

SHORT COMMUNICATION

Preharvest Calcium chloride Application Improves Postharvest Keeping Quality of Tomato (*Lycopersicon esculentum* Mill.)

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ABSTRACT

Developing fruits of local tomato cultivar 'Rajitha' were subjected to different preharvest calcium treatments in order to determine an effective means of extending the keeping quality of the commodity. Treatments were conducted with two calcium concentrations (3% and 5% w/v). There were two spraying protocols; single-application on fruit at 7 days after full bloom (DAFB) and multiple- application viz. weekly spraying starting from 7 DAFB until harvesting. Fruits were harvested at turning stage of colour development and allowed to reach red ripe stage under ambient conditions. Observations were made on the shelf life, titratable acidity (TA), total soluble solids (TSS) content, total calcium content in the outer pericarp and % weight loss during storage. Effect of calcium chloride treatment on susceptibility to fungal diseases was tested on fruits harvested at the 'turning stage' on wounded and non-wounded tissues. Rot development with respect to time was tested for both natural disease development and under artificial inoculation with fungal species isolated from tomato fruits. Calcium chloride treatment resulted in a 2.3 to 3.8-fold extension of shelf life of fruits compared to non-treated ones. Calcium treated fruits also exhibited a significantly higher firmness, total calcium content in the outer pericarp and TSS content but significantly lower fresh weight at harvest and a greater weight loss during storage under ambient temperature (27 ± 2 °C). Calcium treatment has no consistent relationship with the % TA of fruit. *Aspergillus niger* and *Rhizopus stolonifer* were identified as the main postharvest fungal pathogens in tomato. Disease severity was less in Ca²⁺ treated fruits and the significance of this effect varied between pathogenic fungal species. Irrespective of the Ca²⁺ treatment, wounded fruits exhibited higher disease severity under natural conditions and also upon artificial inoculation. The extended shelf life in calcium-treated tomato appears to be through higher retention of firmness and retardation of skin colour development.

Keywords: firmness; quality; shelf life; storage

INTRODUCTION

Calcium ions are known to be involved in many physiological processes in plant tissues (Simon, 1978) and hence are associated with many deficiency disorders in crops. Both preharvest and postharvest applications of calcium on fruits and vegetables have reported to play an important role in maintaining their quality. Calcium strengthens the cell wall structure by getting incorporated into the middle lamella, thereby increasing the firmness of fruit (Sams *et al.*, 1993). Also, increased Ca²⁺ levels reduce respiration and ethylene production rates in a variety of fruit crops, contributing to longer postharvest life (García *et al.*, 1995).

Preharvest calcium sprays have shown to be rather effective than soil applications since Ca²⁺ absorption into roots is poor (Toivonen and Bowen, 1999). Also, calcium moves slowly through the plant due to its relative immobility (Bangerth, 1979). Preharvest Ca²⁺ spray has reported to decrease brown rot development in peach

(Manganaris *et al.*, 2005), reduce scald incident in apples (Kadir, 2005) and cause resistance against *Botrytis* rot in strawberry (Wójcik and Lewandowski, 2003). Effectiveness of the method of CaCl₂ applications as a postharvest treatment differs among crops and the mode of application (Shorter and Joyce, 1998).

Tomato is one of the most widely consumed vegetables in the world. For fruits like tomato, postharvest handling is as critical as production practices due to their delicate nature. Postharvest losses may take place at any stage in the handling system from harvesting through storage and marketing to final delivery to the consumer. Due to its climacteric nature, tomato is highly perishable especially in tropical and subtropical areas. Nearly 30-50% of the produce is lost after harvest because of inadequate handling and preservation (Inaba and Crandall, 1986). In tomato, deficiency of calcium causes blossom-end rot and cracking (Simon, 1978). In general, the direct application of calcium to the fruit is the most effective method for

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increasing fruit calcium content, which could be accomplished by preharvest sprays, postharvest dips or vacuum or pressure infiltration (Trentham *et al.*, 2008). Foliar treatment of CaCl₂ on tomato is known to result in greater flesh firmness, quicker development of red colour, higher weight loss and soluble solids content (Garcia *et al.*, 1995). For green-harvested tomato, vacuum infiltration was found to be effective in rapid reduction of ethylene production and respiration rates, which was also dependent upon vacuum pressure and the concentration of CaCl₂ used (Wills and Tirmazi, 1979; Senevirathna and Daundasekera, 2010). There are no published records related to the effects of direct spray of Ca²⁺ on tomato fruits at pre-harvest level. This study was aimed at investigating the effects of preharvest calcium treatment on developing tomato fruits on postharvest keeping quality with respect to physico-chemical parameters and fungal rot development.

