EFFECTS OF HARVEST STAGES, POSTHARVEST PRE-TREATMENTS AND STORAGE DURATION ON THE QUALITY AND SHELF LIFE OF MINIMALLY PROCESSED LITCHI

by

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DECLARATION

I **ZANELE VERONICA NHLEKO** declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree Master of Science Horticulture has not been previously submitted by me for a degree at this or any other University; that it is my work in design and execution, and that all materials contained herein have been duly acknowledged.

Candidate : Ms ZV Nhleko _____ Signature _____ Date

DEDICATION

I dedicate this study to my supportive family, with love and care.

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ABSTRACT

The most common postharvest limitation of litchi fruit is pericarp browning, which leads to consumer rejection of the solid fruit in the market even when the edible portion is not affected. Previously, sulphur dioxide (SO_2) fumigation was used to control the browning and extend shelf life of litchi fruit. However, SO_2 fumigation leaves undesirable residues, alters the fruit taste and may results in health hazards for consumers. An alternative method, namely, minimal processing was used to control pericarp browning and curb postharvest losses in litchi. Litchi fruit were harvested at two maturity stages (early harvest; 120 days after full bloom (DAFB), late harvest; 130 DAFB), peeled and immersed for two (2) minutes in three (3) solutions that represented treatments, namely 1) 1% citric acid 2) 1% calcium lactate and 3) a combination of citric acid and calcium lactate both at 1% measure. The untreated arils were dipped in sodium hypochloride (NaOCl) solution for 1 minute and represented the control samples. The treated arils were packed in sterilized clamshell containers and stored at 1±0.5°C and 95% relative humidity for 12 days, then held at 10±0.5°C for 2 days for shelf life study. As a result of the interaction effect of harvest stages and postharvest pre-treatments, least mass loss percentage (1.32%), juice leakage (1.8 ml per 120 g of fruit) and pH (4.18) was observed in litchi arils harvested late and treated with 1% citric acid only under cold storage. Under shelf life study, H2 control samples presented lower mass loss (2.8%) and juice leakage (4.2 ml per 120 g of fruit). At the end of cold storage, litchi arils harvested early and treated with 1% citric acid combined with 1% calcium lactate presented better tissue strength (56.0 N) and radical scavenging activity (36.6 mmol AAE/mL), while those harvested late presented higher ascorbic acid content (72.9 µg/mL), least microbial population and total colour change (3.5). However, at the end of shelf life storage, litchi arils harvested early and treated with 1% citric acid combined with 1% calcium lactate presented lower (3.1) total change in colour. Overall, harvesting the fruit late and treating with citric acid alone or combined with calcium lactate showed the potential of maintaining better aril quality with least microbial population for up to 12 days under 1±0.5°C storage, whereas harvesteing the fruit early and treating with citric acid alone or combined with calcium lactate showed the potential of maintaining better aril quality under shelf life storage.

Keywords: *Litchi chinesis sonn*; calcium lactate; citric acid; maturity; fresh-cut.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

The litchi (*Litchi chinensis Sonn.*) fruit is a subtropical crop belonging to the *Sapindaceae* Family native to parts of Southern China. It is currently cultivated commercially in China, India, Thailand, Taiwan, Madagascar and South Africa (Soni and Agrawal, 2017). This exotic fruit is mostly valued in international market for its distinctive sweet acidic taste, attractive red pericarp and its high nutritional value sinceit is rich in vitamin C and phenolic compounds (Anjum *et al*., 2017; Zhao *et al*., 2020).It is normally consumed fresh or processed to produce dried fruit, wine, jellies and juice (Punia and Kumar, 2020). The litchi fruit is non-climacteric with comparatively low levels of ethylene production after harvest. The fruit does not ripen after harvest and ethylene production remains constant at 1-3°C storage temperatures for 30 days (Holcroft and Mitcham, 1996; Aizat *et al*., 2013).

In South Africa, the crop flourishes in subtropical regions, which are hot, humid and frost-free. The litchi industry contributed approximately 6.5% (R264 million) to the total gross value of subtropical crops (R4 billion) during the 2016/17 production season (DAFF, 2018). In addition, 10 647 tons was produced in South Africa during the 2017/18 production season. However, there are several limitations to litchi development, particularly after harvest that hinders the industry from expanding.

According to De Jager *et al*. (2003), postharvest losses of litchi are expected to be 20-30% of the harvested fruit and could reach as high as 50%. The common postharvest physiological disorders associated with the fruit include; pericarp browning, micro-cracking, desiccation and decay. Pericarp browning is the most problematic for marketing because the pericarp turns brown within 2 - 3 days after harvest. As a result,the fruit is rejected by consumers in the market while the edible portion (aril) is still in outstanding condition (Jiang *et al*., 2006; Sivakumar *et al*., 2007; Cronje, 2008).

As a way of addressing this issue, sulphur dioxide fumigation (SO_2) has been widely used to preserve the red colour and inhibit postharvest decay (Underhill *et al*., 1991; Ramma, 2004; Apai *et al*., 2015). However, there has been growing concern about SO_2 residue levels in the fruit and their effect on overall flavour (Lemmer and Kruger,

2001). Additionally, SO_2 is viewed as allergen as the result of its ability to cause irritation in people, principally those vulnerable to asthma (Mphahlele *et al*., 2020). This necessitates a shift from the use of SO_2 to alternative prospectivemethods which can control pericarp browning in litchi.

Due to its sensory quality and customer suitability, minimally processed litchi has the potential to be commercialized as a ready-to-eat produce (Mphahlele *et al*., 2020). Nevertheless, minimally processed litchi is affected by mould growth, discoloration and loss of texture after one week of storage at 4±2°C (Shah and Nath, 2008). In order to preserve and prolong the shelf life of the minimally processed fruit, several studies have been reported on various postharvest pre-treatments including the application of calcium salts (Chiabrando, 2013; Benitez *et al*., 2014; Troyo, 2019), anti-browning agents (Putnik *et al*., 2017; Kumar *et al*., 2018), anti-microbial agents (Tajkarimi and Ibrahim, 2012; Perez-Gago and Palou, 2016) and modified atmosphere packaging (De Reuck, 2010; Caleb *et al*., 2013; Zhang *et al*., 2015).

Nonetheless, the need for a greater understanding of the influence of minimal processing on litchi fruit without the use of sulphur as well as other major anti-browning and firming agents requires attention. In addition to these treatments, harvest time and storage conditions significantly influence the behaviour of the produce during storage and need to be considered.

1.2 Problem statement

The litchi fruit is highly valued in the international market for its attractive red pericarp, nutritional value and desirable flavour (Zhao *et al*., 2020). However, marketing of fruit is limited due to its high perishability and short harvest season (Cronje, 2008). Pericarp browning is the major constraints that restrict the expansion of the industry in litchi exporting countries. It is also the most serious factor for marketing of the fruit since it is rejected by consumers in the market even though the edible arils are not affected (Jiang *et al*., 2006).

Browning of the pericarp develops after harvest at room temperature and low relative humidity (Kaewchana *et al*., 2006). In recent years, SO_2 fumigation has been adopted by the South African Litchi Industry for controlling pericarp browning and prolonging

shelf life. Nevertheless, there have been growing concerns about the residue levels of SO2 present in the fruit and its effect on the overall flavour (Sivakumar *et al*., 2010; Kumar *et al*., 2013).

Due to its sensory quality and customer convenience, minimally processed litchi has the potential to be commercialized as a ready-to-eat produce. Numerous studies have been reported on the minimal processing and the use of pre-treatments in different varieties of litchi to preserve freshness and prolong their shelf life (Shah and Nath, 2006, 2008; Kaushik *et al*., 2014; Phanumong *et al*., 2015, 2016, 2017). However, the need for considerate research on the influence of minimal processing on litchi fruit without the use of sulphur requires consideration.

Additionally, in order to extend the shelf-life of minimally processed fresh product, biological processes such as enzymatic browning reactions, respiration and transpiration rates should be minimized. Various ways of minimizing respiration and transpiration rate have been reported on a range of minimally processed product. These include proper pre and post-harvest management of the crop (Benichou *et al*., 2018; Yousuf *et al*., 2018). Consequently, harvest time and storage conditions significantly impact the behaviour of the produce during storage and need to be considered.

1.3 Motivation of the study

The production of litchi is relatively limited in all parts of the world, but the postharvest physiology of the fruit has been well documented. According to Bolanos *et al*. (2010), although postharvest research has made tremendous progress, pericarp browning of litchi after harvest remains the fundamental constraint to stored fruit. The rise in economic losses and growing concern over food safety and environment pollution, have driven postharvest technology research to develop alternative treatments to replace SO2 fumigation (Kumar *et al*., 2013).

According to Shah and Nath (2008), the adoption of minimal processing technologies in the litchi fruit industry lowers fruit losses due to pericarp browning and decay after harvest. Preservation of litchi into minimally processed product is beneficial for

commercialisation and value addition (Sivakumar and Korsten, 2010). This method creates new marketing opportunities as it provides the potential advantages of minimally processed products, such as a fresh and convenient product with no or minimal pre-consumption preparation time and consistent quality (Phanumong *et al*., 2017).However, minimally processed litchi fruit show rapid quality loss and shorter shelf lifedue to increase in respiration rate and accelerated enzymatic cell membrane degradation (Phanumong *et al*., 2016). To overcome these undesirable changes, several studies have been reported on the minimal processing and treatments of different varieties of litchi to preserve and prolong their shelf life (Dong *et al*., 2004; Kaushik *et al*., 2014; Phanumong *et al*., 2015, 2017). Preservatives such as ascorbic acid (Ozdemir and Gokmen, 2019), citric acids (Fan *et al*., 2018; Moradinezhad, 2020) and calcium solutions (Benitez *et al*., 2014) have been utilized in many parts of the world as suitable preservatives for maintaining quality of minimally processed produce.

Fruit harvested too early or too late in the season is more prone to physiological disorders and has a shorter shelf life than fruit harvested at the appropriate maturation stage (Tilahun, 2013). Therefore, maturity at harvest is the most critical element determining postharvest life and final quality of litchi such as the appearance, texture, flavour, and the nutritive value (Mareike *et al*., 2010). Moreover, postharvest factors such as storage duration and temperature play a crucial role in maintaining the quality of minimally processed fruit. The combined effects of these factors on the quality and shelf life of minimally processed litchi fruit remains crucial.

1.4 Purpose of the study

1.4.1 Aim

Selection of the appropriate harvest stage, postharvest pre-treatments and storage duration that could be beneficial in preserving the quality of minimally processed litchi cv. ‘Mauritius’.

1.4.2 Objective

To determine the combined effects of harvest stage, postharvest pre-treatments and storage duration on the physicochemical properties and shelf life of minimally processed litchi fruit cv. ‘Mauritius’.

1.4.3 Hypothesis

The combined treatment factors; harvest stage, postharvest pre-treatments and storage duration will have significant influence on physicochemical properties and shelf lifeof minimally processed litchi fruit cv. ‘Mauritius’.

CHAPTER 2

LITERATURE REVIEW

2.1 Work done on research problem

2.1.1 South African litchi production

Litchi (*Litchi chinensis Sonn*.) is a non-climacteric fruit belonging to the *Sapindaceae* family cultivated in the tropical and warmer subtropical regions of the world. The litchi fruit is a significant economic crop in South Africa, primarily intended for export. (Malahlela *et al*., 2018). The crop thrives in hot, humid and frost- free regions. The plantings per province and per production region are shown in Figure. 2.1. Mpumalanga represents 67% of the SA industry (1034 ha) and Onderberg is the largest production region in the province with 59% (907 ha) of total plantings. Limpopo represent 28% (441 ha) of SA plantings of which 23% (363 ha) are situated in Letaba area. KwaZulu-Natal makes up 5% of the industry (73 ha) (SALGA, 2020).

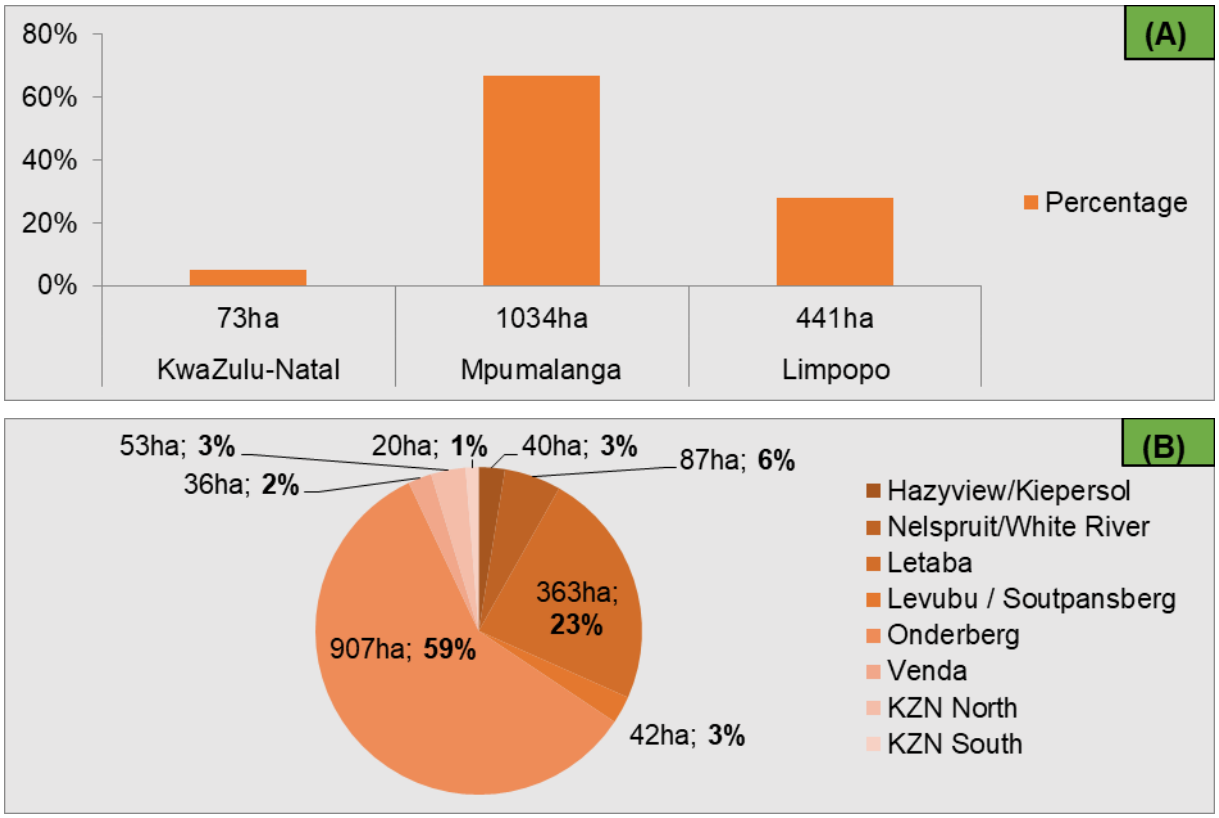

Figure 2.1. Litchi plantings in South Africa by province (A) and per production region (B) (South African Litchi Growers Association 2019/2020 report (SALGA, 2020)).

2.1.2 Litchi pericarp browning

Browning of the pericarp, desiccation, postharvest decay and micro-cracking have all been highlighted as important constraints to the litchi industry's expansion in exporting countries (Reichel *et al*., 2013). Although pericarp browning has no effect on the aril's eating quality, it is the most significant since it degrades the fruit's visual appeal at the export market. Browning is initiated after harvest within 3 to 6 days at room temperature (25–30°C) (Sivarkumar *et al*., 2010). In-depth research has been conducted to determine the biochemical process underlying litchi browning (Jiang, 2000; Qu *et al*., 2021). Bhushan *et al*. (2015) indicated that the pH of the pericarp tissue plays a major role in the browning mechanism since it affects the presence of anthocyanin cells accountable for the red colour of litchi. At higher pH, anthocyanin is converted to a colourless form (carbinol) consequently causing browning of the pericarp. Other mechanisms of litchi pericarp browning are mainly attributed to the oxidation process of phenolics, the degradation of anthocyanin by the enzymes polyphenol oxidase (PPO) or peroxidase (POD) and formation of polymeric browning pigments (o-quinones) (Bhushan *et al*., 2015).

Strategies to mitigate litchi fruit pericarp browning have been previously researched using several postharvest treatments. In recent years, SO_2 fumigation has been implemented by the South African Litchi Industry to control pericarp browning and lengthen fruit shelf life (Fig. 2.2).

Figure 2.2. Twenty four (A) hours after SO_2 fumigation of litchi fruits cv. 'Mauritius' 35th dayof cold storage (B) of SO_2 fumigated litchi fruits cv. 'Mauritius' (Sivakumar *et al*., 2010).

Nevertheless, SO_2 fumigation has certain drawbacks associated with residual toxicity and change in taste of the litchi fruit (Sivakumar *et al*. 2005). Pack house workers have also suffered health related problems during processing of the SO_2 fumigant fruits (Kumar *et al*., 2013). In addition, it has been observed that SO_2 fumigation in litchi intensified micro-cracking of the fruit pericarp (Sivakumar *et al*. 2005). As a result, an alternate postharvest method that is safe for eating, environmentally acceptable and economically viable is needed to overcome litchi pericarp browning while retaining overall fruit quality.

Minimal processing of litchi fruits offers an alternative method of improving the market ability of the fruit and maintains the product fresh and ensures its nutritional quality (Siddiqui *et al*., 2011). The review focused on published literature on minimally processed litchi fruit aimed at developing postharvest treatments that are acceptable to maintain freshness in the postharvest management chain. Due to limited information specifically on minimallyprocessed litchi fruit, the review would also elaborate more on minimally processed/fresh cut fruits.

2.1.3 Postharvest quality of litchi

Impact of minimal processing

Any fruit that has been physically converted from its original form but retains in its freshness has been described as minimally processed (De Oliveira Silva *et al*., 2012). This procedure is required to keep the product fresh while also ensuring its nutritional quality. The advantages that come with minimal processing include low severity of the processing methods, maintaining quality as fresh or close to the fresh prepared products, maintain product's nutritive values, convenient to consumers, reduced labour in preparation before consumption and provide varied shelf-life (Martin-Diana *et al*., 2007; Siddiqui *et al*., 2011).

Processing of fresh-cut fruits requires preliminary steps, such as washing, peeling, shredding, and cutting (Arfin *et al*., 2017). These steps may result in cuts, bruises and injuries to internal tissues and can cause desiccation and wilting as well as microbial and enzymatic spoilage. These injuries fasten the respiration rate, which further triggers the increased production of ethylene, senescence and enzymatic browning

(Artes and Allende, 2005). The impact of minimal processing on the quality of fruits is demonstrated (Fig 2.3).

Deterioration in minimally processed fruits is a result of chemical and enzymatic shifts, microbial deterioration and improper handling, processing and packaging (De Corato, 2020), which largely depend on the composition and nutritional components of the fruit. The available phenolic compounds and coloured pigments in the fruit render antioxidant activity, while tissue sensitivity is also a delicate issue of the fresh fruits as it can be the source of microbiological spoilage activity (Hodges and Toivonen, 2008). Sensory parameters, such as flavour, sweetness, sourness and acidity, largely depend on the post harvesting operations and maturity at harvest while fruit tissuesoftening result from wounding and fruit maturity (Bai *et al*., 2009; Toivonen and Brummell, 2008). However, the postharvest parameters are not clearly related to the presence of flavour compounds, it is mainly dependent on the metabolic and physiological process at the maturity of fruits (Rico *et al*., 2007).

