THE POTENTIAL OF PUTRESCINE POSTHARVEST DIPS AND COLD STORAGE TEMPERATURE ON FRUIT QUALITY AND SHELF-LIFE OF 'SOLO' PAPAYA (*CARICA PAPAYA* L.)

By

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DECLARATION

I, Eulenda Tinyiko Mabunda declare that the mini-dissertation 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. Also, this is my work in design and execution, and related materials contained herein had been properly acknowledged.

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DEDICATION

This study is dedicated to my family and friends.

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ABSTRACT

Cold storage is commonly used to prolong papaya fruit storability. Furthermore, the optimal recommended storage temperature is below 10℃ for export and distant market. However, chilling injury (CI) occurs at 10℃ or lower during prolonged cold storage. This condition hampered consumer acceptance, resulting in economic losses for producers and exporters. Therefore, the study aimed to investigate the potential of postharvest polyamine dips and storage conditions to improve the quality and shelf-life of 'Solo' papaya fruit. The experiment was conducted as 4 x 2 factorial arranged in a completely randomised design (CRD) with eight replications. The fruits were treated with putrescine (PUT) (0 (control), 1, 2 and 3 mM) before storage for 21 days at 7.5 and 13℃ plus 5 days storage at ambient temperature. Additionally, the PUT effect on quality attributes and shelf-life were studied. The results showed that physiological and pathological disorders increased with progressive storage, irrespective of storage temperature. However, PUT treatment reduced the incidence of chilling injury and anthracnose at both 7.5 and 13℃. Additionally, the interaction of treatment and cold storage temperature significantly affected 'Solo' papaya fruit physical and biochemical quality attributes. Furthermore, treatment with 2 and 3 mM PUT concentration reduced changes in colour, mass, firmness, TA, and TSS compared to control. In conclusion, postharvest PUT improved 'Solo' papaya fruit quality and prolonged shelf-life.

Keywords: Biochemical and physical properties; chilling injury; pathological disorders; firmness; PUT postharvest dips; mass loss

CHAPTER 1
INTRODUCTION

1.1. Background

Papaya (*Carica papaya* L.) is a typical climacteric fleshy fruit appreciated worldwide due to its sweetness and characteristic flavour associated with the soft pulp (Harindra *et al*., 2015). Tropical countries from Asia are the main papaya producers, accounting for 56% of worldwide production (FAOSTAT, 2017). However, countries from South America (16%), Africa (10%) and Central America (9%) are also important papaya producers (FAOSTAT, 2017). In South Africa, papaya is cultivated commercially mainly in the Lowveld areas of Mpumalanga province accounting for 61% of the gross farming income from the papaya industry and Limpopo for 37% and the remaining 2% from KwaZulu-Natal (Schulze and Maharaj, 2007). The domestic market has steadily grown by 10% in recent years, presumable due to increasing awareness of the potential health benefit attributed to papaya phytochemicals (Khosroshahi *et al*., 2007).

Papaya ranks first among fruits for vitamin C, vitamin A, riboflavin, folate, calcium, thiamin, iron, niacin, potassium, and fibre (Pawase *et al*., 2018). Moreover, it is an excellent provitamin A (carotenoids) source, which is important for good health, especially, eyesight and it reduces the chances of early blindness in children (Sherwin *et al*., 2012). In addition, papaya fruit contains higher lycopene levels, an important antioxidant aiding in digestion (Harindra *et al*., 2015). However, despite these numerous health benefits, papaya fruits are highly susceptible to mechanical damage and postharvest disorders compromising fruit quality (Patel *et al*., 2016).

Consequently, this makes it an easily perishable fruit with a short shelf-life (Patel *et al*., 2016). Therefore, storing perishable produce at optimum temperature determines its quality and it is a crucial factor during retailing, distribution, and transportation (Proulx *et al*., 2005).

According to Singh and Rao (2011), an optimal papaya storage temperature depends on the harvest maturity stage. Normally, the appearance of yellow string on the blossom-end of the fruit surface indicates the optimum maturity for long-distance transport (Kadder, 2006). Furthermore, fruit harvesting may be delayed for local markets until the fruit surface is ≥50% yellow (Singh and Rao, 2011). Papaya fruit harvested at mature-green to one-fourth yellow stage are recommended to be stored at 13℃ for up to 21 days, whereas partially ripe fruit showing one-fourth to one-half yellow colour is stored at 10℃ (Kadder, 2006). According to Ahmad and Siddiqui (2015), temperature storage is a common technique used to improve the longevity and quality of many fruits. However, storage below critical temperature often causes poor fruit quality and chilling injury (CI) in tropical and subtropical crops (Jaw *et al*., 2012). Consequently, the climacteric nature of papaya fruit frequently encounters postharvest storage issues such as rapid respiration, ethylene (C_2H_4) production, firmness loss, quality deterioration and CI when stored below 10℃ (Ahmad and Siddiqui, 2015). However, exogenous polyamines (PAs) are emerging as an effective tool in maintaining quality and extending the shelf-life of fruit crops under cold storage conditions (Jaw *et al*., 2012). Research evidence indicates that PAs act as an anti-senescence agent, which reduce respiration rate, delay C_2H_4 production, retard colour change, maintain firmness, and reducing mechanical and chilling damage (Valero *et al*., 2002a). In addition, PAs accumulation induces defence resistance against CI

through increased arginine decarboxylase activity, consequently preserving membrane integrity (Koushesh-Saba *et al*., 2012).

In nature, polyamines are positively charged aliphatic amino acids present amongst all living cells and involved in numerous biological processes including plant growth and development, flowering, ripening, stress response and senescence (Valero *et al*., 2002b). In horticultural fruit crops, postharvest treatment with PAs have been demonstrated to influence ripening processes, fruit quality and shelf-life (Hanif *et al*., 2020). Several studies showed that polyamines [Spermidine (SPD), Putriscene (PUT) and Spermine (SPM)] biosynthesis shares a common precursor s-adenosyl methionine (SAM) with ethylene (C_2H_4) (Hanif *et al*., 2020). Ethylene evolution is associated with rapid softening, firmness loss, ripening, and fruit senescence; however, PAs have been reported to induce opposite effects related to fruit ripening and senescence (Valero *et al*., 2002a). In ‘Mauricio’ apricot (*Prunus armeniaca*) fruit treatment with one mM PUT improved the shelf-life of damaged and undamaged fruit by reducing C_2H_4 evolution and respiration rates; and thereby, mitigating the mechanically damaged bruised zones (Martínez *et al*., 2002). Similar results were reported in ‘Kensington pride’ mango (*Mangifera indica*) fruit, whereby, 2 mM PUT application increased fruit shelf-life (Martínez *et al*., 2002). Furthermore, exogenous one mM PUT application on ‘Hayward’ kiwifruit (*Actinidia deliciosa*) reduced respiration rate, ethylene production, consequently, increasing shelf-life and fruit firmness, therefore, maintaining the overall fruit quality attribute (Valero *et al*., 2002a). Unlike most fruit crops, there is no information on PUT potential to maintain papaya fruit quality and extend the shelf-life under cold storage conditions, which provides strong grounds for the study undertaken (Hanif *et al*., 2020). Therefore, the present

work was initiated to evaluate the potential of putrescine postharvest dips and cold storage to improve ‘Solo’ papaya fruit quality and shelf-life.

1.2. Problem Statement

Papaya fruit is highly perishable due to its climacteric characteristics such as; respiratory peak, rapid C_2H_4 production and accelerated softening, limiting quality and shelf-life (Ahmad and Siddiqui, 2015). Consequently, these are the major problems associated with commercialization and distribution as the fruit becomes susceptible to postharvest disorders and decay, resulting in quality loss (Singh and Rao 2011). Furthermore, as papaya fruit has a relatively short shelf-life (about 7 days) compared with other fruit crops, maintaining quality during transportation from production areas to consumers is a challenge (Khosroshahi *et al*., 2007). Therefore, different storage and preservation methods are used to keep the harvested fruits in edible condition (Ayomide *et al*., 2019).

Harvesting methods, harvest maturity, postharvest treatments and storage conditions associated with maintaining papaya fruit quality have been of least interest to many researchers and producers (Chutichudet and Chutichudet, 2014). Consequently, fruits are often of poor quality, with considerable losses. Cold storage and PAs like PUT are developing as approaches for lengthening fruits shelf-life and delaying ripening processes (Koushesh-Saba *et al*., 2012). Putrescine bind to the phospholipid site of the cell membrane to prevent cytolysis and improve cold resistance (Li and He, 2012). Additionally, PUT can reduce colour change and firmness loss, subsequently prompting resistance toward mechanical and chilling damage (Khan *et al*., 2008).

Therefore, research should be done on postharvest PUT dips and low-temperature storage conditions in improving papaya fruit quality.

1.3. Rationale

Papaya is one of the most significant fruit crops and is an excellent source of antioxidant nutrients (flavonoids, carotenes), minerals (potassium and magnesium) and fibre (Mendoza *et al*., 2008). Nevertheless, papaya is highly perishable, and its climacteric nature makes it to be subjected to rapid deterioration because of rapid respiratory activities (Workneh *et al*., 2012). Consequently, the fruit encounters high postharvest losses; therefore, long-distance fruit transportation is a challenge (Widodo *et al*., 2016). Cold storage is a technique used to slow down metabolic activities, maintaining fruit quality (Widodo *et al*., 2016). However, papaya fruits are highly susceptible to low-temperature (below 10℃) storage as they develop chilling, leading to poor quality and short shelf-life (Workneh *et al*., 2012). Therefore, this necessitates finding other technologies for prolonging papaya storability under cold storage. Putrescine as a postharvest treatment has been shown to possess a pronounced capability of increasing storage and maintaining the firmness of numerous fruit crops, for example strawberry (*Fragaria ananassa*), plum (*Prunus domestica*), apricot (*Prunus armeniaca*) and mango (*Mangifera indica*) under cold storage (Malik and Singh, 2005 and Khosroshahi *et al*., 2007).

Recently, it was shown that 2 Mm PUT is the most effective concentration for maintaining `Red Lady` papaya harvested at 25% yellow colour break (Hanif *et al*., 2020). However, there is no information about PUT potential on preserving ‘Solo’

papaya quality harvested at 25% yellow colour-break stage under cold storage conditions (Workneh *et al*., 2012). Therefore, the proposed study focused on PUT postharvest dips and low-temperature storage potential to improve papaya fruit quality and shelf-life.

1.4. Aim and Objectives

1.4.1. Aim

To investigate the potential of postharvest PUT dips and cold storage temperature on the improvement of 'Solo' papaya fruit quality and shelf-life.

1.4.2. Objective

To determine whether postharvest PUT dips and storage conditions will improve 'Solo' papaya fruit physico-chemical properties and shelf-life.

1.5. Hypothesis

The application of postharvest PUT dips and storage conditions had no effect on 'Solo' papaya fruit quality and shelf-life.

1.6. Structure of mini-dissertation

Chapter 1: The introduction provides the study background and the importance of papaya fruit crop. Furthermore, it includes problem statement, motivation, the scope of work, and study aims and objectives.

Chapter 2: Reviews previous and relevant work about papaya fruit crops, their climacteric nature, uses and postharvest quality. Moreover, it comprises a detailed

description of PUT and cold storage's role in the improvement of the quality and shelf-life of various fruit crops.

Chapter 3: Evaluates quality parameters such as titratable acidity (TA), total soluble solids (TSS), colour, firmness and mass with the main objective to link such parameters to fruit ripening and quality in reference to the treatments applied.

Chapter 4: This chapter aims to interpret and discuss results clearly to outline the difference amongst treatments based on mean separation to determine the best treatment dose.

Chapter 5: Aims to provide a general summary, conclusion and recommendations of the study. It also specifies key findings and recommends on how to maintain quality and extend the shelf-life of papaya fruits. Furthermore, it indicates the research gaps for future research.

CHAPTER 2
LITERATURE REVIEW

2.1. Introduction

In tropical and subtropical zones, papaya (*Carica papaya* L.) is popular; and economically important fruit crop (Silva *et al*., 2007). In general, papaya fruit is rich in antioxidant nutrients (carotenes, vitamin C and flavonoids), B vitamins (folate and pantothenic acid), minerals (potassium and magnesium), and fibre (Evans and Ballen, 2012). Therefore, papaya consumption is considered for its medicinal benefits, particularly digestion enhancement and relief from constipation (Jayathunge *et al.,* 2011). However, being a climacteric fruit, marketing papaya is a great problem because of its short shelf-life, which leads to high postharvest losses (Archbold *et al*., 2003; Lanka *et al*., 2011; Marpudi *et al*., 2011).

After harvest papaya fruit has a limited storage-life due to adverse physicochemical changes such as mass loss, fruit softening, quality deterioration and changes in sugars and acid content, which may differ between different cultivars (Parven *et al*., 2020). According to Jayathunge *et al*. (2011), ‘Rathne’ papaya fruit shelf-life varies from 3 to 6 days under tropical climatic conditions. Additionally, papaya's short shelf-life may be attributed to rapid ripening and presents a serious constraint for efficient handling and transportation (Jayathunge *et al*., 2011). Therefore, low storage is one of the techniques used to prolong fruit storability by reducing excessive desiccation and decay (Waskar *et al*., 2015).

Cold temperature storage is the most common technique to extend papaya fruit shelf-life (Ayele and Bayleyegn, 2017). In general, cold storage temperature reduces fruit respiration rate, mass, firmness loss, and decay (El Otmani *et al*., 2011; Maul *et al*., 2011). Furthermore, cold storage temperature also maintains fruit biochemical properties such as total soluble solids and organic acids (Rab *et al*., 2015; Marcilla *et al*., 2009). However, most tropical, and subtropical fruits are sensitive to cold damage, especially when exposed to low temperatures above freezing point (Gross *et al*., 2016). Generally, 'EX 15' papaya fruit is injured when stored below their critical temperature, ranging between 10 and 13℃ for most varieties (Proulx *et al*., 2005). In recent years, polyamines postharvest (PAs) treatment has been the emerging technique used to improve the shelf-life of fruits under cold storage temperature by inhibiting chilling damage, ethylene (C_2H_4) biosynthesis and delaying the ripening process (Koushesh-Saba *et al*., 2012).