MATERIALS AND METHODS

Tomato cultivar 'Rajitha' was planted at the Horticultural Crops Research and Development Institute, Gannoruwa, Peradeniya (7° 16' 60N, 80° 34' 60E). Hundred seedlings of 21 days of age were transplanted in plastic pots (diameter - 28 cm, height - 24 cm) and then arranged in a randomized complete block design. Plants were grouped into 5 batches each with 20 replicates. Fertilization was done according to the recommendations of the Department of Agriculture (DOA Publication, 2005).

Calcium treatments included two distinct CaCl₂ concentrations as 3% and 5% (Garcia *et al.*, 1995). Each treatment was split into two different patterns of calcium application; single application on fruits 7 days after full bloom (DAFB) and multiple applications (weekly spraying on fruits starting 7 DAFB until harvesting). There were 5 treatments including T1: 5% spraying 7 DAFB, T2: 5% spraying weekly starting 7 DAFB until harvesting, T3: 3% spraying 7 DAFB, T4: 3% spraying weekly starting 7 DAFB until harvesting and T5: control fruits were treated with distilled water. Each 100 ml of CaCl₂ solution was mixed with 20 µl of a surfactant, Tween 20, in order to facilitate even spreading on fruits.

Experiment 1: Effect of CaCl₂ treatment on the shelflife and physico-chemical parameters of tomatoes

Fruits were harvested at turning stage of colour development (more green than yellow). Fruits without any defects and diseases were selected and transported to Plant Pathology Laboratory, Department of Botany, University of Peradeniya and stored under ambient temperature 27 ± 2 °C) and

relative humidity (65 ± 2 %), until they reached red-ripe stage.

Shelf life

Shelf life was estimated as the number of days taken to reach the red ripe stage of the peel. The peel colour was rated visually using a self-prepared scale of 0-5 based on the classification introduced by the U.S. Department of Agriculture (Anon, 1975). The colour development during ripening was 1 - turning; 2 - one third pink; 3 - two third pink; 4 - orange; 5 - red.

Red-ripe fruits from each treatment were randomly selected for the analysis of physico-chemical parameters including total calcium content of outer pericarp, firmness, titratable acidity (TA), total soluble solids (TSS) content, and loss of fresh weight of fruits.

Total calcium content of tomato tissues

The amounts of calcium in fruit tissues were determined by the method described by Shorter and Joyce (1998), with slight modifications. The outer pericarp region of fruits from each treatment were separated and dried at 110°C and ground into a powder. The powder was ashed at 625°C in a muffle furnace and then digested with 20% HCl. Digested ash was filtered and volumerized up to 50 ml. Ca content was estimated by atomic absorption spectrophotometer and the tissue Ca²⁺ concentration data were expressed as mg Ca²⁺/g dry weight.

Under other physico-chemical parameters tested, titratable acidity was measured by titrating 10 ml aliquot from extracted fruit juice with 0.1M NaOH using Bromothymol Blue as the indicator (AOAC, 1980). The total soluble solids (TSS) content (Brix°) in tomato juice was determined using a hand-held refractometer [Model, 121, Yagami International Ltd, Japan] (Wills and Ku, 2001). The weight difference between turning stage (at harvest) and red ripe stage was expressed as a percentage weight loss during storage. Fruit firmness was measured using a hand-held penetrometer (Forestry Suppliers Inc., U.K). One reading from each replicate fruit was taken from the middle part along the long axis of the fruit, and the mean was calculated.

Experiment 2: Effect of Ca²⁺ treatment on fungal rot development

Isolation of postharvest fungal pathogens of tomato

Fungal pathogens were isolated on to potato dextrose agar (PDA) from the diseased tomato fruits (Meredith, 1968) collected from the open market in Kandy, Sri Lanka. Koch's postulates were performed to confirm the pathogenicity of the isolates (Tortora *et al.*, 1997).

Effect of Ca²⁺ treatment on fungal disease development

Fungal disease development under natural conditions and artificial inoculation was investigated either on artificially wounded or non-wounded fruits. For artificial inoculation, fruits were inoculated with 25 µl of conidial suspension (10⁵ conidia ml⁻¹) of fungal species isolated from tomato fruits. All the fruits were incubated in a moisture chamber at room temperature (27±2 °C) and visual estimation of % disease area was reported daily.

Experiment design and data analysis

For analysis of physico-chemical parameters, 10 replicate fruits from each treatment were taken. Six replicate fruits for each treatment were used for disease-related experiments. Fruits were arranged in a complete randomized design and data analysis was done by ANOVA. Treatment means were separated using Tukey's pairwise comparisons at 5% level of significance ($p=0.05$).