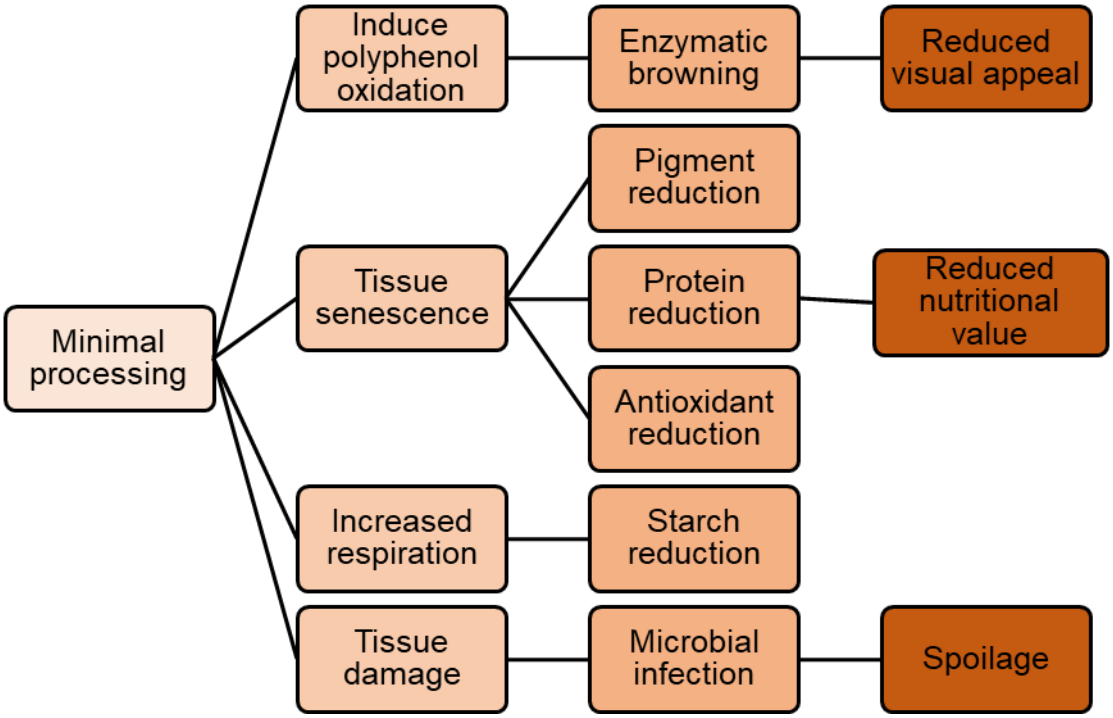

Figure 2.3. Diagram representation of the consequences of minimal processing on quality of fruits (Wiley and Yildiz, 2017).

In addition to sensory attributes, nutritional and health functional components also determine minimally processed product's key quality parameters. These further depend on the climatic conditions, harvesting operations and methods of harvesting

as well as the processing steps used, such as cutting, shaping, packaging, speed of operations such as cooling and mixing (Wiley and Yildiz, 2017). Other important factor of the post processing is the packaging techniques.

Furthermore, proper sanitation, storage and transportation conditions such as temperature and relative humidity need to be considered (Plotto *et al*., 2006). The use of modern preservation technologies, which monitor undesirable changes during storage, ensures the protection and success of minimal fruit processing (De Corato, 2020). The following are some of the preservation methods used in minimally processed fruits: the application of edible coating (Olivas and Barbosa-C, 2005), use of chemical and bio preservatives (Singh and Alam, 2012), antioxidant treatments (Ozdemir and Gokmen, 2019), mild heat treatments (Maghoumi *et al*., 2013), and vacuum packaging (Yousuf *et al*., 2018).

Impact of harvest stage/time

In any given fruit, whole or minimally processed, maturity at harvest is vital since it influences important processes and attributes such as respiration rate, appearance, colour, taste and texture changes (Soliva-Fortuny *et al*., 2004; Reichel *et al*., 2010). However, processing of fruits at different stages of maturity may come with detrimental trade-offs in the sense that an improvement in one quality attribute results in reduction in another attribute (Bai *et al*., 2009). Benichou *et al*. (2018) indicated that any fruit picked either too early or too late in its season is more susceptible to physiological disorder and has shorter shelf life than fruit picked at proper maturity stage.

Research has been conducted to determine the effect of harvesting time on the quality of fruits under cold storage (Turk, 1988; Zhao *et al*., 2021). A study conducted by Oms-Oliu *et al*. (2009) indicated that an advanced ripeness stage at processing could be a limiting factor on the quality and shelf-life of fresh-cut pears (*Pyrus commus L*.). This was in accordance with reports by Barrett *et al*. (2010), that any fruit harvested immature or mature green give overall better quality than fully mature fruit since they can withstand mechanical damage during postharvest handling, and has excellent visual quality. Ngamchuachit *et al*. (2015) reported that for commercial operations, less mature fruits are usually selected for fresh-cut processing due to the ease of shipping, handling and storability of the whole fruits, and minimal change in

visual and textural quality of the fresh-cut products. Gorny *et al*. (2000) conducted a study to determine quality changes in fresh-cut pear slices as affected by ripeness stage (ripe, partially ripe and mature green). The results obtained indicated that slices made from mature-green (early harvest) and partially ripe pear slices exhibited significantly less cut surface darkening at 0°C. Likewise, better quality in fruits harvested at firm ripe maturity stage (early harvest) was observed in minimally processed kiwifruit (*Actinidia* species) stored at 4°C for 10 days (Beirao-da-Costa *et al*., 2006) and in mango (*Mangifera indica*) cubes stored at 4°C for 14 days (Beaulieu and Lea, 2003).Therefore, from these studies, it can be assumed that fruits harvested at an early stageof maturity show better quality.

On the contrary, several studies have shown the advantageous effects of harvesting the fruits at a later stage of maturity, Hodges and Toivonen (2008) indicated that fully mature fruit give better quality since less ripe fruits are insufficiently ripe to satisfy consumer liking in firmness or provide acceptable volatiles and flavour/aroma attributes. Fully mature fruit with declining metabolic activity are at an optimal stage for harvest given that post-harvest changes occur more slowly and storage life is improved (Kader and Mitcham, 2008; Streif *et al*., 2009). Better quality in fruits harvested at a soft ripe stage (late harvest) was observed in red raspberries stored at 16°C for 4 days (Wang *et al*., 2009) and in mango slices stored at 5°C and 10°C for 8 days (Allong *et al*., 1999).

2.1.4 Postharvest treatments affecting quality attributes of minimally processedfruits

Storage conditions and Packaging

Minimally processed products are generally much more perishable than intact products because they have been subjected to physical stress as a result of peeling, chopping or slicing (Arfin *et al*., 2017). O' Connor-shaw *et al*. (1994) compared the shelf life of fresh cut honeydew (*Cucumis melo L.*), kiwifruit, papaya (*Carica papaya*), pineapple (*Ananas comosus*) and cantaloupe (*Cucumis melo var. cantalupensis*) fruits at the temperatures recommended for whole fruit. It was determined that fresh cut fruit had longer shelf life at 4°C than at the whole fruit recommended temperature when these were greater than 4°C. Therefore, the minimally processed fruits should be stored at lower temperatures than those recommended for intact commodities.

The optimum storage temperature for minimally processed fruits is 0 - 3°C, but several fruits are prepared, transported and stored at 5°C (Watada *et al*., 1996; Morga *et al*., 2004). Bolanos *et al*. (2010) conducted a study on the effect of storage temperature and time on quality in minimally processed litchi fruit. The results showed that storing minimally processed litchi cv. Racimo Rojo at 2°C for up to 18 days maintains the fruit in a condition essentially unchanged from when fresh, indicating this to be a promising method for prolonging fruit shelf life.

Any increase in temperature during storage could trigger water loss and the hydrolysis of starch and other polysaccharides into soluble sugars (Alam *et al*., 2013). Moreover, the prepared fruits must be properly packed in order to get the most benefit from the minimal processing method. This is because the type of storage packaging chosen has a significant impact on the quality of minimally processed fruits. Montero-Calderon *et al*. (2008) conducted a study on influence of packaging conditions on fresh-cut 'Gold' pineapple shelf-life during 20 days of storage at 5°C. Fresh-cut fruit pieces were packed in polypropylene trays and enfolded with polypropylene film under active (high 40% or low oxygen, 11.4%) or passive modified atmospheres (air or cut fruit coated with 1%, w/v alginate). From the microbial point of view, the shelf-life of 'Gold' fresh-cut pineapple was limited to 14 days by mesophilic bacterial growth.

On the contrary, Sothornvit and Rodsamran (2008) conducted a study on the effect of a mango edible film and storage conditions on minimally processed mangoes shelf-life. The shelf-life of unwrapped minimally processed mangoes kept in cellophane bags at room temperature (30°C) and cold storage (5°C) were 2 and 4 days, respectively. When the minimally processed mangoes were wrapped in a mango film and kept in cellophane bags, the shelf-life was extended to 5 and 6 days, when stored at 30 and 5°C, respectively.

Therefore, different fruits require diverse packaging type and a good packaging material must have good permeability qualities (Lamikanra, 2002). A greater percentage of fresh-cut products are stored and marketed in modified atmosphere packaging (MAP) in conjunction with chilled storage (Bai *et al*., 2001). The MAP predominantly preserves the quality of fresh-cut products by matching the oxygen transmission rate (OTR) of the packaging film to the respiration rate of the packaged product.

However, using MAP protocols for fresh-cut fruits has frequently led to the generation of anaerobic conditions and high CO2 levels which eventually have a damaging effect on product quality through production of ethanol, acetaldehyde, off-flavours and odours (Hodges and Toivonen, 2008).

Packaging systems popularly used for preserving minimally processed litchi fruit include polystyrene clamshell box (Fig. 2.4A) (Phanumong *et al*., 2015, 2019), snap on lid containers (Fig. 2.4B) and polystyrene trays overwrapped with a polypropylene film (Fig. 2.4C) (Shah and Nath, 2006; 2008). These rigid trays protect the products during handling and storage. Mphahlele *et al*. (2020) conducted a study on the effects of packaging and duration on quality of minimally processed litchi cv. 'Mauritius' packed inside clamshell trays with different perforation sizes: 0 (P-0), 1.1 mm (P-1), and 5.4 mm (P-2) and stored at 1°C for 15 days. The results showed least mass loss % and highest TSS in fruit packaged under P-0 while fruit packed in P-2 (5.4 mm perforation) had the highest firmness compared to samples from other packages. It was further recommended that for minimally processed litchi fruit, non-perforated clamshell containers can be used during storage and present better overall quality.

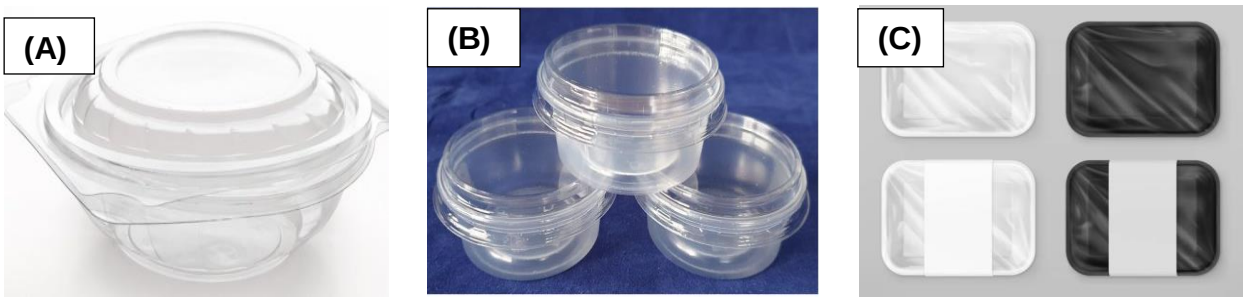

Figure 2.4. Non-perforated clamshell container (A), snap on lid plastic container (B) and polystyrene trays overwrapped with a polypropylene film (C).

Chemical treatments

Calcium salts

Calcium salts have been used extensively as a postharvest chemical pre-treatment to preserve the quality and extend shelf life of minimally processed fruits (Kumar and Shukla, 2017; Martin-Diana *et al*., 2007; Waghmare and Annapure, 2013). However, the effect of calcium on the quality of minimally processed fruit depends on the type of

calcium salts (Aguayo *et al*., 2008), calcium concentration (Luo *et al*., 2011) and dipping time (Manganaris *et al*., 2007). According to Martin-Diana *et al*. (2007), calcium salts that suitable to be used for perseveration of minimally processed include; calcium chloride ($CaCl_2$), calcium phosphate ($Ca_3(PO_4)_2$), calcium lactate ($C_6H_{10}CaO_6$), calcium gluconate ($C_{12}H_{22}CaO_{14}$) and calcium propionate ($C_6H_{10}CaO_4$). Several published work indicated that the most effective calcium salt concentrations used in minimally processed fruit range from 0.5 – 3% and the dipping time ranges from 1 to 5 minutes (Barbagallo *et al*., 2012; Thakur *et al*., 2019).

Calcium and its respective salts especially $CaCl_2$ and $C_6H_{10}CaO_6$ has been well known as firming agents involved in delaying the loss of tissue strength of produce. Calcium ions (Ca^{2+}) can react with the carboxylic groups of de-methyl esterified homogalacturonan pectin polysaccharide domains, forming calcium-pectategel, which confers resistance to proteolytic enzymes in plant cells, aiding to maintain the texture and rigidity of the cell wall (Lamikanra and Watson, 2007; Alandes *et al*., 2009). The use of calcium salts as a firming agent has been applied to various minimally processed fruits, such as cantaloupes (Luna-Guzman and Barrett, 2000), kiwifruit (Beirao-da-Costa *et al*., 2014) and in litchi fruits (Shah and Nath, 2008).

Calcium lactate showed a more lasting outcome of firmness preservation than $CaCl_2$ during the storage fresh-cut cantaloupe (Luna-Guzman *et al*., 1999). Likewise, calcium can help to keep the fresh-like appearance of minimally processed fruits longer by preventing the development of browning, through reducing the action of polyphenol oxidase (PPO) along with its respective substrates cut surfaces (Yang *et al*., 2017). Flesh browning has been controlled in minimally processed honeydew chunks treated with 40 mM $CaCl_2$ for 30 s and stored at 10 for 7 days (Saftner *et al*., 2003).

The existing application of the calcium treatment in minimally processed fruits has already been studied on a significant number of commodities, with limited information on minimally processed litchi (Table 2.1). Its application resulted in increased tissue strength in most if not all reported studies, which can be attributed to the calcium ion that maintain the structure of cell wall by increasing the cross linkages with the cell wall and middle lamella pectin.

Inam-ur-Raheem *et al*. (2013) indicated significantly delayed browning in guava (*Psidium guajava*) fruit with $CaCl_2$ treatment. This effect can be attributed to the function of $CaCl_2$ in retarding the rate of respiration and by decreasing the activity of enzymes responsible for browning in fruits. Silveira *et al*. (2011) further elaborated that calcium applications can delay or slow down changes related to respiration and senescence processes and thereby having a direct effect in preserving the functionality of the membranes.

Although some studies reported improved total soluble solids in minimally processed fruits treated with calcium salts, a few studies have reported reduced total soluble solids. Loss in total soluble solids has been reported by Shah and Nath (2006) and Phanumong *et al*. (2015), while Phanumong *et al*. (2016) and Punumong *et al*. (2016) reported improved total soluble solids content. The conflicting results might be influenced by on several factors, such as variety, stage of maturity, the degree of tissue damage and storage conditions. However, Shah and Nath (2008), indicated that the reduction in TSS during storage may be due to utilization of sugars by growth of microbes, whereas Phanumong *et al*., (2015) indicated that the reduction might be due to the dissolution of components in litchi arils into the treatment solution, or the water absorption into the tissue during dipping.

Table 2.1.Studies conducted to determine the effect of calcium salts on quality parameters of minimally processed fruits.

Fruit	Treatment	Packaging and storage duration	Result	Reference
Litchi cv.'Jugkapat'	1% $CaCl_2$, 2% $C_6H_{10}CaO_6$, 2% $C_6H_{10}CaO_4$	Polystyrene clamshell, 2±1°C, 12 days.	$CaCl_2$ retarded the loss of cell turgor, $C_6H_{10}CaO_6$ showed highest firmness and, $CaCl_2$ slightly increased the aril's TSS and TA and reduced pH.	Phanumong *et al*., 2019
	0.5 - 3% of; $CaCl_2$, $C_6H_{10}CaO_6$, $C_6H_{10}CaO_4$.	Polystyrene clamshell box, 2±1°C, 12 days.	All treatments decreased respiration rate by 1.5 to 2 folds, reduced juice leakage and delayed microbial growth.	Phanumong *et al*., 2016
	1% $CaCl_2$	Polyethylene bag (5% O_2 +5% CO_2), 2±1°C, 18 days	$CaCl_2$ reduced juice leakage, retarded increasing ethanol content, total bacteria and yeast-moulds counts and retarded the loss of cell turgor.	Punumong *et al*., 2016
Litchi (cv. 'Rose')	2% $C_6H_{10}CaO_6$	Polystyrene trays, 4±2°C, 20days.	The TSS, pH and sensory scores decreased, drip losses and microbial count increased.	Shah and Nath, 2006
Melon cv.'Galia'	0.4% of; $CaCl_2$, $C_6H_{10}CaO_6$ $C_6H_{10}CaO_4$.	Polypropylene (PP) trays, 5°C for 10 days.	$C_6H_{10}CaO_4$, $CaCl_2$ and $C_6H_{10}CaO_6$ had lower respiration rate, and maintained good firmness.	Silveira *et al*., 2011
Guava	0.9%, 1.8%, 2.7%, 3.6% of; $CaCl_2$, $C_6H_{10}CaO_6$.	Plastic boxes, 5±2°C for 24days.	$CaCl_2$ at 2.7% showed delaying firmness and browning and $C_6H_{10}CaO_6$. 2.7% $CaCl_2$ and 3.6% $C_6H_{10}CaO_6$ exhibited better results than other concentrations with storage.	Inam-ur-Raheem *et al*., 2013
Papaya	2% $CaCl_2$	Polypropylene plastic bags, 5°C for 25 days.	Dipping fresh cut papaya in $CaCl_2$ solution extended shelf-life of the fruits for 15 days.	Thakur *et al*., 2019

Calcium chloride ($CaCl_2$), calcium lactate ($C_6H_{10}CaO_6$), andcalcium propionate ($C_6H_{10}CaO_4$).

Citric acid

Citric acid has been used extensively as a postharvest chemical pre-treatment to preserve the quality and extend shelf life of minimally processed fruits (Bieganska-Marecik and Czapski, 2007; Siriwardana *et al*., 2015; Izzah *et al*., 2015). However, the effect of citric acid on the quality of minimally processed fruit may be influenced by concentration and dipping time. The effect of citric acid on the physicochemical attributes of minimally processed fruits is presented in Table 2.2. Nevertheless, there is limited information reported on the use of citric acid in minimally processed litchi fruit. Studies conducted on minimally processed fruit treated with citric acid observed that dipping the fruit for 1 – 5 minutes widens the effectiveness of the chemical (Cocci *et al*., 2006). He and Luo (2007) indicated that citric acid is typically applied at levels ranging from 0.5% to 2% in fresh-cut products.

