THE EFFECT OF CALCIUM CHLORIDE POSTHARVEST DIPS AND CONCENTRATIONS ON THE IMPROVEMENT OF STORAGE AND SHELF-LIFE OF 'CLASSIC ROUND' TOMATOES (*SOLANUM LYCOPERSICUM,* L.)

BY

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DECLARATION

I, Keitumetse Delician Matsunyane declare that the mini-dissertation report hereby submitted to the University of Limpopo, for the degree Master of Science in Horticulture has not been previously submitted by me or anybody for a degree at this or any other university. In addition, this is my work in design and in execution, and related materials contained herein had been duly acknowledged.

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Signature Date

DEDICATIONS

This study is dedicated to my late grandfather (Lehlohonolo Aaron Matsunyane), my grandmother (Tshokolo Jenette Matsunyane), my mother (Anna Maleane) and my son (Letlotlo Matsunyane).

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ABSTRACT

Tomato is popularly consumed as fresh vegetable or processed product due to its nutritional and health benefits. However, due to its high perishability, tomato cannot be stored for longer duration. Therefore, the aim of this study was the determination of appropriate dipping times into different calcium chloride concentrations to preserve the postharvest quality, storage and the shelf-life of tomato fruit. 'Classic round' tomato fruit were harvested at their pink maturity stage. The experiment was carried out as a completely randomized design (CRD), factorial arranged as 4 × 3 × 8. Treatment factors were: 4 × $CaCl_2$ (0, 0.0045, 0.01 and 0.03%), 3 × dipping times (0, 30 and 60 minutes) and 8 × shelf-life (0 - 7 days). Fruit were stored at 15º C for 30 days, thereafter, held under room temperature for 0 - 7 days of shelf-life while collecting data. During shelf-life period, fruit were evaluated for weight loss, firmness, colour, TSS, TA, pH, physiological and pathological disorders. The interaction between the treatments and dipping times showed a significant effect on weight loss, firmness, colour parameters [L*, b*, chroma and hue angle (º)], total soluble solids (TSS), titratable acidity (TA), pH, decay and black mould occurrence. However, significant interactive effects were not shown on a* colour component and chilling injury. In conclusion, calcium chloride ($CaCl_2$) improved the quality and shelf-life of 'Classic round' tomato fruit. Calcium chloride concentration 0.01% was effective at 30 minutes dipping time, meanwhile, 0.03% $CaCl_2$ was effective at 60 minutes dipping time. Therefore, 0.01 and 0.03% can be recommended for commercial preservation use for tomato fruit quality and shelf-life.

Keywords: Chemical parameters; firmness; physiological disorders; postharvest losses.

CHAPTER 1

GENERAL INTRODUCTION

1.1. Background

Tomato (*Solanum lycopersicum,* L.) belongs to the Solanaceae family and cultivated around the world in the tropical and subtropical regions (Senevirathna and Daundasekera, 2010). Globally, the production of tomato amount to approximately 37.38 million metric tonnes (FAO, 2019) with China contributing 50 million tonnes followed by India with 17.5 million tonnes (Arah *et al*., 2015). South Africa has an annual tomato production at around 600 000 tonnes contributing 24% of the total vegetable production (DAFF, 2016). Tomato is popularly consumed as a fresh vegetable or processed product and there is an increased interest among consumers because of the awareness of its potential health benefits and nutrition (Szabo *et al*., 2018). It is a major contributor of carotenoids (lycopene), phenolics, vitamin C and small amounts of vitamin E in daily diets (Lenucci *et al*., 2006).

However, its shelf-life is limited due to qualitative losses which occur during postharvest handling. This is due to rapid respiration, ethylene evolution, firmness loss, weight loss and susceptibility to both physiological low temperature (chilling injury) and pathological disorders (Arthur *et al*., 2015). According to Serrano *et al*. (2008), tomato fruit ripening is associated with series of degradative biochemical reactions. The biochemical changes include an increase in respiration and ethylene production, fruit acidity changes, starch, and sugar content (Padmanabhan *et al*., 2016). The rapid increase of ethylene and respiration rate can be related to an increase in the fruit's storage temperature and the mechanical damage that occurs during postharvest handling (Martínez-Romero *et al*., 2004). Mechanical damage can serve as an entry point of disease-causing pathogens, which are responsible for causing decays on the fruits (Chakraborty *et al*., 2018).

The quality of tomatoes must be well maintained throughout the postharvest storage (Tolesa and Workneh, 2017). Storing tomatoes under low temperatures of about 10-15°C slows down their metabolic processes. According to Cantwell (2001), higher metabolic rate results in faster ripening, meanwhile, slow ripening process results from lower metabolic rate. Although low temperatures at 10°C favours extended

storage life of tomatoes, it is however regarded as the critical point and any temperatures below has a disadvantage on the fruit quality (Žnidarčič *et al*., 2010). These temperatures cause chilling injuries, which can also lead to uneven ripening and fungal infestation (Lee *et al*., 2008; Babitha *et al*., 2010). To extend the shelf-life and quality of tomato fruit without further decrease in storage temperature, postharvest treatments such as calcium chloride are recommended together with a temperature range of 10-15°C (Bhattarai and Gautam, 2006). Calcium chloride is nontoxic and serves as a detoxifying agent (Dhiman *et al*., 2021)

Calcium is known to be involved in many physiological processes in fruit and vegetables, whilst, playing an important role in maintaining fruit quality (Gao *et al*., 2019). In the cell wall and the exterior of the plasma membrane calcium is bound in an exchangeable form (Mirdehghan and Ghotbi, 2014). It serves as a binding agent in the form of calcium pectate in the cell wall (Arthur *et al*., 2015). Therefore, this helps to maintain the quality and extend the storage life by delaying ripening and senescence as well as reducing respiration rate and physiological disorders (Cheour and Souiden, 2015). Senevirathna and Daundasekera (2010) found that 'Thilina' tomato fruit treated with calcium chloride exhibited firmer texture. According to Arthur (2014), firmer texture on 'Power' tomato fruit was associated with calcium chloride linked to reduced action of polygalacturonase, which is an enzyme that facilitates the degradation of pectate during ripening.

1.2. Problem statement

Due to high perishability caused by its climacteric nature, tomato fruit cannot be stored for longer duration. The increased ethylene production and respiration rate after harvest leads to drastic loss of firmness due to high activity of cell wall degrading enzymes (Maduwanthi and Marapana, 2019). Calcium has been found to improve the rigidity of cell walls and obstruct polygalacturonase from reaching their active sites, thereby retarding tissue softening and delaying ripening (Nirupama *et al*., 2010). Firmness and resistance to softening resulting from the presence of calcium has been attributed to the stabilization of membrane system and formation of Ca-pectates, which increase rigidity of the middle portion and cell wall of the fruit (Das and Kim, 2010). However, calcium uptake and translocation into the fruit is a complex process, consequently always low to facilitate membrane integrity and longer storage. Therefore, research should be done on the appropriate calcium

application concentration in the form of $CaCl_2$, and time of application, on their potential to improve the storage and shelf life of tomato fruit.

1.3. Rationale of the study

Tomatoes have a limited shelf life at ambient conditions and are highly perishable (Haile and Safawo, 2018). Different chemicals are being used during postharvest stage to preserve shelf life and enhance quality (Arah *et al*., 2016). These treatments reduce tomato postharvest losses estimated at 10.2% in South Africa (FAOSTAT, 2014), leading to income loss to the farmers. Among these treatments, calcium has been a successful practice adopted by the food industry due to multiple benefits such as reduced physiological disorders and ethylene production, meanwhile enhancing resistance to pathogen infection (Francis *et al*., 2012). Calcium also has the ability to protect and enhance the tomato fruit quality by stabilizing and strengthening the cell wall (Hocking *et al*., 2016). However, lack of knowledge on the appropriate dipping times at a given concentration reduces the full potential of calcium to react accordingly. Therefore, findings on calcium concentrations with corresponding application times, resulting in efficient translocation of calcium to the fruit tissue, would assist in preserving the tomato quality. Additionally, contributing to postharvest losses reduction and increasing the percentage of tomato's economic contribution to the country.

1.4. Aim

The aim of the study was determination of the appropriate dipping times into different calcium chloride concentrations to preserve the postharvest quality, storage and the shelf-life of tomato fruit.

1.5. Objective

To determine whether calcium chloride concentrations and dipping times had an effect on the storage, shelf-life and quality of tomato fruit.

1.6. Hypothesis

Different calcium chloride concentrations and dipping times had no effect on the storage, shelf-life and quality of tomato fruit.

1.7. Structure of mini dissertation

Chapter 1: Provides the background of the study and the importance of tomato fruit. It also states the problem statement, rationale and the study aim and objective.

Chapter 2: The reviews of previous and relevant work pertaining to the study; calcium transport and translocation in tomato fruit. The effect of calcium on physical, chemical and physiological and pathological disorders.

Chapter 3: Provides materials and methods used during the data collection and analysis.

Chapter 4: Focuses on interpreting the results and discussing them based on findings by other authors.

Chapter 5: Provides summary, conclusions, and recommendations of the study.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

In recent years, tomato production has increased due to economic and nutritional importance of the crop (Nicola *et al*., 2009). In addition to the nutritional benefits derived from tomatoes and tomato-based products, the crop serves as a source of revenue to smallholder and commercial farmers (Sarma, 2018). In South Africa, tomato crop contributes to foreign exports earning, thereby, adding 17% to the Gross Domestic Product (GDP) (DAFF, 2019). However, the major problem with tomato is with its climacteric ripening characteristics, which results in increased ethylene and respiration rate (Valero *et al*., 2002). According to Zhang *et al*. (2009), ethylene triggers the biochemical and physiological processes which induce the fruit softening. Fruit softening has been associated with alterations in the pericarp cell wall; specifically, middle lamella pectin breakdown (Chylińska *et al*., 2017). In general, polygalacturonase (PG) and pectin methylesterase (PME) are the major enzymes involved in the softening process, due to pectin degradation (Wang *et al*., 2018).

Calcium is known to decrease pectin methylesterase (PME) activity, which results in decreasing pectin solubility due to enhanced calcium-binding to de-esterified galacturonic acid units (Anthon *et al*., 2005). Therefore, calcium treatments including calcium chloride ($CaCl_2$) were found to have potential in reducing cell wall degrading enzyme activity, thus, reducing fruit softening. However, there is limited literature on the calcium chloride ($CaCl_2$) concentration required for postharvest application and dipping duration on tomato fruit. Therefore, this chapter would discuss work done and not yet done on research problem.

2.2. Calcium absorption and translocation in the fruit

Calcium is a nutritional element sufficiently available in the soil but deficient in the fruit due to its immobility within plant cells (Saure, 2005). In the cell wall and membrane, calcium ions (Ca^{2+}) play an important role as counteracting ions for inorganic and organic anions in the vacuole (Gao *et al*., 2019). Furthermore, calcium also acts as a crucial second messenger, representing one of the most versatile

signalling molecules in all eukaryotic organisms (Chigri *et al*., 2012). Moreover, calcium plays a very important role in signal transduction pathways, and these calcium messages must be decoded and amplified by calcium-binding proteins (calcium sensors) to carry out the appropriate responses, including the expression of calmodulin (CaM) and calmodulin-like (CML) proteins (Kamthan *et al*., 2015), calcineurin B-like proteins (CBL), and calcium-dependent protein kinases (CDPK) (McCormack *et al*., 2005). However, calcium deficiency has become a problem in fruits causing large economic loss (Gayed *et al*., 2017).

The exogenous application of calcium has been found to be the most effective way to increase calcium amounts in the fruit (Martins *et al*., 2020). Calcium chloride ($CaCl_2$) is the most common calcium source used during fruit postharvest treatments (Kazemi, 2013). According to Bitange *et al*. (2021), $CaCl_2$ had the ability to increase internal calcium amount of mango fruit cv. 'Van dyke' through dipping and vacuum infiltration treatments. In the fruit flesh, increasing calcium affect some changes associated with ripening and senescence (Ferguson, 2006). However, studies have found calcium absorption and translocation in the fruit to be extremely complex (Hocking *et al*., 2016). According to Fernández and Brown (2013), there are many factors that influence calcium uptake from solution and distribution within the plant organs including fruits. Trentham *et al*. (2008) found that ‘Golden delicious' apple fruit surface properties, particularly cuticle, affect absorption and distribution of postharvest applied chemicals. The number of cracks on the fruit surface also determines the calcium uptake amount in the fruit (Saure, 2005). In addition, the calcium absorption does not solely depend on fruit surface permeability but also on the amount retained and the concentration (Schlegel and Schonher, 2002). According to Swietlik and Faust (2011), the reduced capacity of pectins to bind calcium due to high esterification degree also determines the absorbed calcium availability in the fruit.

Subsequent to the absorption of calcium through lenticels and cracks on the cuticle, the calcium signal is decoded, amplified and relayed by calcium sensors; calmodulin (CaM) or cam-like (CML) and carbonyldipyrrinone (CDPK) (Gao *et al*., 2019). The calcium sensors transmit the signal to the downstream targets including, transporters, kinases, transcription factor and metabolic enzymes, therefore, triggering specific responses like, respiration and ethylene rate (Pirayesh *et al*.,

2021). Moreover, calcium as a signal ion, participates in hormone synthesis directly, which could affect the fruit ripening process (Xiong *et al*., 2021).

2.3. Effect of calcium on physical properties

2.3.1. Effect of calcium on fruit weight loss

In fruits, weight loss occurs during the transpiration process through the fruits' surface, which is a normal metabolic process resulting in shrivelling and deterioration (Turmanidze *et al*., 2017). According to Kowalczyk *et al*. (2017), respiration is the chemical process by which fruits and vegetables convert sugars and oxygen into carbon dioxide, water, and heat. Meanwhile, transpiration process is the transport of moisture through the skin of the commodity, the evaporation of moisture from the commodity surface and the convective mass transport of the moisture to the surroundings (Rennie and Tavoularis, 2009). According to Duan *et al*. (2013), the tomato fruit respiration rate is related with mechanical damage, the temperature and humidity. Van-Hung *et al.* (2011) found that 'Chikuyo' eggplant, 'Kyomizori' mizuna and 'Toyomitsu-hime' fig fruit weight loss metabolic process depends on the gradient of water vapour pressure between the surrounding atmosphere and the fruit tissue. In 'Gootya' tomato fruits, calcium treatment was responsible for the delayed senescence and rate of respiration and transpiration (Bhattarai and Gautam 2006). This was due to calcium abilities to bind the polygalactonic acids to each other; and therefore, resulting in strong and rigid cell membranes (Paliyath and Murr, 2008).