In nature, polyamines occur as free molecular bases and have been reported to bind with negatively charged phospholipids or other anionic sites on membranes (Pandey *et al*., 2002). Putrescine (PUT), spermine (SPM) and spermidine (SPD) are biologically active PAs form that can regulate various physical, physiological and biochemical processes of fruit (Malik and Singh, 2005; Khan *et al*., 2007). Putrescine application maintains cell turgor, membrane integrity, and tissue firmness and delays membrane lipid catabolism, extending fruit storage-life. Furthermore, exogenous PUT application imparts other beneficial effects such as; delayed colour change, reduced mechanical damage susceptibility and chilling injury and increased shelf-life of both climacteric and non-climacteric fruit (Khosroshahi *et al*., 2007, Fawole *et al*., 2020). However, there is limited information on the effect of PUT on extending shelf-life, alleviating CI and maintaining papaya fruit quality attributes (Hanif *et al*., 2020). Therefore, this

review aimed to discuss existing knowledge on PUT and cold storage's role in the improvement of fruit crop's quality and shelf-life.

2.2. Cold storage

Cold storage temperature is the most important environmental factor that influences the deterioration of harvested horticultural commodities (Kassim *et al*., 2013). Low-temperature storage is a commonly used technique to extend papaya fruit shelf-life (Ayele and Bayleyegn, 2017). Furthermore, the shelf-life of most perishable commodities is extended at a temperature near 0℃ (Emongor, 2010; Kader, 2013). In general, storing fruit crops at low temperatures decreases metabolic activity, rapid colour development, firmness and flavour loss (Tedesse *et al*., 2015). Therefore, the use of an optimum temperature during transportation, distribution and retailing is a major factor that determines the quality of fruit crops (Kader, 2011).

The optimum storage temperature of banana (*Musa acuminate*) and papaya (*Carica papaya* L) range between 13-14 and 7-13℃, respectively (Zhou *et al*., 2014). Additionally, 'Williams' banana fruit stored at 12℃ and 'Hortus gold' papaya fruit at 7℃ had an extended shelf-life of 8 weeks. In general, the shelf-life of fruits is extended at low-temperature storage because metabolism is retarded by a reduction in respiration rate, ethylene production, colour changes and softening (Gebreslessie, 2003). However, low temperatures for papaya storage are limited due to its susceptibility to chilling injury (Perez *et al*., 2014). Moreover, papaya fruit kept in refrigerated storage for a more extended period is susceptible to fungal decay (Zhou *et al*., 2014). However, Malik and Singh (2005) reported that PUT treatment maintained 'Kensington Pride' mango *(Mangifera indica)* fruit quality under low-temperature storage conditions by

lowering ethylene and respiration rate. Additionally, PUT possesses the ability to reduce colour change and firmness loss, subsequently prompting resistance towards mechanical and chilling damage (Barman *et al*., 2011).

2.3. Polyamines biosynthesis

The biosynthesis pathways for polyamines are well established in many organisms; mammals, fungi, bacteria, and plants (Valero *et al*., 2002a; Kusano *et al*., 2007). In plants, PUT is produced by two alternate pathways; the first one is catalyzed by ornithine decarboxylase (ODC), the second and most common pathway starts with arginine formation because of arginine decarboxylase (ADC) action via agmatine (Kusano *et al*., 2007). Agmatine amidohydrolase (AIH) and *N*-carbamoylputrescine amidohydrolase are collectively two enzymes involved in agmatine conversion into PUT (Kusano *et al*., 2007). Putrescine (PUT) is converted into SPD and then into SPM by the addition of an aminopropyl residue from decarboxylated S-adenosylmethionine (DCSAM), which results from SAM decarboxylation by SAM decarboxylase (SAMDC) (Valero *et al*., 2002a). These reactions are catalyzed by two closely related enzymes; SPD synthase (SPDS) and SPM synthase (SPMS) (Valero *et al*., 2002a).

Ethylene (C_2H_4) and polyamines (SPD and SPM) biosynthesis share a common precursor SAM and are known to exert opposite effects concerning fruit ripening and senescence (Hosseini *et al*., 2018). Therefore, a balance between PAs and C_2H_4 is critical to retard or to accelerate both processes (Pandey *et al*., 2000; Valero *et al*., 2002). During senescence, PAs decrease and are likely a cause of the initiation or acceleration of C_2H_4 production through ACS induction or the increase of tissue sensitivity to C_2H_4 action (Valero *et al*., 2002a). Conversely, PAs (probably in conjunction with other growth-promoting substances) may be required to reduce the

ACS gene(s) expression; thus, inhibiting ethylene production in developing tissues (Fawole *et al*., 2020). The inhibitory effects of exogenous PAs in ethylene production may be ascribed to the competitive biosynthesis mechanism between ethylene and PAs, therefore, ACC synthase and ACC oxidase inhibition (Yahia *et al*., 2001).

2.4. The relationship between endogenous and exogenous PUT

During plant growth, polyamine synthetase (PAS) metabolic enzymes and polyamine content change throughout the stages (Duan, 2007). Endogenous PAs and PAS activity were found to be highest during ovary development in tomato (*Solanum lycopersicum*) and avocado (*Persea americana*) fruit crops and lowest during senescence (Valero *et al*., 2002a; Kushad and Dumbroff, 1991). As senescence progresses, chlorophyll content gradually decreases, with ADC (arginine decarboxylase) and ODC (argininedecarboxylase) activities decrease, while PAO (polyamine oxidase) and hydrolases activities, such as ribonuclease and protease rapidly increase (Valero *et al*., 2002b). All these changes are inhibited by exogenous PAs application (Duan, 2007; Chen *et al*., 2019).

2.5. Effect of PUT and cold temperature storage on physical parameters

Mass

Mass is an important quality parameter as produce price is often determined on a mass basis (Atukuri, 2017). During storage, physiological mass loss occurs due to horticultural produce's transpiration and respiration processes (Lata, 2017). In fresh produce, mass loss is an important index of post-harvest storage-life (Atukuri, 2017). It is mainly attributed to water loss during metabolic processes such as respiration and transpiration (Fawole *et al*., 2020). Furthermore, cellular metabolism during the ripening process also results in substrate and water vapour lost, therefore,

accelerating mass loss (Shiri *et al.,* 2013). Generally, relative humidity and storage temperature at which the product is kept are the primary factors that affect mass loss (Maguire *et al*., 2001; Jourbert, 2016).

Mass loss in fruits and vegetable increases as the storage temperature increases due to the increase in the vapour pressure deficit (VPD), causing higher moisture loss (Díaz-Pérez, 2019). Additionally, 'Malike' tomato fruit stored at 5℃ exhibited lower cumulative mass loss, in contrast, higher mass loss values were observed at 10℃ (Tedesse *et al*., 2015). Similar results of fruit mass loss increase in storage temperature have been reported on different fruits such as 'Conchor' tomato (Žnidarčič, 2006), and 'Hass' avocado fruits (Perez *et al*., 2004). However, PUT (1mM) postharvest dips was shown to reduce mass loss on 'Mrida' pomegranate and 'Langra' mango fruit under low-temperature storage through cell integrity permeability consolidation and reduced chilling damage (Barma *et al*., 2011 and Jawandha *et al*., 2012).

Generally, a significantly reduced mass loss rate was observed in 'Florddaking' peach fruit treated with 2 mM PUT when compared with control fruit at 1℃ (Abbasi *et al*., 2019). Furthermore, smaller mass loss was reported on 'Native' and 'Cavendish' banana fruit treated with 2 mM PUT when compared with control fruit at 2℃ (Hosseini *et al.*, 2018). Similarly, 'Wonderful' pomegranate fruit treated with 3 mM PUT had the lowest mass loss when compared with control fruit (Atukuri, 2017). Additionally, the highest mass loss was found on untreated 'Red lady', whereas 2 mM PUT treated fruit had the lowest mass loss at 12℃ (Hanif *et al*., 2020). Putrescine effect on mass loss could be due to changes in fruits biophysical properties (Abbasi *et al*., 2019). Generally, PUT modify cell wall properties and water tissue permeability in fruit crops

((Martínez *et al*., 2002). Additionally, reduced mass loss in PUT treated fruit could be attributed to stabilization or consolidation of both cell integrity and the permeability of the cell wall tissues (Malik and Singh, 2005). Furthermore, PUT prevents chilling injury, consequently inducing tissue disruption and connection between the skin and the external atmosphere allowing water vapour transference (Hosseini *et al*., 2018).

Fruit firmness

The shelf-life of fruits is dependent on firmness, which changes significantly during ripening and storage (Díaz-Pérez, 2019). During ripening, fruit softening is the major limiting factor for shelf-life (Razzaq *et al*., 2013a). Furthermore, a substantial cell wall pectin portion is converted to a water-soluble form affecting the texture as fruits ripen (Díaz-Pérez, 2019). Generally, the major changes involved in fruit softening are cell wall catabolism and interstellar matrix development containing pectin (Díaz-Pérez, 2019). Additionally, enzymes such as polygalacturonase (PG), pectinmethylesterase (PME), β-galactosidase, and pectatelyase (PL) degrade the polymeric carbon hydrates, mainly pectin and hemicellulose, thus, cell wall weakening (Sañudo-Barajas *et al*., 2019).

Furthermore, excessive production and reactive oxygen species (ROS) accumulation cause oxidative damage, which consequently reduce antioxidant systems' ability to eliminate free radicals such as hydrogen peroxide (H_2O_2), hydroxyl radical (HO) and superoxide (O_2^-) during ripening of fruits (Jimenez et *al*., 2002). The generated ROS induce cell damage, including membrane integrity loss in the tissue and oxidative damage to lipid, DNA and proteins (Hodges *et al*., 2003). According to Botton *et al*. (2019), fruits exhibit firmness loss, possibly due to hydrolase enzyme action, induced by ethylene as the storage time elapse.

Generally, ‘Gaixian’, ‘Yuhuang’ and ‘Aozhou 14’ plum fruit stored at 0 and 2℃ maintained firmness for a longer time, while fruit stored at higher temperature (25℃) showed a drastic softening (Pan *et al*., 2016). Similarly, ‘B10’ carambola fruit at 10°C suppressed cell wall solubilization, thereby, delaying cell wall polyuronides depolymerisation and retarding the increase in PG and PME (cell wall enzymes) activities (Fawole and Opara, 2013). Furthermore, PAs are good anti-senescent compounds that can retard fruit softening and maintain fruit quality during storage (Khan *et al*., 2007).

In untreated ‘Bagheri’ and ‘Asgarabadi’ apricot fruit, firmness decreased while fruit treated with one mM PUT had the highest firmness values at 1℃ (Koushesh-Saba *et al*. 2012). Additionally, the highest fruit firmness was observed in ‘Selva’ strawberry fruit treated with 2 mM PUT at 5℃, whereas lowest firmness rates were recorded in control fruits Khosrohahi *et al*. (2007). Similarly, ‘Angelino’ plum fruit treated with 2 mM PUT was firmer when compared with untreated fruit at 1℃ (Khan *et al*. 2008). Furthermore, ‘Wonderful’ pomegranate fruit treated with 2 mM PUT exhibited high firmness values compared to control fruit at 5℃ (Fawole *et al*., 2020). The main effects of infiltrated PA during the postharvest-life of fruits and vegetables is a net increase in firmness (Valero *et al*., 2002b).

The effect of polyamines on maintaining fruit firmness can be attributed to their cross-linkages to pectin -COO- group substances in the cell wall, blocking the access of degrading enzymes, therefore, reducing softening rate during storage (Valero *et al*., 2002a). Additionally, PAs play a vital role in the antioxidative system and protect membranes against oxidative injury caused by ROS (Verma and Mishra, 2005). In

general, PUT exhibit antioxidant properties in relation to H_2O_2 and O_2 radicals (Li *et al*., 2005). Furthermore, postharvest PUT application has been found to increase the total antioxidant activity in 'Angelino' plum (Khan *et al*., 2008) and 'Lasgerdi' and 'Shahrodi' apricot (Davarynejad *et al*., 2013).

Colour

Fruit colour is an important maturity index that determines the optimum harvesting stage of many fruits (Lata, 2017). According to Díaz-Pérez (2019), fruit colour is influenced by the concentration and distribution of skin pigment such as; anthocyanins, chlorophyll, carotenoids and external factors. In addition, rapid colour development could be due to the tissue oxidation process and water loss during ripening process (Singh *et al*., 2012). Furthermore, carotenoids, such as lycoperne, β- and α- carotene, luteine, and β- and α- cryptoxanthin are also major colour pigments that impart colour to many horticultural commodities such as tomato (*Solanum lycopersicum*), carrot *(Daucus carota)*, mango *(Mangifera indica*) and papaya (*Carica papaya* L) (Díaz-Pérez, 2019). In 'Hongkong' papaya fruit, colour change is ascribed to chlorophyll enzymatic degradation as fruit maturity advances (Ding *et al*., 2007). In 'Hass' avocado fruit, colour can be determined using objective colour parameters such as lightness (L*), chroma (C*), redness and greenness (a*), yellowness and blueness (b*) and hue angle (h^0) and subjective (eye colour) colour parameters (Cox *et al*., 2004). Additionally, low storage temperature influences 'Williams' banana fruit colour indices (Gebreslessie, 2003).

In 'EX 15' papaya fruit, colour indices did not change during 5℃ storage when compared with fruit stored at 15 or 20℃ (Proulx *et al*., 2005). Furthermore, rapid colour development was observed on 'Zhongbai' papaya fruit stored at 16℃, contrarily, in

fruit stored at 1 and 6℃, colour change was delayed (Pan *et al*., 2016). Rapid colour change at higher temperature storage could be attributed to the tissue oxidation process and water loss associated with reduced lightness as fruits ripen (Singh *et al*., 2012). However, PUT may retard chlorophyll degradation in skin tissues by inhibiting peroxidase activity (Hosseini *et al.,* 2018).

Generally, 2 mM PUT significantly lowered a* value in ‘Angelino’ plum fruit when compared with control fruits at 1℃ (Khan et al., 2008). Additionally, higher values of a* and b* were found on untreated ‘Langra’ mango fruit while 2 mM PUT treated fruit had lower a* and b* values at 13℃ (Jawandha *et al.*, 2012). Similarly, a progressive decline in L* values was observed on untreated ‘Native’ and ‘Cavendish’ banana fruit, whereas 2 mM PUT treatment increased L* values (Hosseini *et al*., 2018). Furthermore, L* values were decreased on untreated ‘Santa’ plum fruit whereas, fruits treated with 1 mM PUT maintained high L* values at 13℃ (Serrano *et al*., 2003).