Enzymatic browning is a most important factor contributing to the quality loss of fresh-cut fruits. Browning affects the outer appearance of the fresh-cut fruits and degrades its sensory characteristics and nutritional value. Citric acid has an inhibitory effect on polyphenol oxidase (PPO) through reducing pH and complexing with the copper on the active enzyme centre (Eleni and Theodoros, 2011).

Maketup and Krajayklang (2016) reported a reduction in browning incidences in fresh cut pineapple fruit treated with citric acid. The best results were obtained from the 0.5% citric acid solution which showed reduced browning incidence at day 6. Citric acid has been used as an anti-browning agent in various minimally processed fruits including apples (*Malus domestica*) (Azevedo *et al*., 2018), mango (Chiumarelli *et al*., 2011) and bananas (*Musa*) (Siriwardana *et al*., 2015). However, Chen *et al*. (2016) observed that treatment with citric acid alone aggravated the browning of fresh-cut apples during storage. This contradiction can be related to factors such as concentration of citric acid and storageconditions.

Postharvest practices including peeling and cutting of fruits removes the protecting epidermal layer exposing the produce to contamination by numerous pathogens (Jideani *et al*., 2017). It has been observed that citric acid treatment can retard microbial growth in minimally processed fruits (Ramos *et al*., 2013). For instance,

microbial growth has been successfully retarded in fresh cut apples (Chen *et al*., 2016), mango (Techavuthiporn and Boonyaritthonghai, 2016) and in peeled oranges (*Citrus sinensis*) (Pao and Petracek, 1997) treated with citric acid. In contrary, Latifah *et al*. (2011) observed that microbial growth of fresh-cut pineapple treated with citric acid did not change for samples stored at 2°C, but increased steadily in those stored at 10°C.

Table 2.2. Studies conducted to determine the effect of citric acid on quality parameters of minimally processed fruits.

Fruit	Treatment	Packaging and storage duration	Result	Reference
Mango (cv.'Nam DokMai')	0.5, 1.5 and 2.5% $C_6H_8O_7$ for 3 min	10°C and 90-95% RH for4 days	Significant inhibition of microorganisms growth as indicated by total plate count (TPC). All compounds applied significantly suppressed the colour change.	Techavuthiporn and Boonyaritthonghai, 2016.
Mango (cv.'Tommy Atkins')	0.5% $C_6H_8O_7$	Polyvinyl chloride (PVC) stretch films, 5°C for 15 days	Promotion of colour preservation increased mass loss during storage and effectively reduced respiration rate.	Chiumarelli *et al*., 2011
Pineapple (cv. 'Huaimun')	0.5% and 1.0% $C_6H_8O_7$ solution.	Clamshell trays, 2.5°Cfor 12 days	Better fruit quality, best visual appearance with less browning, delayed senescence and extended storage life.	Maketup andKrajayklang, 2016
Pineapple	1.0, 1.5 and 2.0% $C_6H_8O_7$.	Polypropylene containers, 10 and 2°C for 14 days.	Treatment with 1.5% citric acid was more acceptable.	Latifah *et al*., 2011
Apple (cv.'Fuji')	0.5% $C_6H_8O_7$	PE cling film 5±2°C for 15 days.	Citric acid aggravated the browning, increased the mass loss and reduced bacterial count.	Chen *et al*., 2016
Oranges	0.1, 0.25, 0.5, 1.0% $C_6H_8O_7$	Perforated plastic containers, 4°C for 21 days.	Lowered pH, decreased counts of total aerobic organisms and extended shelf life.	Pao and Petracek, 1997

Citric acid= $C_6H_8O_7$, RH= Relative humidity, cv= cultivar.

Combined chemical treatments

Combining treatments that target different quality parameters presents minimally processed fruits with optimal quality. Shah and Nath (2008) investigated minimally processed litchi treated with anti-browning agents (4.9 g/kg cysteine, 20 g/kg ascorbic acid and 0.134 g/kg 4-hexyl resorcinol) along with osmo-vacuum dehydration packaged in polypropylene film and stored at 4±2°C for 24 days. The combined treatment of anti-browning agents and osmo-vacuum dehydration treatment were found to be most effective in preventing the changes in litchi arils. However, there is limited information reported on the use of combination of calcium lactate and citric acid in minimally processed litchi fruit.

The effect of combined treatments on the quality attributes of minimally processed fruits is presented in Table 2.3. Aslam *et al*. (2018) conducted a study on the effectiveness of firming agent integrated with anti-browning agents on the quality of fresh cut papaya. The results indicated that calcium lactate (2.4%) integrated with either citric acid (1.7%) or ascorbic acid (1.7%) showed storage stability for firmness. Similarly, several studies also reported better firmness with application of combination of treatments (Krishna *et al*., 2018; Chiabrando and Giacalone, 2012).

Combined treatments have proven beneficial in preserving colour of minimally processed fruits. Guan and Fan (2010) investigated the effects of sodium chlorite and calcium propionate, individually and combined, on quality and microbial population of apple slices were “Granny Smith” apple slices. Results showed that combination of calcium propionate and sodium chlorite was able to inhibit apple browning during storage. Overall, the results suggested that combination of sodium chlorite with 0.5%and 1% calcium propionate could be used to inhibit tissue browning. Similarly, anti- browning treatments such as citric acid, ascorbic acid, sodium chlorite and $CaCl_2$ present in the treatment solution maintained the colour of minimally processed apples (Chiabrando and Giacalone, 2012), litchi (MFB and TAA, 2017), papaya (Krishna *et al*., 2018) and mango (De Souza *et al*., 2006).

Microbial growth is one of the main concerns associated with fresh-cut or minimally processed fruits. Microbial activity was minimized in fresh-cut rose apple using sodium

chlorite combined with $CaCl_2$ and calcium ascorbate during storage at 4±2°C for 9 days (Mola *et al*., 2016). Similarly, Ediriweera *et al*. (2012) reported microbial counts within safe-to-consume limits in minimally processed pineapples treated with sodium chloride alone or in combination with $CaCl_2$. It can be assumed that the function performed by a chemical treatment is enhanced or improvedwhen treatment is used in combination.

Furthermore, MFB and TAA (2017) worked on minimally processed litchi treated with a combination of 0.5% ascorbic acid + 2.0% sorbitol + 1.0% $CaCl_2$ + 1.5% calcium lactate, 0.5% citric acid + 2.0% Sorbitol + 1.0% $CaCl_2$ + 1.5% calcium lactate, 0.5% ascorbic acid + 1.0% $CaCl_2$ and 0.5% citric acid + 1.0% $CaCl_2$, and stored in normal refrigerator at 4±10°C. Selected level of 0.5% citric acid+1.0% $CaCl_2$ showed better shelf life (13 days) over other treatments and control.

Table 2.3a. Studies conducted to determine the effect of combined chemical treatments on minimally processed fruits.

Fruit	Treatments	Packaging and storage duration	Results	References
Litchi 'Rose'	4.9 g/kg cysteine, 20g/kg AA and 0.134g/kg 4-hexyl resorcinol) along withosmo-vacuum dehydration	Polypropylene film and stored at 4±2°C for 24 days	A decrease in pH, TSS (°brix), sugars (g/kg), ascorbic acid (g/kg), total phenolics (g/kg), firmness (N), colour (L* value) and sensory characteristics was observed whereas an increase in microbial counts (log cfu/g), acidity (g/kg) and drip losses (ml/kg) was observed. The combined treatment of litchis with anti-browning agents and osmo- vacuum dehydration treatment were found to be most effective in preventing the changes up to 24 days.	Shah and Nath, 2008.
Papaya 'Bombay'	$C_6H_{10}CaO_6$ (2.4%) CA (1.7%), AA (1.7%)	Air tight plastic bag, 4±2°C for 16 days	$C_6H_{10}CaO_6$ integrated with CA along with AA showed significant storage stability for firmness, colour, pH, acidity and weight loss as well as sensory characteristics for as longer as 16 days of storage.	Aslam *et al*., 2018.
'Granny Smith' apple	$C_6H_{10}CaO_4$ 0%, 0.5%, 1%, and 2% 0.05% NaCl	Stored at 3 and 10°C for up to 14days	Samples treated with the combination of NaCl with $C_6H_{10}CaO_4$ did not show any detectable yeast and mould growth during the entire storage period at 3°C.	Guan and Fan, 2010
Litchi 'Bedana'	0.5%, 2%, 1%; AA, sorbitol, $C_6H_{10}CaO_4$, $CaCl_2$	Stored at 4±10°C for up to 15 days.	0.5% CA + 1.0% $CaCl_2$ can retain the color in minimal processed litchi than other treatments satisfactorily up to 13 days of preservation time.	MFN and TAA, 2017

Table 2.3b. Studies conducted to determine the effect of combined chemical treatments on minimally processed fruits**.**

Fruit	Treatments	Packaging and storage duration.	Results	References
Papaya	$CaCl_2$ + CA $CaCl_2$ (1%/2%) CA (2.5%/5%)	Polyethylene film, 5°C for 12 days	Least physiological loss in weight in the cubes treated by $CaCl_2$ (2%) + CA (5%). Sugars, ascorbic acid, total carotenoids content and organoleptic score were highest with minimum browning in the cubes treated with $CaCl_2$ (2%) + CA (5%).	Krishna *et al*., 2018
Pineapple 'Mauritius'	1% NaCl +1% $CaCl_2$	Polystyrene packages, 5-7°Cfor 12 days.	NaCl and a combination of NaCl and $CaCl_2$ resulted in maintaining a better flavour. Microbial counts were within safe to consume limits.	Ediriweera *et al*.,2012
Apple	AA (1%, w/v) + $CaCl_2$ (1%, w/v), CA (1%, w/v) + $CaCl_2$ (1%, w/v).	Polypropylene plastic bags, 4°Cfor 5 days.	Combination of AA, CA and $CaCl_2$ resulted in a reduction of browning and deterioration. These anti-browning agents helped to maintaining the colour of fresh-cut apples during storage. $CaCl_2$ + CA maintained colour and firmness.	Chiabrando andGiacalone, 2012
'Kensington' Mango	O2 (2.5; 21.0%), CO_2 (0, 5, 10, 20, 40%), CA (0, 2.0%), $CaCl_2$ (0, 3.0%), AA (0.5, 1.0%).	Plastic containers with two holes(1mm diameter), 3°C 15 days.	$CaCl_2$ (3%) application was partly effective at controlling darkening. $CaCl_2$ however significantly slowed (but did not stop) loss of tissue firmness.	De Souza *et al*.,2006

Carbon dioxide = CO_2, Oxygen = O_2, $CaC_{12}H_{14}O_{12}$ = Calcium ascorbate; $C_6H_{10}CaO_6$= Calcium lactate; $C_6H_{10}CaO_4$ = Calcium propionate CA=citric acid; AA= Ascorbic acid; $CaCl_2$=Calcium chloride; Sodium chloride = NaCl.

2.2 Work not done on research problem

Maturity of fruit at harvest is the most critical element that determines the final quality such as the appearance, texture,flavour, and the nutritive value. In depth research on the effect of harvesting time on physicochemical properties of minimally processed litchi fruit instead of intact fruit has not been previously investigated. Minimally processed litchi fruit show rapid quality loss and shorter shelf life due to increase in respiration rate which can be overcome using preservatives such as calcium salts and citric acids. However, the effect of these pre-treatments as well as storage duration on development of microbial contaminants on minimally processed litchi fruit remains crucial. Under favourable storage conditions, the combined effect of harvest stagesand postharvest pre-treatments need to be investigated.

2.3 Addressing the identified gaps

In the South African Litchi Industry, as a way of replacing SO_2 fumigation, minimal processing can be used in order to improve marketing and maintain product freshness. The need for a greater understanding of the influence of minimal processing on litchi fruit without the use of sulphur as well as other major anti-browning and firming agents requires attention. This study focuses on the application of minimal processing protocol on the litchi fruit, in this way, the detrimental effects SO_2 fumigation has on human health and overall fruit flavour can be easily avoided. The stage at which the fruit is harvested is the first significant consideration that defines how the fruit will behave after harvest. The effect of harvesting time on quality of whole/intact litchi fruit has been previously investigated (Cronje, 2008). However, postharvest quality of minimally processed litchi as an effect of harvesting time is yet to be investigated and constitute the perceived gap in the research problem. The use of postharvest pre-treatments has shown advantageous effects on the overall quality of minimally processed litchi fruit (Phanumong *et al*., 2015, 2016, 2019). Additionally, while the effect of storage duration on quality and shelf life of minimally processed litchi has been investigate previously (Bolanos *et al*., 2010; Mphahlele *et al*., 2020), the combined effect of harvest stages, postharvest pre-treatments and storage duration remains crucial. Furthermore, the information generated from this study will assist in closing the identified gaps and could lead to the introduction of minimally processed litchi fruit to retailers as value added product.

CHAPTER 3

MATERIASL AND METHODS

3.1 Study location

Mature litchi fruit cultivar ‘Mauritius’ was harvested from Burgershall farm, situated at Mpumalanga province (25° 6' 38.96" S 31°5' 2.04" E). The study was conducted at the Agricultural Research Council - Institute for Tropical and Subtropical crops (ARC-ITSC) in Nelspruit, Mpumalanga, South Africa (25° 29' 56" S 31° 20' 13" E) in December 2019.

3.2 Plant material and preparation

Litchi fruits were harvested manually with care to minimize mechanical injuries in their earlyand late stage of maturity. After harvest, fruit were immediately transported using standard plastic crates to the postharvest laboratory and stored at 10°C pre-cooling room overnight prior to minimal processing (Fig.3.1).

Figure 3.1. Harvested litchi fruits.

Fruit with bruises, sign of infection or deformed fruit were discarded from the lot. The

selected fruit were sorted for uniformity in colour, shape and size. Once sorted, the fruit were washed and disinfected by dipping in a sodium hypochloride (NaOCl) solution (50 mg/L) for 1 minute and blotted dry using sterile tissue paper before processing. All processing unit and distilled water used to prepare the dipping solution were kept at ±16°C for the duration of sample preparation. The processing, packaging and preparation area was first sanitized with 70% (v/v) ethanol (Ferreira *et al*., 2015). To avoid injury to the pulp, the fruit were manually and carefully peeled (Fig.3.2). The peeled litchi arils were immersed in solutions of citric acid (1%) and calcium lactate (1%) separately for 2 min, and combination of citric acid (1%) and calcium lactate (1%).

Figure 3.2. Peeled litchi arils ready for treatment.

After minimal processing, the baseline measurements (day 0) were conducted prior to packaging and storage. The mass of clamshell was recorded at day 0 and labelled accordingly. Treated arils were drained of excess water for 30 sec then packed in sterilized clamshell containers with approximately 6 to eight pieces of arils (120-122 g) (Fig. 3.3). The packed samples were stored immediately at 1±0.5°C and 95% relative humidity (Fig. 3.4). Untreated arils were dipped in NaOCl solution for 1 minute and represented the control samples. During storage, four clamshells per treatment were sampled on days 0, 3, 6, 9 and 12. In addition, on each sampling day additional four

packages were taken and stored for 2 days at 10±0.5°C for shelf life study.

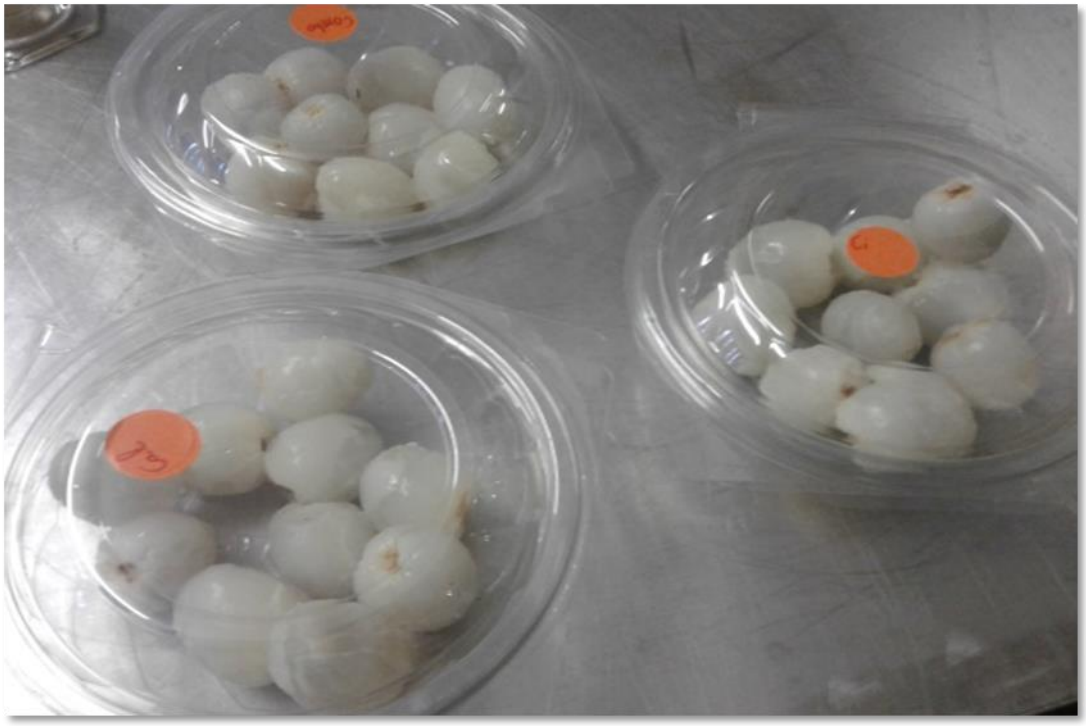

Figure 3.3. Litchi arils packed in clamshell containers.

Figure 3.4. Litchi arils under cold storage.

3.3 Research design and treatments

The experiment was a 2 x 3 x 5 factorial design with 8 replications. Three main treatment factors namely: **harvest stages** (A) [early (H1) - 120 days after full bloom (DAFB) and late (H2) - 130 DAFB], **postharvest pre- treatments** (B) [citric acid (T1), calcium lactate (T2) and citric acid + calcium lactate (T3)] and **storage duration** (C) [0, 3, 6, 9 and 12 days].

3.4 Data collection

3.4.1 Physical analysis

Mass loss and juice leakage

The mass of the clamshell containing fruit was recorded at day 0 and continued for each sampling storage day using digital analytical balance (SBA 16, Scaltec instruments, Germany), with an accuracy of ±0.01 g. Mass loss of litchi fruit was taken after removing and quantifying juice leakage from the clamshell in accordance with Mphahlele *et al*. (2020). The difference between initial and final weight of fruit was considered as totalweight loss during storage interval and results were expressed as percentage (%) (AOAC, 2007). The percentage mass loss was calculated according to equation (1). Juice leakage from the arils was measured per punnet using a 100 ml graduated cylinder and the results expressed as mL/120 g of fruit.

$$\text{Total weight loss (\%)} = \frac{\text{Initial fruit weight} - \text{Final fruit weight}}{\text{Initial fruit weight}} \text{ X } 100\ \% \qquad \text{Eq. (1)}$$

Texture profile

Texture of four fruit per treatment was measured using a computer-controlled automatic fruit texture analyzer (TA.Xt plus; Stable Micro Systems Ltd., Surrey, UK) according to Shah and Nath (2008), by performing a puncture test on flesh with a stainless steel needle of 3 mm diameter. The texture strength was measured at the test speed of 1 mm per sec. Puncture tests were taken from the two opposite equatorial sides of the same fruit. Texture strength measurements were taken as the first peak force value obtained during the test to penetrate the fruit 3 mm, at 1 mm/s.

