freshly prepared nutrient agar medium plates under aseptic condition. Inoculated plates were incubated. The colonies which were similar to ECC (*Ervinia carotova pva carotovora*) i.e. wet colony, convex, round with circular edges and creamy were picked and tested with 3% potassium hydroxide for confirmation of gram negative bacteria.

Black rot (*Alternaria alternata*) and Green mold (*Penicillium digitatum*): Infected tomato fruit samples were mounted on temporary slides. Conidia of the fungus were microscopically observed and identification was confirmed (Alexopoulos and Mims, 1979).

For further confirmation fruit samples were surface sterilized with 0.1% $HgCl_2$ (mercuric chloride), leaves were then rinsed with three exchange of sterile distilled water. A portion of healthy and diseased fruit tissues were cut and transformed to freshly prepared PDA (Potato Doctrose agar medium) plates under aseptic conditions. Inoculated plates were incubated at 28°C. Culture was observed and slides were prepared and conidia of fungus were microscopically observed.

Statistical analysis: The data were analyzed through analysis of variance procedure under Randomized Complete Block Design. Means were compared using LSD test at 0.05 level of probability when F-test was significant (Jan *et al*., 2009).

RESULTS

Fruit calcium content (mg/100 g DW): The Ca sources (CaS) and Ca concentrations (CaC) significantly affected the Ca content of the tomato fruits, while the effect of storage conditions (SC) on Ca content of the fruit was not significant. The interactions CaS x CaC and CaC x SC were significant. However, the rest of the interactions were found to be non significant (Table 2). Among the different CaS, the highest Ca content (13.10 mg/100 g DW) was recorded in fruits treated with calcium chloride, followed by calcium sulphate (12.49 mg/100 g DW). The least Ca content (12.13 mg/100 g DW) was observed in fruits treated with calcium gluconate (Table 2).The Ca content of tomato fruit increased significantly with an increase solution of CaC. The highest Ca content (12.89 mg/100 g DW was observed for 0.75% and 0.5% Ca application followed by the Ca content of tomato fruit (12.41 mg/100 g DW) in fruits dipped in 0.25% Ca solution. The least Ca content (9.20 mg/100 g DW) was recorded in fruits in the control group (Table 2). The CaSxCaC interaction indicated that increasing the CaC, increased the Ca content at all sources of Ca, however the Ca content was lower (11.84 mg/100 g DW) with 0.25% Ca of calcium lactate solution. The maximum calcium content (13.58 mg/100 g DW) was recorded in fruits treated with calcium chloride and 0.75% calcium concentration (Fig. 1). The CaC x SC interaction revealed that increasing Ca concentration increased the Ca content of the fruits regardless of the storage temperature, however the increase was more prominent (12.89 mg/100 g DW) with 0.75% concentration of Ca at low temperature storage (Fig. 2).

Ion leakage (%) from cell membrane: The mean ion leakage from cell membrane was significantly affected by various storage conditions (SC), Ca sources (CaS) and Ca concentration (CaC). However, the interactions of SC x CaS, SC x CaC, CaS x CaC and SC x CaS x CaC were non-significant (Table 2). The mean ion leakage from cell membrane for SC was higher (47.13%) in the fruits stored at lower temperature as compared to ambient temperature (41.58%) (Table 2). Among the various CaS, the ion leakage from cell membrane was the highest (45.78%) in fruit treated with calcium gluconate, followed by 43.78% with calcium lactate treatment. The least ion leakage from cell membrane (37.50%) was recorded in fruits treated with calcium chloride (Table 2). The least ion leakage (40.25%) with 0.75% Ca treatment increased linearly with decrease in Ca concentration followed by ion leakage percentage (41.54%) with 0.5% Ca concentration. Maximum ion leakage (46.83%) was recorded in control group fruits (Table 2).

Ion leakage (%) from cell wall: The ion leakage from the cell wall was profoundly affected by various storage conditions (SC), Ca sources (CaS) and Ca concentration (CaC). All the interactions were found non-significant for the ion leakage from cell wall (Table 2). The ion leakage from the cell wall was higher (27.13%) in the fruits stored at lower temperature compared to 22.64% ion leakage at ambient temperature (Table 2). Among the different CaS, the least ion leakage (19.06%) was recorded with calcium chloride treatment, followed by 20.06% ion leakage treated with calcium sulphate treatment. The ion leakage from cell wall, however, was the highest (27.28%) in fruit treated with calcium gluconate (Table 2). Ion leakage percentage for the cell wall was the least (21.38%) with 0.75% Ca concentration that increased to 22.71 and 24.71% with decreasing

calcium concentration to 0.5 and 0.25%. The highest ion leakage (26.83%) was noted in control fruits (Table 2).