In a study conducted by Kabir *et al*. (2020), weight loss percentage increased over the storage period for all tomato cultivars ('Dabol-large', 'Sense q' and '7160'). Moreover, Mahajan and Dhatt (2004) reported that 'Asian' pear fruit treated with $CaCl_2$ proved to be most effective in reducing weight loss when compared with non-treated fruit during a 75-day storage period. Correspondingly, Shirzadeh *et al*. (2011) found that dipping 'Jonagold' apples in calcium chloride ($CaCl_2$) reduced the weight loss percentage. According to Turmanidze *et al*. (2017), the lower weight loss in 'Killarney' raspberry and 'Red dream' strawberry treated with calcium chloride may be due to increased water holding by the calcium pectate hydrogel formation, and a delay in the dehydration process.

2.3.2. Effect of calcium on firmness

Firmness is the most important factor in the quality of tomato, which is closely associated with ripening stage (Wu and Abbott, 2002). The mechanisms involved in fruit firmness maintenance depend primarily on the species and cultivar (Martínez-Romero *et al*., 2007). According to Kalamaki *et al*. (2006), firmness is associated with a group of polysaccharides in the cell wall called the pectins, which are responsible for the cell wall architecture, functions in signalling, cell-to-cell adhesion and determining the wall porosity. Wang *et al*. (2018) found that the pectin disassembly during ripening which is related to the development of soft fruit texture, through the degradation of pectin by pectin-methylesterase (PME) and polygalacturonase (PG) enzymes.

Calcium acts as intracellular messenger and responsible for maintaining the plant cell wall integrity (Barman *et al*., 2018). Sati and Qubbaj (2020) observed that 'Izmir' tomato fruit treated with 6% $CaCl_2$ maintained higher firmness when compared with control fruit. Similarly, 'Earli Grande' peaches dipped in $CaCl_2$ (1, 2 and 3%) retained firmness more than control fruit (Sohail *et al*., 2015). The results were due to calcium chloride ($CaCl_2$) acting as a binding agent in the cell wall to form calcium pectate, which increases the rigidity of the middle lamella and cell wall (Khaliq *et al*., 2015). Additionally, Pila *et al*. (2010) found that calcium also inhibited cell wall activity degrading enzymes on 'Duke' tomato fruit, so the outer membrane for the cell wall became stronger and rigid. According to Sharma and Pratima (2018), cell wall integrity is also preserved when de-esterified pectic acid residues form cross-bridges between negatively charged carboxylic groups and divalent cations such as calcium, thus minimizing pectic substance solubilisation. Furthermore, calcium chloride application assist in reducing fruit respiration rate thus slowing down the ripening process and maintaining fruit firmness (Sohail *et al*., 2015).

2.3.3. Effect of calcium on colour development

Colour is one of the major visual quality parameter that affects consumer acceptance of fresh products including tomato fruit (Nasrin *et al*., 2008). In tomato fruit, external colour is the result of both flesh and skin colour (Scheerlinck *et al*., 2006). During normal fruit ripening process, rapid chlorophyll degradation occurs in the tissues with increased carotenoids and coloured pigment level. Sadali *et al*. (2019) found that, at an early stage in the ripening process of bell pepper and tomato fruit chloroplast

thylakoid membranes, starch grains and chlorophyll pigments are broken down. Thereafter, new carotenoid pigments such as β-carotene and lycopene, which are responsible for the orange and red colours of tomato, accumulate in the plastid (Yuan *et al*., 2015). Fu *et al*. (2016) found that ripening-associated genes through the characterization of their mutants in 'Micro tom' tomato fruit, including *rin* (ripening-inhibitor), *Cnr* (colourless non-ripening), *gr* (green-ripe), *gf* (green flesh), *hp* (high pigment), and *Nr* (never ripe), affect fruit ripening and colour formation. Similarly, water loss through the membrane also causes the darker appearance of the fruit (Nunes *et al*., 2005).

Tomato fruit colour can be determined using objective colour parameters such as L*, a*, b*, chroma (C*) and hue angle (º) (Batu, 2004). According to Bui *et al*. (2010), lightness factor, L∗, decreased during the 'Savoura' tomatoes ripening stages (turning to red stage). This decrease reflected the tomatoes darkening with carotenoid synthesis and greenness loss. Lopez-Camelo and Gomez, (2004) found that 'Kada' tomato a∗ value (green-red axe) increases during maturation as a consequence of the synthesis of lycopene (red) and depletion of chlorophyll (green), representing a colour change from green to red. The 'Savoura' tomato b∗ value (blue-yellow axe) decreased during ripening showing that the effect of the yellow from the β-carotene was smaller when compared to the red of lycopene (Bui *et al*., 2010). In 'Naomi F1' tomato fruit, chroma value changed slightly during maturation, and thus, it did not show a good correlation with the ripening stages of tomato (Raffo *et al*., 2002). In addition, Hurr *et al*. (2005) observed that 'Florida 47' hue angle values declined with advancing ripening.

Studies have reported that calcium effect on metabolic activities contribute to fruit colour development. Erbaş and Koyuncu (2021) found that postharvest calcium treatment delayed the 'Ziraat' sweet cherries colour deterioration. The delaying colour change or darkening by calcium treatments may be associated with its prevention of senescence (Wang and Long, 2015). Similarly, 'Izmir' tomato colour development was delayed when treated with 6% calcium chloride ($CaCl_2$) (Sati and Qubbaj, 2021). According to Park *et al*. (2018), colour development delay was due to the decreased carotenoids biosynthesis and chlorophyll content preservation. However, cherries cv. 'Vogue' decreased in colour parameters [L*, chroma (C*) and

hue angle (º)], measured after 23 days of storage were not affected by calcium treatment (Tsantili *et al*., 2007).

2.4. Effect of calcium on chemical properties

2.4.1. Effect of calcium on total soluble solids (TSS)

Total soluble solids refer to dissolved solids within a substance (Li *et al*., 2016). According to Beckles (2012), soluble sugars and organic acids are the major components of the soluble solids. These components and their interaction are important for tomato quality and for processed concentrate as they affect sweetness, sourness and flavour intensity (Tigist *et al*., 2013). Kaur *et al*. (2006) found that 'Castle rock' tomato fruit showed an increase in TSS from 4.15 to 6.62 g/100 g as ripening progressed from green to ripe stage. However, Javanmardi and Kubota (2006) observed that at postharvest storage period the 'Clermon' tomato total soluble solids (TSS) showed no significant changes in both room temperature and low temperature of 10-15°C. According to Menéndez *et al*. (2006), TSS increase occur due to starch hydrolysis and polysaccharides of the cell wall, therefore, resulting in soluble sugars. The greater water loss through transpiration when compared with carbohydrates loss produced by respiration causes starch hydrolysis (Hernández-Muños *et al.,* 2006).

There are conflicting results about the effect of various calcium salts on various fruits soluble solids. Li *et al*. (2014) observed that postharvest calcium chloride treatment on 'Lingwu long' jujube fruit did not affect TSS. Similarly, Hussain *et al*. (2012) found that 'Red delicious' apples treated with calcium chloride (0.4, 1, 1.5 and 2%) treatment had no significant effect on preventing the TSS as well as total sugar increase when compared with control. However, Eric *et al*. (2015) observed contradicting results, whereby, 'Power' tomatoes TSS increased with storage days and fruit dipped in $CaCl_2$ (2 and 6%) showed less increase when compared with control. Subsequent to $CaCl_2$ delaying the natural physiological processes such as; ripening, senescence, and respiration, it is also responsible for TSS increase and decrease due to inhibitory effect on the activities of enzymes involved in hydrolysis (Hussain *et al*., 2012).

2.4.2. Effect of calcium on titratable acidity

Titratable acidity is directly related to the concentration of organic acids present in the fruit, which are an important parameter in maintaining fruit quality (Tyl and Sadler, 2017). According to Saddler and Murphy (2010), fruit titratable acidity decreases with increase in shelf-life. Similarly, the 'Holand' tomato titratable acidity showed a reduction after 14 days of storage (Hao *et al*., 2020). A decline in acidity level was attributed to the consumption of organic acid for metabolism activity including respiration in 'Roma vf', 'Melkasalsa and Melkashola' tomato fruit (Tigist *et al*., 2013). In addition, Akhtar *et al*. (2010) suggested that acidity decreases due to fermentation or break up of acids to sugars in 'Surkh' loquat fruit during respiration.

In 'Chinese' jujube fruit, the postharvest calcium nitrate (1%) application increased TA, whereas calcium chloride and calcium sulfate did not affect TA (Moradinezhad *et al*., 2019). Meanwhile, Reyes-Medina *et al*. (2017) observed that 'Cape' gooseberry acidity treated with calcium chloride ($CaCl_2$) increased over time, from the beginning of storage and up to day 14. Thereafter, from day 14 until the last day of the postharvest period, the 'Cape' gooseberry TA decreased (Reyes-Medina *et al.,* 2017). Furthermore, Hussain *et al*. (2019) found that at postharvest, 'Williams' pear fruit treated with calcium chloride ($CaCl_2$) retained lower TA when compared with control. However, contradictive results were observed by Eric *et al*. (2015), whereby, 'Power' tomato fruit dipped in postharvest $CaCl_2$ (2 and 6%) retained higher titratable acidity than the control. According to Genanew (2013), $CaCl_2$ is an ethylene inhibitor and ethylene plays an active role in the tomatoes ripening process (Opiyo and Ying, 2005). In general, ripening is also associated with the conversion of starch and acids to sugar (Paliyath and Murr, 2008). Wani *et al*. (2008) found that in 'Barlett' pear fruit, sugar accumulation during ripening contributes to acidity reduction, as a result, there is an increase in TSS acid ratio.

2.4.3. Effect of calcium on pH

Potential of hydrogen, otherwise known as pH, measures acid levels and alkaline in a substance on a scale from 0 to 14 (Sadler and Murphy, 2010). According to Abu-Goukh and Bashir (2003), a high pH indicates higher alkaline content and a low pH signals higher acidity. Teka (2013) found that 'Merel' tomato acidity can be closely associated with their degree of ripeness. In general, tomato pH ranges from 4.0- 4.5 and the more mature and riper, the lower the acidity, with pH approaching 4.9

(Chinamale, 2014). The acidic content change is due to the combined effects of leaching and organic acids oxidation in the biological matrix (Ordóñez-Santos and Vázquez-Riascos, 2010).

The study conducted by Korkmaz *et al*. (2017) showed the pre-harvest application effect of $CaCl_2$ on the 'El-basha' tomato fruit juice pH, whereby, when the calcium concentration is increased, fruit pH decrease significantly. However, Bhattarai and Gautam (2006) reported that there was no apparent effect of postharvest calcium treatment albeit it was recorded higher in control 'Gootya' tomato fruit and lower in calcium treated fruit. Similarly, Shehata *et al*. (2021) have also reported the significant effect of calcium on '448' tomato juice pH, it was higher in control than in calcium chloride ($CaCl_2$) treated fruit. According to Marschner (2002), the ability of calcium to retain lower pH occurs due to higher calcium concentration in fruits, which enable polygalactunorase activity reduction, and therefore, resulting in smaller increases in the pH levels.

2.5. Effect of calcium on physiological and pathological disorders

2.5.1. Effect of calcium on decay

Fruit decay is a natural phenomenon associated with the ripening process and postharvest diseases (Khaliq *et al*., 2015). According to Moneruzzaman *et al*. (2008), maturity stage, ripening conditions and their combinations were found to have significant effect on tomato decay. In general decay is higher in ripe tomato due to higher respiration rate, higher skin water loss permeability and susceptibility to disease organisms (Prusky, 2011). Zapata *et al*. (2008) found that in 'Rambo' tomatoes, fruit ripening under long-term storage, fungi and bacteria were associated with some enzymatic change processes, which trigger damage to fruits and deteriorate the middle lamella, in particular plant enzymes associated with the senescence process. Thereafter, resulting in fruit decay, which is the major factor for postharvest losses.

The study conducted by Sohail *et al*. (2015) showed that 'Earli grande' peach fruit treated with 1% $CaCl_2$ at postharvest had higher (61.22) decay percentage than fruit treated with 3% $CaCl_2$. Similarly, 'Power' tomato fruit dipped in 6% $CaCl_2$ recorded significantly lower levels of decay than the 2% $CaCl_2$ and the control (untreated) (Eric *et al*., 2015). In addition, Sati and Qubbaj (2021) observed that 'Izmir' tomato

fruit treated with 6% $CaCl_2$ showed lower decay percentage when compared with control. According to Nirupama *et al*. (2010), calcium application assists in maintaining membrane integrity, tissue firmness, cell turgor as well as delaying membrane lipid catabolism; and therefore, decreasing the decaying rates. The incorporation of calcium ions in fruit tissue promotes new cross-links between anionic homogalacturonans, strengthening the cell wall, particularly, the middle lamella, which is responsible for holding cells together (Munoz *et al*., 2008). Thus, increasing cell wall stability and middle lamella of the fruits (Langer *et al*., 2019). Meanwhile, the higher decay content in untreated 'Earli grande' peach fruit was the result of lesser tissue strength and cellular disorganization (Sohail *et al*., 2015).

2.5.2. Effect of calcium on chilling injury

Cold storage is widely adopted to retain quality and prolong the tomato fruit shelf-life (Fagundes *et al*., 2015). However, tomato fruit can develop physiological disorders during cold storage; among the most prominent is chilling injury, which cause significant losses (Arah *et al*., 2015). Chilled tomato fruit develop several symptoms, such as sunken areas on the fruits (blemishes), diseases caused by pathogen, and losing their ability to develop full colour, which lead to substantial degradation of quality (Zhao *et al*., 2009). According to Dreyer and Dietz (2018), chilling injury in fruits and vegetable is closely associated with oxidative stress, resulting from excessive reactive oxygen species (ROS). A large quantity of ROS accumulation alters membrane organization and cause or exacerbate lipid peroxidation, leading to damage to the cell system (You and Chan, 2015).