Colour

The colour of four representative fruits per treatment was measured using a Minolta chromameter (Model CR-300 Minolta; Ramsey, NY). White background (Illuminants C: Y = 83.44, x = 0.3051, y = 0.3202) was used for calibration before measurements were taken. The colour was expressed as browning index (*BI*) and total colour difference (ΔE) which was calculated according to Pathare *et al*. (2013) using equations (2) and (3):

$$BI = 100\left(\frac{X-0.31}{0.17}\right) \quad \text{Eq. (2)}$$

Where,

$$X = \frac{(a^{*}+1.75L)a}{(5.645L+a^{*}-3.012b*)}$$

$$\Delta E = \sqrt{\Delta a\,{*}^{2} + \Delta b\,{*}^{2} + \Delta L\,{*}^{2}} \quad \text{Eq. (3)}$$

3.4.2 Chemical analysis

Total Soluble Solids (TSS), pH and Titratable Acidity (TA)

For chemical analysis, eight pieces of arils per replication were macerated in a juicer and analysed for pH, TA and TSS. Approximately, 50 ml of juice was extracted and used to measure the pH using pH meter (Mettler Toledo, South Africa) at room temperature of 21°C. Litchi fruit juice TSS was measured using a digital refractometer (Atago, Tokyo, Japan) which was calibrated with distilled water at 20°C andexpressed in °Brix.

Two millilitres of juice was extracted and poured into a beaker where 10 ml of distilled water was added. The TA was measured using NaOH (0.1 N) as a standardized titration solution according to Tsegay *et al*. (2013). When the end point of titration was reached, the amount of NaOH used on the burette was read off and recorded to calculate TA. The millilitres of NaOH were used to calculate the TA expressed as percentage citric acid using the formula according to Mitcham *et al*. (1996) using equation (4).

$$TA = \frac{ml\ of\ NaOH\ x\ N\ of\ NaOH\ x\ acid\ milliequivalent\ factor}{ml\ juice\ titrated}\ x\ 100 \qquad \text{Eq. (4)}$$

Radical scavenging activity (RSA)

The ability of litchi juice to scavenge 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical was determined using the method described by Karioti *et al*. (2004). Crude litchi juice (15 µL) was mixed with 735 µL methanol in centrifuge tubes followed by the addition of 0.1 mM solution of DPPH (750 µL) dissolved in the methanol solution. The mixture was incubated in the dark for 30 min at room temperature. The absorbance was measuredat 517 nm using a UV-visible spectrophotometer (Jenway, UK). An ascorbic acid standard curve (y = -0.3262x + 0.6807, and r^2 = 0.98) was used to determine radical scavenging activity (RSA) and results were expressed as micromole ascorbic acid equivalent per millilitre of litchi juice (mmol AAE/mL).

Ascorbic acid (AA)

Ascorbic acid concentration was measured according to the method of Klein and Perry (1982). Crude litchi juice (1 mL) was mixed with 10 mL of 1% metaphosphoric acid and then sonicated in an ice bath for 4 min. The samples were then centrifuged at 4000 g for 5 min. Supernatants (1.0 mL) were pipetted into a tube and mixed with 9 mL of 2, 6 dichlorophenolindophenol dye (0.0025 g). The mixture was incubated in the dark for 10 min and the absorbance was measured at 515nm using spectrophotometer (Jenway, UK). Standard curve of authentic L-ascorbic acid (y = -0.0009x + 0.0897 and r^2 = 0.98) was used to calculate ascorbic acid content. Results were expressed as mass of ascorbic acid equivalents per volume of crude litchi juice (µg/mL).

3.4.3 Microbial evaluation

For microbial analysis, litchi aril that showed signs or symptoms of infection by fungus pathogens were analysed according to the method of Mailafia *et al*. (2017). The fruit were cut into small segments (4 mm in diameter) with a sterilized blade, surface sterilized in 70% ethanol, 10% sodium hypochlorite and sterilized water for 10 to 20 sec and dried between sterile absorbent papers. The pieces were then plated on Potato Dextose agar (PDA) aseptically and then incubated at 27°C for 5 days. A pure

culture was obtained and maintained by sub-culturing each of the different colonies that emerged onto the PDA plates and incubating at 27°C for 5 days. Pure culture was used to identify the fungal pathogens in the fruit. Genomic DNA was extracted from the pure cultures using Zymo Research, Quick-DNA™ Fungal/Bacterial Miniprep Kit (Inqaba Biotechnical Industries (Pty) Ltd., Pretoria, South Africa).

The intergenic spacer (ITS) target was amplified with the primers listed in Table 3.1 using New England BioLabs Inc., OneTaq® Quick-Load® 2X Master Mix (Inqaba Biotechnical Industries (Pty) Ltd., Pretoria, South Africa). The PCR amplicons were run on a gel and the DNA bands on gel were extracted with the Zymoclean™ Gel DNA Recovery Kit (Inqaba Biotechnical Industries (Pty) Ltd., Pretoria, South Africa). The fragments were sequenced in the forward and reverse direction using BrilliantDye™ Terminator Cycle Sequencing Kit (V3.1., BRD3-100/1000, NimaGen BV, The Netherlands) and purified with Zymo Research, ZR-96 DNA Sequencing Clean-up Kit™ (Inqaba Biotechnical Industries (Pty) Ltd., Pretoria, South Africa). The purified fragments were analysed on the ABI 3500xl Genetic Analyser (Applied Biosystems, ThermoFisher Scientific, MA, USA). Each sample reaction was analysed using CLC Bio Main Workbench vr. 7.6, and results obtained via Basic Local Alignment Search Tool (BLASTN + 2.2.31, NCBI) based on Altschul *et al*. (1997).

Table 3.1. Intergenic spacer (ITS) primers sequences for genomic litchi DNA.

Name of primer	Target	Sequence	Reference
ITS1	Small Sub-unit	TCCGTAGGTGAACCTGCGG	White *et al*., 1990
ITS4	Large Sub-unit	TCCTCCGCTTATTGATATGC	White *et al*., 1990

3.5 Statistical analysis

Statistical analysis was carried out using analytical software Genstat for Windows 18th Edition (VSN International, Hemel Hempstead, UK). Data was subjected to a factorial analysis of variance (ANOVA) at 95% confidence interval. Significant means were separated with the Fischer's Least Significant Differences. All data was presented as mean ± standard deviation (SD).

CHAPTER 4
RESULTS

4.1 Physical changes

4.1.1 Mass loss

Changes in mass of packaged minimally processed litchi fruit during storage under cold (1±0.5°C) and cool shelf life (10±0.5°C) conditions are presented in Figure 4.1. Based on statistical analysis, the interaction effects of harvest stages and pre-treatments (A*B) as well as harvest stages and storage duration (A*C) had significant ($P<0.05$) impact on mass loss of the fruit under cold storage (Appendix 4.1). Percentage mass loss increasedcontinuously across all the treatments, however, H2 arils had least mass loss percentage over time when compared to H1 arils. Significantly ($P<0.05$) lower (1.32%) mass loss percentage was observed in H2 fruit treated with 1% citric acid only (H2T1) on storage day 3, the highest (7.65%) was observed in H1 control arils (H1C) throughout storage (Fig 4.1A).

In contrast, under shelf life study, changes in litchi aril mass was significantly ($P=0.0091$) affected by storage duration (Appendix 4.2). An increase in fruit mass was observed with storageprogression, however, H1 fruits treated with 1% calcium lactate (H1T2) presented highest (9.8%) loss in mass on storage day 6+2 of shelf life study, while the lowest (2.8%) was observed in H2 control (H2C) samples on storage day 3+2 (Fig. 4.1B).

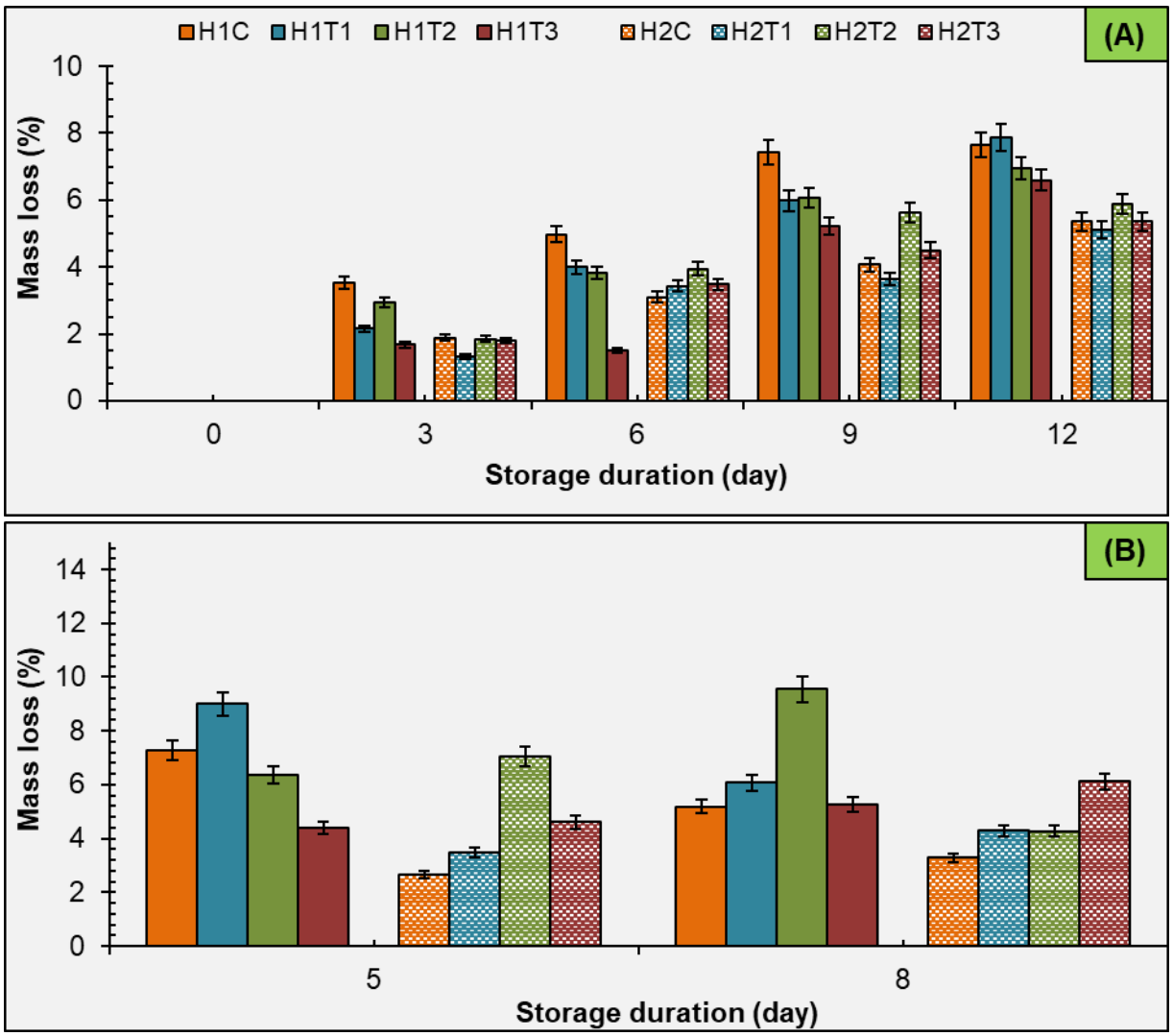

Figure 4.1. Changes in mass loss (%) of litchi cv. 'Mauritius' arils during storage at 1±0.5°C for 12 days (A) and 10±0.5°C for 2 days after cold storage (day 3+2 and day 6+2) (B). Harvest stages [early (H1) and late (H2)], postharvest pre-treatments [citric acid (T1), calcium lactate (T2), citric acid + calcium lactate (T3) and non-treated as control].

4.1.2 Juice leakage

The effects of harvest stages, postharvest pre-treatments and storage time on juice leakage of minimally processed litchi fruit stored at 1±0.5°C for 12 days and at 10±0.5°C for 2 days is presented (Fig 4.2). All experimental factors; harvest stages (A), postharvest pre-treatments (B) and storage time (C) each had significant ($P<0.05$) impacton juice leakage of minimally processed litchi fruit stored at 1±0.5°C (Appendix 4.3). Similarly, the interaction effect of harvest stages and pre-treatments (A*B), as

well as harvest stages and storage duration (A*C) had significant (P<0.05) impact on litchi fruit juice leakage under cold storage (Appendix 4.3). Higher juice leakage was observed in early harvest (H1) compared to fruit harvested late (H2) in the season however, highest (10.6 ml per 120 g of fruit) juice leakage was observed in H1 control samples (H1C) on storage day 9, whereas H2 fruit treated with 1% citric acid only (H2T1) had minimal (1.8 ml per 120 g of fruit) leakage on storage day 3 (Fig. 4.2A).

At shelf life storage, interaction of harvest stages and pre-treatments (A*B) significantly (P=0.0016) influenced aril juice leakage (Appendix 4.4). The highest (7.7 ml per 120 g of fruit) juice leakage was observed in H1 fruit treated with citric acid only on storage day 6+2 of shelf life, the lowest (4.2 ml per 120 g of fruit) observed in H2 control (H2C) samples on storage day 3+2 of shelf life (Fig 4.2B).

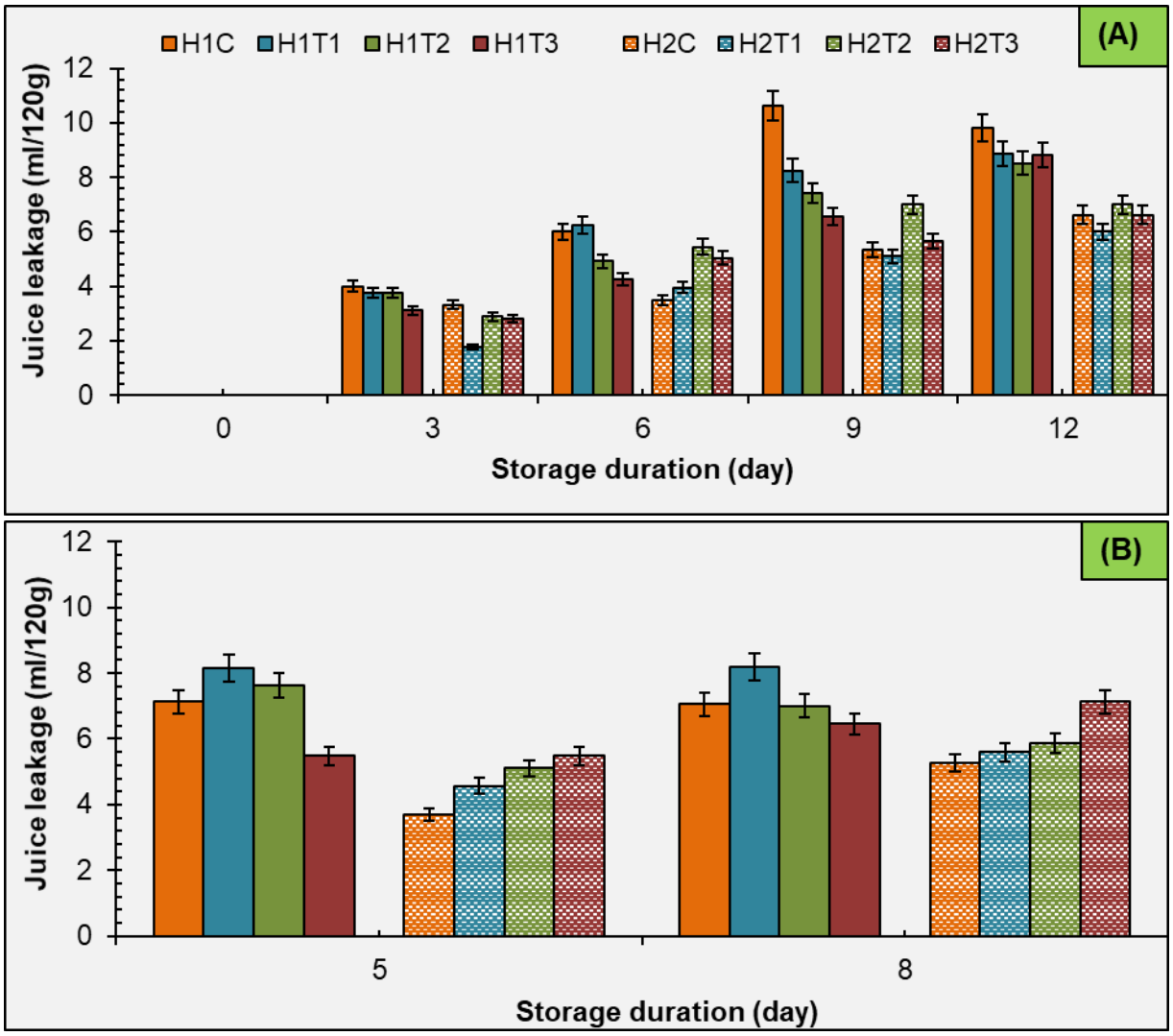

Figure 4.2. Changes in juice leakage of litchi cv. 'Mauritius' arils during storage at

1±0.5°C for 12 days (A) and 10±0.5°C for 2 days after cold storage (day 3+2 and day 6+2) (B). Harvest stages [early (H1) and late (H2)], postharvest pre-treatments [citric acid (T1), calcium lactate (T2), citric acid + calcium lactate (T3) and non-treated as control].

4.1.3 Textural profile

Tissue strength of litchi arils decreased continuously across all treatments as storage progressed under cold storage (Fig. 4.3A). Interaction of harvest stages and storage duration (A*C) had a significant (P=0.0117) impact on changes in textural profile of the fruit (Appendix 4.5). Although the interaction of harvest stage and treatments (A*B) had no significant (P=0.7663) impact on texture, H1 arils treated with a combination of 1% citric acid + 1% calcium lactate (H1T3) achieved highest (56 N) texture strength at the end of cold storage, whereas the lowest (17.8 N) was observed on storage day 9 in H1 conrol arils (Fig 4.3A). Throughout storage, litchi aril harvested early had higher tissue strength compared to late harvested arils.