Soft rot (%) (*Erwinia carotovora*): The incidence of soft rot varied significantly with storage conditions (SC), Ca sources (CaS) and Ca concentrations (CaC). All the interactions except CaS x CaC were non-significant for soft rot incidence (Table 3). The soft rot incidence in relation to storage conditions was higher (21.62%) in tomato fruits stored at ambient temperature as compared to 18.49% soft rot incidence observed with storage at low temperature (Table 3). The means regarding CaS indicated the minimum soft rot incidence (6.11%) of tomato fruits dipped in calcium chloride solution. By contrast, the maximum soft rot incidence (24.84%) was recorded in tomato fruits dipped in calcium gluconate solution, followed by calcium lactate with soft rot incidence of 17.03% (Table 3). The soft rot of tomato fruit increased with decreasing Ca concentration in the dipping solution. The incidence of soft rot was the minimum (11.72%) in fruit dipped in solution containing 0.75% calcium, followed by 13.78% soft rot incidence observed with dipping the fruits in 0.5% Ca solution. The highest (25.17%) soft rot incidence was observed in control fruits (Table 3). The interaction CaS x CaC indicated that increasing Ca concentration decreased the soft rot incidence at all sources of Ca, however the increase was more prominent at 0.25% Ca made of calcium gluconate (32.74%). By contrast, the least soft rot incidence (3.50%) was recorded in tomatoes dipped in calcium chloride containing 0.75% calcium (Fig. 3).

Black rot (%) (*Alternaria alternata*): The black rot incidence of tomato fruits was significantly affected by storage conditions (SC), Ca sources (CaS) and Ca concentrations (CaC). The interaction of CaS x CaC was significant but the rest of the interactions were non-significant (Table 3). The black rot incidence of tomato fruit was higher (21.28%) in the fruits stored at ambient temperature than the 16.63% black rot incidence in the fruits stored at low temperature (Table 3). The means across CaS revealed that the highest (23.11%) black rot incidence was recorded in fruits dipped in calcium gluconate, followed by black rot 18.61% treated with calcium lactate solution. The least black rot incidence (13.17) was recorded by dipping tomato fruits in calcium chloride solution, which was closely followed by 13.39% fruits dipped in calcium sulphate solution (Table 3). The means across calcium concentrations revealed that the black rot incidence decreased with increasing CaC. The least black rot incidence (16.0%) was observed when the fruits were dipped in 0.5% calcium solution, followed by 16.83% for 0.75% Ca. The highest incidence of black rot (20.83%) was observed in fruits of control group (Table 3). The CaS x CaC interaction revealed that increasing Ca concentration decreased the incidence of black rot with calcium sulphate as calcium source but increased with calcium gluconate applied as calcium source. The black rot incidence was the highest (25.0%) when the fruits were dipped in 0.75% Ca solution made of calcium gluconate as calcium source and the least (11%) with 0.5% and 1% Ca from calcium sulphate as calcium source, respectively (Fig. 4).

Green mold (%) (*Penecillium digitatum*): The green mold incidence was significantly influenced by the storage conditions (SC), Ca sources (CaS) and Ca concentrations (CaC). The interaction CaS x CaC was significant and the rest of the interactions were non-significant (Table 3). The Green mold incidence of tomato fruit was higher (24.76%) in the fruits stored at low storage temperature as compared to 20.58% in fruits stored at ambient temperature (Table 3). Among the various Ca sources, the highest incidence of the green mold (21.57%) was recorded in fruit dipped in calcium lactate solution, which was followed by green mold incidence of 17.74% observed with calcium gluconate solution. The Green mold incidence was the least (10.04%) when tomato fruits were dipped in calcium chloride solution (Table 3). The incidence of the green mold also increased with decreasing calcium concentration. The least green mold incidence (13.73%) was observed with dipping the fruits in 0.75% calcium solution that was followed by 15.42% green mold incidence recorded in fruits dipped in 0.5% Ca solution. The maximum green mold incidence (29.93%) was observed in tomato fruits of control (Table 3). The CaS x CaC interaction revealed that increasing Ca concentration decreased the incidence of green mold at all sources of Ca. The increase in incidence was more prominent (28.88%) at 0.25% Ca concentration from calcium lactate source and the lowest (6.38%) in fruits dipped in calcium chloride solution of 0.75% calcium concentration (Fig. 5).