The postharvest calcium application maintains cell turgor, membrane integrity, tissue firmness and delay membrane lipid catabolism, therefore, reducing the physiological disorder incidences (Mirdehghan and Gotbi, 2014). According to Akhtar *et al*. (2010), calcium is effective in terms of membrane functionality and integrity maintenance, which lower phospholipids and proteins losses and reduce ion leakage. Study conducted by Mirdehghan and Ghotbi (2014) showed that calcium chloride ($CaCl_2$) treated 'Malas-e-yazdi' pomegranate fruit had lower chilling injury index when compared with untreated fruit (control). Correspondingly, prohexadione-calcium (Pro-Ca) was found to reduce chilling injury on 'Newton' tomato fruit (Aghdam, 2013). In the plant tissue, the Pro-Ca application induced the flavonoid metabolism alteration when compared with control (Halbwirth *et al*., 2002).

2.5.3. Effect of calcium on black mould

Black mould is a fungal disease caused by several pathogens which include *Alternaria arborescens, Stemphyllinm botryosum* and *Stemphylium consortiale* (Ma *et al*., 2020). According to Gangadhara *et al*. (2021), black mould rot often appears on fruit that has been injured by chilling, calcium deficiency and exposure to high temperatures and humidity. Additionally, infected fruit lesions are initially sunken and later quickly covered with a dark brown to black mould (Shehata *et al*., 2006). Lesions also develop internally if the vascular strand that is connected to the stem scar is infected (Yasser *et al*., 2019). Severe internal bruises greatly predisposes the fruits to infection that forms internal black spots (Ma *et al*., 2020).

Calcium applications enhance the fruit resistance to postharvest pathogens and reduces susceptibility to postharvest diseases and disorders (El-Gali, 2014). According to Decreux and Messiaen (2005), calcium availability influence the defence response eliciting capacity and specificity of protein OGA (O-GlcNacase). Degradation of pectic homogalacturonan backbone generates short-chain molecules known as OGAs, these have been implicated in pathogen defence signalling activation (Hocking *et al*., 2016). The OGA bind to the wall-associated kinase (WAK) only in the presence of calcium and calcium crosslinking forming conditions (Decreux and Messiaen, 2005). Furthermore, a study conducted by Sharma *et al*. (2013) indicated that calcium infiltration reduce the level of pectin degradation by botrytis and the decay level in 'Royal delicious' apples. Lara *et al*. (2004) suggested that improved resistance to fungal attack in calcium treated 'Pajaro' strawberry fruit was associated with the preservation of cell-wall and middle-lamella structures. Calcium ions tightly bind the pectins in the cell walls and produce cationic bridges between pectic acids, or between pectic acids and other acidic polysaccharides (Yaghubi-Akram *et al*., 2019). These bridges make the cell wall less accessible to the action of pectolytic enzymes (El-Gali, 2014).

2.6. Conclusion

Calcium chloride ($CaCl_2$) has been widely utilised as a postharvest treatment on variety of fruits and vegetables including tomato. Researchers generally reported on the ability of calcium chloride to extend fruit shelf-life through maintained firmness, retaining weight loss and effecting the fruit chemical qualities. However, there is a limited information on the required dipping time in $CaCl_2$ solutions 'Classic round'

tomato fruit. Moreover, there are conflicting studies on the precise calcium concentration to be applied at postharvest for tomato fruit quality preservation. Therefore, research interest should focus on investigating the effect of different $CaCl_2$ concentrations and dipping time on extending the 'Classic round' tomato fruit shelf-life.

CHAPTER 3

MATERIALS AND METHODS

3.1. Experimental sites

Field grown ‘Classic round’ tomatoes were harvested at their pink maturity stage based on skin colour at ZZ2 farm in Moeketsi, Limpopo Province, South Africa (23° 35' 41" S, 30° 5' 51" E), 701 m above sea level. The climate in the area is characterized by hot dry summers and cool dry conditions in winter season (Tshiala, 2014). The area receives an annual rainfall of >1000 mm and temperature ranges between 26°C maximum and 13.4°C minimum (Novela, 2016). The harvested tomato fruit were transported at ambient temperature to the Agricultural Research Council - Tropical and Subtropical Crops (ARC-TSC) postharvest laboratory in Nelspruit (25°27′04.8″S, 30°58′09.75″E) for storage and laboratory analysis.

3.2. Treatments, procedures and experimental design

In the laboratory, undamaged fruit without defects were randomly selected and distributed among the treatments. Thereafter, fruit were dipped in different solutions of $CaCl_2$ concentrations (L_1= 0.0045, L_2= 0.01 and L_3= 0.03 %) and control (distilled water) for 0, 30 and 60 minutes, separately. All treated fruit were then allowed to air-dry for 30 minutes under ambient temperature. Subsequently, 30 fruit per treatment were packed in cardboard boxes and stored at 15°C for 30 days. As a third factor, fruit were held under room temperature for 0-7 days of shelf-life while collecting data. Thus, the experiment was carried out as a completely randomized design (CRD) with three factors, factorial arranged as 4 × 3 × 8. The following postharvest variables were determined during the postharvest life:

3.3. Postharvest qualities

3.3.1. Determination of weight loss (%)

Total number of 30 fruit per each treatment factor, were weighed on a digital weighing balance (SBA 61, Scaltec instruments, Heiligen). To determine the weight loss, fruit were weighed before treatment was applied, which served as the initial fruit weight. The weight loss was calculated as the difference between the initial weight of the fruit and the final weight after storage. Weight loss was determined by the following formula and expressed as a percentage (Gharezi *et al*., 2012):

Weight loss (%) = [(Initial weight- fruit weight on the day of observation)/Initial fruit weight)] ×100

Figure 3.1: Digital weighing balance measuring a tomato fruit

3.3.2. Determination of fruit firmness

Fruit firmness was measured from 30 fruit per each treatment factor, using a non-destructive digital densimeter (pulp hardness tester) (Model: 53524, Bareiss, Oberdischingen, Germany) with a 5 mm tip and results were expressed in DM (Densimeter value). The fruit firmness was measured before storage and every day after storage until spoilage was reached.

Figure 3.2: Densimeter used to measure tomato fruit firmness

3.3.3. Determination of colour change

Colour change was determined using the chromameter (Minolta CR-400, Corp, Ramsey, NJ, USA) with an 8 mm diameter light path aperture. The colour parameters readings, L* value (lightness or brightness) and chroma (C*), a* (redness or greenness), b* (yellowness or blueness) and hue angle were automatically calculated and recorded following the method used by Chepngeno *et al*. (2016). The

chromameter device was initially calibrated before use with a Minolta standard white tile.

Figure 3.3: Chromameter used to measure colour parameters (L*, C*, a*, b* and hº) values)

3.3.4. Determination of total soluble solids (TSS)

A handheld refractometer (Model 121, Yagami International Ltd, Japan) was used to determine the TSS (Brix°) from tomato juice. A total number of nine fruit per treatment were used. The TSS was measured using a drop of juice, and values were expressed in °Brix (Pila *et al*., 2010). The total soluble solids were measured before and after storage until the end of the shelf-life. The refractometer was calibrated using distilled water before readings were taken.

Figure 3.4: Refractometer used to measure tomato juice total soluble solids (TSS)

3.3.5. Determination of titratable acidity (TA)

The titratable acidity (TA) was determined using the method previously described by Wills and Ku (2001). A total number of 9 fruit per treatment were used. Briefly, 20 ml of tomato juice was squeezed, diluted with distilled water to 50 ml, and titrated with

0.1 M NaOH using phenolphthalein as an indicator. The appearance of light pink colour marked the end of titration. The acidity was calculated as a percentage of citric acid using the following formula:

Acid (%) = [(titre value × normality× m.eq.wt.of acid)/volume of the sample)] ×100

Where: Milli-equivalent weight (m.eq.wt.) of citric acid = 0.06404

Figure 3.5: Apparatus (burette and flask) used for titration of acid in the tomato juice

3.3.6. Determination of pH

To determine pH of 9 fruit per each treatment factor, 50 ml of tomato juice was squeezed into a beaker and a pH meter (Thermo Scientific™ Orion™ Star A211, Beverly, United States of America) was used. The electrodes were first calibrated using pH 4.01 and 7.00 buffers. The pH electrode was then placed into the electrode stand and rinsed each with distilled water. Afterwards, the probe was placed into the sample, immersing about 1-2 inches into the solution. When the pH meter indicated the reading is stable, pH was recorded to two decimal places as previously described by Sinha *et al*. (2019).

Figure 3.6: Thermo Scientific™ Orion™ Star pH meter used to measure fruit juice pH

3.3.7. Determination of decay (%)

Symptoms of fungal mycelia growth were observed to determine the decay. Decay percentage of the total fruit was calculated by dividing the total number of fruit by the number of original fruit using the formula below:

Decay (%) = [(number of decayed/initial fruit number)]× 100

3.3.8. Determination of chilling injury on tomato fruit

Using a visual scale, chilling injury was determined using a 5-rating scale: 0 = no injury, 1 = slight injury, 10-50% of the surface with discolouration, 2 = medium injury, more than 50% of the surface with larger damage, and 3 = severe injury. The chilling injury index (CII) was calculated using the following formula (Herrera *et al*., 2007):

CII = [∑ (number of fruits in each class × class value)]/ (total number of examined fruits)

3.3.9. Determination of pathological diseases on tomato fruit

Pathological diseases were determined by visual observation. These include black mould which is characterized by small dark blotches and large sunken areas and bacterial soft rot characterized by small, sunken, water-soaked lesions that progress rapidly and liquefy the tissue. Disease incidences were expressed using a scale of 0 to 4, whereby, 0 = indicated no symptoms, 1 = very slight symptoms or covering < 10% of the fruit surface, 2 = slight symptoms or covering > 10% but < 25% of the fruit surface, 3 = moderate symptoms or covering > 25% but 40% of the fruit surface, 4 =

severe decay, covering > 40% of the fruit surface (Shao *et al*., 2019). The results of each disease were expressed in percentage using the following formula:

Disease index (%) = $[(1\times N1+2\times N2+3\times N3+4\times N4)/4\times N)] \times 100$

Where; N was the total number of fruit measured and N1, N2, N3 and N4 are the number of fruit showing the different severities of disease incidence.

3.4. Data analysis

The analysis of variance (ANOVA) was carried out using GenStat® 18th edition computer-based statistical software (VSN International, Hemel Hempsted, U.K.). The treatment means were separated by the Least Significant Difference (LSD) at a 5% (0.05) level of probability.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Results

4.1.1. Physical parameters

Weight loss

The weight loss was not significantly ($P > 0.05$) affected by the treatments, interaction of treatments and shelf-life (days), the interaction of dipping times and shelf-life and the interaction of treatments, dipping times and shelf-life period. However, dipping time, shelf-life and the interaction between treatments and dipping times had a significant effect ($P < 0.05$) on tomato fruit physiological weight loss (Figure 4.1). In general, a gradual increase in physiological weight loss during shelf-life was observed in all fruit, regardless of dipping time and treatments.

With regards to treatments, no significant physiological weight loss differences were observed between fruit dipped for 0 and 30 minutes, irrespective of treatments. Meanwhile, lower weight loss was recorded in fruit treated for 60 minutes when compared with 0 and 30 minutes, regardless of treatments. Moreover, fruit dipped in 0.03% $CaCl_2$ for 60 minutes showed higher physiological weight loss followed by 0.0045% $CaCl_2$. Whereas, fruit treated with 0.01% $CaCl_2$ and distilled water for 60 minutes maintained a lower physiological weight loss when compared with fruit treated with 0.0045 and 0.03% $CaCl_2$ (Figure 4.1).

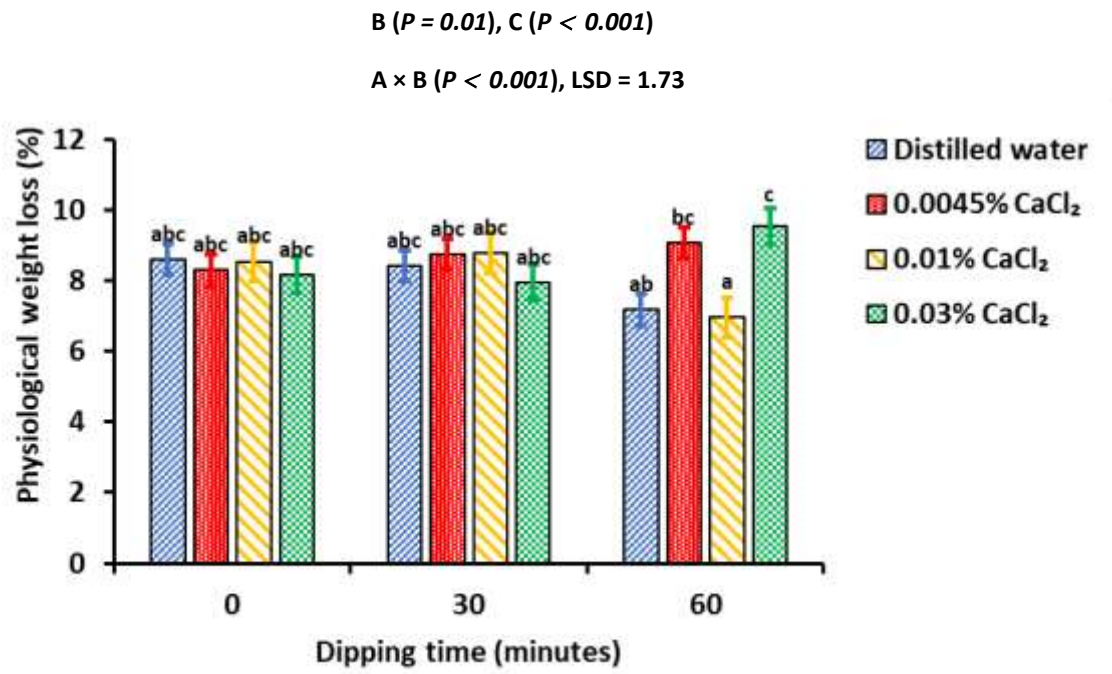


Figure 4.1: Effect of $CaCl_2$ concentrations and dipping times on weight loss of 30 sampled tomato fruit per treatment. Vertical bars represent the means of 10 fruit per treatment at a set dipping time. Values in each bar followed by different letters are significantly different at P ≤ 0.05. B = dipping times, C = shelf-life, A × B = interaction of treatments and dipping time.