Putrescine infiltration effect is to ameliorate chlorophyll breakdown in several plant organs, including fruits, such as 2 mM PUT treated ‘Mauricio’ apricot fruit at 10℃ (Martínez-Romero *et al.*, 2002). Furthermore, PUT treatment was found to delay colour change in ‘Mauricio’ apricot fruit during cold storage, which is an indicator of reduced senescence rate (Martínez-Romero *et al*., 2002). Additionally, exogenous PUT retarded chlorophyll loss in ‘Honey Dew’ muskmelon fruit by reducing the hydrolytic activities acting on chloroplast thylakoid membranes (Lester, 2000).

2.6. Effect of PUT on biochemical parameters

Titratable Acidity (TA)

In fruits, titratable acid (TA) assessment primarily estimates consumption quality and hidden attributes (Gebreslassie, 2003). Furthermore, TA is considered an indicator of fruit maturity or ripeness (Lata, 2017). Generally, acids make an important contribution to fruit post-harvest quality, as taste is mainly a balance between the sugar and acid contents (Hanif *et al*., 2020). Therefore, postharvest acidity assessment is important in taste evaluation on fruit crops (Gebreslessie, 2003). Generally, there is a decrease in total acidity with ripening of commodity (Ngnambah, 2013). In 'Solo' papaya (*Carica papaya* L) fruit, the main organic acids are citric acid, malic acid and quinic acid (Ngnambah, 2013). Furthermore, titratable acid content decreases during ripening and storage in 'Santa Clara' tomato fruit (De Castro *et al*., (2005).

Generally, 'Hortus gold' papaya fruit exhibited a decreased TA trend content at 5.5℃ when compared with fruit at 7, 10 and 22℃ storage temperatures (Gebreselasie (2003). Similarly, a higher TA content was observed on 'Malike' tomato fruit stored at 10℃ when compared with fruit at 5℃ (Žnidarčič and Požr 2006). Generally, higher TA loss in fruit crops could be attributed to higher respiration and ripening rate, whereby, organic acids are used as a substrate in the respiration process (Fawole and Opara, 2013). Furthermore, PUT reduces the titratable acidity of fruits by suppressing respiration (Fawole *et al*., 2020).

In 'Langra' mango fruit, titratable acid decrease was observed by 2 mM PUT treatment when compared with the control fruit at 13℃ (Jawanda *et al.,* 2012). Furthermore, TA decreased in 'Mridula' pomegranate fruit treated with 2 mM PUT when compared with control fruit at 2℃ (Barman *et al.,* 2011). Similarly, 'Native' and 'Cavendish' banana fruit treated with 2 mM PUT retained minimum TA values when compared with control

fruit at 2℃ (Hosseini *et al*., 2018). Additionally, exogenous PUT application delayed respiration rate and ethylene production during storage in 'Wonderful' pomegranate fruit at 5℃ which caused a delay in organic acids, consequently, reducing fruit TA content (Fawole *et al*., 2020).

Total soluble solids (TSS)

Fruits contain many compounds which are water-soluble such as sugars, acids, vitamin C and amino acids (Lata, 2017). These soluble compounds form fruit soluble solids content (Fawole *et al*., 2020). In most ripe fruits, sugars form the main soluble solids component (Gebreslessie, 2003). Generally, total soluble solids increase during fruit maturity and ripening (Fawole *et al*., 2020). Therefore, TSS content is a useful maturity index or ripeness stage (Fawole and Opara, 2013). Furthermore, a refractometer is used to measure fruit total soluble solids content; for papaya, the optimum TSS is 11.5 °Brix (Lata, 2017). Generally, TSS increase with colour, maturity and storage temperature, as demonstrated in 'Malike' tomato fruit at 5℃ (Žnidarčič and Požrl, 2006).

In 'Sai Num Phueng' and 'See Thong' mandarin citrus fruit, low temperature (5℃) storage resulted in higher TSS content when compared with fruit stored at higher temperature (25℃) (Roongruangsri, 2013). Similar results were obtained in 'Hortus gold' papaya fruit, whereby the fruit exhibited relatively higher TSS values at 7℃ when compared with higher storage temperatures (10 and 22℃) Gebreslessie (2003). The increase in TSS can be ascribed to starch hydrolysis products, sucrose and gluconeogenesis synthesis (Fawole and Opara, 2013). Furthermore, an increasing TSS trend was demonstrated on 'Selva' strawberry fruit subjected to 2 mM PUT,

therefore this may be ascribed to ethylene suppression of ethylene biosynthesis which directly affects the metabolism of sugar in fruit (Khosroshahi *et al*., 2007).

Mirdehghan *et al*. (2007) observed no significant effect on TSS of 'Mollar de Elche' pomegranate fruit subjected to 1 mM PUT at 2℃. In contrast, 'Angelino' plum fruit treated with 2 mM PUT exhibited lower TSS content when compared with control fruit at 1℃ (Khan *et al*., 2008). Additionally, lower TSS values were found in 'Native' and 'Cavendish' banana fruit treated with 2 mM PUT than those found in the control fruit at 2℃ (Hosseini *et al*., 2018). Furthermore, 2 mM PUT resulted in minimum TSS accumulation when compared with control fruit at 12℃ in 'Red lady' papaya fruit (Hanif *et al*., 2020). Putrescine role to modify total soluble solutes remain unclear, however, most reports postulated that the PUT may reduce respiration rate and lead to a slow breakdown process of starch to sugars (Malik and Singh, 2006; Hanif *et al*., 2020; Hosseini *et al*., 2018) as well as suppressing consumption of organic acids metabolism (Torrigiani *et al*., 2004). Additionally, it may be attributed to slow metabolic transformation in soluble components and conversion to sugars resulting in reduced TSS content due to retarded ripening process (Bhat *et al*, 2014).

2.7. Effect of PUT pathological and physiological disorders

Chilling injury (CI)

Chilling injury (CI) is a physiological disorder that develops when subtropical and tropical fruits are stored at a temperature ranging from 0-8℃ (Sevillano *et al*., 2009). According to Kok (2011), CI is dependent on the temperature, low-temperature duration, cultivar and maturity stage. Furthermore, CI is the most important obstacle to trade expansion in tropical fruits and the major cause of their typically short postharvest-life (Kadder, 2011). Chilling disorders may appear at low-temperature

storage for some crops but are usually enhanced or more visible when transferred to non-chilling temperatures (El-Hilali *et al*., 2003). Several factors are the primary cause of CI, including a phase transition in membrane lipids and the alteration in the substrate specificity of a regulatory enzyme (Han *et al*., 2006; Aghdam *et al*., 2012).

At a temperature lower than 10℃, papaya fruit may develop CI symptoms such as skin pitting, scald, hard lumps in the pulp around the vascular bundles, water-soaked flesh, abnormal ripening with blotchy discolouration, and increased decay susceptibility (Kadder, 2011). Generally, 'EX 15' papaya fruit at the colour-turning ripeness stage and stored at temperatures below 7℃ less than 14 days ripen normally when transferred to room temperature (Proulx *et al*., 2005). However, CI symptoms occur after 14 days at 7℃ for mature-green 'EX 15' papaya fruit, and after 21 days for 60% yellow 'EX 15' papaya fruit (Proulx *et al*., 2005). Therefore, the use of optimum temperature and postharvest treatments (particularly PUT) during transportation, distribution, and retailing is a major factor that determines the quality of the fresh produce (Kader, 2011).

In general, treating 'Angelino' pomegranate with 3 mM PUT maintained the lowest CI incidence throughout the storage period at 5℃ for 4 months (Fawole *et al*., 2020). Furthermore, a significantly reduced skin browning was observed on 'Mollar de Elche' pomegranate fruit treated with 1 mM PUT at 2℃ after 60 days storage (Mirdehghan *et al.,* 2007). Similarly, the application of 1 mM PUT either alone or in combination with carnauba wax reduced CI and skin browning on 'Mridula' pomegranate fruit after storage at 3℃ for 4 months (Barman *et al*., 2011). Additionally, CI suppression associated with 1 mM PUT treatment was reported in 'Bagheri' and 'Asgarabadi'

apricot fruit compared to control fruits stored at 1℃ for 13 days (Koushhesh-Saba *et al*., 2012).

According to Li and He (2012), putrescine bind to the phospholipid site of the cell membrane to prevent cytolysis and improve cold resistance. However, there are several different viewpoints on the relationship between PUT and plant chilling stress (Wu and Yuan, 2008). Wanga *et al*. (2003), reported that PUT accumulates as a defence response of plants to reduce CI because PUT accumulation was found to be positively correlated with the cold resistance of plants. Additionally, CI reduction may be attributed to PUT's antioxidant activity and membrane-stabilizing effect (Bhut *et al*., 2004). Furthermore, PUT might facilitate the reduction of CI by lowering the permeability of peel tissues to oxygen (Mirdehghan *et al*., 2007).

Anthracnose

Stem-end rot (*Gnomonia comar*), anthracnose (*Glomerella cingulate*), grey mould (*Botrytis cinerea*), soft rot (*Pectobacterium carotovorum*) and rhizopus rot *(Rhizopus stolonifer)* are common papaya postharvest diseases (Lata, 2017). Among these, anthracnose is the most important disease responsible for major losses in papaya after harvest (Gebreslessie, 2003). Anthracnose is caused by *Colletotrichum* species which can directly penetrate the fruit skin or enter through openings such as stomata and wounds or remain as latent infection during postharvest handling (Lata, 2017).

Gomez-Moraes *et al*. (2013) reported that disease symptoms may appear on green unripe fruit under an extended cold storage period. Furthermore, huge economic losses occur by this disease during papaya storage and marketing (Lata, 2017). Round brownish depressed lesions characterize the symptoms of anthracnose, and in

some cases, salmon-coloured areas formed by the conidial masses that cover the lesion (Gomes-Moraes *et al*., 2013). Infection involves softer fruit tissues and eventually, the diseased portion falls out or can be readily separated from the uninfected parts of the fruit. In addition, the disease can also cause pulp off-flavour (Lata, 2017).

A study by Hosseini *et al*. (2018) showed a gradual increase in microbial population (*Penicillium expansum)* on untreated 'Cavendish' and 'Native' banana fruit while in 1 and 2 mM PUT treated fruit; there was a decline in microbial population after withdrawal from 20 days storage at 1℃. Similar results were reported by Khosrashi *et al*. (2007), wherein, 1 and 2 mM PUT treatments decreased microbial population on 'Selva' Strawberry fruit when compared with control fruit when stored for 13 days at 5℃. Furthermore, in 'Red lady' papaya fruit, decay increased with progression in storage regardless of the applied treatments at 12℃; however, it was less noticeable on 2 mM PUT treated 'Red lady' papaya fruit compared to control fruit after 28 days (Hanif *et al*., 2020). In addition, 2 mM PUT was found to be the most effective treatment in substantially reducing the rate of fruit disease or decay incidence in 'Flordaking' peach fruit at 1℃ after 6 weeks storage (Abbasi *et al*., 2019). These results were supported by Khosroshahi *et al*. (2007), who reported that exogenous PUT might have an anti-pathogenic function in 'Selva' strawberry fruit at 5℃. Furthermore, PUT makes strong bond with phenols and hydroxycinnamic acid amide (HCAA) both of which induce resistance against pathogens, ultimately, reducing decay incidence (Walters, 2003). Another factor for reducing the decay of PUT treated 'Red lady' papaya fruit may also be associated with the strong defence mechanism against fungal attacks (Hanif *et al*, 2020).

2.8. Prospects and conclusion

Postharvest treatment of climacteric and non-climacteric fruit with PAs increases their shelf life with good quality. Postharvest PUT to extend shelf-life and postharvest disorders reduction has been widely studied in several fruit crops, including peaches, plum, strawberry, and pomegranate. However, there is limited information on the influence of PUT on alleviating CI, extending papaya fruit shelf-life and maintaining quality attributes, precisely cv. ‘Solo’ harvested at 25% yellow-colour under low (7.5 ℃) temperature conditions which is the recommended storage for the export market. Therefore, research interest should be redirected on investigating the effect of PUT on ‘Solo’ papaya fruit quality and shelf-life under low temperature storage conditions.

CHAPTER 3

MATERIALS AND METHODS

3.1. Description of study area

Matured and uniform-sized ‘Solo’ papaya fruit were harvested at 25% yellow colour break from Kudu farm, Low`s Creek, Nelspruit, Mpumalanga, South Africa (25^{o}58’07’’ S, 31^{o}30’04’’ E). Fruits were transported at ambient temperature to the Agricultural Research Council - Tropical and Subtropical Crops (ARC-TSC) postharvest laboratory in Nelspruit (25^{o}28’0’’ S, 30^{o}58’0’’ E) for postharvest PUT treatment, storage and analysis.

3.2. Postharvest procedures, treatment and design

The fruits were sterilised in water with chlorine prior to treatment application, they were allowed to dry at ambient temperature. Fruit was divided into 10 lots of 16 fruit per treatment for each storage condition. Putrescine was applied as dips treatment in 10 litres solution containing 0 (control), 1, 2 and 3 mM PUT concentration for 60 minutes separately. Thereafter, fruit were placed in a cold storage room set at 7.5 and 13°C and 90±5% relative humidity (RH) for 21 days. Fruits were then transferred to shelf-life conditions (±25°C, 30-40±5% RH) for 5 days (0-5 days). During shelf-life, eight fruit were sampled and evaluated per treatment within each storage condition. The experiment was conducted as 4 x 2 factorial arranged in a completely randomised design (CRD) with 8 replications.

3.3. Data collection

3.3.1 Determination of fruit mass loss

All treatments were weighed using a digital weighing balance (SBA 61, Scaltec instruments, Heiligenstadt, Germany), and the difference in mass between day 0 and final was compared to the initial fruit mass to determine mass loss percentage (Figure 3.3.1) (Hanif *et al*., 2020).

Mass loss (%) = (Initial mass- fruit mass on the day of observation)/Initial fruit mass) ×100

Where M_0 is the initial mass and M_1 is the final mass

Figure 3.1: Digital weighing balance used to measure ‘Solo’ papaya fruit mass

3.3.2 Determination of firmness

The firmness was determined using a Sinclair IQ^{TM} automated desktop machine (Model: 53524, Bareiss, Oberdischingen, Germany). The firmness of each fruit was determined by taking the mean of three readings at the equatorial region and expressing as newton (N) (Hanif *et al*., 2020).