Under the shelf life condition, all factors and their interaction had no significant (P>0.05) impact on the tissue strength of minimally processed litchi aril (Appendix 4.6)

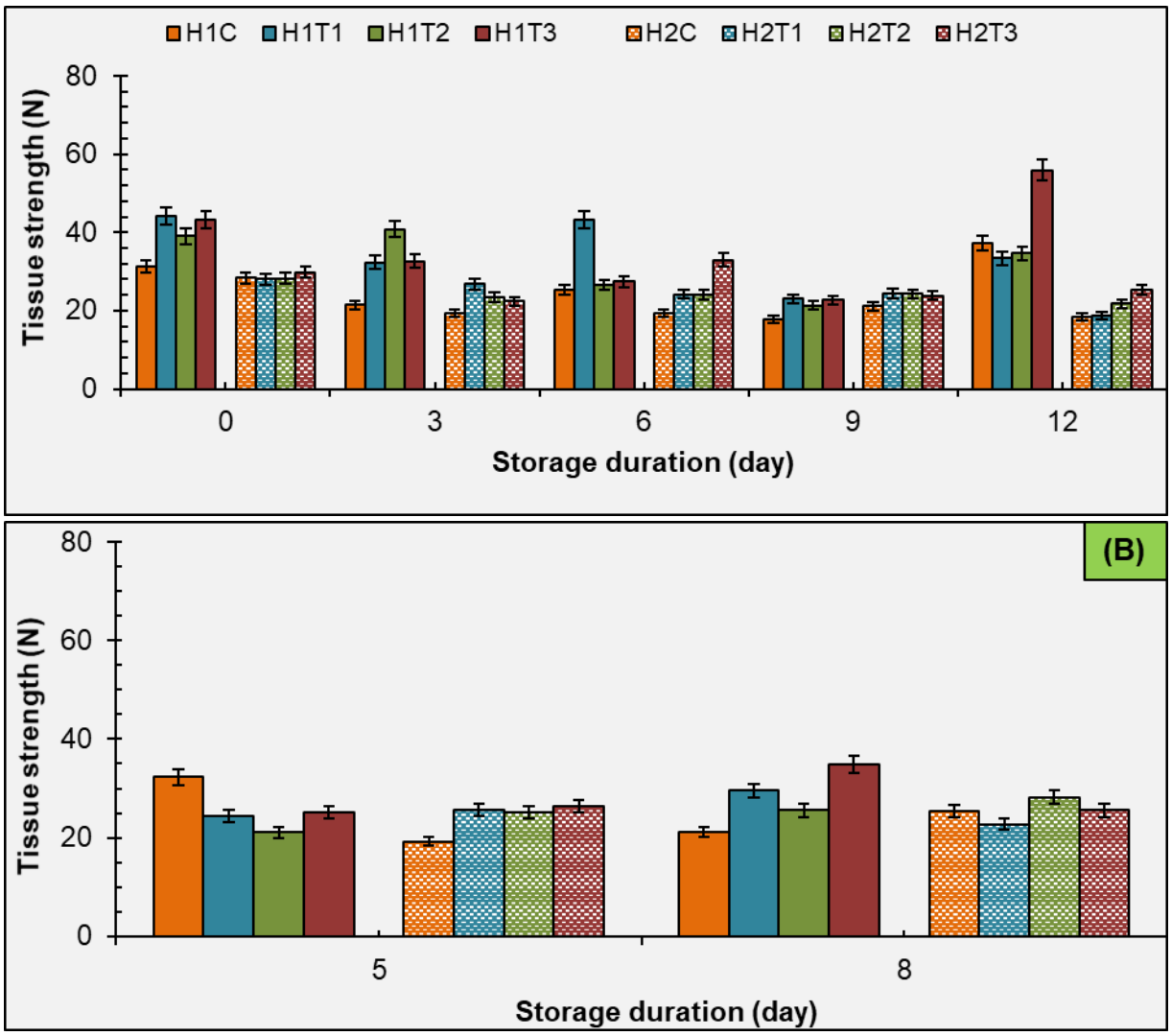

Figure 4.3. Changes in aril tissue strength of litchi cv. 'Mauritius' arils during storage at 1±0.5°C for 12 days(A) and 10±0.5°C for 2 days after cold storage (day 3+2 and day 6+2) (B). Harvest stages [early (H1) and late (H2)], postharvest pre-treatments [citric acid (T1), calcium lactate (T2), citric acid + calcium lactate (T3) and non-treated as control].

4.1.4 Colour parameters

The interaction effect of pre-treatments and storage duration (B*C) as well as harvest stage and pre-treatments (A*B), had significant ($P<0.05$) impact on browning index (BI) of minimally processed litchi arils stored at 1±0.5°C (Appendix 4.7). Notably, H2 litchi fruit presented average lower BI as compared to H1 fruit. Early harvest arils treated with 1% citric acid in combination with 1% calcium lactate (H1T3) achieved the

lowest (-534.5) browning index at the end of storage, the highest (-299.0) was observed in H2 arils treated with 1% calcium lactate (H2T2) (Table 4.1).

At shelf life study, BI of litchi arils was significantly (P=0.0279) affected by harvest stages, and the interaction of harvest stages and storage duration also affected BI significantly (P=0.0393) as presented in Appendix 4.8. Highest (-412.4) BI was observed on storage day 3+2 of shelf life study in H1 arils treated with 1% calcium lactate only (H1T2), the lowest (-534.1) was observed on storage day 6+2 in H2 arils treated with calcium lactate only (H2T2).

Total colour difference (ΔE) of minimally processed litchi fruit stored at 1±0.5°C is presented in Table 4.1. Interaction effect of pre-treatments and storage duration (B*C) had significant (P=0.0145) effect on colour change of minimally processed litchi arils during storage (Appendix 4.9). The highest (8.0) change in colour was observed in H2 arils treated with calcium lactate (1%) at the end of cold storage. Late harvest arils treated with a combination of citric acid (1%) + calcium lactate (1%) (H2T3) achieved the lowest (3.5) colour change at the end of storage.

Undershelf life study, interaction of harvest stages and postharvest treatments (A*B) significantly (P=0.0069) influenced the change in colour of litchi arils during storage (Appendix 4.10). Similarly, the interaction of harvest stages and storage duration (A*C) had significant (P=0.0392) impact on total colour change of litchi arils during storage (Appendix 4.10). The lowest (3.1) change in total colour was observed on storage day 3+2 in H1 arils treated with a combination of calcium lactate (1%) + citric acid (1%).

Table 4.1a. Changes in browning index (BI) and total colour difference (ΔE) of litchi cv. 'Mauritius' arils stored at 1±0.5°C.

Day	Harvest stage	Treatments	Browning index (BI)	Colour difference (ΔE)
0	1	C	-467.9^{b-e}	0.0^{j}
		T1	-415.1^{b}	0.0^{j}
		T2	-477.1^{b-e}	0.0^{j}
		T3	-476.0^{b-e}	0.0^{j}
	2	C	-563.7^{fg}	0.0^{j}
		T1	-476.7^{b-e}	0.0^{j}

Table 4.1b. Changes in browning index (BI) and total colour difference (ΔE) of litchi cv. 'Mauritius' arils stored at 1±0.5°C **(continue)**.

Day	Harvest stage	Treatments	Browning index	Colour difference
0	2	T2	-522.4^{c-g}	0.0^{j}
		T3	-506.5^{c-g}	0.0^{j}
3	1	C	-503.6^{c-g}	3.3^{f-i}
		T1	-462.5^{b-e}	2.4^{ih}
		T2	-478.5^{b-e}	4.1^{d-i}
		T3	-458.2^{b-d}	4.5^{c-h}
	2	C	-568.8^{g}	3.6^{f-i}
		T1	-508.0^{c-g}	3.7^{f-i}
		T2	-541.6^{e-g}	2.0^{ij}
		T3	-503.5^{c-f}	2.6^{g-i}
6	1	C	-489.1^{bc}	7.0^{ab}
		T1	-526.6^{c-g}	2.7^{g-i}
		T2	-422.2^{b}	3.6^{f-i}
		T3	-519.5^{c-g}	3.0^{f-i}
	2	C	-518.1^{c-g}	3.0^{f-i}
		T1	-502.9^{c-g}	3.8^{e-i}
		T2	-525.4^{c-g}	2.8^{f-i}
		T3	-507.4^{c-g}	2.7^{g-i}
9	1	C	-527.1^{c-g}	7.0^{ab}
		T1	-507.9^{c-g}	6.6^{abc}
		T2	-447.4^{bc}	4.9^{b-g}
		T3	-512.9^{c-g}	3.8^{e-i}
	2	C	-508.1^{c-g}	4.0^{d-i}
		T1	-503.5^{c-g}	2.7^{g-i}
		T2	-530.7^{d-g}	3.9^{e-i}
		T3	-525.3^{c-g}	3.4^{f-i}
12	1	C	-492.3^{b-g}	4.6^{b-h}
		T1	-448.5^{bc}	5.1^{b-f}
		T2	-414.4^{b}	6.4^{a-d}
		T3	-534.5^{d-g}	6.1^{a-e}
	2	C	-500^{c-d}	7.4^{a-d}
		T1	-502.8^{c-g}	4.7^{b-h}
		T2	-299.0^{a}	8.0^{a}
		T3	-485.2^{b-f}	3.5^{f-i}

Means (n = 8) within the same column followed by a different letter are significantly different at $p < 0.05$. Harvest stages [early (H1) and late (H2)], postharvest pre-treatments [citric acid (T1), calcium lactate (T2), citric acid + calcium lactate (T3) and non-treated as control].

Table 4.2. Changes in browning index (BI) and total colour difference (ΔE) of litchi cv. 'Mauritius' arils stored at 10±0.5°C.

Day	Harvest stage	Treatments	Browning index	Colour difference
5	1	C	-475.3^{a-c}	5.3^{bc}
		T1	-502.8^{bc}	4.8^{c}
		T2	**-412.4^{a}**	3.2^{c}
		T3	-460.0^{ab}	**3.1^{c}**
	2	C	-514.5^{bc}	4.8^{c}
		T1	-522.9^{bc}	2.6^{c}
		T2	-506.6^{bc}	3.2^{c}
		T3	-508.1^{bc}	4.3^{c}
8	1	C	-527.1^{bc}	7.7^{ab}
		T1	-531.1^{c}	**8.1^{a}**
		T2	-477.5^{a-c}	4.7^{c}
		T3	-501.0^{bc}	4.3^{c}
	2	C	-531.9^{c}	3.9^{c}
		T1	-499.3^{bc}	3.2^{c}
		T2	**-534.1^{c}**	3.4^{c}
		T3	-478.0^{a-c}	4.9^{bc}

Means (n = 8) within the same column followed by a different letter are significantly different at $p < 0.05$. Harvest stages [early (H1) and late (H2)], postharvest pre-treatments [citric acid (T1), calcium lactate (T2), citric acid + calcium lactate (T3) and non-treated as control].

4.2 Chemical changes

4.2.1 Total soluble solids (TSS**)**

The TSS of packaged minimally processed litchi stored at 1±0.5°C remained relatively constant, with statistically significant differences over storage time. The interaction effect of harvest stages and storage duration (A*C) had significant (P=0.0067) effect on measured TSS (Appendix 4.11). Similarly, harvest stages (A) and pre-treatment (B) separately had significant (P=0.0001) impact on measured TSS (Appendix 4.11).

Late harvest arils (H2) maintained slightly high TSS content compared to H1 samples, however, H2 arils treated with 1% citric acid (H2T1) maintaining the highest (18.9ºBrix) TSS on storage day 0 of cold storage (Fig 4.4A). The lowest (16ºBrix) TSS was observed in H1 arils treated with 1% calcium lactate + 1% citric acid (H1T3) on storage day 0 (Fig 4.4A).

Under shelf life observation, the interaction effect of harveststages and pre-treatments (A*B) had a significant (P=0.0087) impact on TSS (Appendix 4.12). Slight TSS variation was observed in all treaments during shelf life but remained between 15.8ºBrix to 18.9ºBrix (Fig 4.4B). The highest (19.0ºBrix) TSS was observed in H1 arils treated with citric acid only (H1T1) on storage day 6+2 of shelf life, H1 control arils (H1C) had the lowest (16.0ºBrix) measured TSS on the same storage day (6+2) (Fig 4.4B).

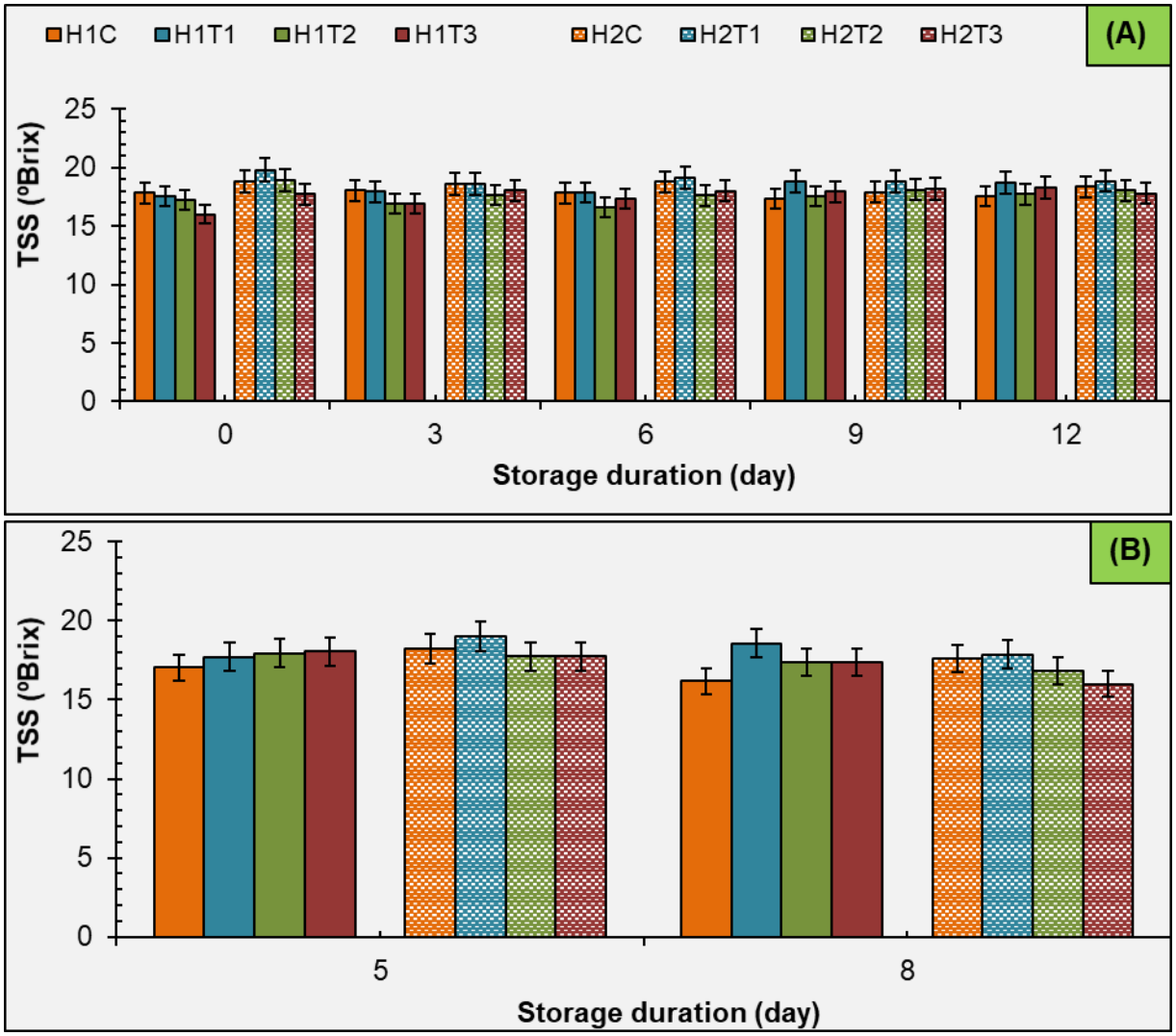

Figure 4.4. Changes in total soluble solids of litchi cv. 'Mauritius' arils during storage at 1±0.5°C for 12 days (A) and 10±0.5°C for 2 days after cold storage (day 3+2 and day

6+2) (B). Harvest stages [early (H1) and late (H2)], postharvest pre-treatments [citric acid (T1), calcium lactate (T2), citric acid + calcium lactate (T3) and non-treated as control].

4.2.2 Titratable acidity (TA)

The TA of packaged minimally processed litchi arils under cold storage was significantly (P=0.0001) influenced by the interaction of harvest stages and pre-treatments (A*B), as well as harvest stages and storage duration (A*C) (Appendix 4.13). Similarly, the interaction of harvest stages, pre-treatments and storage duration (A*B*C) had significant (P=0.0457) impact on measured TA (Appendix 4.13). Early harvest litchi fruit (H1) had significantly lower TA compared to the late harvest (H2). However, TA content of H1 litchi arils increased with an increase in storage duration until day 9, and thereafter declined slightly on day 12. On the other hand, TA of litchi arils from H2 batch declined as storage progressed until day 9, and then increased marginally on the last day (Fig.4.5A). Late harvest litchi arils treated with citric acid (1%) (H2T1) presented the highest (0.64% citric acid) measured TA at day 0, followed by early harvest arils (0.62% citric acid) treated with 1% calcium lactate (H1T2) at day 12 (Fig 4.5A).

During the shelf life observation all experimental factors and their interactions had significant (P<0.05) impact on TA of litchi arils, except for the interaction of harvest stages, pre-treatments and storage duration (A*B*C), as well as pre-treatments and storage duration (B*C), which were not significant (P>0.05) (Appendix 4.14). The highest (0.46% citric acid) TA was observed on storage day 3+2 of shelf life study, in H2 arils treated with a combination of 1% calcium lactate and 1% citric acid (H2T3), on storage 3+2 of shelf life, H1 arils treated with a combination of 1% calcium lactate and 1% citric acid (H1T3) had the lowest (0.03% citric acid) TA (Fig 4.5B).

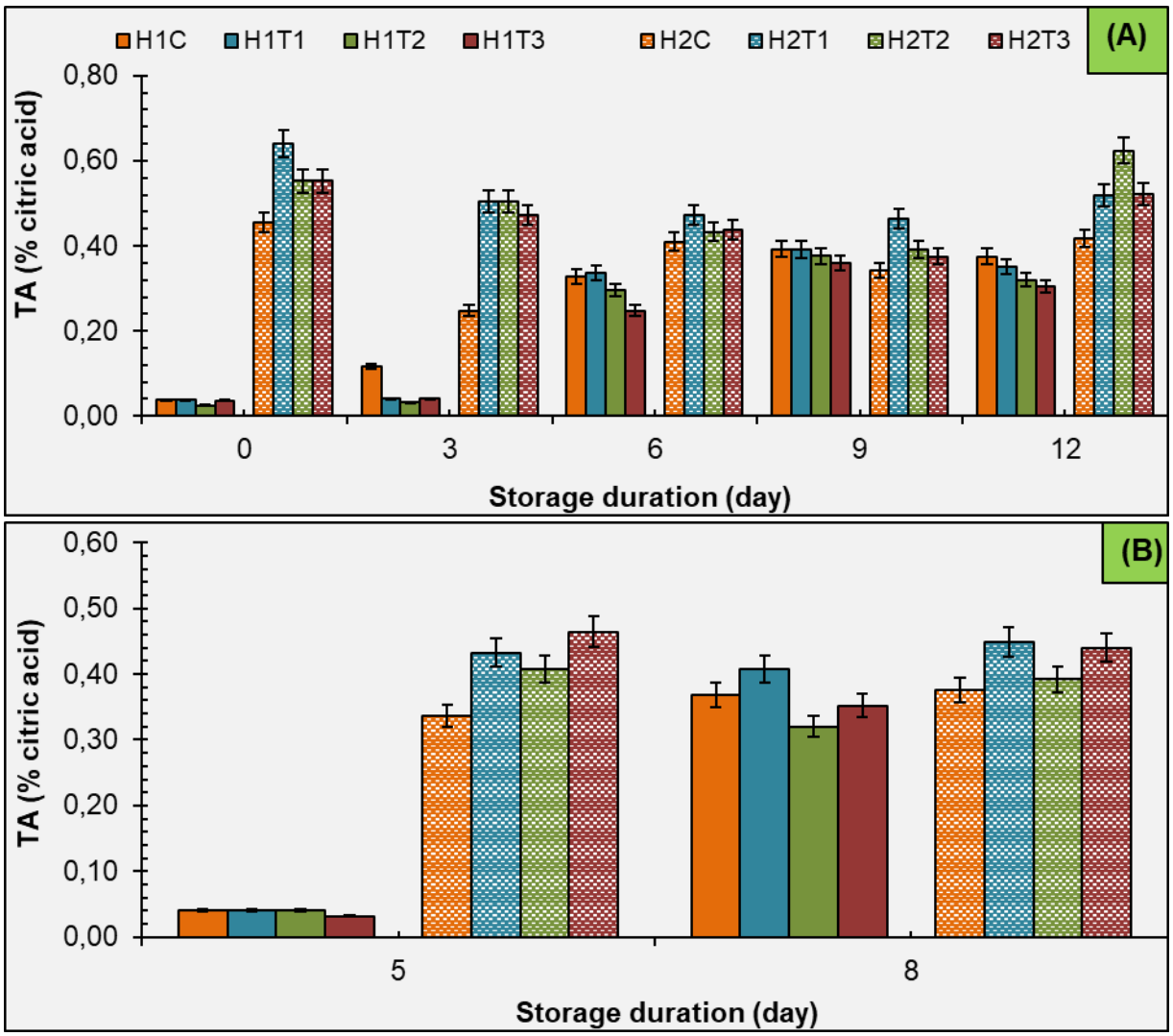

Figure 4.5. Changes in titratable acidity of litchi cv. 'Mauritius' arils during storage at 1±0.5°C for 12 days (A) and 10±0.5°C for 2 days after cold storage (day 3+2 and day 6+2) (B). Harvest stages [early (H1) and late (H2)], postharvest pre-treatments [citric acid (T1), calcium lactate (T2), citric acid + calcium lactate (T3) and non-treated as control].