Table 2. Influence of calcium sources and concentration on calcium content (mg/100g DW), cell membrane ion leakage (%), and cell wall ion leakage (%) of tomato fruit.

Treatments	Calcium content (mg/100g DW)	Cell membrane ion leakage (%)	Cell wall ion leakage (%)
Storage Conditions			
Ambient Temperature	12.05	41.58	22.64
Low Temperature Storage	12.02	47.13	27.13
Significance	NS	*	*
Calcium Concentrations (%)			
0	9.20 [c]	46.83 [d]	26.83 [d]
0.25	12.14 [b]	43.83 [a]	24.71 [a]
0.5	12.41 [b]	41.54 [b]	22.71 [b]
0.75	12.89 [a]	40.25 [c]	21.38 [c]
$LSD_{(0.05)}$	**0.164**	**2.78**	**2.11**
Calcium Sources			
Calcium chloride	13.10 [a]	37.50 [d]	19.06 [d]
Calcium gluconate	12.13 [c]	45.78 [a]	27.28 [a]
Calcium lactate	12.20 [c]	43.78 [b]	24.94 [b]
Calcium sulphate	12.49 [b]	40.44 [c]	20.44 [c]
$LSD_{(0.05)}$	**0.189**	**3.21**	**2.44**
Interactions			
Source x Concentration	Fig 1	---	---
Significance	***	NS	NS
Source x Storage	---	---	---
Significance	NS	NS	**NS**
Concentration x Storage	Fig 2	---	---
Significance	**	NS	NS
Control x Storage			
Significance	NS	NS	NS
Source x Concentration x storage	---	---	---
Significance	NS	NS	NS

Means followed by similar letter(s) in column do not differ significantly from one another
NS = Non-significant and *, ** = Significant at 5 and 1% level of probability, respectively.

Table 3. Influence of calcium sources and concentration on soft rot (%), black rot (%) and green mold (%) of tomato fruit.

Treatments	Soft rot (%)	Black rot (%)	Green mold (%)
Storage Conditions			
Ambient Temperature	21.62	21.28	20.58
Low Temperature Storage	18.49	16.63	24.76
Significance	*	NS	*
0	25.17 [a]	20.83 [a]	29.33 [a]
0.25	19.31 [b]	18.38 [b]	18.87 [b]
0.5	13.78 [c]	16.00 [c]	15.42 [c]
0.75	11.72 [c]	16.83 [c]	13.73 [c]
LSD at α 0.05	2.81	1.85	2.76
Calcium Sources			
Calcium chloride	6.11 [d]	13.17 [c]	10.04 [d]
Calcium gluconate	24.84 [a]	23.11 [a]	17.74 [b]
Calcium lactate	17.03 [b]	18.61 [b]	21.57 [a]
Calcium sulphate	11.78 [c]	13.39 [c]	14.67 [c]
LSD at α 0.05	3.245	2.13	3.19
Calcium Concentrations (%)			
Interactions			

Source x Concentration	Fig 3	Fig 4	Fig 5
Significance	*	*	*
Source x Storage Conditions	---	---	---
Significance	NS	NS	NS
Concentration x Storage	---	---	---
Significance	NS	NS	NS
Control x Storage			
Significance	NS	NS	NS
Source x Concentration x storage	---	---	---
Significance	NS	NS	NS

Means followed by similar letter(s) in column do not differ significantly from one another
NS = Non-significant and *, ** = Significant at 5 and 1% level of probability, respectively.