Figure 3.2: Sinclair IQ™ automated desktop machine used to measure 'Solo' papaya fruit firmness

3.3.3 Determination of colour

The determination of papaya fruit colour was accomplished using a handheld Minolta chromameter (Minolta CR-400 Corp, Ramsey, NJ, USA) with a white calibration plate (Y = 87.00; x = 0.3146; y = 0.3215) L* = lightness, a* = greenness/redness and b* = yellowness/blueness and thereafter, converted to chroma and hue angle (*h*°) using the necessary equations according to McGuire (1992).

Figure 3.3: Chromameter used to measure objective colour parameters [lightness (L*), a*, b*, chroma (C*) and hue angle (*h*°)]

3.3.4 Determination of total soluble solids (TSS) and titratable acidity (TA)

Total soluble solids (TSS) in papaya juice samples were measured with a digital refractometer (121, Yagami International Ltd, Tokyo, Japan), and expressed as % °Brix (Lata, 2017). The method used to determine titratable acidity (TA) was described by Fadanelli *et al*. (2019). In brief, 10 grams of papaya juice were diluted in 40 ml of distilled water and titrated with 0.1N sodium hydroxide (NaOH) to pH 8.1. TA was expressed as g citric acid/kg papaya, using the following equation: TA (g citric acid/kg of papaya) = (V × 0.1 × 1000 × 0.064)/m

Where: 0.1 is the normality of NaOH (N), 0.064 is the conversion factor for citric acid, V is the volume of NaOH required (mL) and m is the mass of papaya juice sample used (g).

3.3.5 Determination of chilling injury

The chilling injury index (CII) was used to evaluate the chilling injury. Using a visual scale, fruit were classified into three categories: 0 = no injury; 1 = slight injury with up to 10% of the surface damaged; 2 = medium injury with 10-50% of the surface damaged; and 3 = severe injury with more than 50% of the surface damaged. The CII was calculated using the following formula (Herrera, 2007):

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

3.3.6 Determination of pathological disorders

Fruit were assessed for pathological disorders namely anthracnose and black dry spots after 5 days of shelf-life condition. Anthracnose was characterised by round brownish depressed lesions. Whereas black dry spot was characterised by irregular dark brown to black fungal spots. Using a visual scale, fruit were classified into four categories: 0= no mould growth; 1= slightly visible mould growth; 2= 10-40% surface area covered with mould growth and 3; when greater than 40% fruit surface area of the fruit was covered with mould growth (Lata, 2017).

3.3.7 Data analysis

The analysis of variance (ANOVA) was carried out using GenStat® 18th version computer-based statistical software (VSN international Hemel Hempsted, UK). The significant difference between treatments means was separated using Least Significant Difference (LSD) at $P \leq 0.05$.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1. Physical Parameters

Fruit mass loss

The results showed that the treatment, cold storage temperature and shelf-life (days) significantly affected ‘Solo’ papaya fruit mass loss after 21 days cold storage plus 5 days shelf-life (Figure 4.1). Furthermore, fruit mass loss was significantly affected by the interaction between treatment and storage temperature, the interactive effect between storage temperature and shelf-life, and the interaction between storage temperature and shelf-life (Figure 4.1). However, the interaction between treatment, cold storage temperature shelf-life (days) had no significant effect ($P < 0.05$) on ‘Solo’ papaya fruit mass loss. Generally, fruit mass loss increased with shelf-life, irrespective of the treatment and cold storage temperature (Figure 4.1).

Overall, cold storage temperature had a significant ($P < 0.05$) effect on ‘Solo’ papaya fruit storage and shelf-life with a reduced mass loss at 7.5℃ when compared with 13℃ storage (Figure 4.1). Furthermore, different cold storage temperatures (7.5 and 13℃) increased mass loss, irrespective of treatment and shelf-life days (Figure 4.1). Regarding treatments, the control fruit showed the highest mass loss rate compared with fruit treated with PUT throughout shelf-life after withdrawal from 7.5℃ storage. Contrary, the lowest cumulative mass loss of 8.32 and 9.39% were recorded on fruit treated with 3 mM PUT at day 4 and 5 of shelf-life at 7.5℃, respectively. Similarly, the highest mass loss was recorded on the control fruit compared with fruit treated with

PUT throughout ripening days at 13℃. Interestingly, fruit treated with 2 mM PUT had the lowest mass loss (10.98%) at day 4 shelf-life at 13℃.

P (A) < 0.01 P (B) < 0.01
P (C) < 0.01 P (AxB) < 0.01
P (AxC) = 0.01 P (BxC) < 0.01
P (AxBXC) = 0.09, LSD = 2.84

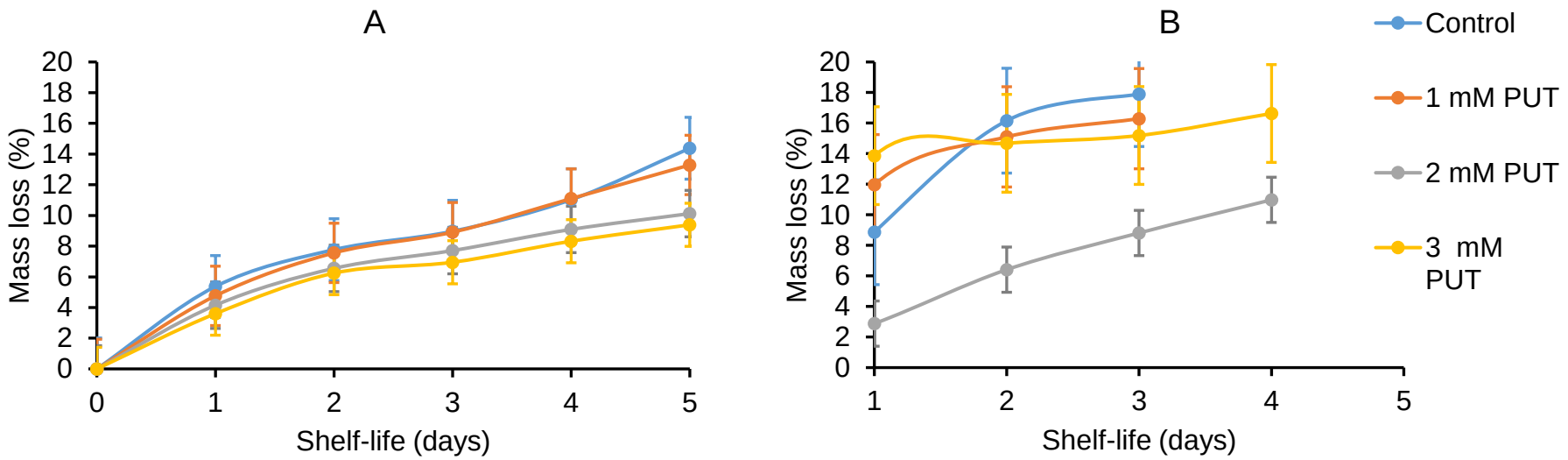

Figure 4.1: Mass loss (%) of PUT treated 'Solo' papaya fruit after 21 days storage at (A) 7.5 and (B) 13℃ plus 5 days shelf-life (n=8). Error bars indicate ±SE of means at P ≤ 0.05. A= treatment, B= cold storage temperature, C = Shelf-life days, A x B = interaction of treatment and storage temperature, A x C= interaction of treatment and shelf-life, B x C = interaction between storage temperature and shelf-life days and A x B x C = the interaction of treatment, storage temperature and shelf-life days.

Firmness

The interaction between treatment, storage temperature and shelf-life had no significant effect ($P > 0.05$) on papaya fruit firmness after 21 days of cold storage plus 5 days shelf-life (Figure 4.2). However, papaya fruit was significantly affected by treatment, cold storage temperature, shelf-life, and the two-way interaction of all the three factors. As presented in Figure 4.2; firmness decreased with shelf-life days, irrespective of treatment and storage temperature. However, the interaction between treatment and storage temperature significantly affected fruit firmness ($P < 0.05$). In general, fruit stored at 7.5℃ had higher firmness values compared with fruit stored at 13℃, regardless of the treatments.

In general, control fruit exhibited the lowest firmness values throughout shelf-life days when compared with fruit treated with PUT at 7.5℃. Contrary, 2 mM PUT retained the highest firmness values throughout shelf-life days compared to those found in control fruit at 7.5℃. Similarly, control fruit had the lowest firmness values when compared with PUT at 13℃ from day 1 to 3 shelf-life days. Contrarily, the highest firmness values were observed on fruit subjected to 2 mM when compared with control after storage at 13℃.

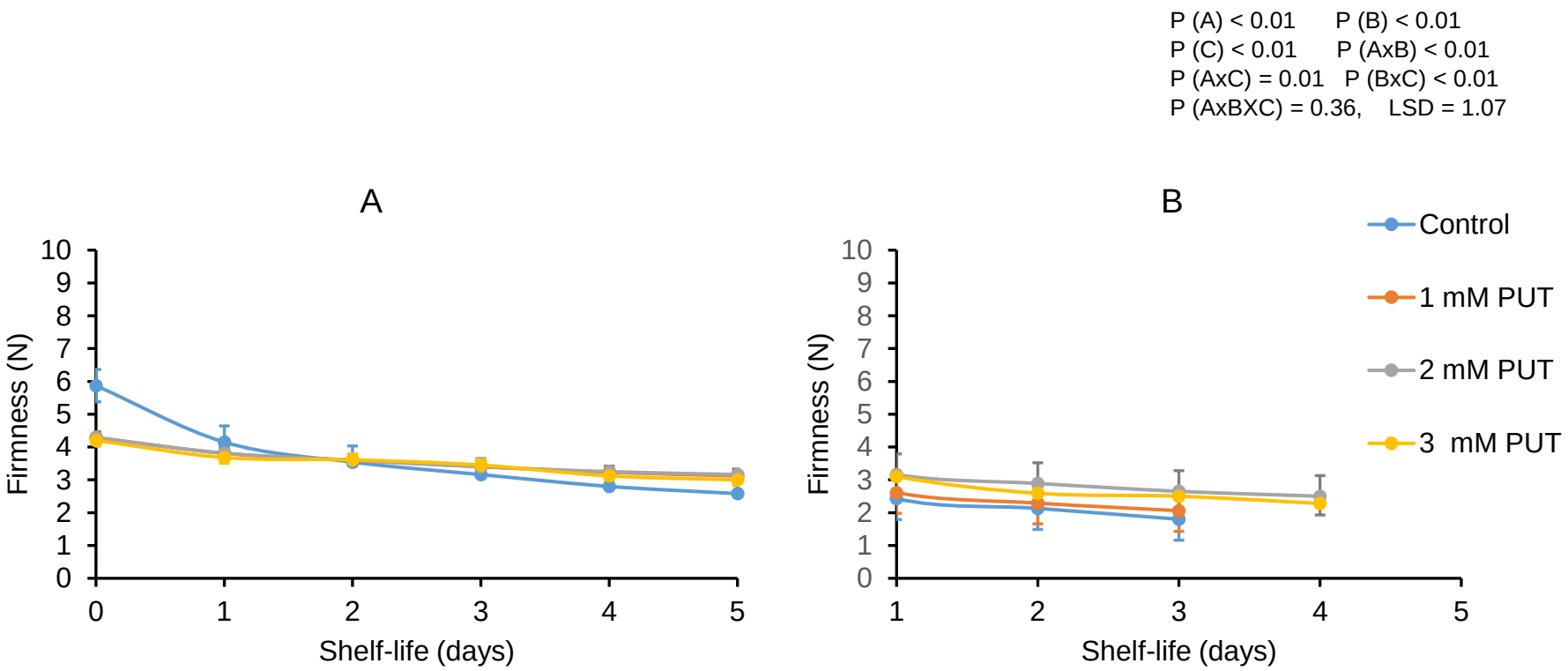

Figure 4.2: Firmness (N) of PUT treated 'Solo' papaya fruit after 21 days storage at (A) 7.5 and (B) 13℃ plus 5 days shelf-life (n=8). Error bars indicate ±SE of means at P ≤ 0.05. A= treatment, B= cold storage temperature, C = Shelf-life days, A x B = interaction of treatment and storage temperature, A x C= interaction of treatment and shelf-life days, B x C= interaction between storage temperature and shelf-life days and A x B x C = the interaction of treatment, storage temperature and shelf-life days.

Colour measurement

Skin colour redness (a)*

Treatment, storage temperature and shelf-life (days) had no significant effect ($P > 0.05$) on papaya fruit exocarp redness after 21 days cold storage plus 5 days shelf-life. In contrast, the interaction between treatment and cold storage temperature had a significant effect ($P < 0.01$) on the fruit redness (Figure 4.3). Generally, a* values increased with shelf-life days, irrespective of the treatment and storage temperature. However, fruit stored at 13℃ had the highest a* values when compared with fruit stored at 7.5℃ (Figure 4.3). Regarding treatment effect, control fruit had the highest a* value compared with fruit treated with PUT after withdrawal from 7.5℃ storage. In contrast, fruit treated with 2 mM PUT recorded the lowest a* values throughout shelf-life (days) at 7.5℃. Interestingly, PUT significantly maintained lower a* values in contrast to control exhibited higher a* values throughout evaluation days at 13℃. Additionally, fruit treated with 2 mM PUT had the lowest a* values compared with control fruit at 13℃.

Blueness (b)*

The interaction between treatment, shelf-life and storage temperature had no significant effect ($P > 0.05$) on papaya fruit skin colour blueness after 21 days of cold storage plus 5 days shelf-life (Figure 4.4). Furthermore, the results showed that the interaction between treatment and storage temperature had no significant effect on b* component of the fruit. In general, b* values showed a decreasing trend, irrespective of treatment and storage temperature (Figure 4.4). Generally, fruit treated with 2 mM PUT exhibited the lowest b* values when compared with control fruit after withdrawal

from 7.5℃ storage (Figure 4.5). However, there was no significant difference between control fruit and PUT treated fruit in terms of b* irrespective of the shelf-life days at 7.5℃. Interestingly, PUT significantly retained the lowest b* values when compared with control fruit at 13℃. Furthermore, the lowest b* values was recorded on fruit treated with 2 mM PUT when compared with control fruit at 13℃.