4.2.3 pH

Slight variation in pH was observed for all treatments but remained within 4 – 5 range during storage at 1±0.5°C (Fig. 4.6A). The treatment factors as well as their interaction had significant ($P<0.05$) impact on aril pH (Appendix 4.15). Throughout storage, fruit pH was marginallyhigher in early harvest arils (H1) than in those harvested late (H2). However, the highest (4.98) pH was recorded at day 6 in early harvested arils treated with citric acid (1%) + calcium lactate (1%) (H1T3), whereas lower (4.18) pH was observed in H2 litchi arils treated with 1% citric acid (H2T1) at day 9 (Fig 4.6A).

At shelf life, the pH of litchi arils was significantly (P<0.05) affected by the individual treatment factors and their interaction, except for the interaction of harvest stages and pre-treatments (A*B), as well as harvest stages and storage duration (A*C), which did not have significant (P>0.05) impact on measured pH (Appendix 4.16). Litchi arils harvested early and treated with a combination of 1% calcium lactate and 1% citric acid (H1T3) presented the highest (5.0) pH value on storage day 3+2 of shelf life whereas H2 arils treated with a combination of 1% calcium lactate and 1% citric acid presented the lowest (4.2) pH value on storage day 6+2 of shelf life (Fig 4.6B).

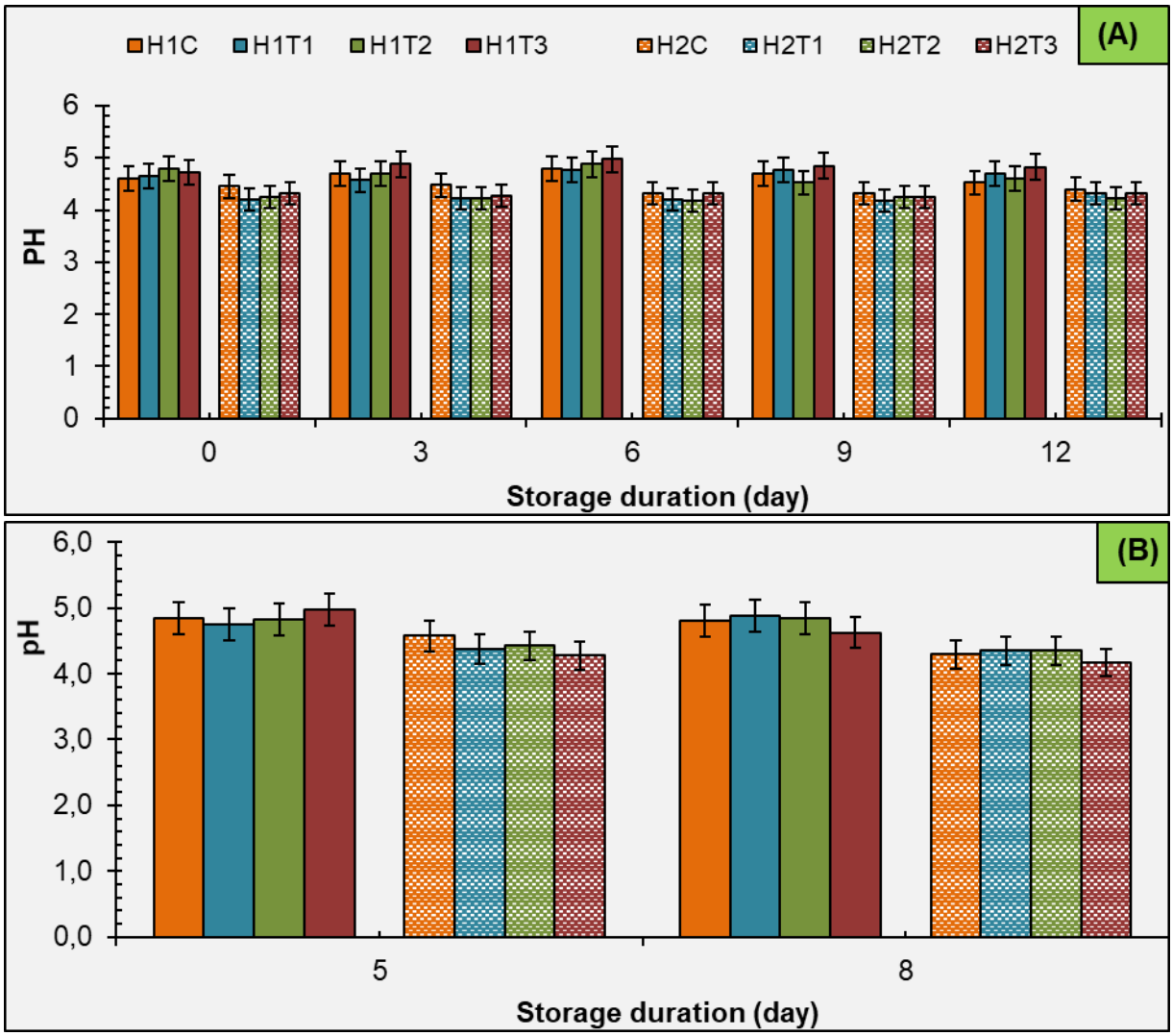

Figure 4.6. Changes in pH of litchi cv. 'Mauritius' arils during storage at 1±0.5°C for 12 days (A) and 10±0.5°C for 2 days after cold storage (day 3+2 and day 6+2) (B). Harvest stages [early (H1) and late (H2)], postharvest pre-treatments [citric acid (T1), calcium lactate (T2), citric acid + calcium lactate (T3) and non-treated as control].

4.2.4 Ascorbic acid (AA)

Results obtained showed that the treatment factors and their interaction significantly ($P<0.05$) influenced fruit ascorbic acid content (Appendix 4.17). However, harvest stage independently had no significant ($P>0.05$) impact on AA content (Appendix 4.17). Ascorbic acid content decreased continually during storage across all the treatments (Fig. 4.7A). Throughout the storage period, litchi fruit harvested early (H1) presented significantly higher ascorbic acid content compared those harvested late (H2). At day 12 of cold storage, H2 arils treated with a combination of 1% citric acid and 1% calcium lactate (H2T3) maintained higher (72.9 µg/mL) ascorbic acid content, whereas the lowest (40.0 µg/mL) ascorbic acid content was observed on storage day 6 in H1 arils treated with 1% calcium lactate (H1T2) (Fig 4.7A).

During shelf life investigation, treatments interactions had significant ($P<0.05$) effect on ascorbic acid content ofpacked litchi arils (Appendix 4.18). Ascorbic acid content in control samples declined from 72.67 µg/mL to 34.67 µg/mL in early harvest litchi compared to late harvest (75.16µg/mL to 68.33 µg/mL) at the end of shelf life study. However, treated samples with citric acid only better maintained ascorbic acid content (89.83) (Fig 4.7B). On day 6+2 of shelf life, the highest (89.93 µg/mL) ascoric acid content was observed in H2 arils treated with citric acid only, whereas the lowest (34.67 µg/mL) was observed on day 6+2 in H1 control arils (Fig 4.7B).

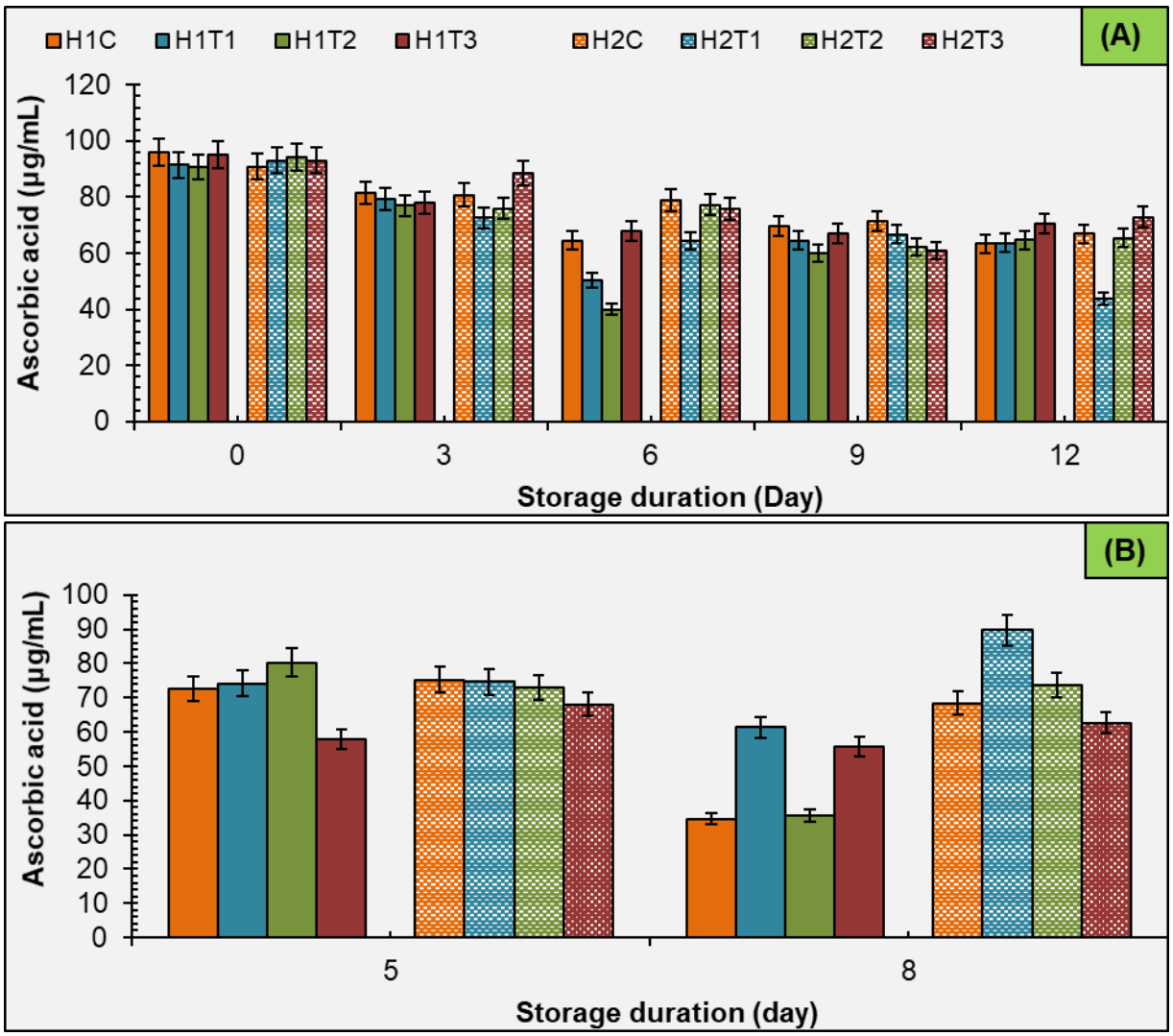

Figure 4.7. Changes in ascorbic acid content in litchi cv. 'Mauritius' arils during storage at 1±0.5°C for 12 days (A) and 10±0.5°C for 2 days after cold storage (day 3+2 and day 6+2) (B). Harvest stages [early (H1) and late (H2)], postharvest pre-treatments [citric acid (T1), calcium lactate (T2), citric acid + calcium lactate (T3) and non-treated as control].

4.2.5 Radical scavenging activity (RSA)

The results on RSA showed significant ($P<0.05$) individual and interaction effect of harvest stages, postharvest treatments and storage duration (Appendix 4.19). A continuous and significant ($P<0.05$) reduction in RSA was observed across all treatments and control samples (Appendix 4.19). However, H1 fruit samples maintained relatively higher RSA until day 3 during cold storage, whereas for the rest of the storage period RSA was slightly higher H2 fruit samples, irrespective of storage day and the treatment used. Greater (36.6 mmol AAE/mL) average RSA was observed

in H1 arils treated with the a combination of calcium lactate (1%) + citric acid (1%) (H1T3) on cold storage day 12, whereas H2 control samples presented the lowest (25.8 mmol AAE/mL) RSA on cold storage day 6.

For samples transferred to shelf life condition, RSA ranged from 25.89 to 49.36 mmol AAE/mL (Fig. 4.8B). Results obtained under shelf life showed significant ($P<0.05$) interaction effects of harvest stage and storage duration (A*C), harvest stages and pre-treatments (A*B), as well as pre-treatments and storage duration (B*C) on the RSA of the litchi arils stored at 10±0.5°C (Appendix 4.20). Fruit RSA increased during shelf life investigation from day 3+2 to 6+2, with highest (50.67 mmol AAE/mL) RSA in H1 arils treated with calcium lactate only on storage day 6+2 of shelf life. However, the lowest (25.83 mmol AAE/mL) RSA was observed in H1 arils treated with citric acid only on day 3+2 of shelf life (Fig. 4.8B).

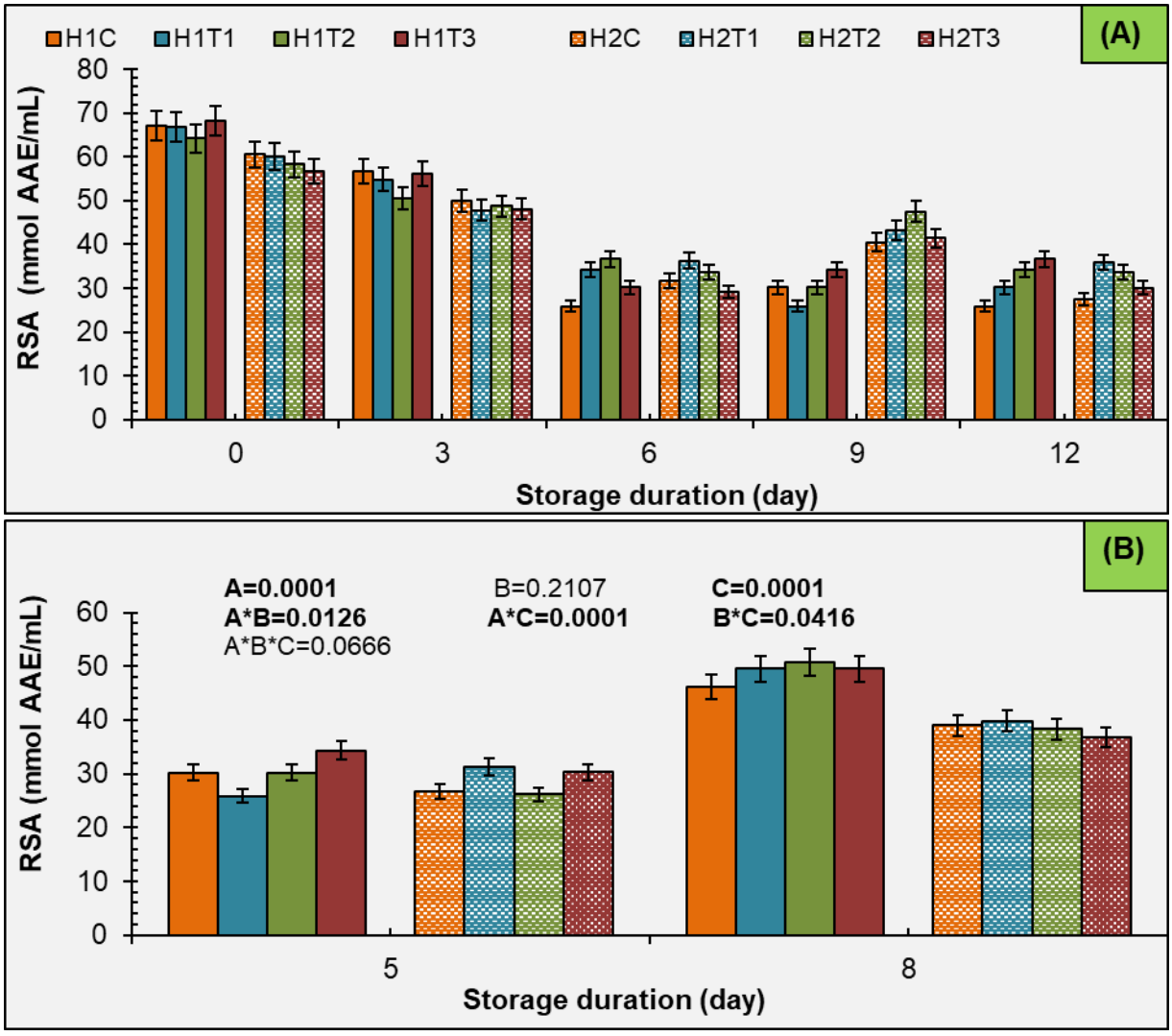

Figure 4.7. Changes in radical scavenging activity in litchi cv. 'Mauritius' arils during

storage at 1±0.5°C for 12 days (A) and 10±0.5°C for 2 days after cold storage (day 3+2 and day 6+2) (B). Harvest stages [early (H1) and late (H2)], postharvest pre-treatments [citric acid (T1), calcium lactate (T2), citric acid + calcium lactate (T3) and non-treated as control].

4.3 Microbial contamination

The intergenic spacer (ITS) sequencing reported a total of 28 fungal species across all treatments during cold storage (Table 4.3). In general, it was observed that there was a shift in the fungal profile as a function of harvest stages, pre-treatments applied and during cold storage. Furthermore, based on individual fungi isolate, a total of 25 and 14 fungal species were found in the early and late harvested minimally processed litchi fruit, respectively (Fig. 4.9). Prior to cold storage, in the early harvested litchi fruit, *Colletotrichum gloeosporroides* was found to be the most dominant, followed by *Phomopsis sophorae*, *C. boninense* and *Alternaria alternata*. However, *Diaporthe sp*. were found to be the most dominant fungi (9 species) during cold storage (Fig. 4.9A). In addition, change in colour to pink was observed in the control samples on storage day 9 in the late harvest samples, which may be an indicator of yeast growth. Consistent with the observed colour change on day 9, fermenting yeast, *Hanseniaspora sp*. was most abundant isolate (Fig. 4.9B). It is noteworthy that *Alternaria spp*. were most prevalent in the early harvest than the late ones. Litchi arils treated with citric acid (1%) + calcium lactate (1%) had the least (9 culturable fungal microflora isolated) microbial infections when compared to the other treatments. Overall, number of microbial fungi isolated were higher in the early harvest batch of litchi fruit in comparison to the late harvest sample (Fig 4.9B).

Table 4.3. Major culturable fungi found on treated and non-treated (control) minimally processed litchi during 12 days storage at 1±0.5°C using ITS sequencing.