Fruit firmness

The interaction of treatments and shelf-life and the interaction between treatments, dipping times and shelf-life period had a non-significant effect (P > 0.05) on the fruit firmness. However, treatments, dipping times, shelf-life, the interaction of treatments and dipping time and the interaction between dipping times and shelf-life had a significant effect (P < 0.05) on the fruit flesh firmness (Figure 4.2). Generally, tomato fruit firmness decreased progressively during storage and shelf-life days, irrespective of dipping times and treatments.

Among the studied treatments, fruit dipped for 60 minutes recorded significantly high firmness when compared with fruit dipped for 0 and 30 minutes, regardless of the treatments. Furthermore, fruit dipped in 0.03% $CaCl_2$ for 0 minutes were firmer than

fruit dipped in distilled water, 0.0045 and 0.01% $CaCl_2$ (Figure 4.2). While, fruit dipped in distilled water, 0.0045 and 0.01% $CaCl_2$ for 0 minutes showed no statistically significant difference in firmness. Furthermore, fruit dipped in 0.01% $CaCl_2$ for 30 minutes maintained significantly higher flesh firmness when compared with fruit treated with 0.0045% $CaCl_2$ and distilled water. Whereas, the flesh firmness of fruit dipped in 0.03% $CaCl_2$ was not significantly different from fruit dipped in 0.01% $CaCl_2$. Additionally, no significant difference existed among the treatments at 60 minutes dipping time with respect to firmness.

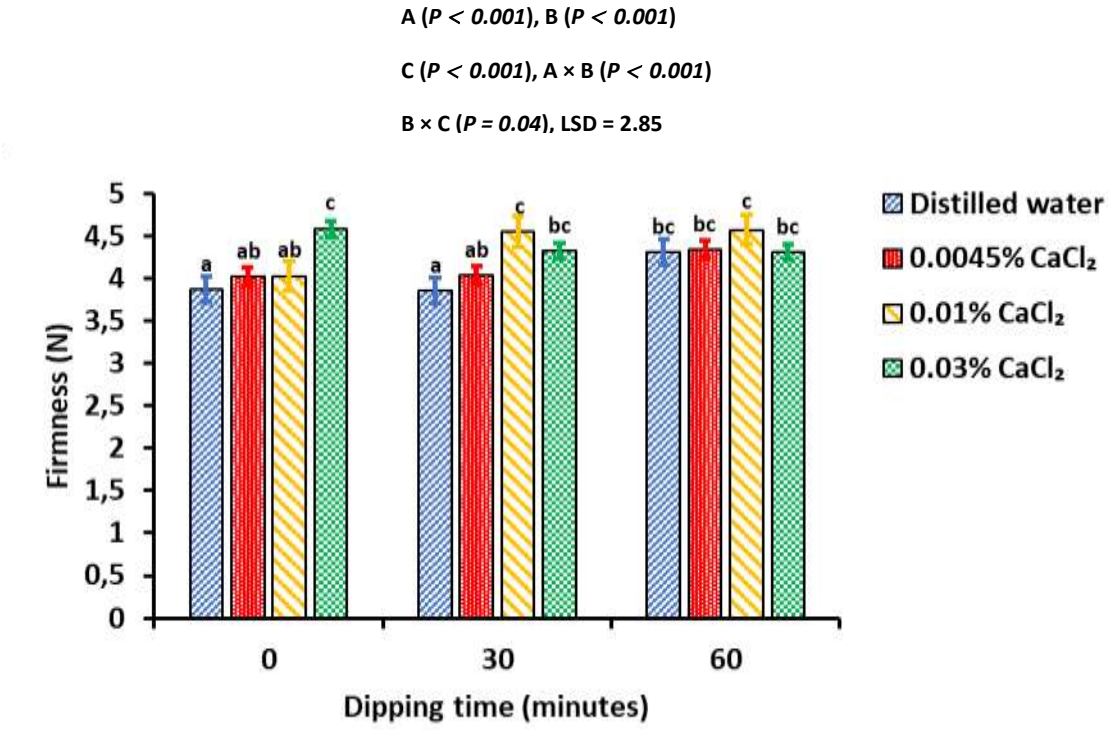


Figure 4.2: Effect of $CaCl_2$ concentrations and dipping times on changes in firmness of 30 sampled tomato fruit per treatment. Vertical bars represent the means of 10 fruit per treatment at a set dipping time. Values in each bar followed by different letters are significantly different at P ≤ 0.05. A = treatments, B = dipping times, C = shelf-life (days), A × B = interaction of treatments and dipping times, B × C = interaction of dipping times and shelf-life (days).

Colour parameters

Redness (a* component)

The results showed that dipping times, interaction between treatments and shelf-life and interaction between treatments, dipping times and self-life had a non-significant effect ($P > 0.05$) on the fruit redness (a*). Moreover, fruit redness was influenced by treatments, shelf-life and both the treatments and dipping time interaction as shown in Figure 4.3. Generally, the fruit redness showed the gradually increasing trend throughout the shelf-life period, irrespective of the dipping times and treatments. With respect to treatments, fruit dipped in 0.01 and 0.03% $CaCl_2$ for 30 and 60 minutes showed higher a* values when compared to control and 0.0045% $CaCl_2$ (Figure 4.3).

Blueness (b* component).

The b* component of the tomato exocarp colour was not significantly ($P > 0.05$) influenced by dipping time, interaction of treatments and shelf-life (days), interaction between dipping times and shelf-life and the interaction between treatments, dipping times and shelf-life. However, treatments, shelf-life and the interaction between treatments and dipping times had a significant effect on the exocarp b* component (Figure 4.3). Generally, there was no significant change in b* component during the shelf-life period, regardless of the treatments and dipping time.

With respect to treatments, the b* values of treated fruit were not significantly different at 0 minute dipping time. Fruit dipped in 0.01% $CaCl_2$ for 30 minutes maintained a high b* value mean (27.64) followed by 0.03, 0.0045% $CaCl_2$ and control (distilled water) with the least mean value (25.79). Furthermore, fruit dipped in 0.01% $CaCl_2$ for 60 minutes exhibited higher b* component value mean followed by 0.03 and 0.0045% $CaCl_2$. Whereas, fruit dipped in distilled water for 60 minutes maintained a lower b* value mean as compared to 0.0045, 0.01 and 0.03% $CaCl_2$ (Figure 4.3).

Chroma

The interaction between treatments and shelf-life, interaction between dipping time and shelf-life and the interaction of treatments, dipping times and shelf-life (days) had a non-significant effect ($P > 0.05$) on the tomato exocarp chroma. In contrast,

treatments, dipping times, shelf-life and the interaction of treatments and dipping times had a significant effect ($P < 0.05$) on the chroma values of the exocarp chroma (Figure 4.3). In general, the chroma of the fruit increased a day after storage and afterwards, decreased with shelf-life period, regardless of dipping times and treatments.

With respect to treatments, it was observed that fruit dipped for 60 minutes exhibited higher chroma values, followed by 0 and 30 minutes. Moreover, fruit dipped in distilled water for 0 minutes maintained high chroma mean value (31.87), followed by 0.0045, 0.03 and 0.01% $CaCl_2$ with the least mean value (30.44) (Figure 4.3.). Interestingly, fruit dipped in 0.01% $CaCl_2$ for 30 minutes recorded a significantly high chroma value as compared to fruit dipped in distilled water, 0.0045 and 0.03% $CaCl_2$. In addition, fruit dipped in distilled water for 60 minutes recorded high chroma mean value, followed by 0.01 and 0.03% $CaCl_2$. Whereas, fruit dipped in 0.0045% $CaCl_2$ maintained the lowest chroma mean value when compared with distilled water, 0.01 and 0.03% $CaCl_2$.

Lightness

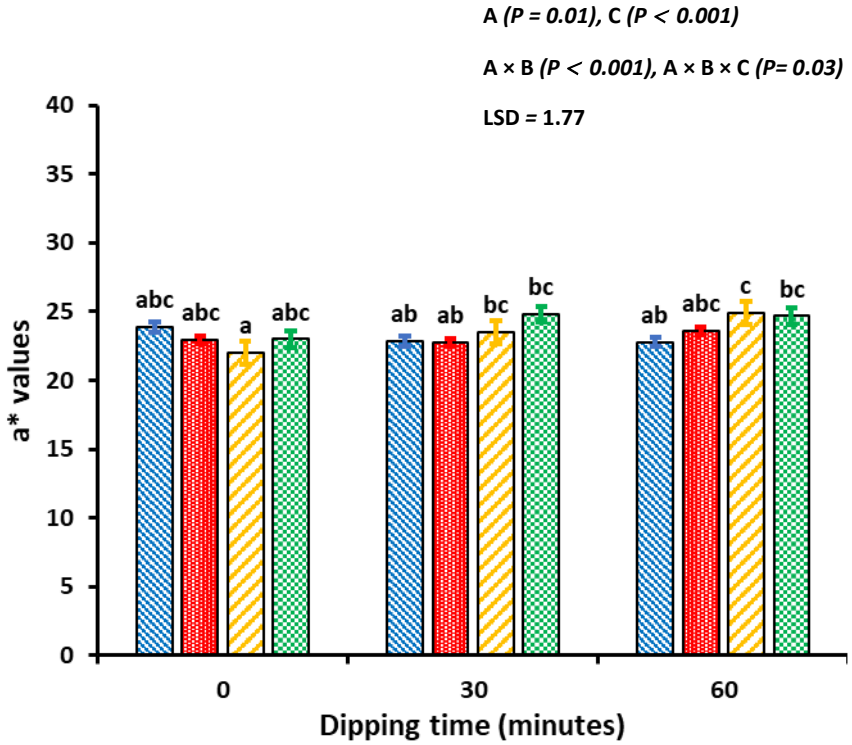
The results of the current study presented that treatments, dipping times, interaction between treatments and shelf-life, interaction between dipping time and shelf-life and the interaction of treatments, dipping times and shelf-life (days) had no significant effect ($P > 0.05$) on the tomato exocarp lightness. However, shelf-life, interaction of treatments and dipping times significantly ($P < 0.05$) affected the tomato exocarp lightness (Figure 4.3). Generally, treated fruit exocarp lightness decreased progressively with shelf-life, irrespective of dipping times and treatments.

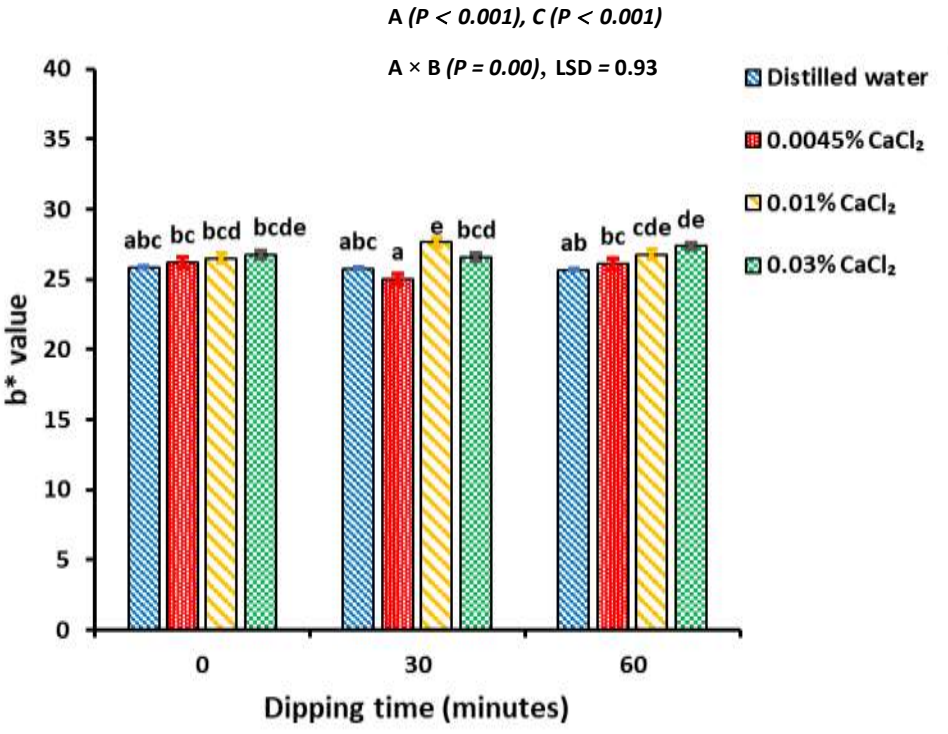
The studied treatments did not show significant differences at 0 dipping time. Fruit treated with distilled water for 30 minutes recorded high lightness values, followed by 0.01 and 0.0045% $CaCl_2$ (Figure 4.3). Whereas, the lowest lightness mean value was observed in fruit dipped in 0.03% $CaCl_2$ for 30 minutes when compared with distilled water, 0.0045 and 0.03% $CaCl_2$. Moreover, fruit exposed to distilled water for 60 minutes dipping time exhibited a high lightness value mean followed by 0.03, 0.01 and 0.0045% $CaCl_2$, respectively.

Hue angle (°)

Treatments, interaction of treatments and shelf-life and the interaction between treatments, dipping time and shelf-life (days) had a non-significant effect ($P > 0.05$) on fruit exocarp hue angle. However, dipping times, shelf-life, interaction of dipping time and shelf-life and the interaction between the treatments and dipping times had a significant effect ($P < 0.05$) on exocarp hue angle. Generally, the hue angle of the treated fruit decreased gradually with the shelf-life, irrespective of dipping times and treatments.

With respect to treatments, no differences existed among the treatments at 0 minutes dipping time. High hue angle value mean (36.07) was observed on fruit dipped in distilled water for 30 minutes, followed by 0.01, 0.0045 and 0.03% $CaCl_2$ with the least hue angle mean value of 34.07 (Figure 4.3). Furthermore, the highest hue angle mean value (36.74) was observed in fruit treated with distilled water for 60 minutes, followed by 0.03, 0.0045 and 0.01% $CaCl_2$ with the least mean value (34.33).