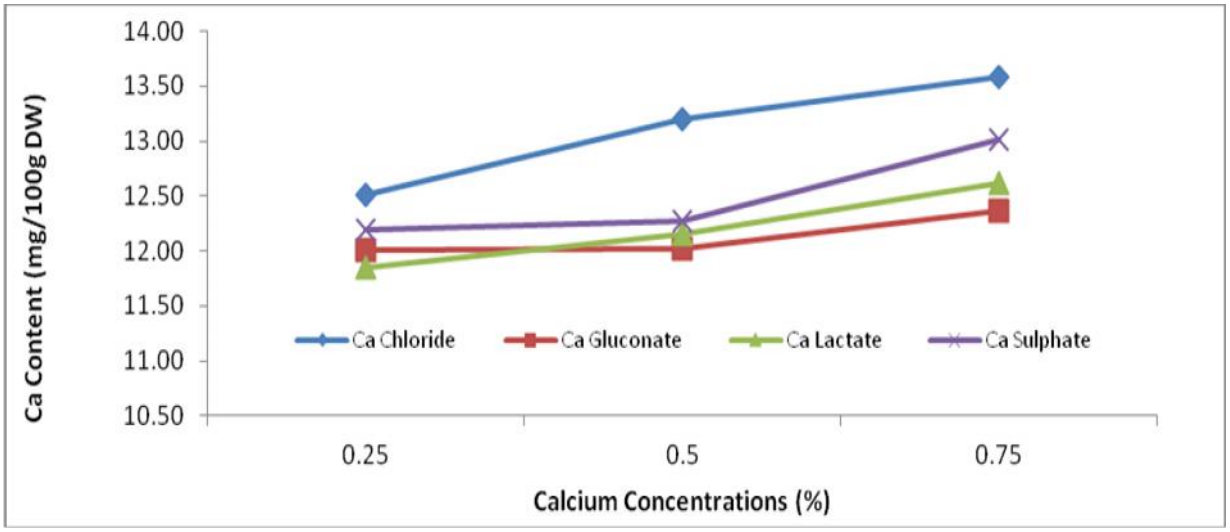

Figure 1: The interaction of calcium sources and concentrations for Ca content of tomato fruit. The vertical bars represent standard error.

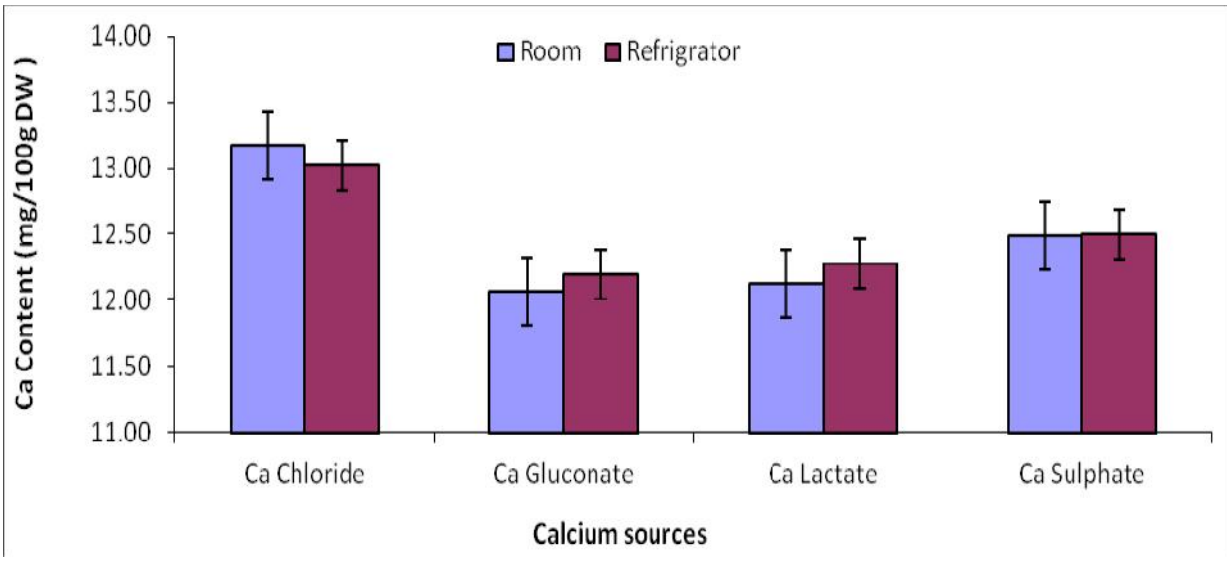

Figure 2: The interaction of calcium sources and storage conditions for Ca content of tomato fruit. The vertical bars represent standard error.