Major microbiota	Identity (% accuracy)	Accession No.	Request ID	Reference
Alternaria alternate	100	MF422133.1	8VS4XPKC014	Rosenzweig *et al*., 2017
Alternaria angustiovoidea	100	MK910060.1	SFNKMZR8016	Disayathanoowat, 2019
Alternaria gaisen	100	MK684064.1	8VS4XPKC014	Ma and Wu, 2019
Alternaria infectoria	98	KM516086.1	SFPWTMKV013	Djisalov *et al*., 2015
Alternaria porri	100	MK905450.1	8VS4XPKC014	Hariprasad *et al*., 2019
Candida oleophila	98	KY102255.1	SFRACE9M01R	Vu *et al*., 2016
Chaetomium globosum	95	MZ727031.1	SFRN4V76016	Shirazi, 2021
Cladosporium tenuissimum	94	MF473304.1	8VMGDZSN016	Bensch *et al*., 2018
Cladosporium cladosporioides	98	KM265457.1	8VMGDZSN016	Alfonzo *et al*., 2014
Colletotrichum boninense	100	KJ619456.1	8VPZ7PAP014	Zhang, 2014
Colletotrichum gloeosporroides	100	MT300326.1	SFY75X9N016	Cara *et al*., 2020
Coriolopsis polyzona	94	FJ904854.1	SFYDM9V5013	Lapmak, 2009
Diaporthe sp	95	KY962983.1	8VSGVP0501R	Poitevin and Duin, 2017
Filobasidium magnum	98	MH197140.1	8VSD1CW6014	Sipiczki,M. and Selim, 2018
Hanseniaspora uvarum	95	MN378470.1	6F2U8BPM016	Bueno, 2019
Nigrospora Oryzae	98	KT966519.1	8VN2EERG016	Monclova-Santana *et al*., 2015
Nigrospora sphaerica	100	LC514689.1	8VMVHVMB016	Firmansyah and Trianto, 2019
Nigrospora lacticolonia	100	MT043787.1	8VMVHVMB016	Mohd Zaini *et al*., 2020
Sarocladium implicatum.	100	MT102934.1	8VMN18XY016	Lyu and Wang, 2020
Sarocladium terricola	100	MG980071.1	8VMN18XY016	Ghule *et al*., 2018
Trametes polyzona	97	KC589124.1	8W0BDJM1014	Douanla-Meli and Langer, 2013

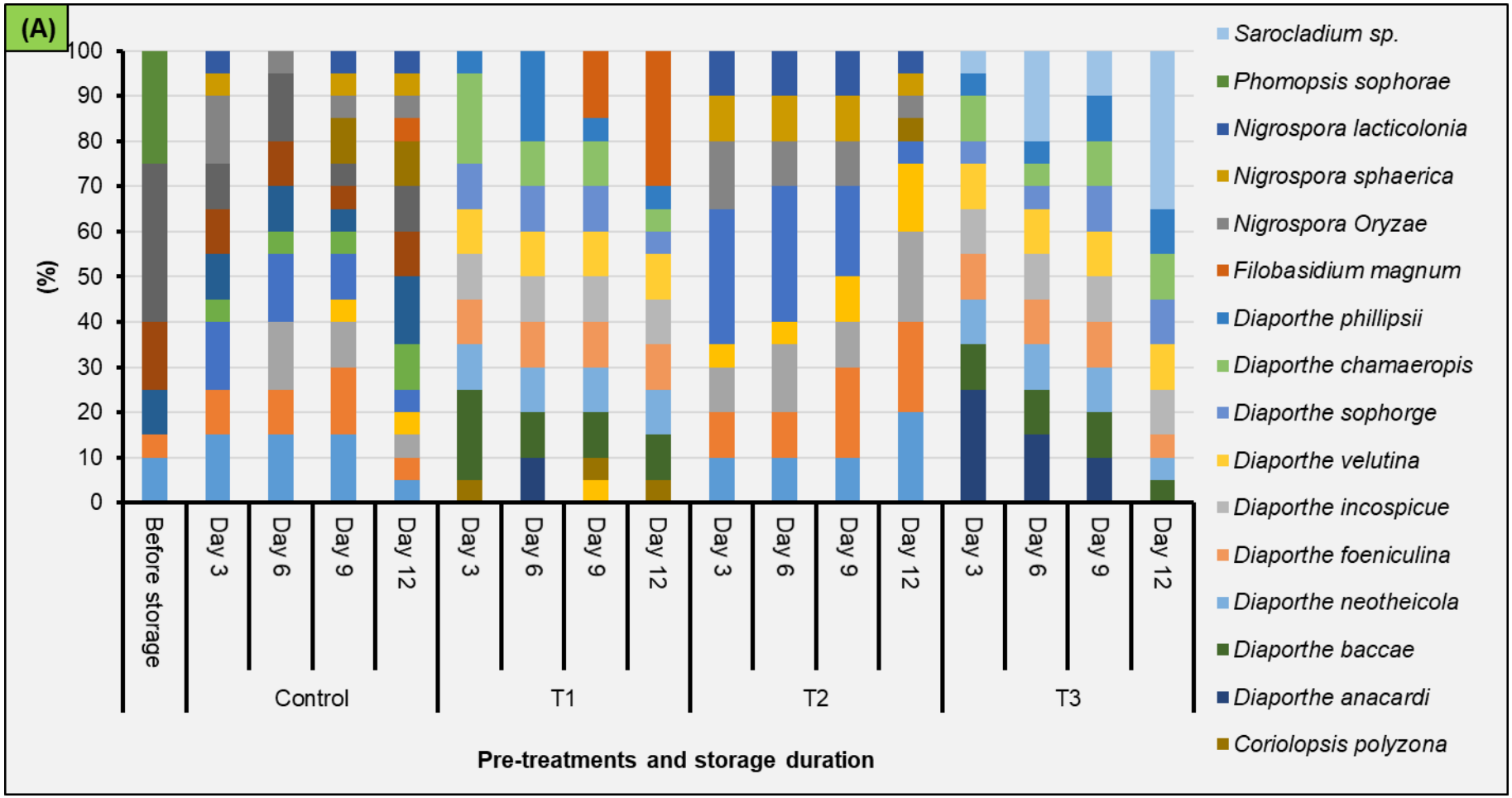

Figure 4.9A. Culturable fungal microflora isolated from minimally processed litchi, early harvest before storage and from treated and non-treated (control) during storage at 1±0.5°C for 12 days. Postharvest treatments (citric acid (T1), calcium lactate (T2) and citric acid + calcium lactate (T3) and non-treated as control.

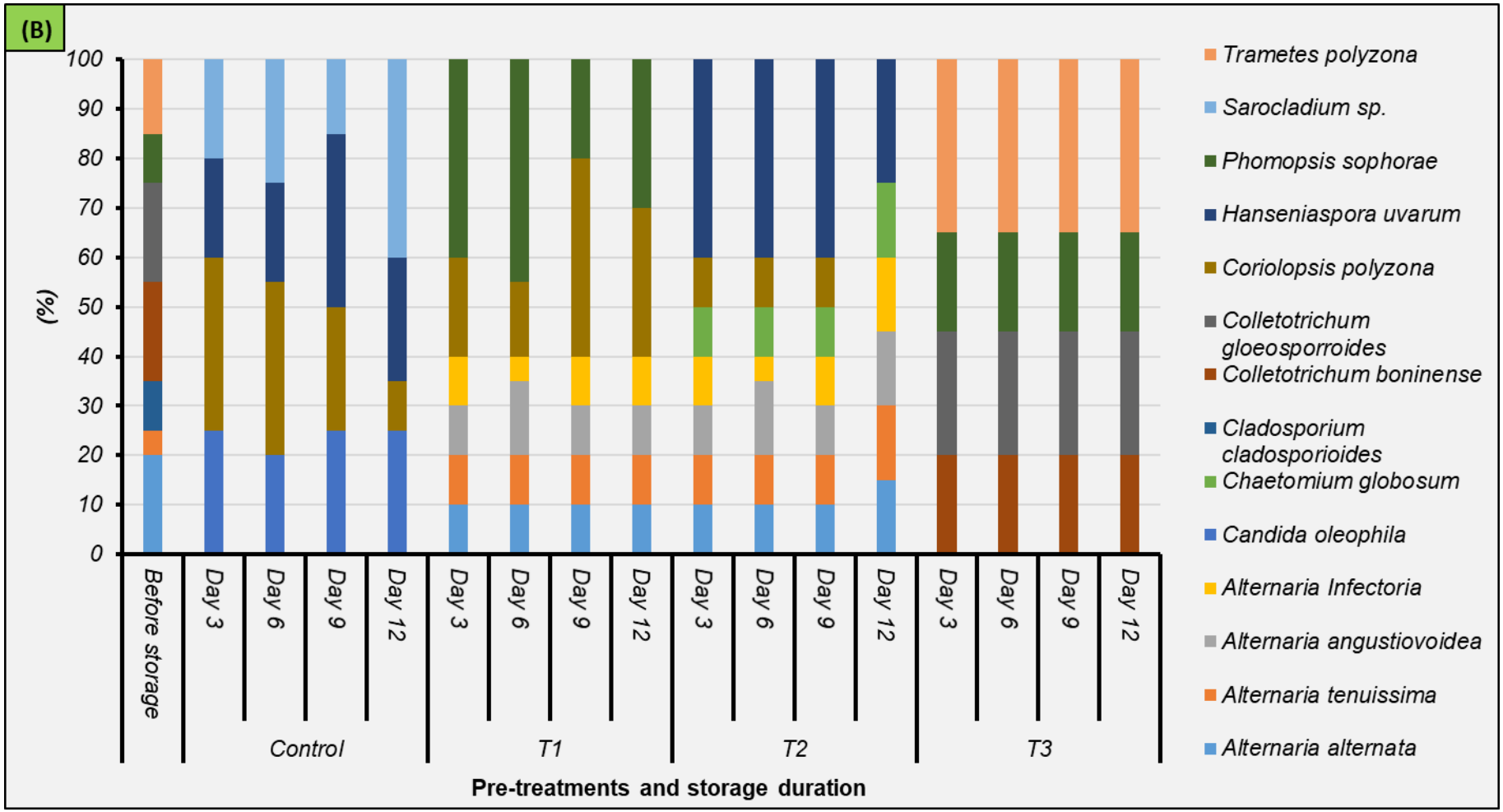

Figure 4.9B. Culturable fungal microflora isolated from minimally processed litchi, late harvest before storage and from treated and non-treated (control) during storage at 1±0.5°C for 12 days. Postharvest treatments (citric acid (T1), calcium lactate (T2) and citric acid + calcium lactate (T3) and non-treated as control.

CHAPTER 5
DISCUSSION

5.1 Physical attributes

5.1.1 Mass loss

Fresh product mass loss is an important parameter since it results in economic losses (Zhong, 2008). The increase in mass loss in minimally processed litchi fruit during cold storage and shelf life observed in this study corresponded to the findings by Phanumong *et al*. (2015) in minimally processed litchi cvs. 'Honghuay', 'Gimseng' and 'Jugkapat' treated with peroxyacetic acid solution, packaged in polystyrene clamshell and stored at 4°C for 12 days. Similarly, Inam-ur-Raheem *et al*. (2013) also indicated that during storage the mass loss of fresh cut guava sliced increased in un-treated and treated (treated with calcium chloride and calcium lactate with concentration 0.9%, 1.8%, 2.7% or 3.6%) different varieties stored at 5°C ± 2°C. The increase in mass loss during cold storage and shelf life was due to the absence of a protective pericarp, which, by increased respiration, makes the delicate fruit more predisposed to dehydration (Dong *et al*., 2004; Phanumong *et al*., 2015). In this study, treatment with citric acid was observed to be more effective in minimizing mass loss in H2 litchi arils. Similar observation was reported by Shahkoomahally and Ramezanian (2014) that citric acid conferred a physical barrier to moisture loss and therefore retarding dehydration and fruit shrivelling. Likewise, Eman *et al*. (2015) in fresh-cut guava and Latifah *et al*. (2010) in fresh-cut pineapple observed that lower mass loss was steadily shown with the fruit treated with citric acid ranging from 0.4 to 1.4% during cold storage.

5.1.2 Juice leakage

Juice leakage is an important indicator for deterioration of minimally processed produce in response to experimental treatment (Shah and Nath, 2008). Increase in juice leakage of minimally processed litchi fruit during cold storage and under shelf life, corresponded with the results by Shah and Nath (2008), Kaushik *et al*. (2014) and Phanumong *et al*. (2015). Kaushik *et al*. (2014) indicated that the structural changes that occur duringprocessing result in the rupturing of the fruit tissue causing

the leaching of the internal fluids.Thus, damage to the fruit tissue as a result of peeling increased juice leakage throughthe vacuole during storage (Phanumong *et al*., 2015). Furthermore, Shah and Nath (2008) indicated that the increased juice leakage is as result of loss of cellular sap as a result of biochemical alterations. Lower juice leakage observed under cold storage and shelf life study, in fruit harvested late and treated with citric acid only can be attributed to the presence of citric acid, which has been found to be effective in conferring a physical barrier to moisture loss, hence reducing aril juice leakage during storage (Eman *et al*., 2015).

5.1.3 Texture profile

Generally, tissue strength of minimally processed fruits is very tightly linked to tissue deterioration and can be used as an index of freshness and quality decline (Cantwell and Soslow, 1999; Toivonen and Brummel, 2008). The decline in aril tissue strength is consistent with observation reported by Ngamchuachit *et al*. (2014). The loss of textural integrity could be attributed to mechanical membrane damage during minimal processing, water loss and cellular degradation due to decay and biochemical changes (Hussein *et al*., 2015). In this study, the presence of citric acid and calcium lactate in the solution was found to be beneficial in reducing loss of tissue strength. This observation is corroborated by other reports that tissue strength can be increased by the addition of calcium salts (Inam-ur-Raheem *et al*., 2013), citric acid (Eman *et al*., 2015), and/or combination of calcium lactate and citric acid (Aslam *et al*., 2018). Therefore, combination of citric acid and calcium lactate can be recommended for maintaining tissue strength of minimally processed litchi fruit during storage.

5.1.4 Colour parameters

In most fruits, fruit colour is one of the key characteristics that distinguish their freshness (Rico *et al*., 2007). One of the main causes of quality loss in minimally processed goods is colour variations on the exposed surface (Garcia and Barrett, 2002). In this study, treatment with citric acid + calcium lactate proved to be effective in preventing browning of the H1 fruit on the last day of cold storage. Similar results have been reported by Krishna *et al*. (2018) in minimally processed papaya cubes treated with 2% of $CaCl_2$ +5% of citric acid stored at 5°C for 12 days. Reduction in degree of

browning in fruits can be attributed to the reduced activity of polyphenol oxidase activity and oxygen concentration, which might be due to the effect of chemicals used in pre-treatments (Techavuthiporn and Boonyaritthonghai, 2016).

According to Patras *et al*. (2011), totalcolour change (ΔE) specifies the degree of colour degradation during storage. The findings in this study suggested that an increase in total colour change during cold storage can be attributed to development of pink discolouration found in litchi fruit (Kaushik *et al*., 2014). According to Chandler and Clegg (1970) pink discolouration is due to the hydrolysis of condensed tannin to catechin and lencoanthocyanin concentration, which was found to be more prevalent in canned pear fruit. On the contrary, there was a decline in colour change for fruit packaged under non-perforated clamshell and stored at 1±0.5°C and 95% relative humidity for 15 days (Mphahlele *et al*., 2020).

5.2 Chemical attributes

5.2.1 Total Soluble Solids

According to Maness and Perkins-Veazie (2003), after harvest, soluble carbohydrates are utilized in major respiration pathways to provide energy to fruit tissues. Thus, the relatively high TSS content observed in cold storage (H1) and shelf life (H2), in litchi fruit treated with citric acid only can beattributed to low metabolic process. The slightly higher TSS recorded in litchi fruit harvested late can be attributed to maturation progression as the fruit ripened, since the TA decreases when TSS increases (Holcroft and Mitcham, 1996). However, contradictory, Shah and Nath (2008) observed a decrease in TSS during storage and further explained that it may be due to utilization of sugars by growth of microbes.

5.2.1 Titratable Acidity

Acidity is a very important quality indicator for litchi, and its reduction is a crucial indicator for good taste change and shelf life (Zhong, 2008). It also indicates the content of soluble sugars in a fruit which explains consumer perception on the sweetness of the fruit. According to Anthon andBarrette (2012), increase in TA could be due to the presence of pectin methylesteraseenzyme activity. On the other hand, the reduction in

TA of early harvest fruit prior ripening stage could be due to high metabolic demand and reduction in organic acids (Tsegay *et al*., 2013). In the present study, high TA levels were observed in fruit harvested late and treated with citric acid only under cold storage as well as in arils harvested late and treated with citric acid + calcium lactate under shelf life study. Similar results have been reported by Ediriweera *et al*. (2012) in minimally processed pineapples dipped in pre-treatment solutions (citric acid 1%) which showed high TA levels stored under 5-7°C, indicating the low usage of organic acids and slow senescence process. In contrast to our results, Mphahlele *et al*. (2020) reported a significant decline in TA in packaged litchi arils cv. 'Mauritius' treated with sodium hypochlorite and stored for 15 days at 1°C. This study further highlights the potential of citric acid pre-treatment to maintain TA of litchi arils.

5.2.2 pH

According to Schmidl and Labuza (2000), pH is dependent on both total quantity and strength of acids present in fruits, and variation of these over time may be the reason for this change with time. The changes in pH values during storage may be associated with growth of microorganisms and subsequent production of organic acids (Heard, 2002). Treatment of the litchi fruit with citric acid lowered pH levels only on storage day 9, when compared with those treated with calcium lactate or a combination of both. Similar results have been reported by Waghmare and Annapure, (2013) in fresh-cut papaya dipped in a solution of citric acid (2% w/v) and stored at 5°C for 25 days, in which the addition of citric acid during the dip pretreatment lowered the pH of treated samples against the non-treated samples. Citric acid is effective in reducing superficial pH of cut fruits since it is a reducingagent (Antoniollo *et al*., 2012).

5.2.3 Ascorbic acid

Litchi fruit is a good source of ascorbic acid (vitamin C) with an average of 27.6mg/100g (Sivakumar *et al*., 2010). The concentration of ascorbic acid in both harvest stages followed a decreasing trend irrespective of the chemical pre-treatments applied under cold storage. Similarly, Kaushik *et al*. (2014) and Mphahlele *et al*. (2020) reported significant decline in ascorbic acid (vitamin C) in minimally processed litchi 'Bombai' stored at 5°C for 12 days and 'Mauritius' stored at 1°C for 15 days,

respectively. According to Mahmood *et al*. (2017), the reduction in ascorbic acid content during storage may be associated with the oxidation mediated by enzymes. Additionally, tissue damage during processing and juice leakage may have contributed to the decline in ascorbic acid content in the litchi arils (Shah and Nath, 2008). In the present study, combination of calcium lactate with citric acid best maintained the highest ascorbic acid content.