Chroma (C)*

The results showed that the interaction between treatment, storage temperature and shelf-life (days) had no significant effect ($P > 0.05$) on papaya fruit skin chroma after 21 days cold storage plus 5 days shelf-life. However, treatment, storage temperature and treatment interaction and the interaction between treatment and shelf-life (days) had a significant effect ($P < 0.01$) on fruit skin chroma. Overall, fruit stored at 7.5℃ showed higher C* values when compared with fruit stored at 13℃ on treated, untreated and control fruit (Figure 4.5). In terms of treatments, the lowest C* values were observed on fruit treated with 2 mM PUT when compared with control fruit at 7.5℃. However, there was no significant difference between control and 2 mM PUT in terms of C* values at 7.5℃. Similarly, C* values in control fruit were significantly higher than those found in PUT treated fruit at 13℃. In contrast, fruit treated with 2 mM PUT exhibited the lowest C* values compared with control fruit throughout shelf-life days at 13℃.

Hue angle (h^o)

The interaction between treatments, cold storage temperatures and shelf-life days had no significant effect ($P > 0.05$) on papaya fruit hue angle after 21 days cold storage plus 5 days shelf-life. However, treatment, storage temperature, the interaction

between treatment and storage temperature and the interactive effect between treatment and shelf-life days had a significant effect (P = 0.01) on the hue angle of the fruit. In general, h^o values showed a decreasing trend, irrespective of storage temperature and treatment (Figure 4.6). Overall, fruit stored at 7.5℃ exhibited higher h^o values when compared with fruit stored at 13℃, irrespective of the treatment (Figure 4.6). Generally, treatment had a significant effect on 'Solo' papaya fruit hue angle in both storage temperatures. Furthermore, fruit treated with 2 mM PUT exhibited higher h^o values when compared with control fruit throughout shelf-life (days) at 7.5℃. Similarly, the highest h^o values were recorded on fruit treated with 2 mM PUT when compared with control fruit at 13℃, irrespective of the shelf-life days.

Lightness (L)*

The interaction between treatment, storage temperature and shelf-life (days) had no significant effect (P < 0.05) on papaya fruit exocarp lightness after 21 days cold storage plus 5 days shelf-life (Figure 4.7). However, L* values decreased with shelf-life days, irrespective of storage temperature and treatment. In general, L* values for 'Solo' fruit stored at 7.5℃ were significantly reduced when compared with fruit stored at 13℃, regardless of the treatments (Figure 4.7). Furthermore, the interaction between storage temperature and treatment had no significant effect (P > 0.05) on the *L** component of the fruit. However, treatment had a significant effect (P < 0.05) on the L* component of the fruit. The results showed that PUT significantly reduced rapid lightness increase throughout shelf-life days when compared with control at 7.5℃. Furthermore, the lowest L* values were recorded on fruit subjected to 2 mM PUT when compared with control fruit at 7.5℃. Similarly, PUT maintained a decreasing lightness trend when compared with control after withdrawal from 13℃. Interestingly, fruit

subjected to 3 mM PUT exhibited lower L* values when compared with control fruit and fruit treated with water at 13℃.

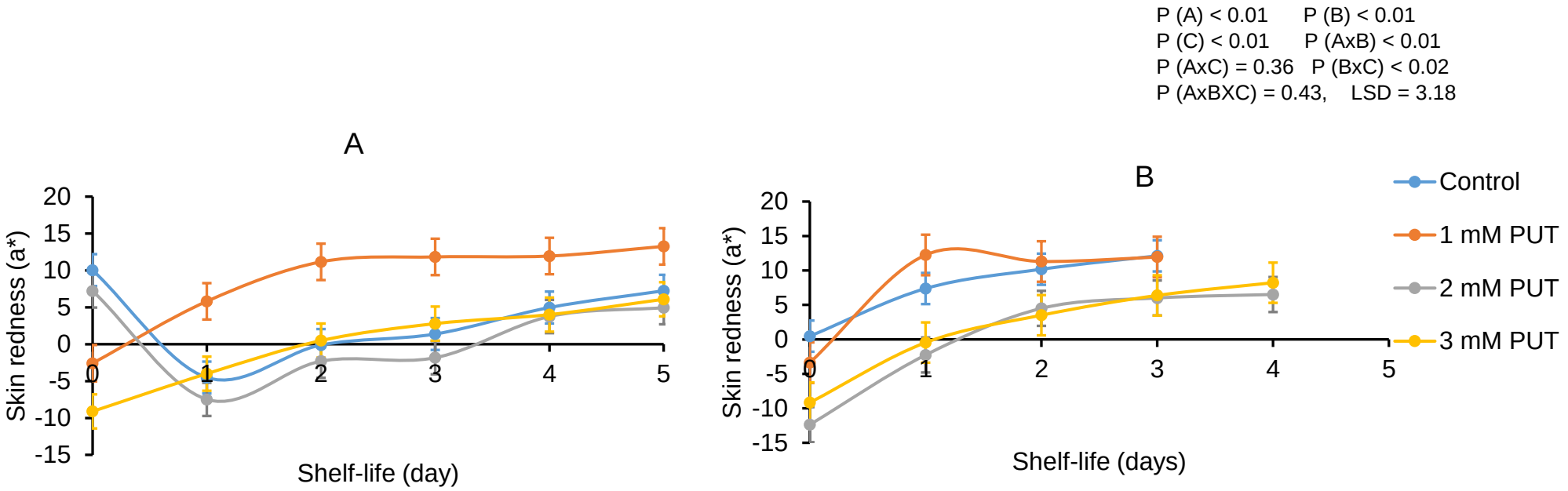

Figure 4.3: Colour (a*) of PUT treated 'Solo' papaya fruit after 21 days of storage at (A) 7.5 and (B) 13℃ plus 5 days shelf-life (n=8). Error bars indicate ±SE of means at P ≤ 0.05. A = treatment, B= cold storage temperature, C = Shelf-life days, A x B =interaction of treatment and storage temperature, A x C= interaction of treatment and shelf-life days, B x C= interaction between storage temperature and shelf-life days and A x B x C= the interaction of treatment, storage temperature and shelf-life days

P (A) < 0.01 P (B) = 0.02
P (C) < 0.01 P (AxB) < 0.10
P (AxC) = 0.01 P (BxC) < 0.26
P (AxBXC) = 0.75, LSD = 3.28

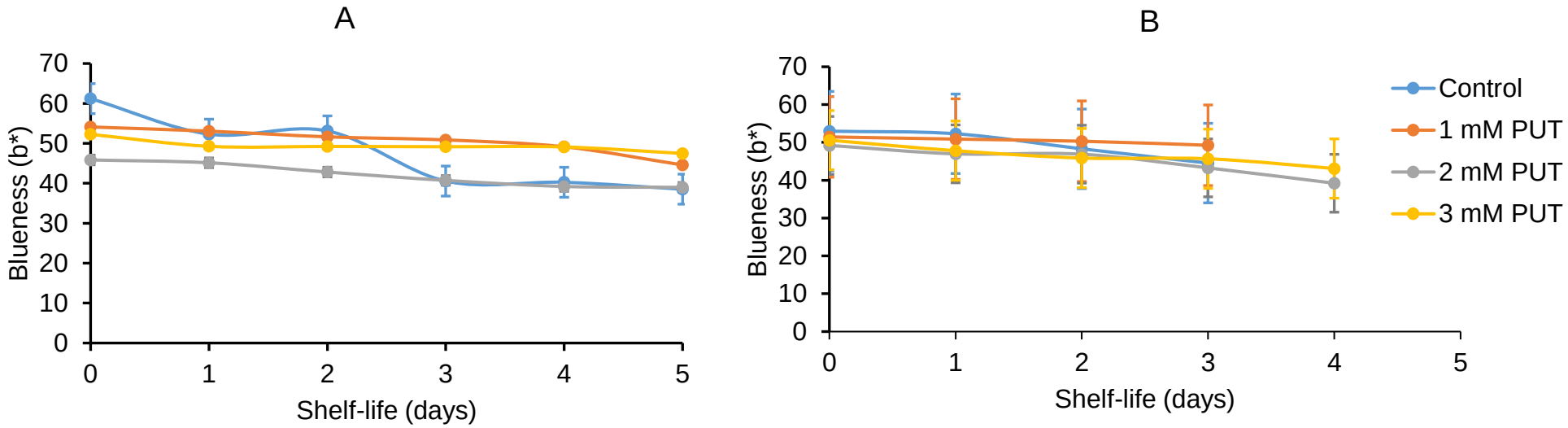

Figure 4.4: Colour (b*) of PUT treated 'Solo' papaya fruit after 21 days of storage at (A) 7.5 and (B) 13℃ plus 5 days shelf-life (n=8). Error bars indicate ±SE of means at P ≤ 0.05. A = treatment, B = cold storage temperature, C= Shelf-life days, A x B= interaction of treatment and storage temperature, A x C= interaction of treatment and shelf-life days, B x C= interaction between storage temperature and shelf-life days and A x B x C= the interaction of treatment, storage temperature and shelf-life days

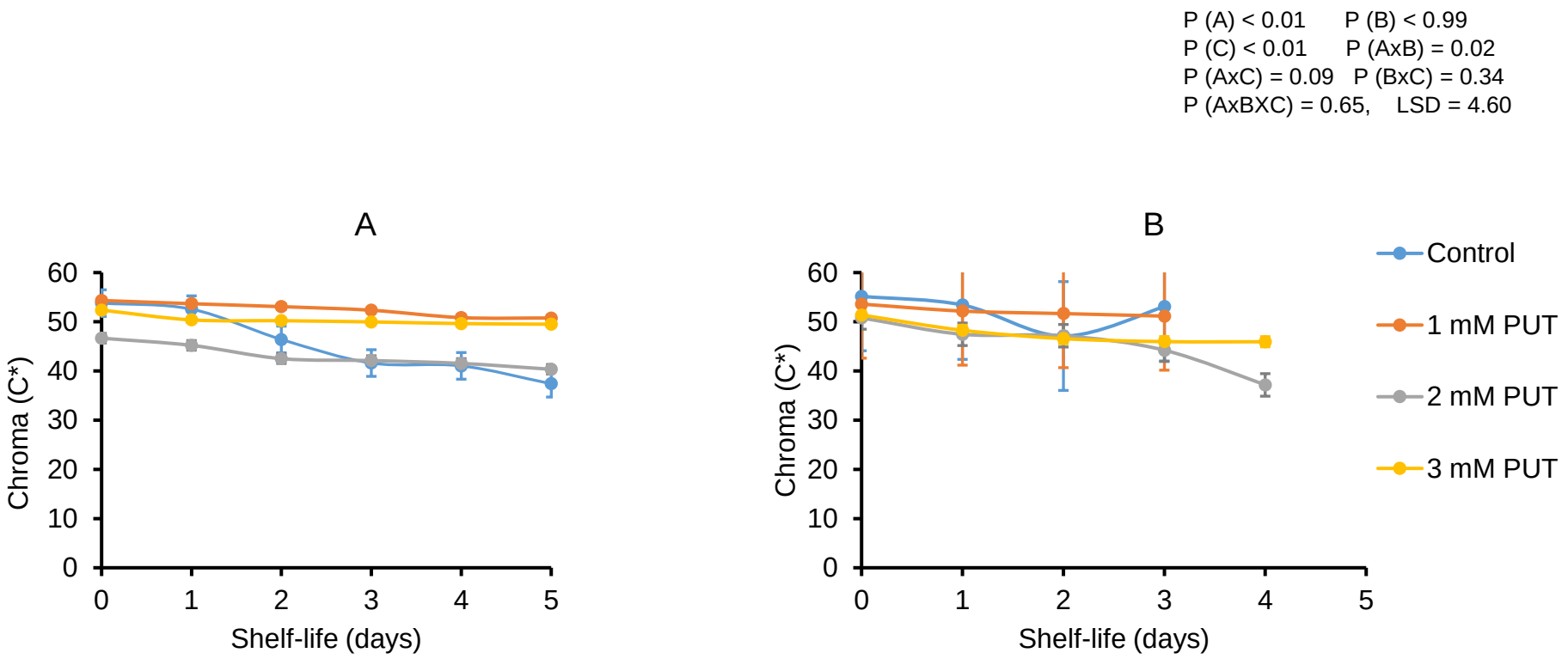

Figure 4.5: Chroma (C*) of PUT treated 'Solo' papaya fruit after 21 days of storage at (A) 7.5 and (B) 13℃ plus 5 days shelf-life (n=8). Values are means of 8 fruits. Error bars indicate ±SE of means at P ≤ 0.05. A= treatment, B = cold storage temperature, C = Shelf-life days, A x B = interaction of treatment and storage temperature, A x C = interaction of treatment and shelf-life days, B x C= interaction between storage temperature and shelf-life days and A x B x C= the interaction of treatment, storage temperature and shelf-life days

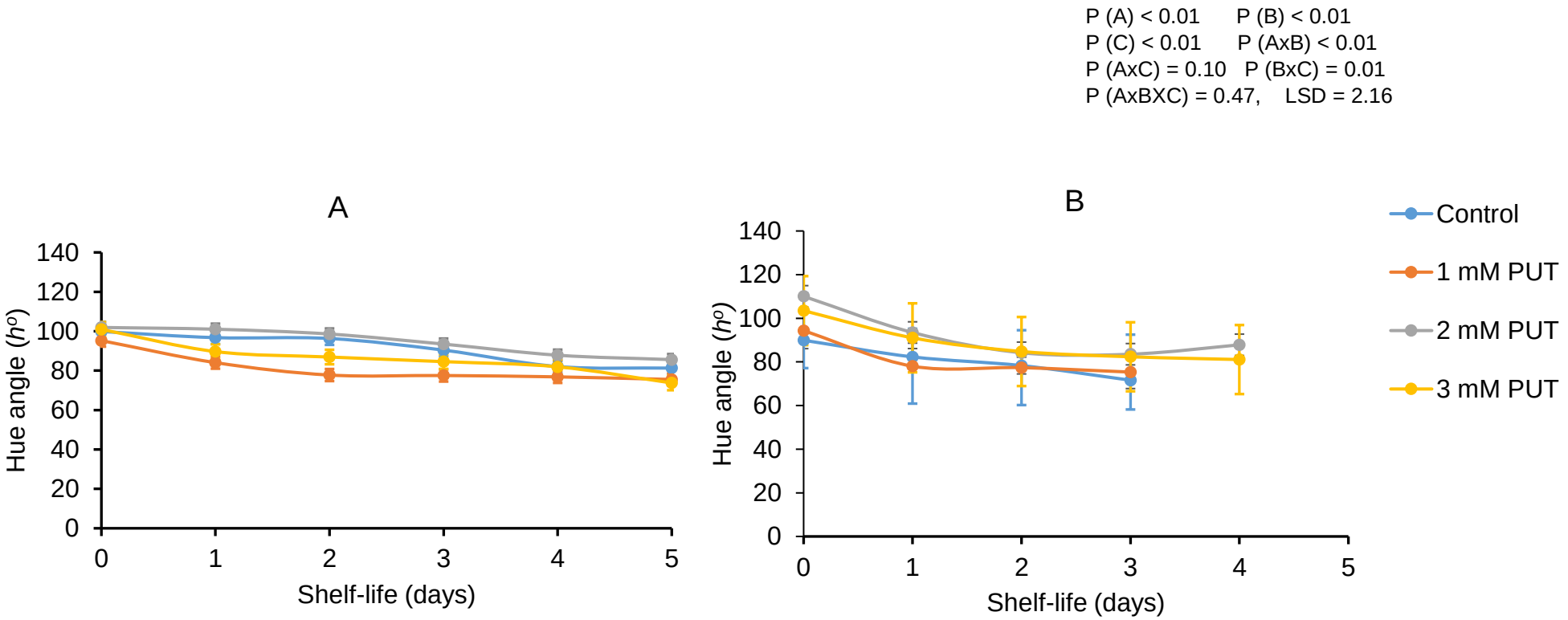

Figure 4.6: Hue angle (h^o) of PUT treated 'Solo' papaya fruit after 21 days of storage at (A) 7.5 and (B) 13℃ plus 5 days shelf-life (n=8). Values are means of 8 fruits. Error bars indicate ±SE of means at P ≤ 0.05. A= treatment, B= cold storage temperature, C= Shelf-life days, A x B = interaction of treatment and storage temperature, A x C= interaction of treatment and shelf-life days, B x C = interaction between storage temperature and shelf-life days and A x B x C= the interaction of treatment, storage temperature and shelf-life days