RESULTS

Shelf life

All CaCl₂-treated fruits exhibited a significantly ($p < 0.05$) longer shelf life with respect to untreated fruits under ambient storage conditions (Table 1). However, single dose of Ca²⁺ application was more effective than multiple doses, in extending the shelf life, irrespective of the concentrations used. Application of 3% CaCl₂ 7 DAFB (T3) resulted in the longest shelf life (42 days) of tomato.

Fruit firmness

CaCl₂ treated fruits showed significantly ($p < 0.05$) higher firmness with compared to untreated fruits at the end of the shelf life (Table 1). However, there was no consistent relationship between fruit

firmness and different Ca²⁺ concentrations used.

Total calcium content of tomato tissues

The total calcium contents in the outer pericarp region of CaCl₂-treated fruits were significantly ($p < 0.05$) higher than those of untreated fruits (Table 1). However, there was no clear relationship between total calcium levels in tomato tissues and different Ca²⁺ concentrations or frequency of Ca²⁺ application.

Titrate acidity and total soluble solids content

There was no consistent relationship between the concentration of applied Ca²⁺ and the TA of fruits (Table 1). A significant ($p < 0.05$) increase in TSS content was evident in all Ca²⁺ treated fruits against non-treated ones.

Weight loss during storage

All Ca²⁺ treated fruits exhibited a significantly ($p < 0.05$) higher loss in fresh weight at the end of shelf life (Table 2). Also, Ca²⁺ treatment resulted a significant ($p < 0.05$) reduction of fruit weight as evident by the fresh weight data at harvest. The lowest fruit yield could be observed with the fruits treated with 3% CaCl₂ 7 DAFB (T3), which also exhibited the longest shelf life (Table 1).

Effect of Ca²⁺ treatment on fungal rot development

Mainly, two postharvest fungal pathogens, *Aspergillus niger* and *Rhizopus stolonifer* were identified. Also, a *Fusarium sp.* was isolated once during this study. Apparently, all Ca²⁺-treated fruits were less sensitive to fungal rot development than the non-treated ones (Table 3). However, this effect was significant ($p < 0.05$) only in fruits that were artificially inoculated with *R. stolonifer*. In general, wounded fruits exhibited significantly ($p < 0.05$) higher degree of disease severity irrespective of the Ca²⁺ treatment, both under natural incubation and artificial inoculation (Table 3).

Table 1. Variation in physico-chemical characteristics and shelf life in tomato fruits of cultivar 'Rajitha' with respect to different pre-harvest CaCl₂ treatments.

Treatment	Total Calcium content in the outer pericarp (mg/g dry weight)	TA	TSS Brix ^o	Firmness (g/cm ²)	Shelf life (days)
T1	6.02 ^a	5.59 ^a	4.83 ^a	0.7 ^a	41.66 ^a
T2	5.97 ^a	5.28 ^a	5.93 ^b	1.0 ^a	28.67 ^b
T3	6.70 ^b	4.83 ^b	5.73 ^b	0.9 ^a	42.67 ^a
T4	2.33 ^c	6.39 ^c	5.66 ^b	1.0 ^a	26.00 ^b
T5 (control)	1.36 ^d	5.90 ^a	4.06 ^c	0.5 ^b	11.00 ^c

T1- 5% CaCl₂ single application; T2- 5% CaCl₂ multiple application; T3- 3% CaCl₂ single application; T4- 3% CaCl₂ multiple application

Mean values in each column followed by different letters are significantly different at $p < 0.05$ level ($n = 10$)

Table 2: Variation in fresh weight at harvest and % weight loss during storage of tomato fruits of cultivar 'Rajitha' in relation to different preharvest CaCl₂ treatments.

Treatment	Fresh weight at harvest (g)	% weight loss during storage
T1	55.67 ^a	11.13 ^a
T2	62.98 ^{ab}	11.64 ^a
T3	42.43 ^b	9.92 ^a
T4	74.60 ^c	9.18 ^a
T5 (control)	79.63 ^c	6.20 ^b

T1- 5% CaCl₂ single application; T2- 5% CaCl₂ multiple application; T3- 3% CaCl₂ single application; T4- 3% CaCl₂ multiple application

Mean values in each column followed by different letters are significantly different at $p < 0.05$ level ($n = 10$)

Table 3: Effect of preharvest Ca²⁺ treatment and postharvest wounding on fungal disease development at 14 days of incubation.

Treatment	% area of disease development		
	Natural	Artificial inoculation with	
		<i>A. niger</i>	<i>R. stolonifer</i>
Ca ²⁺ treated, wounded	49.7 ^{Aa}	57.0 ^{Aa}	11.5 ^{Aa}
Ca ²⁺ treated, not wounded	5.5 ^{Ab}	2.2 ^{Ab}	1.8 ^{Ab}
Ca ²⁺ not treated, wounded	73.3 ^{Aa}	58.3 ^{Aa}	27.5 ^{Ba}
Ca ²⁺ not treated, not wounded	18.5 ^{Ab}	6.5 ^{Ab}	5.8 ^{Bb}

Values in each column followed by different letters are significantly different at $p < 0.05$ level ($n = 6$).