5.2.4 Radical scavenging activity (RSA)

Overall, radical scavenging acticity (RSA) of the fruits gradually decreased with advancement in storage period. However, H1T3 arils had higher RSA as compared to H2 arils at the beginning of storage. In constrast, opposite was true during later stages of storage. Similar results on continuous decline of RSA have been reported by Mphahlele *et al*. (2020) and Duan *et al*. (2011). Mphahlele *et al*. (2020) showed that RSA declined progressively in minimally processed litchi fruit packed in perforated clamshell trays and stored at 1°C for 15 days. According to Duan *et al*. (2011), there is a linear relationship between antioxidant content and RSA. Moreover, Lana and Tijskens, (2006) reported that minimal processing played a crucial role in the decline if antioxidant activity in fresh-cut tomatoes harvested at three different stages of maturity and stored at 5°C. Reduction in RSA during storage could be linkedto the decrease in ascorbic acid concentration. Hence, the reduction in RSA of minimally processed litchi arils can be attributed to processing and the decline in ascorbic acid.

5.3 Microbial contamination

In general, this study observed that there was a shift in the fungal profile as a function of harvest stages, pre-treatments applied and cold storage duration. In the early harvested litchi fruit before storage *C. gloeosporroides* was found to be the most dominant, followed by *P. sophorae*, *C. boninense* and *A. alternata*. However, *Diaporthe sp*. were found to be the most dominant fungi (9 species) during cold storage. Previous study have reported the following fungi on the surface of litchi fruit in, *A. alternata*, *C. gloeosporioides*, *Dothiorella* sp., *F. oxysporum*, *L. theobromae*, *P. expansum*, *P. guepinii*, *Phoma spp*., *Phomopsis sp*., *R. stolonifer* and *T. harzianum*

(De Jager *et al*., 2003). The observed differences could be attributed to the removal of the litchi pericarp in this study prior to the isolation of the fungi.

Among the treatments, minimum growth in fruit treated with citric acid + calcium lactate was observed. Similarly, Krishna *et al*. (2018) reported least fruit spoilage in minimally processed papaya treated with 2% $CaCl_2$ + 5% citric acid and stored at 5°C for 12 days. The chemical pre-treatments rinse off enzymes and substrates released by disrupted cells, thus reducing microbial spoilage, excessive tissue softening and tissue browning. Furthermore, Silveria *et al*. (2011) indicated that application of calcium on fresh-cut fruit also reduces microbial growth, due to calcium increasing the rigidity of the cell wall and resistance to fungal enzymes; while Ramos *et al*. (2013) observed that citric acid is one of the organic acids that play a significant role in the reduction of pH that impacted microbial growth. Therefore, treatment with 1% calcium lactate in combination with 1% citric acid has a potential to be used for preserving the quality of minimally-processed litchi fruit.

CHAPTER 6

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary and Conclusion

The pH, ascorbic acid content and radical scavenging activity of litchi fruit were significantly affected by the interaction of the three treatment factors during storage. The results of this study demonstrated that the texture strength, radical scavenging activity and ascorbic acid content of minimally processed litchi fruit significantly decreased while mass loss and juice leakage increased during 12 days storage at 1±0.5°C irrespective of treatments applied. The total soluble solids content and pH of packaged minimally processed litchi fruit remained relatively constant, with statistically significant differences over storage time. Arils of fruit harvested late presented better mass loss, juice leakage, browning index, total colour change, total soluble solids, pH and microbial contamination. Arils of fruit harvested late in the season and treated with 1% citric acid had least mass loss percentage, juice leakage and increased TSS. Treatment with 1% calcium lactate in combination with 1% citric acid was significantly better in reducing browning and microbial infections and diversity. Under shelf life study, H2 control samples presented lower mass loss and juice leakage. At the end of shelf life storage, litchi arils harvested early and treated with 1% citric acid combined with 1% calcium lactate presented lower total change in colour. Litchi arils can be stored at 10±0.5°C for up to 8 days, however the microbial quality need to be further investigated. In conclusion, harvesting the fruit late and treating with citric acid alone or combined with calcium lactate showed the potential of maintaining better aril quality with least microbial population for up to 12 days under 1±0.5°C storage. Moreover, harvesteing the fruit early and treating with citric acid alone or combined with calcium lactate showed the potential of maintaining better aril quality under shelf life storage.

6.2 Recommendation

It can be recommended that harvesting the fruit at a later stage and treatment with 1% citric acid alone or in combination with 1% calcium lactate has the potential to preserving the quality of minimally-processed litchi fruit for up to 12 days at 1±0.5°C.

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Appendix 4.1. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on mass loss of minimally processed litchi stored for 12 days at 1±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	38.51406	38.5140	26.41	<.0001
Pre-treatments	3	15.49318	5.16439	3.54	0.0168
Harvest stages x Pre-treatments	3	27.28268	9.09422	6.24	0.0006
Storage duration	4	815.6210	203.905	139.81	<.0001
Harvest stages x Storage duration	4	25.01375	6.25343	4.29	0.0028
Pre-treatments x Storage duration	12	11.08900	0.92408	0.63	0.8100
Harvest stages x Pre-treatments x Storage duration	12	15.67325	1.30610	0.90	0.5535
Model	39	948.6869	24.3253	16.68	<.0001
Error	120	175.0075	1.42839		
Corrected Total	159	1123.6944			

Appendix 4.2. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on mass loss of minimally processed litchi stored for 2 days at 10±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	76.125625	76.125625	7.38	0.0091
Pre-treatments	3	43.588750	14.529583	1.41	0.2518
Harvest stages x Pre-treatments	3	42.925625	14.308541	1.39	0.2581
Storage duration	1	0.1406250	0.1406250	0.01	0.9075
Harvest stages x Storage duration	1	0.3025000	0.3025000	0.03	0.8647
Pre-treatments x Storage duration	3	11.970625	3.9902083	0.39	0.7630
Harvest stages x Pre-treatments x Storage duration	3	57.858750	19.286250	1.87	0.1473
Model	15	232.91250	15.527500	1.51	0.1412
Error	48	495.12500	10.315104		
Corrected Total	63	728.03750			

Appendix 4.3. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on juice leakage of minimally processed litchi stored for 12 days at 1±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	87.02500	87.0250	83.77	<.0001
Pre-treatments	3	10.09050	3.36350	3.24	0.0247
Harvest stages x Pre-treatments	3	41.71450	13.9048	13.38	<.0001
Storage duration	4	1253.172	313.293	301.56	<.0001
Harvest stages x Storage duration	4	35.45187	8.86296	8.53	<.0001
Pre-treatments x Storage duration	12	13.26137	1.10511	1.06	0.3967
Harvest stages x Pre-treatments x Storage duration	12	20.83362	1.73613	1.67	0.0816
Model	39	1461.549	37.4756	36.07	<.0001
Error	120	124.6700	1.03891		
Corrected Total	159	1586.219			

Appendix 4.4. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on juice leakage of minimally processed litchi stored for 2 days at 10±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	50.587656	50.587656	33.25	<.0001
Pre-treatments	3	5.8942187	1.9647395	1.29	0.2881
Harvest stages x Pre-treatments	3	27.022968	9.0076562	5.92	0.0016
Storage duration	1	6.6951562	6.6951562	4.40	0.0412
Harvest stages x Storage duration	1	5.2326562	5.2326562	3.44	0.0698
Pre-treatments x Storage duration	3	3.1429687	1.0476562	0.69	0.5634
Harvest stages x Pre-treatments x Storage duration	3	0.4329687	0.1443229	0.09	0.9625
Model	15	99.008593	6.6005729	4.34	<.0001
Error	48	73.027500	1.5214063		
Corrected Total	63	172.03609			

Appendix 4.5. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on tissue strength of minimally processed litchi stored for 12 days at 1±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	4272.328	4272.32	19.34	<.0001
Pre-treatments	3	1912.395	637.465	2.89	0.0368
Harvest stages x Pre-treatments	3	252.9525	84.3175	0.38	0.7663
Storage duration	4	3610.947	902.736	4.09	0.0033
Harvest stages x Storage duration	4	2931.236	732.809	3.32	0.0117
Pre-treatments x Storage duration	12	2009.686	167.473	0.76	0.6929
Harvest stages x Pre-treatments x Storage duration	12	1952.669	162.722	0.74	0.7145
Model	39	16942.21	434.415	1.97	0.0014
Error	200	44178.07	220.890		
Corrected Total	239	61120.28			

Appendix 4.6. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on tissue strength of minimally processed litchi stored for 2 days at 10±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	92.63010	92.63010	0.56	0.4554
Pre-treatments	3	168.8728	56.29093	0.34	0.7951
Harvest stages x Pre-treatments	3	240.8978	80.29927	0.49	0.6918
Storage duration	1	69.53010	69.53010	0.42	0.5176
Harvest stages x Storage duration	1	2.975104	2.975104	0.02	0.8934
Pre-treatments x Storage duration	3	183.0778	61.02593	0.37	0.7744
Harvest stages x Pre-treatments x Storage duration	3	716.6511	238.8837	1.45	0.2342
Model	15	1474.634	98.30899	0.60	0.8689
Error	80	13170.86	164.6357		
Corrected Total	95	14645.49			

Appendix 4.7. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on browning index of minimally processed litchi stored for 12 days at 1±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	87225.63	87225.6	21.44	<.0001
Pre-treatments	3	64166.50	21388.8	5.26	0.0015
Harvest stages x Pre-treatments	3	33715.67	11238.5	2.76	0.0424
Storage duration	4	40119.78	10029.9	2.47	0.0453
Harvest stages x Storage duration	4	31163.72	7790.93	1.92	0.1080
Pre-treatments x Storage duration	12	110798.2	9233.18	2.27	0.0093
Harvest stages x Pre-treatments x Storage duration	12	63622.98	5301.91	1.30	0.2159
Model	39	430812.5	11046.47	2.72	<.0001
Error	280	1139095.4	4068.198		
Corrected Total	319	1569907.9			

Appendix 4.8. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on browning indexof minimally processed litchi stored for 2 days at 10±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	21702.256	21702.256	4.97	0.0279
Pre-treatments	3	26104.321	8701.4404	1.99	0.1194
Harvest stages x Pre-treatments	3	29191.971	9730.6571	2.23	0.0890
Storage duration	1	15750.906	15750.906	3.60	0.0602
Harvest stages x Storage duration	1	19000.314	19000.314	4.35	0.0393
Pre-treatments x Storage duration	3	11286.848	3762.2827	0.86	0.4638
Harvest stages x Pre-treatments x Storage duration	3	1671.8377	557.27924	0.13	0.9436
Model	15	124708.45	8313.8971	1.90	0.0300
Error	112	489543.95	4370.9281		
Corrected Total	127	614252.40			

Appendix 4.9. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on total colour change of minimally processed litchi stored for 12 days at 1±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	64.08200	64.0820	10.68	0.0012
Pre-treatments	3	16.08062	5.36020	0.89	0.4449
Harvest stages x Pre-treatments	3	12.18175	4.06058	0.68	0.5668
Storage duration	4	1085.554	271.388	45.24	<.0001
Harvest stages x Storage duration	4	45.48768	11.3719	1.90	0.1113
Pre-treatments x Storage duration	12	154.4209	12.8684	2.15	0.0145
Harvest stages x Pre-treatments x Storage duration	12	115.3398	9.61165	1.60	0.0904
Model	39	1493.147	38.2858	6.38	<.0001
Error	280	1679.637	5.66870		
Corrected Total	319	3172.784			

Appendix 4.10. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on total colour change of minimally processed litchi stored for 2 days at 10±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	60.50000	60.50000	8.44	0.0044
Pre-treatments	3	58.52812	19.50937	2.72	0.0478
Harvest stages x Pre-treatments	3	91.44937	30.48312	4.25	0.0069
Storage duration	1	39.60500	39.60500	5.52	0.0205
Harvest stages x Storage duration	1	31.20500	31.20500	4.35	0.0392
Pre-treatments x Storage duration	3	7.396875	2.465625	0.34	0.7936
Harvest stages x Pre-treatments x Storage duration	3	9.594375	3.198125	0.45	0.7206
Model	15	298.2787	19.88525	2.77	0.0011
Error	112	803.0000	7.169643		
Corrected Total	127	1101.278			

Appendix 4.11. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on total soluble solids of minimally processed litchi stored for 12 days at 1±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	26.00156	26.0015	35.58	<.0001
Pre-treatments	3	26.00118	8.66706	11.86	<.0001
Harvest stages x Pre-treatments	3	0.305187	0.10172	0.14	0.9364
Storage duration	4	2.183375	0.54584	0.75	0.5619
Harvest stages x Storage duration	4	10.91187	2.72796	3.73	0.0067
Pre-treatments x Storage duration	12	15.63412	1.30284	1.78	0.0584
Harvest stages x Pre-treatments x Storage duration	12	3.977625	0.33146	0.45	0.9374
Model	39	85.01493	2.17987	2.98	<.0001
Error	12	87.68750	0.73072		
Corrected Total	159	172.7024			

Appendix 4.12. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on total soluble solids of minimally processed litchi stored for 2 days at 10±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	0.180625	0.180625	0.23	0.6357
Pre-treatments	3	11.33062	3.776875	4.75	0.0056
Harvest stages x Pre-treatments	3	10.36562	3.455208	4.35	0.0087
Storage duration	1	8.122500	8.122500	10.22	0.0025
Harvest stages x Storage duration	1	2.560000	2.560000	3.22	0.0790
Pre-treatments x Storage duration	3	2.273750	0.757916	0.95	0.4223
Harvest stages x Pre-treatments x Storage duration	3	2.866250	0.955416	1.20	0.3191
Model	15	37.69937	2.513291	3.16	0.0012
Error	48	38.15000	0.794791		
Corrected Total	63	75.84937			

Appendix 4.13. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on titratable acidityof minimally processed litchi stored for 12 days at 1±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	2. 56365	2.56365	1150.04	<.0001
Pre-treatments	3	0.089317	0.02977	13.36	<.0001
Harvest stages x Pre-treatments	3	0.118678	0.03955	17.75	<.0001
Storage duration	4	0.714641	0.17866	80.15	<.0001
Harvest stages x Storage duration	4	1.378099	0.34452	154.55	<.0001
Pre-treatments x Storage duration	12	0.048401	0.00403	1.81	0.0539
Harvest stages x Pre-treatments x Storage duration	12	0.049833	0.00415	1.86	0.0457
Model	39	4.962621	0.12724	57.08	<.0001
Error	120	0.267502	0.00222		
Corrected Total	159	5.230123			

Appendix 4.14. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on titratable acidity of minimally processed litchi stored for 2 days at 10±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	0.724626	0.724626	333.56	<.0001
Pre-treatments	3	0.028517	0.009505	4.38	0.0084
Harvest stages x Pre-treatments	3	0.022742	0.007580	3.49	0.0226
Storage duration	1	0.430664	0.430664	198.24	<.0001
Harvest stages x Storage duration	1	0.420876	0.420876	193.74	<.0001
Pre-treatments x Storage duration	3	0.010179	0.003393	1.56	0.2108
Harvest stages x Pre-treatments x Storage duration	3	0.003317	0.001105	0.51	0.6780
Model	15	1.640923	0.109394	50.36	<.0001
Error	48	0.104275	0.002172		
Corrected Total	63	1.745198			

Appendix 4.15. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on pH of minimallyprocessed litchi stored for 12 days at 1±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	8.055062	8.05506	1014.81	<.0001
Pre-treatments	3	0.373687	0.12456	15.69	<.0001
Harvest stages x Pre-treatments	3	0.335187	0.11172	14.08	<.0001
Storage duration	4	0.109000	0.02725	3.43	0.0108
Harvest stages x Storage duration	4	0.302750	0.07568	9.54	<.0001
Pre-treatments x Storage duration	12	0.231000	0.01925	2.43	0.0074
Harvest stages x Pre-treatments x Storage duration	12	0.283250	0.02360	2.97	0.0012
Model	39	9.689937	0.24845	31.30	<.0001
Error	120	0.952500	0.00793		
Corrected Total	159	10.64243			

Appendix 4.16. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on pH of minimally processed litchi stored for 2 days at 10±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	3.468906	3.468906	298.67	<.0001
Pre-treatments	3	0.130468	0.043489	3.74	0.0170
Harvest stages x Pre-treatments	3	0.074218	0.024739	2.13	0.1087
Storage duration	1	0.131406	0.131406	11.31	0.0015
Harvest stages x Storage duration	1	0.012656	0.012656	1.09	0.3018
Pre-treatments x Storage duration	3	0.189218	0.063072	5.43	0.0027
Harvest stages x Pre-treatments x Storage duration	3	0.132968	0.044322	3.82	0.0157
Model	15	4.139843	0.275989	23.76	<.0001
Error	48	0.557500	0.011614		
Corrected Total	63	4.697343			

Appendix 4.17. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on ascorbic acidcontent of minimally processed litchi stored for 12 days at 1±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	367.7850	367.785	3.33	0.0697
Pre-treatments	3	3256.214	1085.40	9.82	<.0001
Harvest stages x Pre-treatments	3	858.5814	286.193	2.59	0.0541
Storage duration	4	30209.23	7552.30	68.31	<.0001
Harvest stages x Storage duration	4	2801.015	700.253	6.33	<.0001
Pre-treatments x Storage duration	12	3105.211	258.767	2.34	0.0079
Harvest stages x Pre-treatments x Storage duration	12	2976.245	248.020	2.24	0.0112
Model	39	43574.28	1117.28	10.11	<.0001
Error	200	2213.308	110.566		
Corrected Total	239	65687.59			

Appendix 4.18. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on ascorbic acid content of minimally processed litchi stored for 2 days at 10±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	4802.5104	4802.510417	43.36	<.0001
Pre-treatments	3	2796.6145	932.204861	8.42	<.0001
Harvest stages x Pre-treatments	3	289.11458	96.371528	0.87	0.4602
Storage duration	1	3325.2604	3325.260417	30.02	<.0001
Harvest stages x Storage duration	1	3863.3437	3863.343750	34.88	<.0001
Pre-treatments x Storage duration	3	2708.3645	902.788194	8.15	<.0001
Harvest stages x Pre-treatments x Storage duration	3	1890.1145	630.038194	5.69	0.0014
Model	15	19675.322	1311.68819	11.84	<.0001
Error	80	8860.8333	110.76042		
Corrected Total	95	28536.156			

Appendix 4.19. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on radical scavenging activity of minimally processed litchi stored for 12 days at 1±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	0.40017	0.40017	0.03	0.8708
Pre-treatments	3	176.182	58.7274	3.89	0.0099
Harvest stages x Pre-treatments	3	355.059	118.353	7.84	<.0001
Storage duration	4	35822.8	8955.70	593.52	<.0001
Harvest stages x Storage duration	4	3189.43	797.359	52.84	<.0001
Pre-treatments x Storage duration	12	960.278	80.0232	5.30	<.0001
Harvest stages x Pre-treatments x Storage duration	12	386.327	32.1939	2.13	0.0164
Model	39	40890.4	1048.47	69.49	<.0001
Error	200	3017.82	15.08912		
Corrected Total	239	43908.3			

Appendix 4.20. Analysis of variance table for effect of harvest stages, postharvest treatments and storage duration on radical scavenging activity of minimally processed litchi stored for 2 days at 10±0.5°C.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Harvest stages	1	858.0104	858.0104	63.63	<.0001
Pre-treatments	3	62.28125	20.76041	1.54	0.2107
Harvest stages x Pre-treatments	3	155.5312	51.84375	3.84	0.0126
Storage duration	1	4945.010	4945.010	366.69	<.0001
Harvest stages x Storage duration	1	481.5104	481.5104	35.71	<.0001
Pre-treatments x Storage duration	3	116.0312	38.67708	2.87	0.0416
Harvest stages x Pre-treatments x Storage duration	3	100.5312	33.51041	2.48	0.0666
Model	15	6718.906	447.9270	33.22	<.0001
Error	80	1078.833	13.48541		
Corrected Total	95	7797.739			