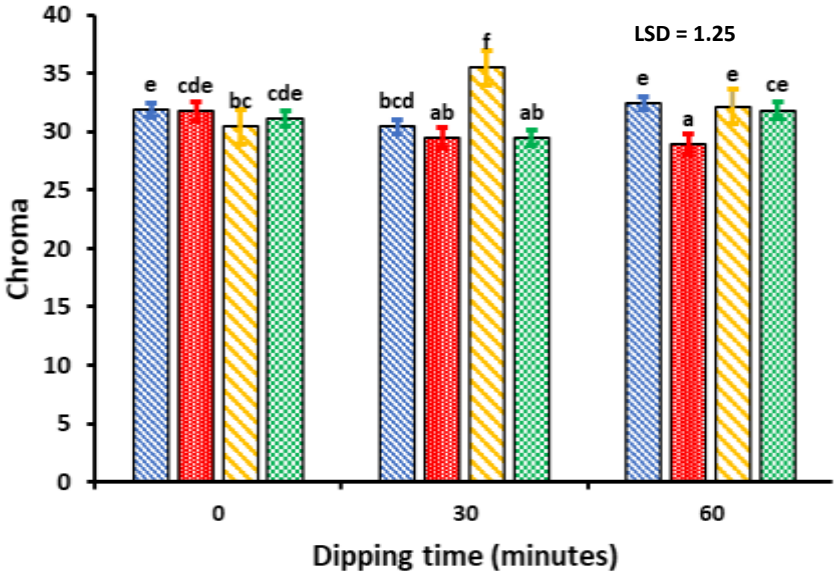

A (P = 0.01), C (P < 0.001)
A × B (P < 0.001), A × B × C (P= 0.03)
LSD = 1.77
a* values
Dipping time (minutes)
0
30
60
abc abc a abc
ab ab bc bc
ab abc c bc


A (P < 0.001), C (P < 0.001)
A × B (P = 0.00), LSD = 0.93
b* value
Dipping time (minutes)
Distilled water
0.0045% CaCl2
0.01% CaCl2
0.03% CaCl2
abc bc bcd bcde
abc a e bcd
ab bc cde de

A ($P < 0.001$), C ($P < 0.001$)

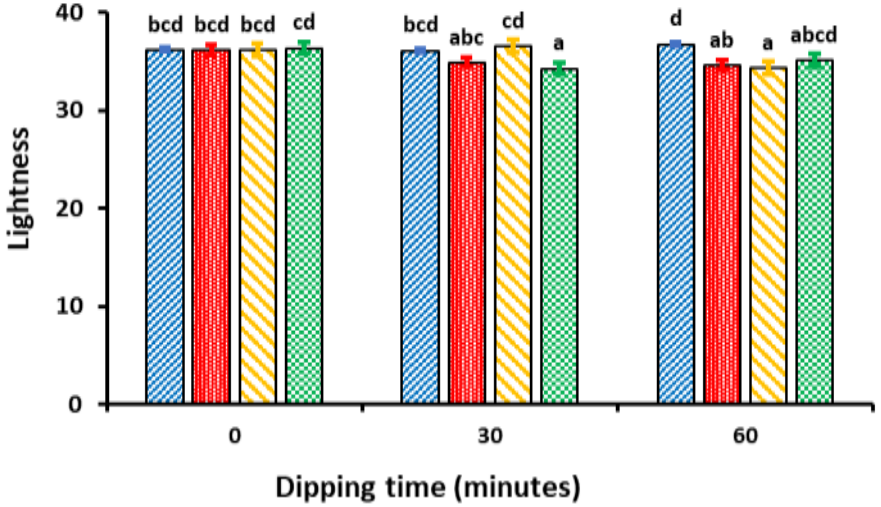
B ($P = 0.002$), A × B ($P < 0.001$)

LSD = 1.25



C ($P < 0.001$), A × B ($P = 0.01$)

LSD = 1.52



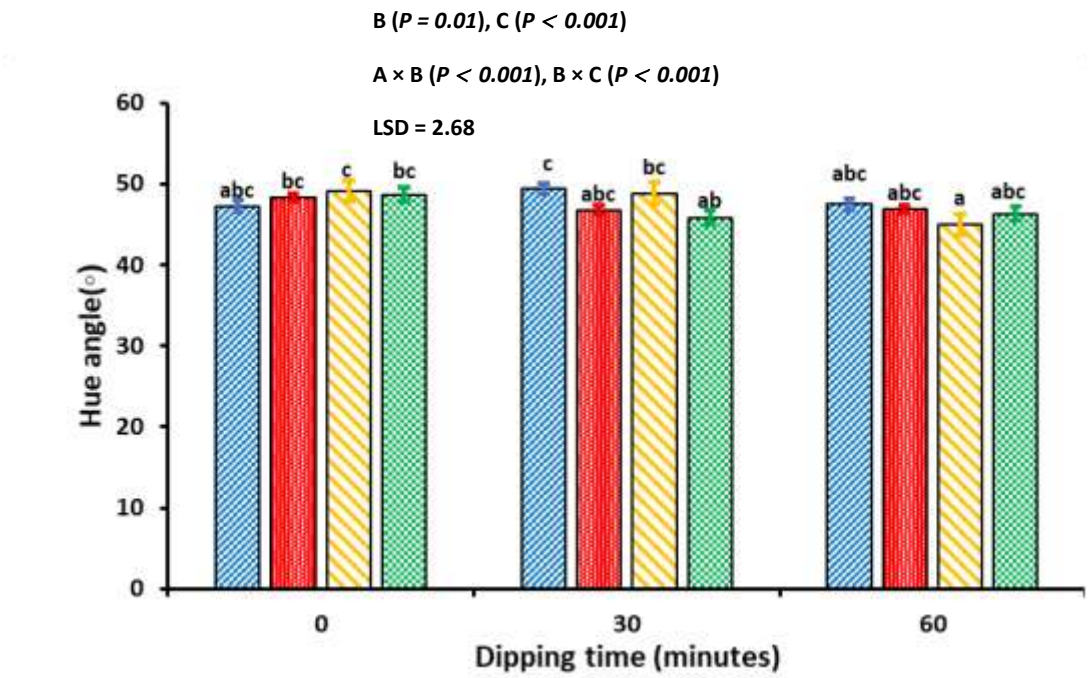


Figure 4.3: Effect of $CaCl_2$ concentrations and dipping times on changes in colour parameters [a*, b*, chroma, lightness and hue angle (°)] of 30 sampled tomato fruit per treatment. Vertical bars represent the means of 10 fruit per treatment at a given dipping time. Values in each bar followed by different letters are significantly different at P ≤ 0.05. A= Treatments, B= Dipping times, C= Shelf-life (days), A × B= Interaction of treatments and dipping times, A × C= interaction of treatments and shelf-life (days), B × C= interaction of dipping times and shelf-life and A × B × C= interaction of treatments, dipping times and shelf-life (days).

4.1.2. Chemical parameters

Total soluble solids (TSS)

There was a significant ($P < 0.05$) effect of interactions between treatments, dip times, and shelf-life on tomato TSS (Figure 4.4). Furthermore, the current study indicated that treatments and dipping times also significantly ($P < 0.05$) affected tomato fruit TSS (Figure 4.4). Generally, tomato fruit TSS increased gradually over the shelf-life period, irrespective of the treatments and dipping times (Figure 4.4). The analysis of variance for the TSS showed significant ($P < 0.05$) differences in fruit dipping times. In this study, fruit dipped for 60 minutes had higher TSS than fruit dipped for 0 and 30 minutes, regardless of the treatments.

In terms of treatments, fruit treated with distilled water for 0 and 30 minutes recorded higher TSS, followed by 0.0045% $CaCl_2$ from day 0 to day 4 of shelf-life. In contrast, fruit dipped in 0.01 and 0.03% $CaCl_2$ exhibited lower TSS when compared with distilled water and 0.0045% $CaCl_2$ (Figure 4.4). From day 0 to day 4, fruit dipped in 0.03% $CaCl_2$ for 60 minutes displayed higher TSS when compared with control fruit (distilled water). While fruit treated with 0.01 and 0.0045% $CaCl_2$ had lower TSS. Further, fruit dipped in 0.03% $CaCl_2$ for 60 minutes showed higher TSS on the last day of shelf-life (day 7), followed by 0.01 and 0.0045% $CaCl_2$ (Figure 4.4).

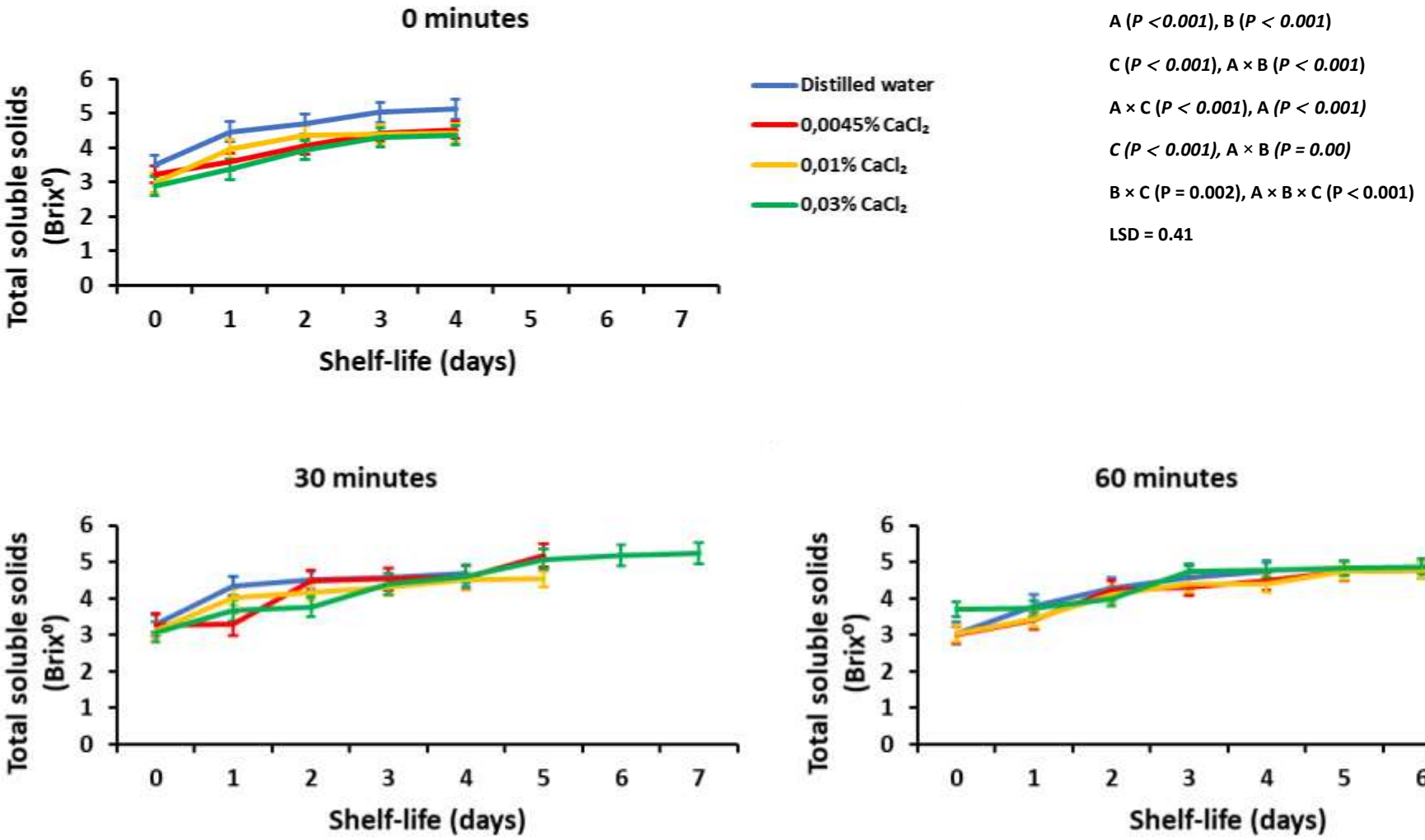


Figure 4.4: Effect of $CaCl_2$ concentrations and dipping times on changes in total soluble solids of tomato fruit per treatment. Values are the means of 9 fruit per treatment factor and error bars indicate ± SE of means at P ≤ 0.05. A = Treatments, B = Dipping times, C = Shelf-life (days), A × B = Interaction of treatments and dipping times, A × C = interaction of treatments and shelf-life (days), B × C = interaction of dipping times and shelf-life and A × B × C = interaction of treatments, dipping times and shelf-life (days).

Titratable acidity (TA)

The results of the present study indicated that the interaction of treatments, dipping times and shelf-life days significantly ($P < 0.05$) affected the tomato juice acidity (Figure 4.5). Moreover, the interaction between dipping times and treatments also had a significant ($P < 0.05$) influence on tomato juice acidity (Figure 4.5). In general, treated fruit acidity decreased over shelf-life period, independent of the dipping times and treatments (Figure 4.5). Moreover, TA was higher in fruit dipped for 0 and 30 minutes when compared with fruit dipped for 60 minutes.

With respect to treatments, fruit dipped in 0.03% $CaCl_2$ for 0 and 30 minutes resulted in a higher TA, followed by 0.01% $CaCl_2$ from day 0 to day 4 of shelf-life. In contrast, fruit that were dipped in distilled water and 0.0045% $CaCl_2$ had lower TA content than fruit that were dipped in 0.03 and 0.01% $CaCl_2$ (Figure 4.5). The fruit dipped in 0.01% $CaCl_2$ for 60 minutes from day 0 to 4 of shelf-life had a higher TA than that dipped in 0.0045% $CaCl_2$. Fruit treated with distilled water and 0.03% $CaCl_2$ displayed lower TA than fruit treated with 0.01 and 0.0045% $CaCl_2$. Additionally, fruit treated for 60 minutes with 0.0045, 0.01 and 0.03% $CaCl_2$ did not show a significant difference in TA after 5 to 7 days of shelf-life (Figure 4.5).