P (A) < 0.01 P (B) = 0.32
P (C) < 0.01 P (AxB) = 0.13
P (AxC) = 0.55 P (BxC) = 0.12
P (AxBXC) = 0.31, LSD = 2.20

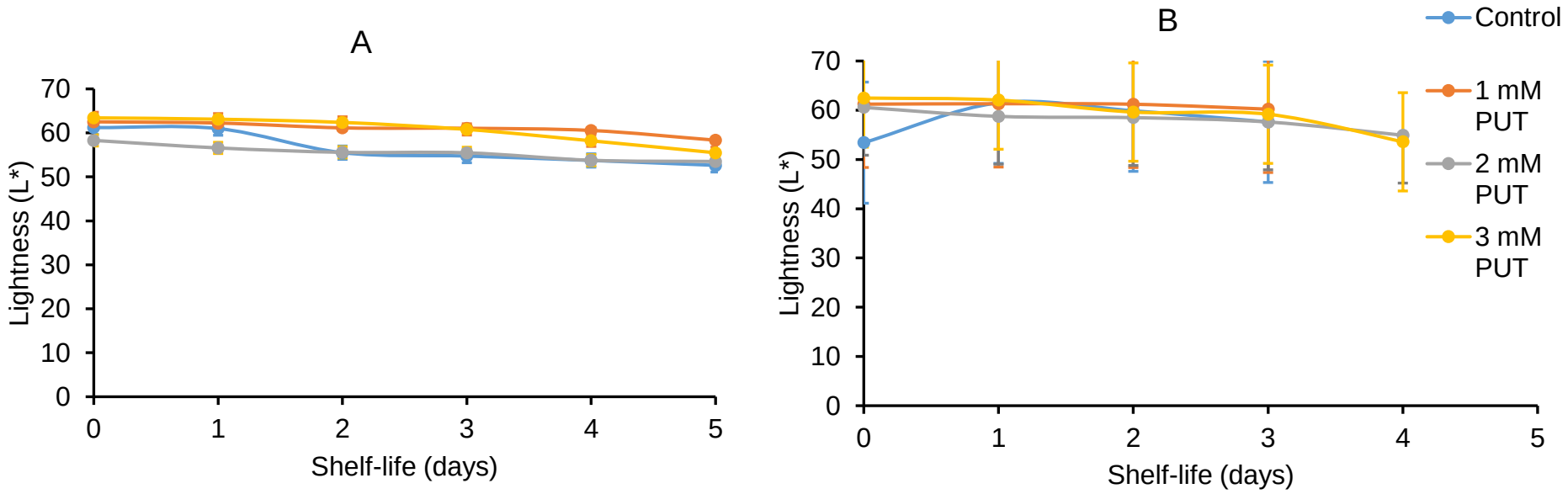

Figure 4.7: Colour (L*) of PUT treated 'Solo' papaya fruit after 21 days of storage at (A) 7.5 and (B) 13℃ plus 5 days shelf-life (n=8). Error bars indicate ±SE of means at P ≤ 0.05. A= treatment, B= cold storage temperature, C= Shelf-life days, A x B= interaction of treatment and storage temperature, A

x C= interaction of treatment and shelf-life days, B x C= interaction between storage temperature and shelf-life days and A x B x C= the interaction of treatment, storage temperature and shelf-life days

4.1.2. Biochemical Properties

Titratable acid (TA)

The results showed that storage temperature had no significant effect on papaya fruit titratable acid content. However, treatment, shelf-life (days), two- and three-way interaction of treatment, cold storage temperature had a significant effect ($P < 0.05$) on papaya fruit titratable acid content of after 21 days cold storage plus 5 days shelf-life (Figure 4.8). Generally, there was a gradual decrease in titratable acid content with an increase in storage duration under all the treatments, irrespective of storage temperature. Overall, TA was higher on fruit stored at 13℃ when compared with fruit stored at 7.5℃, across all treatments. Overall, maximum TA accumulation was recorded on control fruit when compared with fruit treated with PUT throughout shelf-life days at 7.5℃ (Figure 4.8). In contrast, fruit treated with 1 mM PUT retained the minimum TA values when compared with control fruit throughout the storage period at 7.5℃. Interestingly, maximum TA was observed on control fruit from day 1 to 3 of shelf-life when compared with fruit treated with PUT at 7.5℃. In contrast, fruit treated with 1 mM PUT exhibited the minimum TA content when compared with control fruit and fruit treated with water at day 3 shelf-life at 13℃.

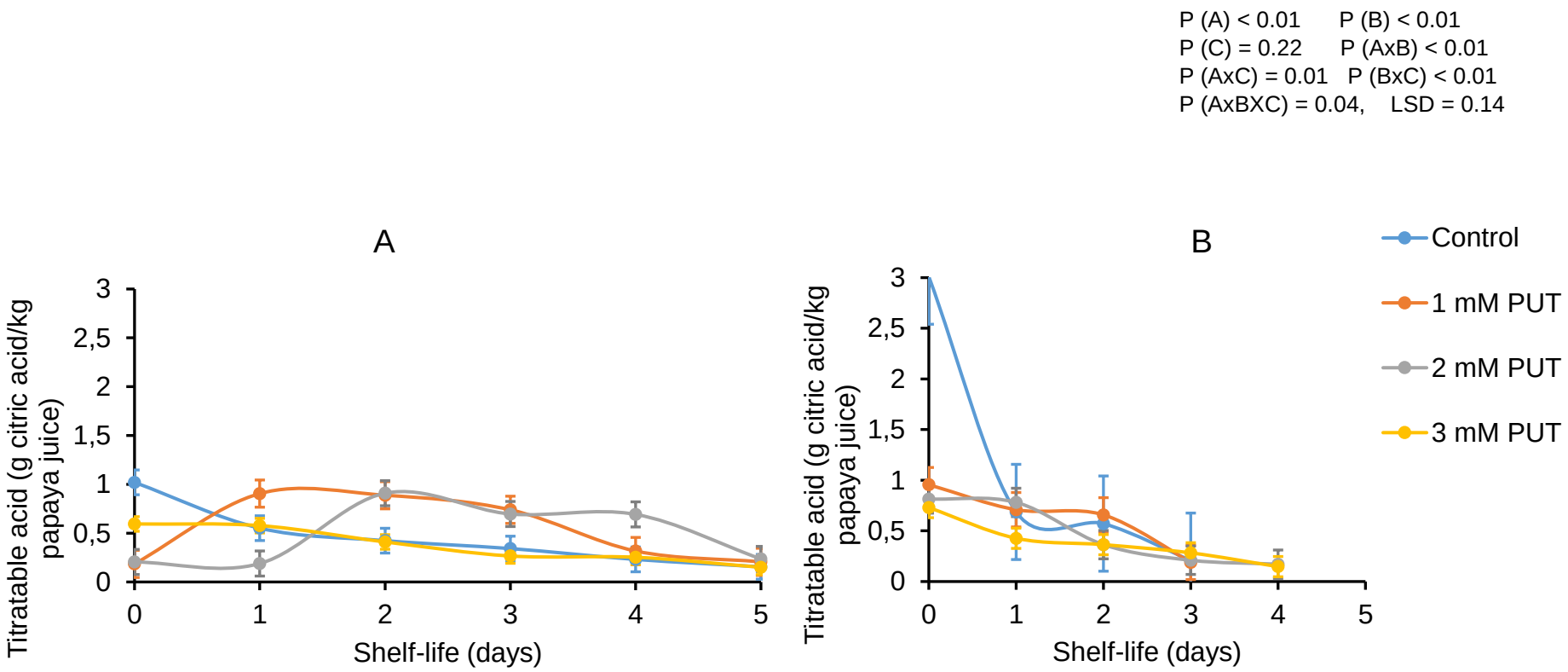

Figure 4.8: Titratable acid (TA) of PUT treated 'Solo' papaya fruit after 21 days of storage at (A) 7.5 and (B) 13 plus 5 days shelf-life (n= 8). Error bars indicate ±SE of means at P ≤ 0.05. A= treatment, B= cold storage temperature, C= Shelf-life days, A x B= interaction of treatment and storage temperature, A xC= interaction of treatment and shelf-life days, B x C= interaction between storage temperature and shelf-life days and A x B x C= the interaction of treatment, storage temperature and shelf-life days

Total Soluble Solids (TSS)

The interaction between treatment, storage temperature and shelf-life (days) had a significant effect ($P < 0.05$) on papaya fruit total soluble solids (TSS) fruit after 21 days cold storage plus 5 days shelf-life. It was observed that the TSS content of papaya fruit increased throughout shelf-life days, irrespective of treatment and storage temperature. In general, TSS was higher on fruit stored at 13℃ when compared with fruit stored at 7.5℃. Regarding treatment effects, TSS was maximum on control fruit throughout shelf-life days when stored at 7.5℃ (Figure 4.9). Contrarily, fruit treated with 2 mM PUT had a minimum TSS throughout storage and shelf-life at 7.5℃ when compared with and control fruit. Similarly, the lowest TSS value was recorded on fruit treated with PUT 2 mM when compared with control fruit after withdrawal from 13℃. In contrast maximum, TSS was recorded on control fruit from day 1 until day 3 shelf-life days when compared with fruit treated with PUT at 13℃. Furthermore, the highest TSS value was recorded on fruit treated with 2 mM PUT when compared with fruit treated with 3 mM PUT at 13℃ on day 4 of shelf.

P (A) < 0.01 P (B) < 0.01
P (C) < 0.01 P (AxB) < 0.01
P (AxC) = 0.01 P (BxC) = 0.01
P (AxBXC) = 0.03, LSD = 0.20

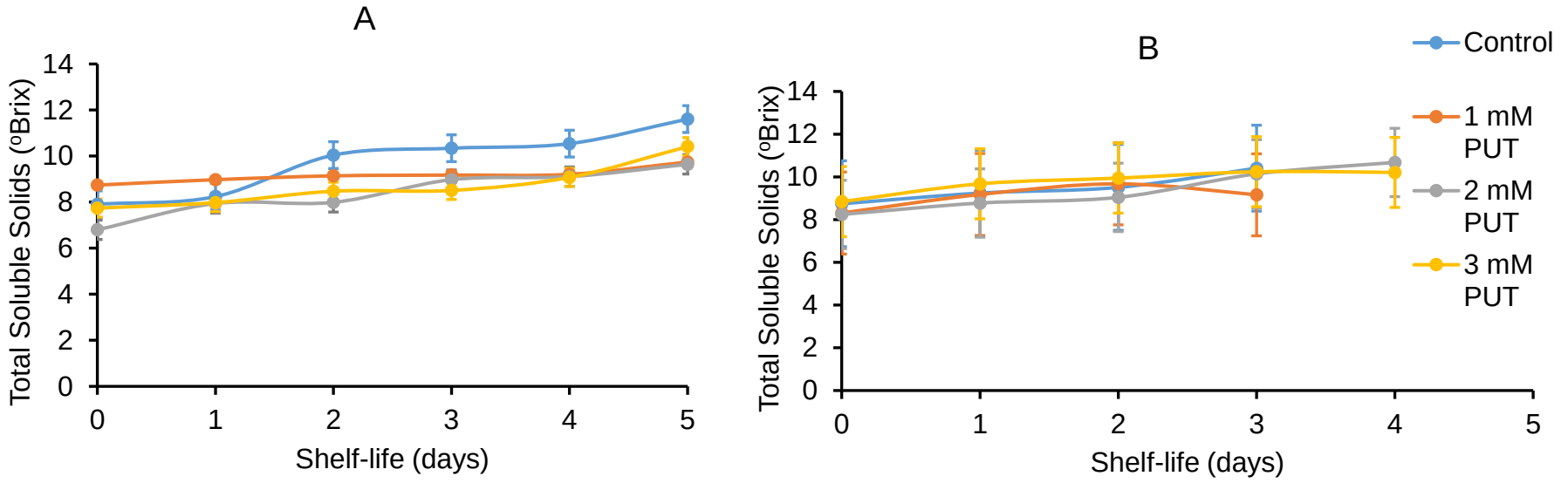

Figure 4.9: Total Soluble Solids (TSS) of PUT treated 'Solo' papaya fruit after 21 days of storage at (A) 7.5 and (B) 13 plus 5 days shelf-life (n=8). Error bars indicate ±SE of means at P ≤ 0.05. A= treatment, B= cold storage temperature, C= Shelf-life days, A x B= interaction of treatment and storage temperature, A x C= interaction of treatment and shelf-life days, B x C= interaction between storage temperature and shelf-life days and A x B x C= the interaction of treatment, storage temperature and shelf-life days

4.1.3. Physiological and pathological disorders

Chilling Injury Index (CII)

The interaction between treatment and storage temperature had no significant effect ($P > 0.05$) on papaya fruit chilling injury index (CII) after 21 days cold storage plus 5 days shelf-life. However, the results showed that treatment exhibited a significant effect ($P < 0.05$) on minimizing chilling injury of the fruit during storage and shelf-life. In general, fruit stored at 13℃ showed a higher chilling injury index when compared with fruit stored at 7.5℃ (Figure 4.10). The data presented in Figure 4.10 indicated that the highest chilling injury index was observed on control fruit when compared with fruit treated with PUT at 7.5℃. In contrast, fruit treated with 2 and 3 mM PUT exhibited the lowest chilling injury index when compared with control fruit when stored at 7.5℃. Contrarily, the highest chilling injury index was observed on control fruit when compared with fruit treated with w PUT at 13℃ . Interestingly, the lowest chilling injury index was also observed on fruit treated with 2 and 3 mM PUT at 13℃.

