EFFECTS OF EDIBLE COATINGS AND MORINGA EXTRACTS ON POSTHARVEST QUALITY OF PAPAYA FRUITS

By

Sabeliwe Langa (211526424)

Submitted in fulfilment of the requirements for the degree

MASTERS IN AGRICULTURE

In the

Discipline of Horticultural Science

School of Agricultural, Earth and Environmental Sciences

University of KwaZulu-Natal

Pietermaritzburg

South Africa

Supervisor: Dr S.T. Tesfay

Co-Supervisor: Dr A Mditshwa

June 2018

DECLARATION

I, Sabeliwe Langa, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

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Student: Sabeliwe Langa Supervisor: Dr Samson Z. Tesfay

DEDICATION

I dedicate this work to my beloved mother, Zanele Mchunu.

ACKNOWLEDGEMENTS

I would like to express my gratitude to the all mighty God for carrying me throughout this journey.

I would like to pass a special acknowledgement to the following people:

The College of Agriculture, Engineering and Science at UKZN, for financial support.

My supervisor, Dr Samson Tesfay, for his guidance, support, constant encouragement and ensuring the success of this project. Co-supervisor Dr Asanda Mditshwa for his guidance.

Dr Nokwazi Mbili for her contribution and guidance with the pathology part of the project.

Mr Kwanda Sithole, UKZN alumni and a friend, for his consistent support and encouragement.

My siblings for their support and motivation at times of giving up, especially my sister Nobuhle Langa who became a shoulder to cry on.

Miss Senamile Dlangalala and Miss Ayanda Ngema, UKZN MSc student, for their support with accommodation during the course of this project.

The Microscopy and Microanalysis Unit staff for laboratory assistant.

Mr Thokozani Nkosi, UKZN laboratory technician, for laboratory assistant and support.

The Nazareth Tertiary Student Association (NATESA) brothers and sisters for their support and always praying for me.

ABBREVIATIONS

AA: Ascorbic acid.

ABTS: 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid.

ANOVA: Analysis of variance.

CA: Controlled atmosphere.

Cel: Cellulose.

CH: Chitosan.

CHE: Chitosan incorporated with pomegranate peel extract.

CI: Chilling injury.

CMC: Carboxymethylcellulose.

CO_2: Carbon dioxide.

CS: Corn starch.

DPPH: 2,2-diphenyl-1-picrylhydrazyl.

EOs: Essential oils.

GAE: Gallic acid equivalents.

GRAS: Generally Recognized As Safe.

HPLC: High performance liquid chromatography.

HPLC-RID: High-performance liquid chromatography–refractive index detector.

LC: Liquid chromatography.

MA: Modified atmosphere.

MAP: Modified atmosphere packaging.

MAHP: Modified atmosphere humidity packaging.

1-MCP: 1-methylcyclopropene.

MLE: Moringa leaf extract.

MLEE: Moringa leaf ethanolic extract

MLWE: Moringa leaf aqueous extract

MSE: Moringa seed extract.

MSEE: Moringa seed ethanolic extract.

MSWE: Moringa seed aqueous extract.

NaOH: Sodium hydroxide.

O_2: Oxygen.

PDA: Potato dextrose agar

PE: Pectinestarage

PG: Polygalacturonase

PL: Pectate lyase

QE: Quercetin equivalents

RDA: Recommended Daily Allowance

ROS: Reactive oxygen species

SEM: Scanning electron microscope

TFC: Total flavonoid content

TSS: Total soluble solids

TTA: Total titratable acidity

UKZN: University of KwaZulu Natal

UV C: Ultraviolet C

Vis: Visible

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ABSTRACT

Carica papaya L., known as papaya is a member of the small family *Caricaceae.* It is an important fruit for both fresh and processed products. It is a good source of vitamin A, lycopene, polysaccharides and proteins. High consumption of papaya is known to contribute to the prevention of the chronic diseases such as cardiovascular disease. About 30-50% of the harvested papaya is reported to never reach the consumers due to postharvest spoilage. Postharvest spoilage can be attributed to the fact that it is perishable after harvesting. The level of spoilage depends on the management of pre-harvest (environment and cultural practices) and postharvest factors (handling, environmental conditions). The factors contribute largely to papaya quality deterioration by stimulating physiological/biochemical processes (respiration, transpiration) and microbial growth. Also, some of the factors affect papaya fruit quality at maturity stage, time of harvest and the harvest method. Various fungicides have been used to reduce postharvest spoilage. However, the negative effects on human health and the environment, accompanied with high costs, residues in plants has encouraged development of alternative approaches. The development of new natural preservatives and antimicrobials has increased as alternatives for fruit quality preservation. Edible coatings are amongst the natural methods of fruit quality preservation and protecting perishable food products from deterioration by retarding dehydration, suppressing respiration, improving textural quality, helping retain volatile flavour compounds and reducing microbial growth.