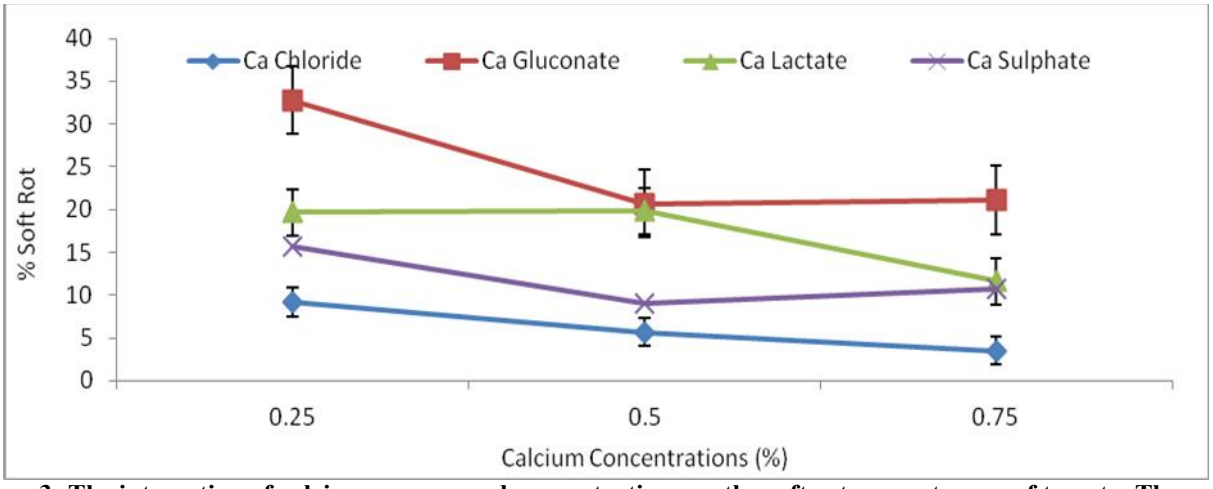

Figure 3: The interaction of calcium sources and concentrations on the soft rot percentage of tomato. The vertical bars represent standard error.

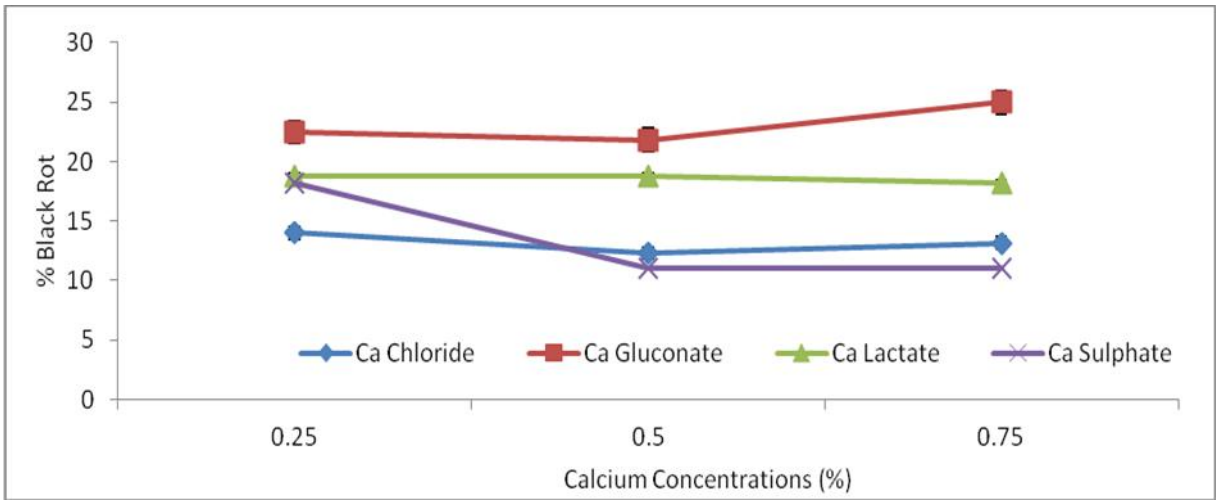

Figure 4: The interaction of calcium sources and concentrations on the black rot percentage of tomato. The vertical bars represent standard error.