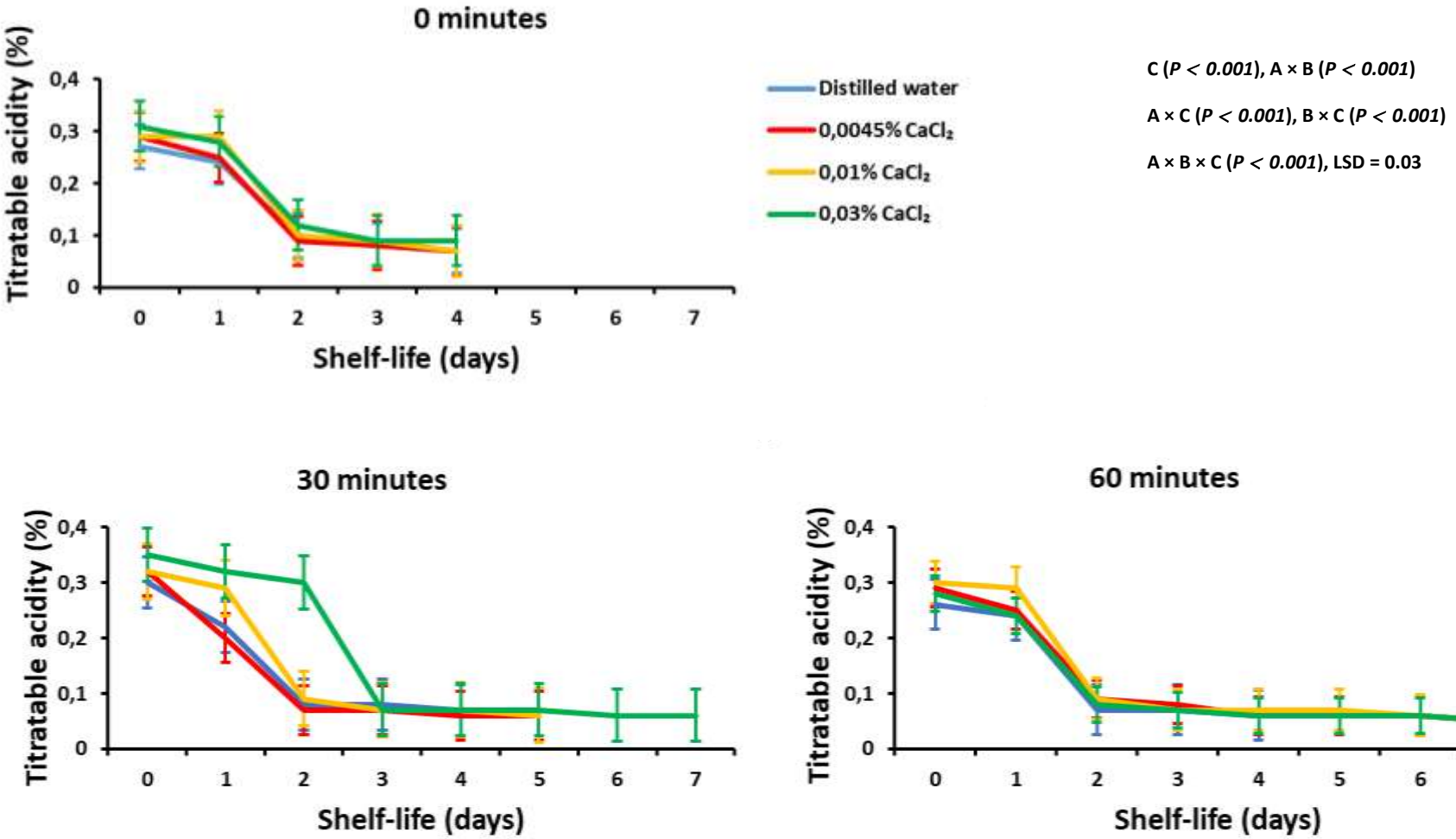


Figure 4.5: Effect of $CaCl_2$ concentrations and dipping times on changes in titratable acidity of tomato fruit per treatment. Values are the means of 9 fruit per treatment factor and error bars indicate ± SE of means at P ≤ 0.05. A = Treatments, B = Dipping times, C = Shelf-life (days), A × B = Interaction of treatments and dipping times, A × C = interaction of treatments and shelf-life (days), B × C = interaction of dipping times and shelf-life and A × B × C = interaction of treatments, dipping times and shelf-life (days).

pH

The pH levels of tomato fruit were significantly ($P < 0.05$) affected by the interaction between treatments, dipping times and shelf-life (Figure 4.6). Similarly, the interaction between treatments and dipping times also had a significant effect ($P < 0.05$) on fruit pH. In general, pH increased with shelf-life, regardless of treatments and dipping times (Figure 4.6). The pH values varied among the three dipping times, the pH values of fruit dipped for 0 minutes were higher when compared with fruit dipped for 30 and 60 minutes (Figure 4.6).

The fruit dipped in distilled water for 0 and 30 minutes recorded higher pH followed by 0.0045% $CaCl_2$ from day 0 to 4 of shelf-life. Meanwhile, fruit dipped in 0.01 and 0.03% $CaCl_2$ showed lower pH when compared with distilled water and 0.0045% $CaCl_2$ (Figure 4.6). The pH of fruit dipped in distilled water for 60 minutes was higher, followed by 0.03% $CaCl_2$, from day 0 to 4 of shelf life. In contrast, fruit treated with 0.01 and 0.0045% $CaCl_2$ maintained a lower pH than distilled water and 0.03% $CaCl_2$. Furthermore, 0.03% $CaCl_2$ for 60 minutes contained the highest pH value followed by 0.01 and 0.0045% $CaCl_2$, respectively, from day 5 to day 7 of shelf-life (Figure 4.6).

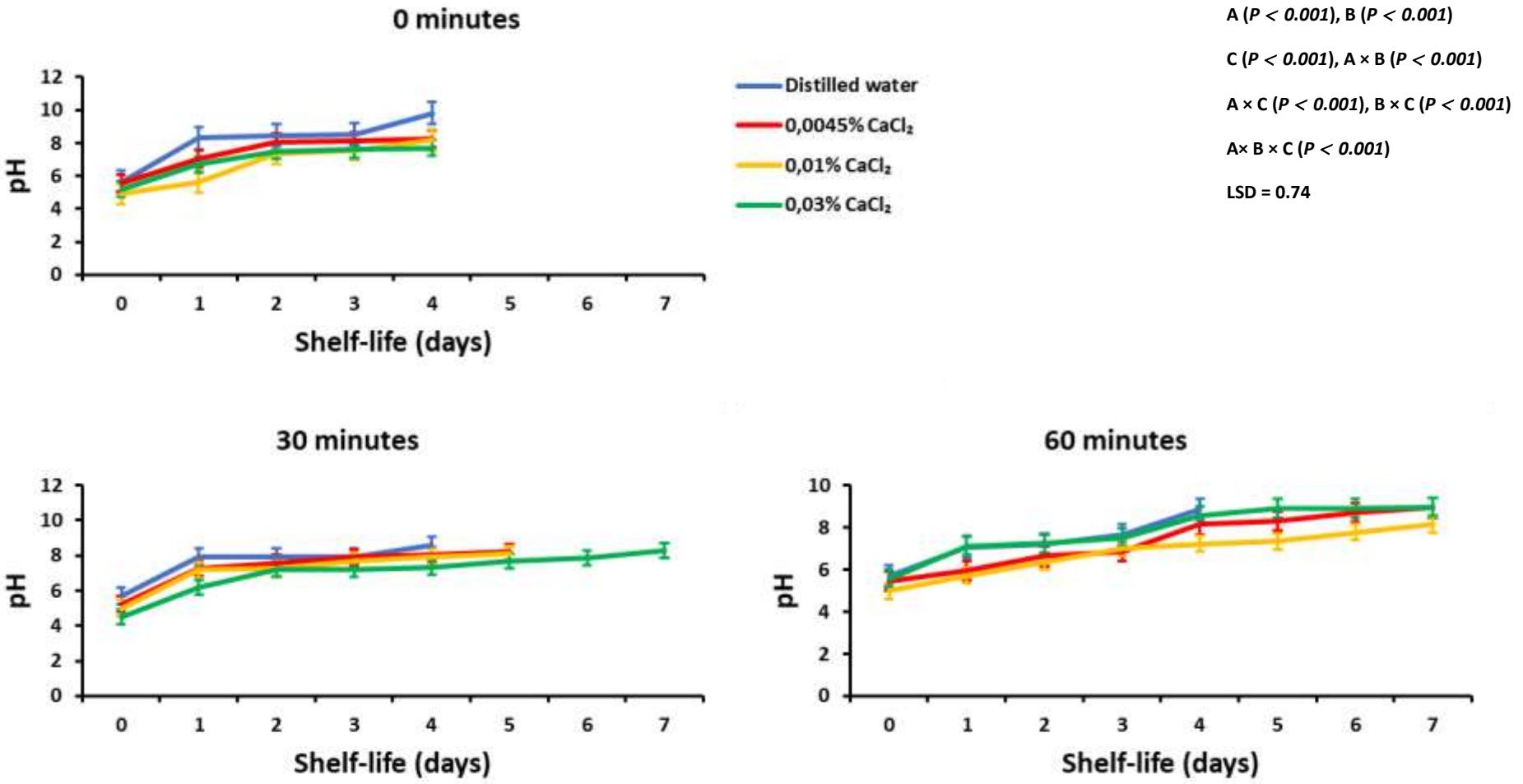


Figure 4.6: Effect of $CaCl_2$ concentrations and dipping times on changes in pH of tomato fruit per treatment. Values are the means of 9 per treatment factor and error bars indicate ± SE of means at P ≤ 0.05. A = Treatments, B = Dipping times, C = Shelf-life (days), A × B = Interaction of treatments and dipping times, A × C = interaction of treatments and shelf-life (days), B × C = interaction of dipping times and shelf-life and A × B × C = interaction of treatments, dipping times and shelf-life (days).

4.1.3 Physiological and pathological disorders

Decay (%)

The decay (%) was significantly ($P < 0.05$) affected by the treatments and dipping time (Figure 4.7). Generally, 'Classic round' tomato fruit exposed to treatments for 0 and 30 minutes recorded high decay percentages when compared with fruit treated for 60 minutes, irrespective of the treatments. The results from Figure 4.7 showed that fruit dipped in distilled water (control) for 0, 30 and 60 minutes had a significantly high decay percentage when compared with 0.0045, 0.01 and 0.03% $CaCl_2$.

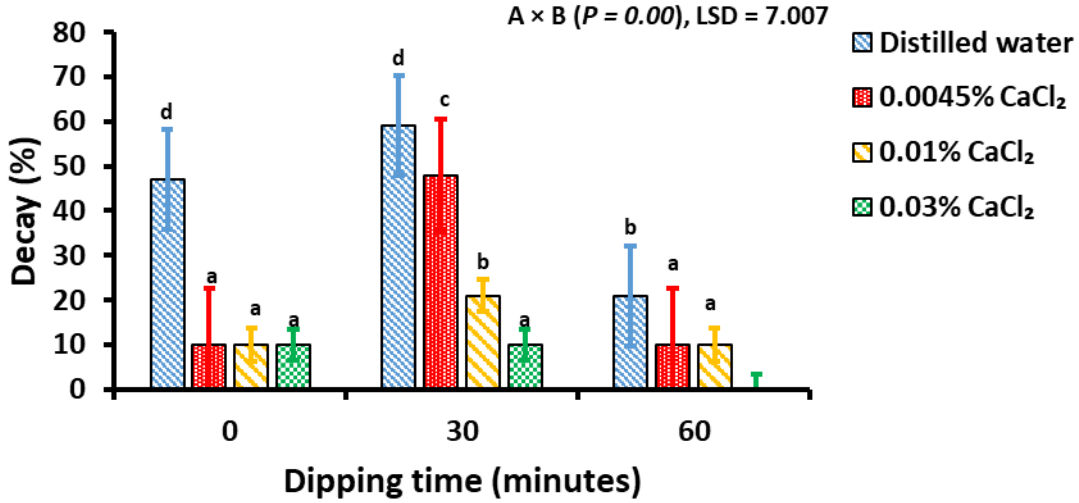


Figure 4.7: Effect of distilled water and $CaCl_2$ solution on the decay of tomato fruit. Vertical bars represent the means of fruit with disease incidence per treatment at a set dipping time. Values in each bar followed by different letters are significantly different at $P \leq 0.05$. A × B = Interaction of treatments and dipping times.

Chilling injury

The main effects of treatments and the interaction between treatments and dipping times on chilling injury were non-significant ($P > 0.05$) (Figure 4.8). However, significant differences were observed among dipping times, fruit exposed to treatments for 0 minutes showed significantly high chilling injury followed by 30 and 60 minutes. With regard to treatments effect, fruit dipped in 0.01% $CaCl_2$ for 0 minutes, showed significantly high chilling injury symptoms followed by 0.0045, 0.03% $CaCl_2$ and distilled water with the least effect. However, fruit dipped in 0.0045, 0.01 and 0.03% $CaCl_2$ for 30 minutes maintained low chilling injury effects when

compared with fruit dipped in distilled water (control) (Figure 4.8). Similarly, fruit dipped in 0.0045, 0.01 and 0.03% $CaCl_2$ for 60 minutes, recorded significantly low chilling injury symptoms when compared with fruit treated with distilled water (control) (Figure 4.8).