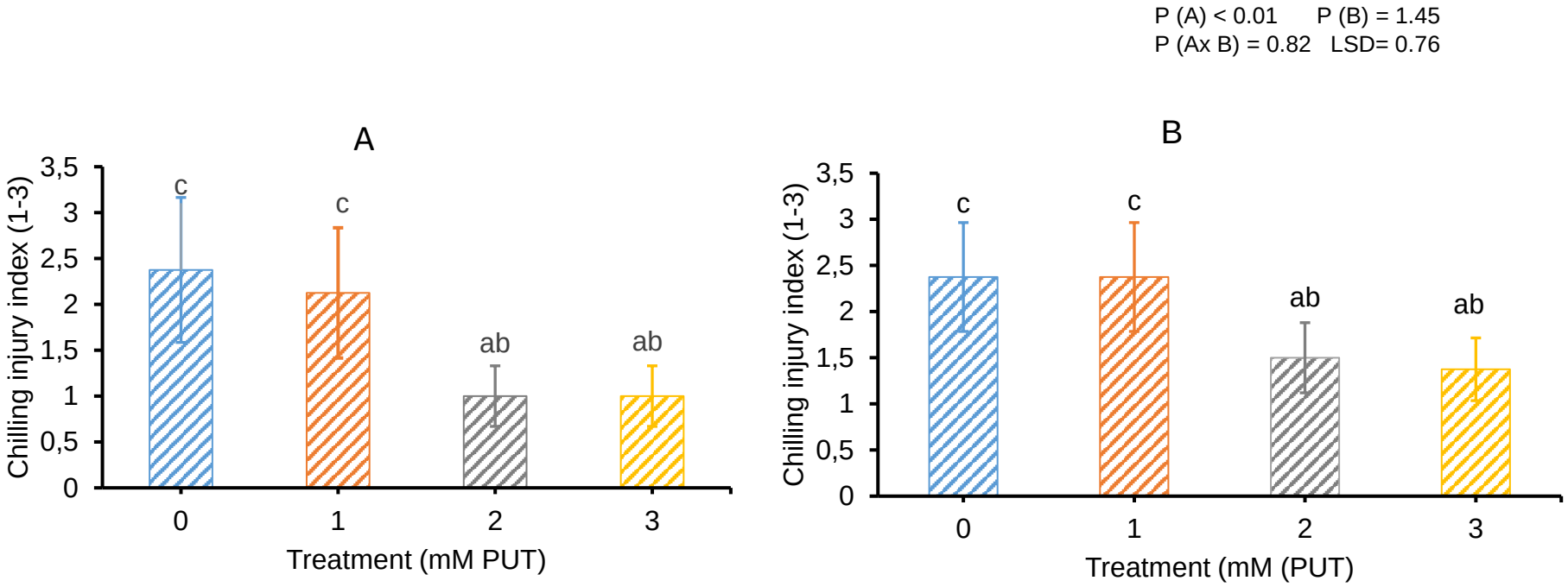

Figure 4.10: Chilling injury index of PUT treated 'Solo' papaya fruit after 21 days of storage at (A) 7.5 and (B) 13℃ plus 5 days shelf-life (n=8). Values are means of 8 fruits. Error bars indicate ±SE of means at P ≤ 0.05. A= treatment, B= cold storage temperature, C= Shelf-life days, A x B= interaction of treatment and storage temperature

Anthracnose incidence

Anthracnose severity on papaya fruit was not significantly affected by the interaction between treatment and storage temperature ($P > 0.05$) fruit after 21 days cold storage plus 5 days shelf-life. However, treatment significantly affected ($P < 0.05$) anthracnose severity on the fruit throughout storage and shelf-life days. Overall, fruit stored at 13℃ showed higher incidence of anthracnose when compared with fruit stored at 7.5℃, irrespective of the treatment applied (Figure 4.11). Regarding treatment effects, control fruit showed higher anthracnose incidence when compared with fruit treated PUT at 7.5℃ (Figure 4.11). In contrast, fruit treated with 2 mM PUT recorded the lowest anthracnose incidence when compared with control fruit after withdrawal from 7.5℃. Interestingly, fruit treated with 2 mM PUT recorded the lowest anthracnose incidence when compared with control fruit and fruit treated with water and 1 mM PUT at 13. In contrast, fruit treated with PUT (2 and 3 Mm) recorded the lowest anthracnose incidence when compared with control fruit PUT after withdrawal from 13℃. Contrarily, control fruit showed higher anthracnose incidence when compared with fruit treated with PUT at 13℃.

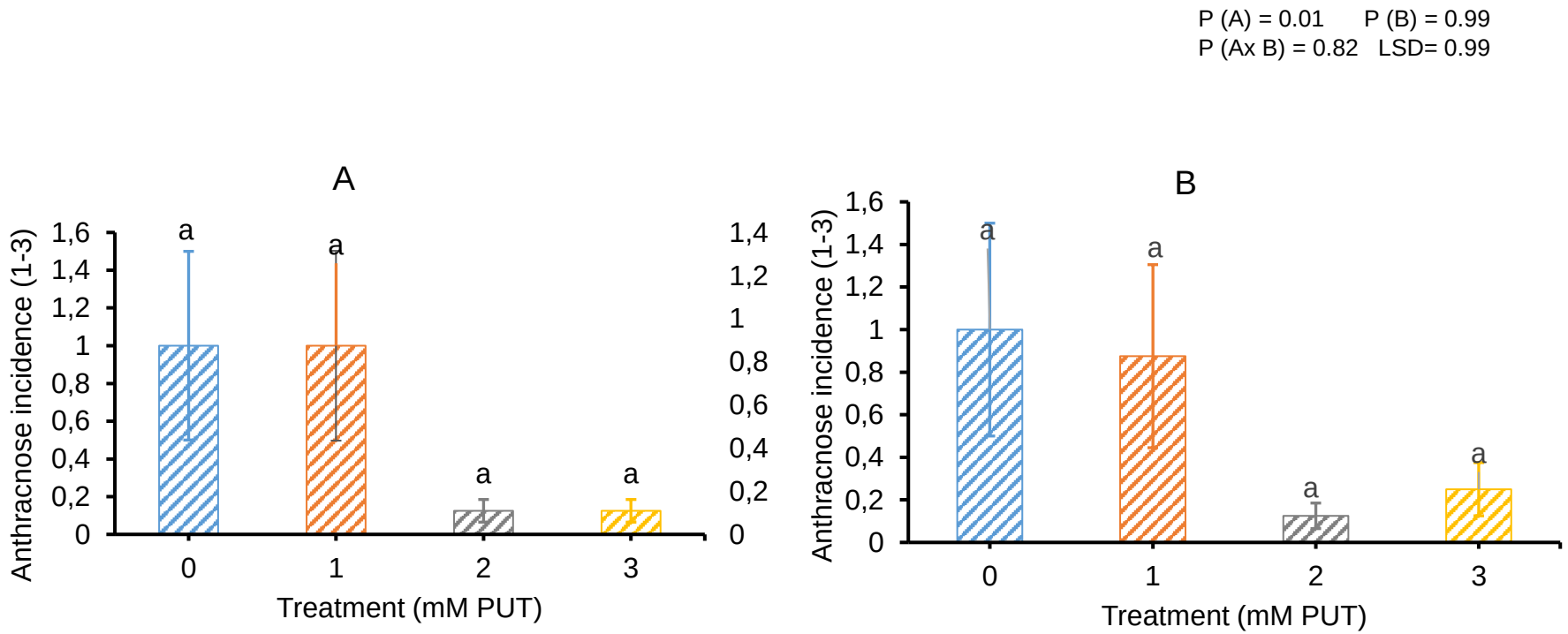

Figure 4.11: Anthracnose incidence of PUT treated 'Solo' papaya fruit after 21 days of storage at (A) 7.5 and (B) 13℃ plus 5 days shelf-life (n=8). Values are means of 8 fruits. Error bars indicate ± SE of means at P ≤ 0.05. A= treatment, B= cold storage temperature, C= Shelf-life days, A x B= interaction of treatment and storage temperature

4.2. DISCUSSION

4.2.1. Physical properties

Mass loss

In fruits, moisture content is an important consideration before consumption (Hosseini *et al*., 2018). According to Hanif *et al*. (2020), fruit moisture loss is related to fruit freshness during and after a longer duration. Fruit moisture loss via transpiration and respiration occurs rapidly after harvest, promoting decay (Kassim *et al*., 2013). The reasons behind mass loss could be rapid respiratory activities, water loss through the skin and the consumption of stored metabolites during metabolic activities (Fawole *et al*., 2020). In the present study, the interaction between treatments, cold storage temperature and shelf-life days significantly increased ‘Solo’ papaya fruit mass loss percentage.

According to Maguire *et al*. (2001), relative humidity and storage temperature are the primary factors that affect mass loss during produce storage. For instance, ‘Solo’ papaya fruit stored at 13℃ exhibited a high mass loss percentage compared to those kept at 7.5℃ in the present study (Figure 4.1). The findings of this study agreed with Žnidarčič and Požrl (2006), whereby a higher mass loss rate was recorded on ‘Malike’ tomato fruit stored at 10℃ than those stored at 5℃. This could be due to high respiration and transpiration rate due to storing fruit under high temperatures (Alferez *et al*., 2005; Rab *et al*., 2015).

The current study's findings showed that the mass loss rate on ‘Solo’ papaya fruit treated with PUT was significantly lower than control fruit mass loss rate, irrespective of cold storage temperature (Figure 4.1). However, 3 mM PUT was more effective

when compared with control in terms of mass loss reduction at 7.5℃. Similar results were reported on 'Wonderful' pomegranate fruit, whereby, lowest mass loss was obtained on fruit treated with 3 mM PUT at 5℃ (Fawole *et al*., 2020). In contrast, 2 mM PUT was efficient in decreasing mass loss on 'Solo' papaya fruit when compared with untreated and control fruit at 13℃ (Figure 4.1). These results were in line with Hanif *et al*. (2020), who found the least mass loss on 'Red lady' papaya fruit treated with 2 mM PUT at 12℃. The effect of PUT on reducing mass loss can be attributed to its anti-senescence properties (Makkar *et al*. 2007). The reduced mass loss due to PUT treatments during storage may be due to comparatively lower rates of respiration and increased fruit firmness in treated 'Verna' lemon fruit compared to control (Valero *et al*., 1998). Furthermore, PUT may have modified the properties of cell wall and the tissue permeability in 'Mauricio' apricot fruit (Martínez *et al*., 2002).

Firmness

Fruit firmness is the main feature of quality (Hosssein *et al*., 2008). According to Hosseini *et al*. (2018), fruit firmness is related to cell wall structure. Fruit softening results from cell compartment structure alteration (such as decrease in hemicellulose and galactose) and pectin dissolution (Mirdehghan *et al*., 2007). Additionally, the activity of hydrolyzing enzymes such as polygalacturonase (PG), and rapid production of reactive oxygen species (ROS) soften fruit tissues during storage (Cheng *et al*., 2008). In the current study, the interactive effect between treatments, cold storage temperatures, and shelf-life days significantly affected 'Solo' papaya fruit firmness (Figure 4.2). The results showed that 'Solo' papaya fruit firmness decreased regularly throughout cold storage temperature storage and shelf-life days.

Cold storage temperature had a significant effect on ‘Solo’ papaya fruit firmness, irrespective of the treatments (Figure 4.2). However, ‘Solo’ papaya fruit stored at 7.5℃ had higher firmness values when compared with fruit stored at 13℃ in both treated and untreated fruit in the current study (Figure 4.2). In ‘Cochoro’ tomato fruit, firmness was also found to be higher in lower temperature (4℃) in comparison with fruit stored at higher temperature (20℃) (Tedesse *et al*., 22015). This reduced softening at lower temperatures could be due to retarded metabolic activity of the fruit as lower temperature inhibits a wide range of metabolic processes including those associated with fruit softening and the deterioration of various textural attributes (Valero *et al*., 1998).

In the present study, PUT treated ‘Solo’ papaya fruit showed significantly higher firmness during ripening when compared with untreated and the control fruit (Figure 4.2). Furthermore, 2 mM PUT application was efficient in maintaining ‘Solo’ papaya fruit firmness when compared with the untreated and control fruit at 7.5 and 13℃. The results were similar to Hanif *et al*. (2020), who found the highest firmness on ‘Red lady’ papaya fruit treated with 2 mM PUT at 12℃. The effect of polyamines (PAs) on fruit softening or firmness reduction augmentation is thought to be a result of their bonds with pectin in the cell wall leading to a physically stabilized cell wall, which is detectable immediately after treatment (Hosseini *et al*., 2018). The bonds between PAs and pectin also inhibit the activity of wall-degrading enzymes, such as pectinesterase (PE), pectinmethylesterase (PME) and polygalacturonase (PG) and reduce fruit softening during storage (Valero *et al*., 2002a).

Furthermore, polyamines play very important roles in the antioxidant system and in protecting plasma membrane phospholipids against ROS damages (Verma and

Mishra, 2005). In mango (*Mangifera indica*) fruit, it has been reported that the antioxidant defense system and the reactive oxygen species (ROS) decrease and increase, respectively, at the beginning of fruit ripening and during senescence (Davarynejad *et al*., 2013). Applying different polyamines concentrations such as PUT has been reported to increase the activity of the antioxidant system in 'Mollar de Elche' pomegranate (Mirdehghan *et al*., 2007; Razzaq *et al*., 2014) as well as 'Lasgerdi' and 'Shahrodi' apricot fruit (Davarynejad *et al*., 2013).