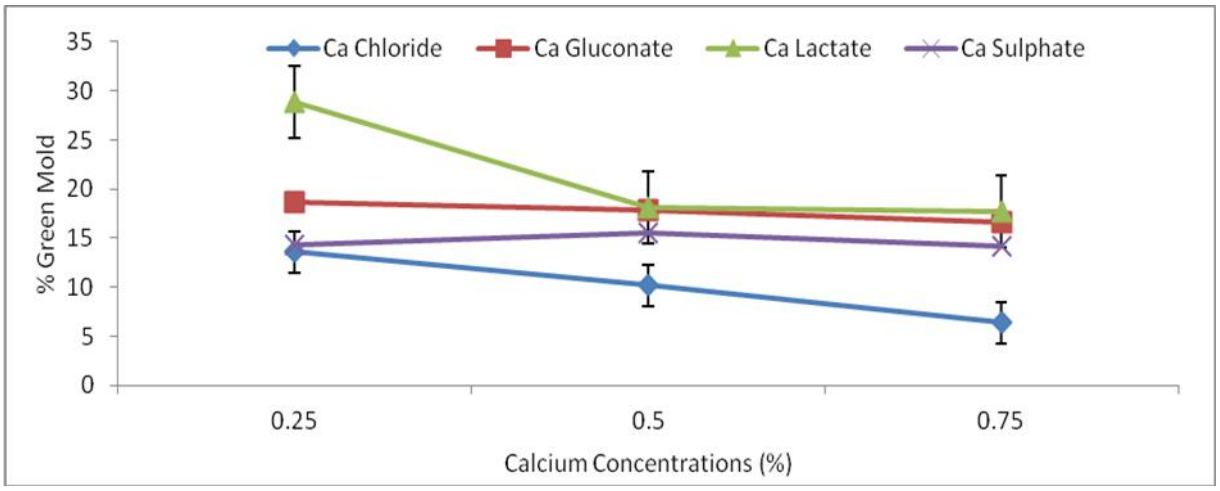

Figure 5: The interaction of calcium sources and concentrations on the green mold percentage of tomato. The vertical bars represent standard error.

DISCUSSION

Calcium content (mg/100 g DW): There was no significant effect of storage temperature on the Ca content of the fruits. The Ca content of the fruit was higher when treated with calcium chloride as compared to the rest of the Ca sources used. Ca contents increased linearly with increase in Ca concentration from 0 to 0.75%. The CaS x CaC interaction indicated that increasing Ca concentration and treating the tomato with calcium chloride as calcium source increased the Ca content of fruit significantly. Likewise, the CaC x SC interaction revealed that increasing Ca concentration increased the Ca content of the fruits regardless of the storage temperature. Calcium concentration in most plants ranges from 0.5-3% of dry matter (Peter, 2005). Calcium is required to promote resistance against biotic stresses (White and Broadly, 2003). Calcium is an important mineral constituent of middle lamellae. Softening of fruits is mainly due to weakening of middle lamellae during repining. Calcium helps to bind polygalacturonic acids with each other and increase the strength and rigidity of membrane. Thus, calcium may have delayed senescence and rate of respiration and transpiration in tomato fruits. Calcium treatments have been commercially used in apples to increase the shelf life and reduce the postharvest disorders (Sharma *et al*., 1996). Increased Ca^{+2} ions are found to decrease respiration and ethylene production rates in many fruit crops including tomato (Garcia *et al.,* 1995). Calcium is an important constituent of cell wall and cell membrane structure, co-factor of enzymes and required for nitrogen metabolism (Thompson and Fry, 2001; Peter, 2005). Being immobile in plants and high soil pH make it unavailable to the fruit so both pre and post harvest calcium applications are required to overcome the deficiencies. Three fold's increase in calcium content was reported by Wills *et al*. (1977) with 12% calcium chloride for a prominent retardation in tomato ripening. Calcium chloride uptake during dipping is through lenticles (Dewey, 1980). Senevirathna and Daundasekra (2010) found more calcium in tomato inner mesocarp and mentioned that stem end scar is the main entry point for calcium. Vacuum infiltration has also been found effective to promote the calcium content in mango and avocado (Shorter and Joyce, 1998). Storage temperature was found to be non-significant in increasing calcium content of tomato. This might be attributed to the calcium absorption occurred only during dipping duration up to the surface drying of fruit.