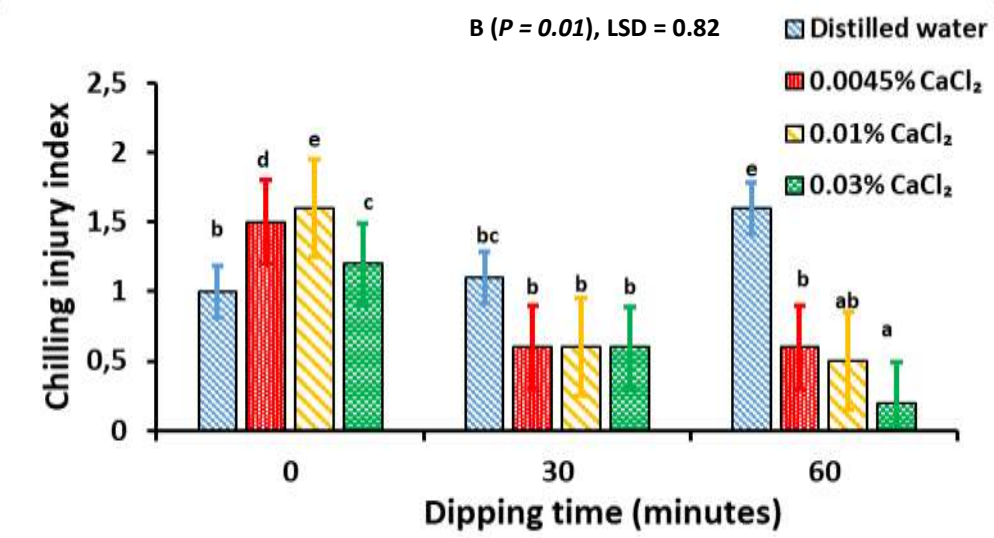


Figure 4.8: Effect of distilled water and $CaCl_2$ solution on the incidence of chilling injury on tomato fruit. Vertical bars represent the means of fruit with disease incidence per treatment at a set dipping time. Values in each bar followed by different letters are significantly different at $P \leq 0.05$. B = Dipping times.

Black mould (%)

The treatments, dipping time and their interaction significantly ($P < 0.05$) had an effect on black mould occurrence on calcium treated and non-treated tomato fruit (Figure 4.9). Overall, fruit treated for 0 minutes showed significantly high black mould occurrence when compared with fruit treated for 30 and 60 minutes. With regard to treatments effect, fruit dipped in distilled water (control) for 0, 30 and 60 minutes developed severe black mould than $CaCl_2$ treatments (0.0045, 0.01 and 0.03%). Whereas, fruit treated with 0.01 and 0.03% $CaCl_2$ for 60 minutes were not affected by black mould (Figure 4.9).

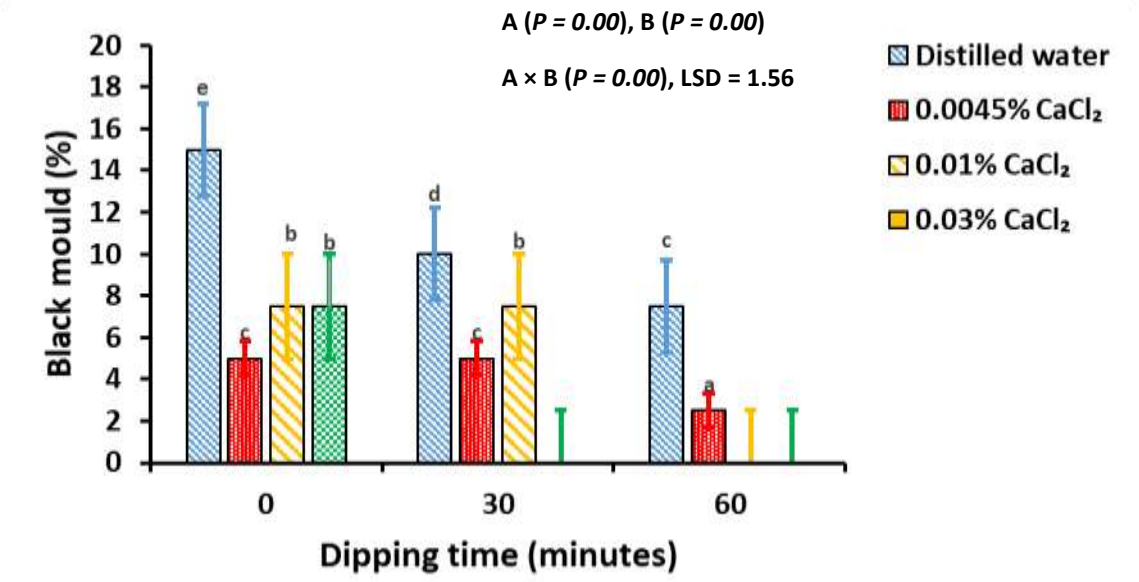


Figure 4.9: Effect of distilled water and $CaCl_2$ solution on the incidence of black mould on tomato fruit. Vertical bars represent the means of fruit with disease incidence per treatment at a set dipping time. Values in each column followed by different letters are significantly different at P ≤ 0.05. A = treatments, B = dipping times and A × B = interaction of treatments and dipping times.

4.2. Discussion

4.2.1. Physical parameters

The effect of calcium chloride ($CaCl_2$) on fruit weight loss

In fruits and vegetables, fruit weight loss is related to fruits senescence, desiccation and it is usually used as a quality index postharvest-life (Nasrin *et al*., 2008). In this study, fruit weight loss gradually increased with shelf-life period, irrespective of treatments and dipping time. In general, fruit weight loss is attributed to fruit moisture loss through transpiration and respiration processes (Lufu *et al*., 2019). According to Ben-Yehoshua and Rodov (2002), transpiration and respiration processes promote water and respiratory substrates depletion, respectively. Therefore, resulting in fruit mass loss through moisture and carbon dioxide diffusion and heat energy dissipation to the atmosphere (Mishra and Gamage, 2020).

In fruit treated with calcium chloride, lower weight loss was recorded for 60 minutes when compared with 0 and 30 minutes (Figure 4.1). These results were in agreement with Eric *et al*. (2015), whereby, 'Power' tomato fruit dipped for 20 and 30

minutes recorded a significantly lower ($P < 0.05$) weight loss than fruits treated for 10 minutes. According to Ullah, (2009), dipping 'Heirloom' tomato fruit for 60 minutes might have retarded respiration and transpiration rate, which are known to be the major weight loss cause. Therefore, it means that calcium chloride application for 60 minutes dipping time increased calcium absorption in treated fruit, which decreased metabolic actions and resulted in lower weight loss (Hussain *et al*., 2019).

In terms of treatments, fruit treated with 0.01% $CaCl_2$ for 60 minutes recorded a lower physiological weight loss when compared with control (distilled water) (Figure 4.1). Similar results were reported by Shirzaden *et al*., (2011), whereby, 'Jonahgold' apple fruit treated with 2% $CaCl_2$ recorded lower weight loss when compared with control (distilled water). In addition, 0.01% $CaCl_2$ was more effective in retaining fruit physiological weight than 0.03% $CaCl_2$ at 60 minutes dipping time (Figure 4.1). Similarly, Mahajan *et al*. (2011) reported that 'Allhad safeda' guava fruit treated with 2% $CaCl_2$ showed lower weight loss when compared with fruit treated with 3% $CaCl_2$. The lower weight loss recorded by $CaCl_2$ treated fruit could be related to the network formation by Ca and pectin in the fruit cell wall to restrict moisture loss (Genanew, 2013).

The effect of calcium chloride ($CaCl_2$) on fruit firmness

In tomato, firmness is an important quality related attribute and may be considered as a final quality index by which the consumer use when deciding to purchase fresh tomato (Batu, 2004). In fruits, firmness change is influenced by factors including; water loss, variation in cell turgor, cell wall composition and microstructural changes in cells and tissues (Paniagua *et al*., 2013). In the current study, firmness decreased progressively with shelf-life in all treatments and dipping times. The decrease in firmness in all the treatments during shelf-life period may be attributed to the faster cell wall weakening (Olveira- Bouzas *et al*., 2021). According to Mahajan *et al*. (2011), cell wall weakening could be due to breakdown of insoluble protopectins into soluble pectins or by enzymatic carbohydrates hydrolysis.

In this study, an interaction between treatments and dipping times had a significant effect ($P < 0.05$) on the fruit flesh firmness. However, fruit dipped for 60 minutes recorded significantly high firmness when compared with fruit dipped for 0 and 30 minutes (Figure 4.2). According to Saure (2005), calcium entry in the fruit depends

on factors such as maturity degree, fruit structure, the dose and the immersion time in the solution. Therefore, the current results showed that 60 minutes dipping time might have been an adequate time for calcium absorption by the fruit; thus, higher firmness was retained during storage and shelf-life.

With respect to treatments, fruit dipped in 0.03 and 0.01% $CaCl_2$ obtained higher firmness when compared with fruit dipped in distilled water (control) at 0 and 30 minutes dipping times. Likewise, Erbas and Koyuncu (2020) indicated that 'Zirrat' sweet cherry fruit treated with calcium had higher firmness when compared with control (distilled water). These results were also in accordance with those reported by Akhtar *et al*. (2010), whereby, 'Lindl' loquat fruit $CaCl_2$ dips (2 and 3%) maintained high firmness when compared with control (distilled water). In this study, 0.01% $CaCl_2$ was more effective than 0.03% $CaCl_2$ on retaining tomato firmness at 30 and 60 minutes dipping time (Figure 4.2). These results were in close relation to Eric *et al*. (2015), who reported that 'Power' tomato fruit treated with 2% $CaCl_2$ for 10 minutes retained higher firmness than fruit treated with 6% $CaCl_2$ for 10 minutes. High concentration of calcium chloride (0.03%) might have changed the cytoplasmic calcium concentration, which may cause membrane damage and enhance respiratory intensity thus lesser firmness (Gao *et al*., 2019).

In general, firmness retention for calcium treated fruit might be due to its accumulation in the cell walls leading to facilitation in cross-linking pectic polymers, which increases the cell wall strength and cell cohesion (Sohail *et al*., 2015). According to Reyes-Medina *et al*. (2017), calcium binds to the negatively charged esterified uronic acid residues generated by the enzyme pectin methyl esterase (PME) during ripening; and thereby, increasing tissue mechanical strength. Additionally, calcium inhibit cell wall activity degrading enzymes; therefore, the outer membrane for the cell wall becomes stronger and rigid (Khaliq *et al*., 2015). The cell wall degrading enzymes are inhibited through the calcium interaction with pectin in the cell wall to form calcium pectate complexes and reduce the cell wall degrading enzymes activity (Pila and Gol, 2010).

The effect of calcium chloride ($CaCl_2$) on fruit colour parameters (lightness, a*, b*, chroma and hue angle).

In most vegetables and fruits, exocarp colour is an important attribute, along with texture, that characterizes the freshness (Rico *et al*., 2007). Additionally, it is the first sensory parameter for acceptance by consumers (Nasrin *et al*., 2008). The color parameters L* (lightness), b* (yellow/blue), chroma and hue angle were significantly ($P < 0.05$) affected by the calcium chloride treatments and dipping times. Therefore, $CaCl_2$ treatments significantly ($P<0.05$) delayed tomato color development from pink stage to red. The delay in color change in tomato fruits treated with $CaCl_2$ treatments might have resulted from decreased carotenoid biosynthesis (Sati and Qubbaj, 2021). Calcium treatments inhibited ethylene production by reducing aminocyclopropane carboxylic acid (ACC) content and ethylene-forming enzyme (EFE) activity (Cheverry *et al*., 2010). The ACC induces lycopene and α-, β- and δ-carotene accumulation by inducing phytoene synthase (*Psy1*), and repressing lycopene β-cyclase (β-Lcy1) and β-carotene hydroxylase (*Crtr-β2*) (Su *et al*., 2015). These transcriptional responses are responsible for lycopene accumulation, which is responsible for tomato fruit red colour.

Lightness (L*)

The tomato fruit lightness (L*) is one of the most important colorimetric parameters that strongly affect shelf-life (Seyed *et al*., 2021). Lightness parameter is a sign of fruit darkening with carotenoid synthesis and greenness loss (Wang *et al*., 2020). In the current study, a linear gradual decrease in tomato fruit lightness (L*) was observed during shelf-life period. These results agreed with Shehata *et al*. (2021), whereby, tomato fruit (cv 448) lightness decreased with increasing storage period, showing fruit darkness and less yellowing. According to Perdones *et al*. (2016), exocarp L* reduction during shelf-life period could be related to the surface dehydration which decrease surface glossiness. However, the dipping times (0, 30 and 60 minutes) did not show significant difference, irrespective of the treatments. Fruit treated with distilled water (control) maintained higher lightness than $CaCl_2$ treated fruit at 30 and 60 minutes. The low lightness retained by $CaCl_2$ may be attributed to calcium ability to reduce metabolism activities thus reducing the carotenoid synthesis (Seyed *et al*., 2021).

Redness (a*) and blueness (b*)

The a* and b* values represent the chromaticity. Red colour is the most attractive visual parameter to consumers (Arias *et al*., 2000). According to Wu and Kubota (2008), red color is gradually developed during fruit ripening and maturation due to the degradation of chlorophyll and lycopene pigment formation in 'Durinta' tomato fruit. The results from the present study showed gradual increase in tomato exocarp a* values with increasing shelf-life period, showing redder and mature fruit. Goncalves *et al*. (2020) found that the higher the component a* levels, the higher are lycopene levels. However, Sati and Qubbaj (2021) reported that gradual increase or slow changes in a* values occur when 'Izmir' tomato fruit are at mature breaker stage (pink or light red). In this study, no significant difference existed among the dipping times and treatments. Therefore, the $CaCl_2$ treatments did not significantly affect the red colour of the tomato at the pink stage.

The positive number for the chromatic descriptor b* indicates strong influence of yellow, being connected to the amount of β-carotenes. In tomatoes, carotene quantity present is associated with the quantity of light intercepted by the fruit (Gautier *et al*., 2008), with variations depending on the cultivar (Grolier *et al*., 2001). In this study, there was no significant changes in the b* component during the shelf-life period, regardless of the treatments and dipping time.

Chroma (C*)

The C* value provides a proportion of the pure predominant tone and concern the difference perception given on a colour relatively to white or grey, with opaque tones close to zero and with more lively to those close to 60 (Preczenhak *et al*., 2014). The higher chroma parameter value, the more saturated and intense is the fruit colour (Borguini and Silva, 2005). The current study showed an increase in the exocarp chroma a day after storage; and afterwards, decreased with shelf-life period, regardless of dipping times and treatments. Lopez-Camelo and Gomez (2004) found a similar trend on eleven tomato cultivars including 'Kada' tomato; whereby, chroma increased as the tomatoes changed from pink to light red to finally decline at the red stage.