The study evaluated the effect of edible coatings on papaya fruit quality, and antifungal activity of plant extracts against fungal pathogens that affect postharvest quality of fruits. In the first section of the study, *Moringa oleifera* extracts (leaf and seed) incorporated with chitosan and CMC (MLE+CH, MSE+CH, MLE+CMC and MSE+CMC) were used as an alternative for synthetic fungicides. The quality parameters were measured to observe the effect of treatments. The quality parameters that were assessed under cold and ambient storage conditions included

pH, total titratable acidity, total soluble sugars, weight loss, firmness, peel colour, vitamin C, total flavonoids, total phenols, antioxidants and soluble sugars. Inhibitory effects of *Moringa oleifera* aqueous and ethanolic leaf and seed extracts (MLWE, MSWE, MLEE and MSEE) was evaluated *in-vitro*. Treatments applied (MLE+CH, MSE+CH, MLE+CMC and MSE+CMC) maintained papaya fruit quality compared to the control under both ambient and cold storage conditions. Treatment MSE+CMC showed better fruit quality maintenance compared to other treatments. MLWE, MSWE, MLEE and MSEE had relatively high inhibitory potential in all tested concentrations (10%, 20% and 30%) compared to the control treatment. A 100% mycelial growth inhibition in PDA agar amended with moringa extracted with ethanol was observed.

Keywords: *Carica papaya*; quality; *Moringa oleifera*; postharvest, edible coatings, fungi

CHAPTER ONE

GENERAL INTRODUCTION

1.1. Background

Papaya (*Carica papaya* L.) is a member of Caricaceae family consisting of four genera, namely Carica, Cylicomorpha, Jarilla and Jacaratia (Ali *et al.,* 2010). It is native to the lowlands of eastern Central America, Mexico and Panama (Ikram *et al.,* 2015). Papaya cultivation is across different continents, such as Brazil, India, Mexico, Nigeria, Indonesia and others, after Spanish colonization of the Americans (Bautista-Baños *et al.,* 2013). Papaya is amongst the most important fruits of tropical and subtropical regions of the world (Julianti *et al.,* 2014; Vij and Prashar, 2015). Papaya is an herbaceous tree-like plant widely cultivated for its edible fruits (Canini *et al.,* 2007). Parts of the plant are used in tropical diets as a fruit and vegetable, for fresh and processed products for both local and international markets (Ali *et al.,* 2010, Canini *et al.,* 2007). Amongst 38 common fruits, papaya was ranked fifth as a nutritionally beneficial fruit based on nutritional scores, percentage Recommended Daily Allowance (RDA) for pro-vitamin A, ascorbic acid (AA), potassium, folate and fibre (Ikram *et al.,* 2015). It is a good source of vitamin A, lycopene, polysaccharides and proteins (Waghmare and Annapure, 2013). The plant is also possesses several medicinal properties (Anuar *et al.,* 2008).

The findings of epidemiological studies have shown that high consumption of fruits and vegetables can contribute to prevention of chronic disease, such as cardiovascular disease and certain types of cancer (Sancho *et al.,* 2011; Ali *et al.,* 2013). Therefore, the dietary habits of the community are changing towards fruits and vegetables (Yahia, 2006). In the last 2 decades, the market of tropical fruits has increased due to this change in dietary habits and other factors, such as increased demand for exotic food products and the use of improved technologies for storage and transportation of fresh produce (Yahia, 2006). This includes papaya fruit, which is

a tropical fruit possessing important antioxidant properties (Ali *et al.*, 2013). The antioxidants are both of hydro-soluble and lipid-soluble types (Ali *et al.,* 2013). The high amounts of antioxidants, including vitamins C, E and A, have many health benefits ranging from reduced risks of developing cardiovascular diseases, macular degeneration to protection against cancer (Ali *et al.,* 2013). Other antioxidant compounds include polyphenols and carotenoids which are associated with reduction of oxidative stress produced by free radicals (Sancho *et al.*, 2011). Most papaya plant parts (stems, leaves, seeds, roots, and latex) are used for health benefits and medical applications (Canini *et al.,* 2007, Huerta-Ocampo *et al.,* 2012). Papaya leaves are known to contain phenolic compounds, including flavonoids, saponins, cardiac glycosides, anthraquinones, and alkaloids (Julianti *et al.*, 2014). The reported alkaloids include carpaine, pseudocarpaine and dehydrocarpaine I and II (Julianti *et al.*, 2014). Papaya is also used for the production of papain and chymopapain, which are valuable proteolytic enzymes used to tenderize meat (Huerta-Ocampo *et al.,* 2012). According to Ali *et al.* (2013) in 2010, 11.2 million metric tonnes of papaya was produced from 433.500 ha in several countries. This is despite the fact that it is a climacteric fruit which becomes very perishable after harvesting (Chien *et al.,* 2013, Li *et al.,* 2013). The papaya fruits encounter considerable postharvest problems during handling and storage due to increased perishability (Vyas *et al.,* 2014). High perishability results to more wastage and less fruits that reach to the consumers (Vyas *et al.*, 2014). Also, the papaya fruits have a short postharvest life due to factors such as weight losses, rapid pulp softening, and the presence of microbial growth (Waghmare and Annapure, 2013).