Cell wall and cell membrane ion leakage (%): The cell wall and cell membrane ion leakage was higher when fruits were stored at low temperature as compared with storage at ambient temperature. The calcium gluconate treated fruits had higher cell wall and cell membrane ion leakage as compared to rest of the sources. The cell wall and cell membrane ion leakage decreased with increasing Ca concentration from 0 to 0.75%. Non freezing temperature below 10°C causes chilling injuries in many plants including tomato (Saltveit and Morris, 1990). Symptoms like increased water loss, ion leakage, abnormal ripening, ethylene production and disease susceptibility develop slowly during chilling but rapidly developed when removed from chilling to non chilling temperature (Saltveit *et al*., 2004). Chilling is characterized by changes in protein (Hausman *et al*., 2000) and membrane structure resulting in more permeability and increased electrolyte leakage (Friedman and Rot, 2006). Techniques used to reduce the ion leakage in vegetables and fruits include plant tissue temperature, calcium dips, heat treatments, atmosphere with high CO_2 and low oxygen levels during chilling and storage at high humidity (Saltveit *et al*., 2004). Since some crucial membranes at chilling temperature are involved in causing chilling injury, fatty acids of membranes need to be altered for decreasing ion leakage. Calcium ions are known to enhance membrane stability and decrease plant cells senescence (Leshem, 1992; Torre *et al*., 1999; Rubinstein, 2000). Calcium applications decrease ion leakage and ultimately cell wall stability and integrity is increased (Mortazavi *et al*., 2007). Meng *et al*. (2009) explained the reason of low electrolyte leakage as less disruption in the plasma lemma membranes. Demarty *et al*., (1984) mentioned that improved cohesion of cell membranes by calcium treatment is the reason of decreased ion leakage. Cell wall tightening by Ca^{++} is due to cross linking between pairs of negatively charged homogalacturonans (Picchion *et al*., 1998). Severity of ion leakage is reduced by post harvest application of calcium in peaches (Wade, 1981), tomatoes (Moline and Teasdale, 1981) and avocado (Chaplin and Scott, 1980). Post harvest calcium application in plum was found effective in terms of integrity maintenance and membrane function with reduced losses of protein and phospholipids and decreased ion leakage (Lester and Grusak, 1990). In cold stress, calcium ions move from vacuoles and intercellular spaces to the cytoplasm. It is perceived that more calcium content present in the cytoplasm enhances the plasma membrane integrity to increase tolerance against cold stress. The results of this research are in line with Akhtar *et al*. (2010) who recorded lowest electrolyte leakage in loquat stored at 4°C with 3% calcium chloride dips. Mirdehghan and Ghotbi (2014) recommended 2% calcium chloride for decreased browning index in pomegranate, a typical sign of chilling injury.

Soft rot (%): The incidence of soft rot was higher in the fruits stored at ambient temperature compared to low temperature storage conditions. The incidence of soft rot was also lower when fruits were treated with calcium chloride compared to other Ca sources. Increasing Ca

concentration from 0 to 0.75% decreased the soft rot percentage on tomato fruits. The interaction CaS x CaC indicated that increasing Ca concentration from calcium chloride decrease the soft rot of tomato. *Erwinia carotovora* is the causal organism of tomato's soft rot. A glue called pectate holds the plant cells together and is broken by pathogen that liquefies fruit tissue (Bartz *et al*., 2012). Tomato tissue becomes watery, soft, foul smelling and slimy in tomato soft rot (Wood, 1998). Calcium plays a vital role in improving resistance against bacterial pathogen in plants (Berry *et al*., 1988). Higher calcium content provided more resistance against bacterial diseases. Calcium ions are involved in enhancing the integrity of plant cell wall components and improve the cell structure which ultimately results in increased resistance to disease involving maceration of tissues (Berry *et al*., 1988). Calcium pre and post harvest application is found to be effective in delaying ripening or reduce post harvest decay and control diseases in perishable horticultural commodities like vegetables and fruits (Poovaiah, 1986). Hajhamed *et al*. (2007) explored that salt compounds namely ammonium sulphate, potassium sulphate and calcium chloride significantly decreased the bacterial soft rot severity in potato. Calcium propionate and calcium chloride lowered tissue maceration in potato tubers due to *E. carotovora* attack (Biggs *et al*., 1997; Droby *et al*., 1997; Olivier *et al*., 1998). Warm temperature at about 30°C favors growth of pathogen and disease development (Perombelon and Kelmon, 1980), while low temperature at about 6°C limits pathogen growth especially those of bacteria. Although tomato storage at 6°C prevents bacterial soft rot, unfortunately tomato is chilling sensitive and prolong exposure to cold temperature caused injuries (Hardenberg *et al*., 1986). It is evident from this investigation that soft rot percentage was significantly declined at low temperature storage.

Black rot (%): The fruits stored at low temperature had lower incidence of black rot compared to the fruits stored at ambient temperature. The black rot incidence was lower in the fruits treated with calcium chloride. Increasing calcium concentration from 0 to 0.75% reduced incidence of black rot. The CaS x CaC interaction revealed that increasing Ca concentration and treating tomato fruit with calcium sulphate significantly decreased the incidence of black rot. Black rot appears as tiny lesions on the shoulders or around stem scar of tomato where cuticle cracks or some damages are found. Initially water soaked lesions are formed, turn to brown black with small pimple like structures having a covering of olive green or dark grey spores (Bartz *et al*., 2012). Black rot in tomato at very high frequency is caused by *Alternaria alternata* (Akhtar *et al*. 1994). Calcium, boron, and phosphorus play a vital role in protecting tomato against fungal as well as bacterial diseases (Graham, 1983). Mahmud *et al*. (2008) reported that calcium chloride applied at different concentration are found to be effective in controlling fungal diseases in stored fruits. Fungal decay in apple by *Botrytis cinerea* (Lien *et al*. 1997) and in peaches by *Monilinia fructicola* (Conway *et al*., 1987) was significantly reduced by calcium post harvest treatments. Basically calcium interferes with pectolytic enzymes activity of fungi and increase the resistance against diseases (Conway *et al*., 1992), decrease the maceration of cell walls caused by polygalacturonase cell wall degrading enzyme because of increase calcium content of cell wall and improved structural integrity (Conway *et al*., 1988). Saftner *et al*. (1997) found that increase calcium content of apple cell wall decreased the ploygalacturonase enzymes activity extracted from *P. expansum* culture. Role of Ca^{++} ions can be explained by its binding ability with intercellular pectic acids to form pectate chloride. Pectate chloride is resistant to polygalacturonase, a fungal pectolytic enzyme (Conway and Sams, 1984).