Colour measurement

In papaya fruit, colour is the most important criterion which increases the visual fruit appearance; and thereby, resulting in easier marketing and consumer acceptability (Lata, 2017). The progressive increase in tissue softness together with a change in exocarp colour and a wide spectrum of aroma compounds production are some of the most easily recognizable changes that accompany ripening in climacteric fruit (Gebreslassie, 2003). However, rapid colour development is not desirable on papaya fruit during storage duration (Lata, 2017). In the present study, 'Solo' papaya fruit exocarp colour change was significantly increased by the interactive effect between treatments, cold storage temperature and shelf-life days (Figure 4.3,4, 5, 6 and 7). In papaya fruit, colour change occurs due to chlorophyll loss, carotenoids development (yellow, orange and red colours), and anthocyanins synthesis and development (Irtwange, 2006).

In this study, the results showed that papaya exocarp colour change was significantly affected by cold storage temperature. Furthermore, 'Solo' papaya fruit stored at 13℃ exhibited rapid peel colour change when compared with fruit stored at 7.5℃. Similarly,

rapid colour change was observed on ‘Reed’ avocado fruit stored at 5.5℃ when compared with fruit at 2.0℃ (Shikwambana, 2016). Furthermore, similar results were reported by Tedesse *et al*. (2015) on ‘Cochoro’ tomato fruit, whereby, colour change at (20℃) increased during storage time, therefore, showing enhanced colour development when compared with fruit stored at 4℃ (Tedesse *et al*., 2015).

The current study showed that changes in ‘Solo’ papaya fruit exocarp colour parameters were influenced by PUT concentration and storage duration. In control and untreated ‘Solo’ papaya fruit, exocarp colour retained higher a*, b*, C^*, h^o and L^* (Figure 4.3, 4, 5, 6 and 7). In contrast, fruit treated with PUT recorded minimum higher a*, b*, C^*, h^o and L^* values (Figure 4.3, 4, 5, 6 and 7). Therefore, PUT postharvest treatment was efficient in delaying ‘Solo’ papaya fruit colour change, irrespective of the storage temperatures. Jawandha *et al*. (2012), also reported that untreated ‘Langra’ mango fruit retained higher values of a* and b* during storage while fruit treated with 2 mM PUT recorded minimum values at 13℃. Similarly, a marked h^o reduction and an increase in a* value was observed on control fruit when compared with 2 mM PUT treated `Flordaking` peach fruit at 1℃ (Abbasi *et al*., 2019).

The effect of PUT on delaying colour change during storage by reducing senescence rate has also been reported in ‘Mauricio’ apricot fruit at 10℃ (Martínez-Romero *et al*., 2002). A decrease in hue angle is characteristic of ripening development and colour change from green to yellow due to chlorophyll degradation and carotenoids synthesis during storage (Abbasi *et al*., 2019). The positive effect of PUT treatment on colour change inhibition might be due to respiration rate and ethylene production suppression; and consequently, a delayed senescence (Razzaq et *al*., 2014). Additionally, PUT contains two amino groups: Spd and Spm synthetic precursors (Xu

et al., 2020). Furthermore, PUT and ethylene shares a common precursor SAM, however, PUT treatment resulted in reduced or slow colour change due to inhibited ethylene emission and respiration in `Wonderful` pomegranate fruit at 5℃ (Fawole *et al*., 2020).

4.2.2. Biochemical properties

Titratable Acid (TA)

Acidity is another important parameter that determines fruit quality from the consumer's perception (Hanif *et al*., 2020). Organic acids are the main contributors to titratable acidity and are reported to be the major substrates for 'Wonderful` pomegranate respiration during storage (Fawole *et al*., 2020). In this study, 'Solo' papaya titratable acid decreased rapidly during cold storage and shelf-life days, irrespective of the treatments. Acidity reduction along with storage might be due to acid utilization during respiration process (Zokaee *et al*., 2007 and Ishaq *et al*., 2009).

The findings of these study showed that cold storage significantly affected titratable acid content on treated, control and untreated 'Solo' papaya fruit. However, 'Solo' papaya TA content decreased rapidly during storage at 13℃ when compared with fruit stored at 7.5℃. Furthermore, a decrease in TA was slightly higher in 'Malake' tomato fruit at 10℃ than at 5℃ (Žnidarčič and Požrl, 2006). Additionally, similar results were reported on 'Cochoro' tomato fruit by Tedesse *et al*. (2015), whereby, at higher temperature (20℃), respiration and ripening rate, was higher than at low temperature (4℃). Therefore, this might be the reason why the highest TA percentage was recorded on 'Solo' papaya fruit stored at chilling temperature (7.5℃) as demonstrated on 'Cochoro' tomato fruit by Tedesse *et al*. (2015).

In the present study, ‘Solo’ papaya fruit titratable acidity content declined during the entire storage duration and shelf-life days; however, the highest TA content was observed on control fruit when compared with fruit treated with PUT, irrespective of the cold storage temperatures (Figure 4.8). The results agreed with Fawole *et al*. (2020), who found the highest TA percentage on untreated `Wonderful` pomegranate fruit at 5℃. The highest TA obtained on untreated ‘Solo’ papaya fruit could be due to increased acid concentration from mass loss, therefore, increasing TA content as demonstrated on ‘Wonderful’ pomegranate fruit (Fawole *et al*. 2020). Contrarily, treating ‘Solo’ papaya fruit with 1 mM PUT was efficient in maintaining the lowest TA percentage at 7.5 and 13℃. Similarly, a decrease in acidity was reported by Jawandha *et al*. (2012), for ‘Langra’ mango fruit treated with 1 mM PUT and stored at 13℃. According to Habibi and Ramezanian (2017), exogenous application of PUT delayed the respiration rate and ethylene production during storage at 5℃ of ‘Blood’ orange fruit which caused a delay in organic acids reduction.

Total Soluble Solids (TSS)

Total Soluble Solids is an important characteristic for optimum edible quality and are generally related to fruit sugar content (Lata, 2017). In ‘Cavendish’ and ‘Native’ banana fruits, TSS increased as the fruit ripens and it is responsible for sweetness (Hosseini *et al.,* 2018). In this study, TSS increased in both treated and untreated fruits during the whole storage period and shelf-life (Figure 4.9). The increase in TSS during the storage period can be related to several factors, including starch decomposition into sugars (Fawole and Opara, 2013), increased respiration rate and sugar transformation into carbon dioxide and water (Eshghi *et al*., 2014). Additionally, a gradual increase in

TSS can be due to cell wall polysaccharide hydrolysis (Comabella and Lara, 2013), and an increased dry matter percentage due to water loss (Dong *et al*., 2004).

In ‘Solo’ papaya fruit, total soluble solids were significantly affected by cold storage temperature, regardless of the treatments (Figure 4.9). However, TSS was slightly higher in fruit stored at 13℃ when compared with fruit at 7.5℃ (Figure 4.9). Similar results were reported on ‘Malike’ tomato fruit whereby, higher TSS values were obtained at 10℃ than those stored at 5℃ (Žnidarčič and Požrl, 2006). This could be attributed to the potential of low storage temperature (<10℃) to slow down respiratory metabolism, therefore, retaining TSS in fruit crops (Tasesse *et al*., 2015).

The findings of this study showed that the total soluble solids (TSS) on ‘Solo’ papaya fruit was significantly affected by the treatments, irrespective of the cold storage temperature (Figure 4.9). However, total soluble solids on ‘Solo’ papaya fruit were not significantly affected by PUT postharvest treatment at 13℃. In contrast, 3 mM PUT significantly retained lower TSS on ‘Solo’ papaya fruit at 7.5℃. Similarly, the lowest TSS accumulation was reported on ‘Red lady’ papaya fruit subjected to 3 mM PUT fruit at 12℃ (Hanif *et al*., 2020). Khan *et al*. (2008) also reported that ‘Angelino’ plum fruit treated with 2 mM PUT stored at low temperature (0℃) exhibited lower soluble solid content. The reduction in TSS due to PUT treatment is ascribed to suppression in ethylene production, which consequently affected the acid and sugar metabolism in ‘Samar Bahisht Chaunsa’ mango fruit during ripening and cold storage (Razzaq *et al*., 2014). Additionally, exogenously applied PUT increased the endogenous PAs level in ‘Babygold 6’ peach fruit that might reduce respiration, consequently, reduced TSS accumulation (Valero *et al*., 1998).

4.2.3. Pathological and physiological disorders

Chilling Injury Index (CII)

In crops, chilling injury (CI) is a physiological disorder that affects chilling sensitive fruit stored at low temperatures (Fawole, 2020). Papaya fruit, like other tropical fruits, are sensitive to chilling temperatures (usually lower than 10℃) and may develop chilling injury (CI) symptoms such as skin pitting, scald, hard lumps in the pulp around the vascular bundles, flesh water soaking, abnormal ripening with blotchy discolouration and increased susceptibility to decay (Proulx *et al*., 2003). Chilling symptoms are the result of oxidative stress at the tissue level (Lukatkin, 2002). Furthermore, under conditions that induce chilling disorders, plasma membrane lipid fraction loses fluidity, which causes a series of changes in cellular membrane permeability (Abbasi *et al*., 2019).

The findings of the study showed that CI developed when 'Solo' papaya fruit was stored either at 7.5 or 13℃, and symptoms were visible as external discolouration on the fruit peel (Figure 4.10). However, fruit stored at 13℃ showed higher chilling indices when compared with fruit stored at 7.5℃ on all treatments (Figure 4.10). Similarly, storage at a lower temperature (-0.5℃) for 28 days under 85-90% RH plus 7 days at 20°C resulted in less chilling injury compared with fruit were stored at relatively higher temperature (4.5℃) for the same storage period in 'Eureka' lemon fruit (Siboza and Bertling, 2013). In this study, chilling injury symptoms were more prominent on control fruit rather than PUT treated fruit, irrespective of storage temperature (Figure 4.10). Therefore, postharvest PUT treatment profoundly influenced the CII of the fruit.

Generally, these findings agreed with Mirdehghan *et al*. (2007), who observed a significantly reduced skin browning on 1 mM PUT treated 'Mollar de Elche'

pomegranate fruit stored at 2℃ after 4 months. Similarly, 1 mM PUT either alone or in combination with carnauba wax reduced CI and skin browning on 'Mridula' pomegranate fruit at 3℃ after 60 days storage (Barman *et al*., 2011).

Putrescine plays a very significant role in alleviating chilling injury symptoms in fruits (Barman *et al*., 2011). Furthermore, exogenous PUT induces cold adaptation, which results in membrane fluidity being maintained at low temperature, therefore, minimizing electrolyte loss and skin browning (Barman *et al*., 2011). Additionally, PUT primarily inhibit lipid peroxidation and thereby preserve the membrane from physical state conversion (Mirdehghan *et al*., 2007).

Anthracnose

The effect of fungal pathogens such as *Glomerella cingulate* (anthracnose) is a major problem of papaya during the postharvest period, along with physical damages during transportation (Lata, 2017). Generally, cold storage significantly affected anthracnose severity on 'Solo' papaya fruit, regardless of the treatment (Figure 4.11). However, anthracnose incidence was increased on 'Solo' papaya fruit stored at 13℃ when compared with fruit at 7.5℃. These results agreed with Shikwambana (2016), who observed higher soft rot incidence on 'Reed' avocado fruit stored at 2.0℃ when compared with fruit stored at 5.5℃. In general, low temperature inhibit microbial growth and suppress metabolic changes, thus maintaining fruit quality during storage in fruit crops (Asiche *et al*, 2017). Additionally, PUT possesses the ability to promote resistance towards mechanical damage and pathological disorders under cold storage temperatures (Khan *et al*., 2008).

Anthracnose infection on 2 mM PUT-treated ‘Solo’ papaya fruit was less than control treatments, therefore, PUT was effective in reducing anthracnose infection, irrespective of the cold storage temperature (Figure 4.11). Similarly, 2 mM PUT was effective in substantially reducing disease incidence in ‘Flordaking’ peach fruit at 1℃ after 6-week cold temperature storage (Abbasi *et al*., 2019). Additionally, PUT metabolism has long been known to be altered responding to biotic stress and to undergo profound changes in plants interacting with fungal pathogens in plants, (Hosseini *et al*., 2018). Furthermore, PUT conjugated to phenolic compounds and hydroxycinnamic acid amides have been shown to accumulate in cells in interactions between plants and a variety of pathogens (Walters, 2003).

CHAPTER 5
SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1. Summary

This study investigated the effect of cold storage temperature and postharvest putrescine on the improvement of 'Solo' papa fruit shelf-life and quality. The results showed that the interaction between these two treatment factors showed a significant effect on 'Solo' papaya fruit mass loss, firmness, object colour parameter (skin redness (a*), Chroma (C*), Hue angle (h^o), total soluble solids (TSS) and titratable acids (TA). However, the interactive effect between cold storage temperature and PUT had no significant effect on the fruit exocarp lightness, blueness and external chilling injury as well as anthracnose incidence. Overall, the findings of the study showed that 7.5℃ was an effective storage temperature with regards to reducing fruit changes in physical and biochemical quality parameters, physiological and pathological disorders. Furthermore, putrescine postharvest significantly maintained 'Solo' papaya fruit quality and shelf-life under cold storage temperature. Furthermore, 2 mM PUT treated fruit had good firmness and maintained TSS and TA until the 5th day of storage at ambient temperature activity after withdrawal from 7.5 and 13℃. Moreover, mass loss, yellow colour development and disease incidence were minimum in 2 mM PUT irrespective of the cold storage temperature.

5.2. Conclusion

Exogenous putrescine application on 'Solo' papaya fruit, especially at higher concentrations (2 and 3 mM) reduced the incidence of anthracnose and chilling injury severity under cold storage temperatures. Therefore, the present study indicates that cold storage temperature and PUT could be a promising technique to significantly

increase the post-harvest storage life of papaya. In conclusion, 2 mM PUT postharvest spray application can be used to delay the fruit ripening process with acceptable fruit quality during ripening at ambient temperature or to extend the storage life at 7.5 and 13℃ of papaya fruit up to 5 weeks with minimum fruit quality losses.

5.3. Recommendations and future research

The present investigation could further be extended to know the effect of PUT to modify total soluble solutes and titratable acid as well as the enzymatic mechanisms responsible for shelf-life extension of papaya fruit. Furthermore, the role of PUT on physical and biochemical quality attributes improvement under cold storage should be studied in different papaya ripening stages. Additionally, the study can be improved by directing research interest towards the translocation of PUT on papaya fruit exocarp.

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