In terms of treatments, fruit dipped for 60 minutes exhibited higher chroma values, followed by 0 and 30 minutes. While, control (distilled water) fruit dipped for 0 and 60

minutes, recorded higher chroma values when compared with $CaCl_2$ treatments (0.0045, 0.01 and 0.03%). However, 0.01 and 0.03% $CaCl_2$ fruit exhibited higher chroma values than control (distilled water) at 30 minutes dipping time. Therefore, it is possible to assume that fruit treated with distilled water accumulated higher amount of lycopene and carotene, which are responsible for the red colour (Alessi, 2010). This is due to rapid respiration in the control fruit and ethylene production, which caused an increased chloroplast-to-chromoplast conversion resulting in accumulation of the red colour pigment (lycopene) (Fu *et al*., 2016).

Hue angle (h˚)

According to Eleni and Theodoros (2011), when the calcium treated 'Bunner' cabbage colour became darker, a decrease in hue angle (h˚) was observed. In this study, hue angle values decreased gradually with shelf-life, irrespective of treatments. Moreover, high hue angle values were observed in control fruit (distilled water) when compared with $CaCl_2$ treatments. These results were similar to results of other researchers (Varela *et al*., 2007; Apintanapong *et al*., 2007) working on 'Lady Finger' banana slices and minimally processed 'Fuji' apple, whereby, calcium chloride as an intermediate inhibitor retained high hue angle values. The action of calcium chloride might be due to the polyphenol oxidase (PPO) inhibition by chloride ions, thus, preventing dark pigment formation in fruit (Garcia and Barrette, 2002).

4.2.2. Chemical parameters

The effect of calcium chloride ($CaCl_2$) on total soluble solids (TSS)

Fruit soluble solid concentration is a good index for determining fruit quality and maturity (Rutkowski *et al*., 2008). According to Kader (2008), TSS increase with maturity and ripening. The changes in soluble sugars of stored fruits and vegetables are due to the balance between anabolic and catabolic processes (Sanchez-Mata *et al*., 2003). In general, ripening involves a combination of both synthesis as well as degradation processes (Kasim and Kasim, 2015). All these processes involve interconversions of different array carbohydrates. According to Ruelas-Chacon *et al*. (2017), soluble solids content changes in tomatoes are correlated with hydrolytic changes in polysaccharides (hemicellulose and pectin). In this study, there was a significant effect ($P < 0.05$) between treatments, dipping times and shelf-life period. The level of TSS increased gradually with shelf-life, irrespective of treatments and

dipping times (Figure 4.4). Similarly, Hussain *et al*. (2010) reported that the 'Red delicious' apples TSS increased with storage period, irrespective of treatments.

In general, fruit dipped for 60 minutes had higher TSS than fruit dipped for 0 and 30 minutes, regardless of the treatments. Therefore, the present study results indicated that calcium absorption was less effective in 60 minutes dipping time. In contrast, Suriati *et al*. (2021) reported that longer immersion of fruit in calcium chloride can increase the calcium content in the tissue, which in turn can inhibit the respiration rate of the fruit. The slow rate of respiration causes slow starch conversion to glucose. Furthermore, less effectiveness of 60 minutes dipping time might have been due to other factors that influence the calcium entry such as; maturity degree, fruit structure and the dose (Saure, 2005).

In terms of treatments, fruit dipped in distilled water (control) for 0 and 30 minutes, exhibited higher TSS than calcium chloride treatments (0.0045, 0.01 and 0.03%) (Figure 4.4). These results were in agreement with Moradinezhad *et al*. (2019) who reported that the highest soluble solid values were found in control samples of 'Chinese' jujube fruit and the lowest in 1% calcium chloride treatment. Pila *et al*. (2010) also reported that dipping 'Duke' tomato fruit in calcium solution decreased the total soluble solids. Similarly, previous studies have been reported on the reduction of TSS in 'Camarosa' strawberry fruit (Aguayo *et al*., 2006) and 'Cristal' tomato fruit (Paliyath *et al*., 2008) as a result of dipping the fruit in calcium chloride. The high TSS level in distilled water treated fruit might be due to weight loss during storage, which occurs mainly due to water loss and that lead to higher concentration of sugars in fruit (Woolf and Ferguson, 2000). However, reduced TSS in calcium chloride treated fruit may be due to decreased respiration and slow synthesis and the use of metabolites in the fruit tissue (Pila *et al*. 2010). Calcium ions bind to the pectic acid of the fruit to form Ca-pectate, which inhibits the respiration process and promote sugars into organic acids. In contrast, fruit dipped in 0.03% $CaCl_2$ for 60 minutes showed higher TSS when compared with control (distilled water) fruit (Figure 4.4). This might be due to high concentration of $CaCl_2$ that caused surface damage and led to induced respiration and more accumulation of sugars (Manganaris *et al*., 2005).

The effect of calcium chloride ($CaCl_2$) on titratable acidity (TA)

Titratable acidity (TA) is the most essential chemical parameter for evaluating fruits quality during storage (Cordenunsi *et al*., 2003). According to Akhtar *et al*. (2010), titratable acidity is directly related to organic acid concentration present in the fruit. In the current study, TA decreased over time independent of treatments and dipping times (Figure 4.5). In the study by Moradinezhad and Jahani (2016), it was observed that the postharvest use of 2% calcium chloride increased the TA of the fruit at the end of the shelf life, which contradicts with the present results on tomato fruit. Hussain *et al*. (2012) found that 'Red delicious' apples acid loss was largely due to the organic acids utilization as respiratory substrates and new compounds synthesis during ripening. Moreover, Mahajan *et al*., (2011) reported that the 'Allahabad Safeda' guava fruit decrease in TA was attributed to an increase in malic enzyme and peruvate decarboxylation reaction during climacteric period. Additionally, sugar accumulation during ripening contributes to acidity decrease due to increase in TSS: acid ratio (Hussain *et al*., 2012).

Generally, fruit exposed to treatments for 0 and 30 minutes recorded high titratable acidity than fruit treated for 60 minutes. Furthermore, 0.03% $CaCl_2$ was more effective than control (distilled water) at 0 and 30 minutes dipping time (Figure 4.5). These results were in agreement with Safa *et al*. (2015), whereby, 'Kiona' sweet cherry fruit immersion of fruits in a 70 mM solution of calcium chloride before storage, preserved the TA during the shelf-life period time compared with the control. Moradinezhad *et al*. (2019) found that, the use of calcium chloride on 'Chinese' jujube fruit may lower the fruit metabolism by reducing the ethylene production and respiration rate that reduces the TA.

Calcium chloride is an ethylene inhibitor and plays an active role in the tomatoes ripening process (Opiyo and Ying, 2005) and ripening is associated with the conversion of starch and acids to sugar (Oms-Oliu *et al*., 2011). In this study, 0.01% $CaCl_2$ was more effective at 60 minutes dipping time in maintain the TA when compared with 0.03% $CaCl_2$. This may be attributed to sufficient dip time, which enabled the $CaCl_2$ to effectively retard metabolic processes; and therefore, accounted for change in titratable acidity and other nutrients. Whereas, the high

calcium chloride might have caused surface damage, which resulted into rapid respiration, thus, less acidity accumulation (Manganaris *et al*., 2005).

pH

In fruits and vegetables, pH plays an important preservation and development role (Khalil *et al*., 2012). In this study, pH was significantly affected by the calcium treatments, dipping times and shelf-life period. Generally, pH increased with an increase in shelf-life period, regardless of the treatments and dipping times (Figure 4.6). According to Etienne *et al*. (2013), fruit acidity increase is due to the loss of organic acids, malic and citric acids as the main acids found in most ripe fruits. Ramirez *et al*. (2005), observed that increase in 'Guimba' pineapple guava fruit pH during storage increased concomitant with respiration rates, and other metabolic processes.

Among the dipping times, fruit exposed to treatments for 0 minutes recorded higher pH values when compared with fruit treated for 30 and 60 minutes. Furthermore, control (distilled water) exhibited higher pH levels at 0 and 30 minutes when compared with $CaCl_2$ treatments (0.0045, 0.01 and 0.03%) (Figure 4.6). In 'Italia' table grapes the calcium ability to retain lower pH levels was due to its role in reducing the polygalacturonate activity; and therefore, marginal pH level increases occur (Nigro *et al*., 2006). According to Sasanuma and Suzuki (2015), calcium cross-linkages between polygalacturonic acid chains favour gel formation which enable calcium ability to interact with the substrate of the enzyme, and thereby, reducing polygalacturonase activity.

4.2.3 Physiological and pathological disorders

The effect of calcium chloride ($CaCl_2$) on decay (%)

In the current study, the lowest mean cumulative decay was recorded in the fruit treated 60 minutes. The control and 0.0045% $CaCl_2$ fruit recorded high decay when compared with 0.01 and 0.03% $CaCl_2$ fruit at 0, 30 and 60 minutes dipping time. In general, fruit decay decreased with an increase in $CaCl_2$ concentration (Figure 4.7). Similarly, Mahajan *et al*. (2011) also found that 'Allahabad safeda' guava fruit spoilage treated with $CaCl_2$ decreased with an increase in $CaCl_2$ concentration. The current study demonstrated that $CaCl_2$ application had merit effect in reducing tomato fruit decay. The decay reduction may be due to the positive role of calcium in

delaying the fruit senescence (Hernández-Muños *et al*., 2008). Therefore, maintaining cell wall integrity; and thus, lowering the decay.

The effect of calcium chloride ($CaCl_2$) on chilling injury

Originally, tomatoes are tropical fruit, and their cold storage is limited by chilling injury (CI) risk (Aghdam and Mohammadkhani, 2014). Chilled tomato fruit develop several symptoms, such as sunken areas on the fruits (blemishes), diseases caused by pathogen and losing their ability to develop full colour, which lead to substantial produce quality degradation (Zhao *et al*., 2009). In this study, chilling injury was more severe in fruit treated with calcium chloride for 0 minutes when compared with 30 and 60 minutes. Furthermore, control fruit exhibited high chilling injury symptoms when compared with $CaCl_2$ treatments. These results were similar to Aghdam (2013), whereby, 'Newton' tomato fruit treated with 50 and 100 µM calcium (Pro-ca) showed low chilling injury symptoms when compared with control. The application $CaCl_2$ induced the flavonoid metabolism alteration in the fruit peel when compared with control (Halbwirth *et al*., 2002). Flavonoids are secondary metabolites, which are responsible for biological processes such as pigmentation of flowers, fruits and vegetables, plant-pathogen interactions, fertility and protection against UV light (Bovy *et al*., 2007). According to Roemmelt *et al*. (2003), flavonoids scavenge reactive oxygen species (ROS), decrease the freezing point and reduce the oxidative stress. Flavonoids are hydrophilic and are primarily located in the cytosol and vacuole (Albrecht *et al*., 2004).

The effect of calcium chloride ($CaCl_2$) on black mould (%)

Black mould caused by *Alternaria alternata*, is among the most common diseases in tomato fruit (El-Garhy *et al*., 2020). In fruits and vegetables, black mould causes significant post-harvest quality losses; thereby, rendering large amounts of tomato fruits unfit for marketing and consumption (Asai and Shirasu, 2015). In this study, fruit dipped in distilled water (control) for 0, 30 and 60 minutes developed more black mould when compared with $CaCl_2$ treatments (0.0045, 0.01 and 0.03%). Whereas fruit treated with 0.01 and 0.03% $CaCl_2$ for 60 minutes were not affected by black mould (Figure 4.9). The calcium ability to reduce the fruit postharvest diseases development has been attributed mainly to the formation of calcium cross-linkages in the cell wall, resulting in decreased effectiveness of cell wall-macerating enzymes

secreted by the pathogen (Stosic *et al*., 2014). According to Boumaaza *et al*. (2015), the high external Ca^{2+} concentrations may increase the concentration of Ca^{2+} in the cytosol, which can be toxic to the fungus.

CHAPTER 5

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

The study investigated the appropriate dipping times into different calcium chloride concentrations to preserve the postharvest quality, storage and the shelf-life of 'Classic round' tomato. The interaction between the two factors showed a significant effect on physiological weight loss, firmness, colour parameters [L*, b*, chroma and hue angle (º)], total soluble solids (TSS), titratable acidity (TA), pH, decay and black mould occurrence. However, significant interactive effects were not shown on a* colour component and chilling injury.

The calcium chloride application at 30 and 60 minutes were effective in maintaining the physical properties of 'Classic round' tomato fruit when compared with 0 minutes. Generally, 0.1% $CaCl_2$ efficiently delayed physiological weight loss and retained fruit firmness at 60 minutes dipping time. Whereas, 0.3% maintained higher fruit firmness at 30 minutes dipping time. In addition, $CaCl_2$ treatments delayed colour change from pink to red when compared with control. However, no significant difference existed among the $CaCl_2$ concentrations in regards to colour change.

Total soluble solids, titratable acidity and pH were effectively retained in fruit treated for 0 and 30 minutes when compared with fruit treated for 60 minutes. The application of 0.03% $CaCl_2$ resulted in lower TSS and high TA and pH at 0 and 30 minutes dipping time. Whereas, 0.01% $CaCl_2$ was more effective in maintaining low TSS and high TA and pH at 60 minutes dipping time. The physiological and pathological disorders were significantly reduced on fruit treated for 30 and 60 minutes when compared with 0 minutes. Both 0.01 and 0.03% effectively reduced the development of decay, chilling injury and black mould when compared to 0.0045 and control (distilled water).

5.2 Conclusion

Calcium chloride improved the quality and shelf-life of 'Classic round' tomato fruit. High $CaCl_2$ concentrations (0.03%) were more effective on maintaining 'Classic round' tomato quality at a short dipping period (30 minutes). Whereas low concentration (0.01%) effectively work at a longer dipping period (60 minutes).

5.3 Recommendations

Based on the current findings, 0.01 and 0.03% $CaCl_2$ can be used at 30 and 60 minutes dipping time, respectively, to preserve the quality and shelf-life of tomato fruit commercially. Therefore, these concentrations will assist in reducing the tomato postharvest losses on the supply chain. Moreover, this will increase the contribution of tomato to the vegetable gross domestic product (GDP). The future studies should focus on investigating the effect of 0.1 and 0.03% $CaCl_2$ on other physiochemical parameters including ascorbic acid, taste and vitamin C to sum the calcium ability on the overall quality of 'Classic round' tomato. Additionally, the current efficient concentrations can be investigated on other tomato cultivars and fruits with high postharvest losses.

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