These results are in conformity with Irshad *et al*. (2014) who recommended 3.5% calcium chloride for control of postharvest fungal diseases up to 14 days. Lima (2000) recommended 6% calcium chloride treatment with 16°C storage temperature for reduced weight loss, respiration and control of peroxidase activity which ultimately delay ripening and postharvest decay in tomato. Torres *et al*. (2009) recommended 6% calcium chloride and 40°C heat treatment for 20 minutes of pine apple up to 6th day consumption. El-Mougy *et al*. (2008) recommended 2% calcium chloride for decreased incidence of grey mold in strawberry.

Green mold (%): Green mold incidence was higher in the fruits stored at low storage temperature compared to ambient temperature. The fruits treated with calcium chloride had lower incidence of green mold compared to the rest of the sources. The incidence of green mold decreased with increasing Ca concentration from 0 to 0.75%. The interaction of CaS x CaC revealed that increasing Ca concentration from calcium chloride decreased the green mold percentage of tomato fruits. Green mold initially appears as water soaked and slightly discoloured spot. Mycellium first appears white and with advancement enlarged in size of about 2 inches, turns to olive green colour spores easily spread with the air current (El-Gali, 2014). The *Penecilium digitatum* grows on temperatures ranging from 15 to 30°C. Mortality occurs when temperature is below 15°C and above 40°C (Sharma and Razak, 2003). The relative humidity required for *P. digitatum* growth is 87%, above 91-100% RH mycelial growth ceased. Calcium contribution in maintaining fruit firmness can be linked with disease resistance of plant (Maas, 1998). Exogenous or added calcium decrease susceptibility to postharvest disorders and diseases through enhancing resistance (El-Gali,

2014). Calcium increase resistance of fruit to plant pathogen through interaction with cell wall component. Pectolytic enzymes which are produced by postharvest pathogens caused softening of tissues (Conway *et al*., 1994). Calcium forms cationic bridges between pectic acids or between acidic polysaccharides and pectic acids by tightly binding to the cell walls pectin. Cell wall becomes resistant and less accessible to the pectolytic enzymes action (Moline, 1994; Conway *et al*. 1994). Pathogenic fungi use acids and sugars from host for their growth and release cell wall degrading enzymes at the same time (Prusky *et al*. 2001). Naradisorn (2013) mentioned that the higher the calcium content of apple fruit, the more resistance of cell wall to degrading enzymes, polygalacturonase and vice versa will be produced. Maouni *et al*. (2007) recommended 4–6% $CaCl_2$ for reduction of fruit decay in pear by *Penecilium expansum* and *Alternaria*. El-Gali (2014) found 6% calcium chloride for 90% control of *P. digitatum* in orange while lower concentration less than 2% was found less effective. Tain *et al*. (2002) recommended 2% calcium chloride for inhibition of *R. solonifer* spores germination and growth. Kaile *et al*. (1992) reported that 100–200 mM calcium ions showed decreased viability of *Botrytis* species isolates compared to 10 mM calcium ions. Likewise, Droby *et al*. (1997) reported that 272 mM (4% wt/vol) concentration of calcium chloride resulted in pronounced inhibition of *P. digitatum* spores' germination.

Conclusions: It was concluded that tomato fruits kept in cold storages (10±2°C and RH 60±5%) with $CaCl_2$ application at 0.75% decreased incidence of soft rot, black rot and increased fruit Ca content, followed by calcium sulphate. Tomato fruits stored at room temperature (32±2°C with RH 60±5%) showed better results in retaining ion leakage and incidence of green mold. Calcium gluconate proved to be poor in retaining the studied attributes. Hence, $CaCl_2$ at 0.75% is recommended for retaining postharvest losses in tomato fruits in cold storages.

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