Uppercase letters denote Ca²⁺ treatment effect and the lowercase letters denote wounding effects.

DISCUSSION

Pre-harvest spray of calcium is known to extend the keeping quality of a number of soft fruits including cherry (Alonso *et al.*, 1995), peach (Manganaris *et al.*, 2005), strawberry (Singh *et al.*, 2006) and apricot (El-Motty *et al.*, 2005). The present study revealed that the direct application of calcium on developing fruits is effective in retarding the colour development and thereby the shelf life. Our observations contradicts with that of García *et al.* (1995) where they observed a quicker development of fruit colour upon preharvest foliar application with calcium. These different results may be due to differences in cultivar and/or the plant organ, which was subjected to calcium treatment.

Calcium treatment was consistently effective in increasing the total calcium levels in tomato pericarp tissues. Reasons for the retardation of skin colour development may be an effect of CaCl₂ on the ethylene generating cycle, which affects the synthesis of pigment lycopene during the process of ripening (Njoroge *et al.*, 1998). The change in

tomato fruit colour also depends upon the mode of calcium application, maturity of fruits and storage temperature (Moline, 1980).

CaCl₂ treated tomato fruits exhibited a significantly ($p < 0.05$) higher firmness than non-treated ones. This stability of the cell walls has been attributed to the formation of cation cross bridges between uronic acid groups (Sams *et al.*, 1993) as evident with elevated firmness levels of apples (Wienke, 1980). Generally, calcium is found in the cell walls in the form of calcium pectate (Agusti *et al.*, 2004). The higher firmness at red-ripe stage of tomato may be due to retarded action of polygalacturonase enzyme (Doesburg, 1975) which mediates the degradation of pectic material during ripening.

Effects of calcium treatment on fruit sensory parameters including TA and TSS have found to be commodity dependent (Kadir, 2005). In the present study, % TA of fruits did not exhibit a consistent relationship with the concentration or frequency of calcium application. However, a significant ($p < 0.05$) reduction of % TA was observed with the fruits with the longest shelf life

(T3: single application of 3% CaCl₂). This may probably due to the reduction of TA levels with significantly prolonged storage period (Dris and Niskonen, 1999). TSS content in calcium-treated fruits was significantly higher than untreated fruits, which is in accordance with the responses against both foliar application (García *et al.*, 1995) and postharvest vacuum infiltration (Senevirathna and Daundasekera, 2010) with tomato. The elevated levels of TSS content could be due to the acceleration of α -amylase activity in fruit tissues at elevated calcium levels (Marschner, 1986).

Although calcium treatment was reported to increase the individual fruit weight and thereby the yield of many crops (Singh *et al.*, 2006), calcium-treated tomato cv. 'Rajitha' fruits exhibited a significant reduction of fresh weight. Though the reason behind this observation is not clear, in certain plant parts, like photosynthesizing leaves, elevated calcium levels could decrease the permeability to sucrose thereby suppressing the phloem loading process (Anderson, 1983). Also, % weight loss (moisture loss) is significantly higher in calcium-treated fruits during storage. Since calcium-treated fruits took longer time to reach red-ripe stage, storage under ambient conditions until such time may have provided higher chances of direct transpiration from fruit surface leading to higher fresh weight loss.

There are number of postharvest fungal pathogens which have been recorded in tomatoes including *Alternaria solani* which causes Alternaria rot and *Colletotrichum* sp. which causes anthracnose disease (Snowdon, 1992). But in this study, only *Aspergillus niger* and *Rhizopus stolonifer*, both wound pathogens, were identified as main causative organisms of postharvest rots. This could be due to the application of fungicides at preharvest stage which may have led to less postharvest disease incidence. It is also possible that this cultivar of tomato is resistant for the above diseases.

Ca²⁺ treatment has lowered disease development by fungal pathogens in tomato. However, the degree of the effectiveness appears to be dependent on the pathogen species. This could be due to the increased stability of the cell walls upon Ca²⁺ treatment. However, significantly higher level of fungal growth on artificially wounded fruit may be due to free passage into fruit tissues and easy access to nutrients (Elad and Evensen, 1995).

Overall, this study reveals that the preharvest calcium sprays on immature fruits could be used in extending the postharvest longevity of tomato mainly through increased firmness, delayed skin colour development and to some extent, through

retarded fungal rot development in fruits. However, the reduced fresh weight of tomato fruits at harvest can be considered as a negative effect associated with the calcium treatments used.

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