1.2. Rationale

Papaya fruit is susceptible to numerous diseases, physical disorders and faster ripening (Ali *et al.,* 2010, Gonzalez-Aguilar *et al.,* 2003; Perez-carrillo and Yahia, 2004). The diseases are mainly caused by various microorganisms, particularly fungi (Hasan *et al.*, 2012). *Colletotrichum*, *Phomopsis*, *Phytophthora*, *Rhizopus*, *Stemphylium* and *Fusarium* are amongst

the genera of fungi that are responsible for enormous fruit losses after harvest (Hasan *et al.*, 2012). Anthracnose caused by *Colletotrichum gloeosporioides* is the most important fungal disease that affects papaya fruit and has been reported extensively (Gonzalez-Aguilar *et al.*, 2003; Li *et al.*, 2013; Sivakumar *et al.*, 2002). This fungal disease leads to extensive postharvest losses during handling and storage (Gonzalez-Aguilar *et al.*, 2003). Factors such as poor keeping quality, difficulties in long distance transportation, and poor or lack of preservation storage facilities result in huge losses of the papaya fruit (Sharmin *et al.*, 2015). These factors create favourable conditions for pathogens to grow (Hamim *et al.*, 2014). The pathogens can cause a remarkable damage and may render the fruit unmarketable (Hamim *et al.*, 2014). About 30-50% of the harvested papaya has been reported not to reach the consumers due to postharvest spoilage (Sharmin *et al.*, 2015). Postharvest spoilage results in huge losses for the fruit and vegetable industry and in South Africa a 44% loss has been reported, which results in price hike (World Wide Fund (WWF), 2017; Hamim *et al.*, 2014). The losses significantly affect farmers' and traders' income and food security (Gwa and Nwankiti, 2017). Therefore, pre-storage treatments and technologies should be improved to reduce losses of papaya fruit (Padmanaban *et al.*, 2014).

Postharvest diseases are normally controlled by synthetic fungicides, such as thiabendazole, imazalil and sodium ortho-phenyl phonate (Arowora and Adetunji, 2014). However, their excessive may have negative effects on human health and the environment, accompanied by high costs, residues in plants, and development of resistance (Arowora and Adetunji, 2014; Nkya *et al.*, 2014; Sahab and Nawar, 2015; Mvumi *et al.*, 2017). Pre-storage treatments, such as temperature reduction and oxygen, modified atmosphere packaging, edible coatings and gamma-irradiation and high pressure have been used to increase the biological stability and thereby extend the shelf life of the products (Niazmand *et al.*, 2009; Sivakumar and Bautista-Banos, 2014; Padmanaban *et al.*, 2014). Bio- control agents and food preservatives such as

sodium carbonate, sodium bicarbonate, potassium sorbate, ozone exposure, heat treatments, methyl jasmonate and salicylic acid are amongst some of the important treatments (Sivakumar and Bautista-Banos, 2014). However, the choice of treatment must be selected with consideration of increased consumer demand for chemical-free, high quality food and an extended fruit shelf life (Misir *et al.,* 2014). Moreover, the selection of treatment is important as there are increasing concerns about use of chemicals in food and the environment (Yousef *et al.,* 2015). In other countries, restrictions on chemical treatments such as postharvest fungicides to avoid negative effects during human food consumption are being applied (Chávez-Sánchez *et al.,* 2013). Hence, the need to identify and develop non-chemical alternative treatments (Yousef *et al.,* 2015). Development of new natural preservatives and antimicrobials and improved storage techniques is increasing (Misir *et al.,* 2014). Storage techniques are helpful as they extend the shelf life and quality of the fruit, which is a key attribute for marketing (Misir *et al.,* 2014). Edible coatings are also amongst the most important technologies for preservation and to extend shelf life of fruits and vegetables (Misir *et al.,* 2014). Edible films and coatings are an environmentally-friendly alternative approach for extension of storage life of fresh and minimally processed fruits and vegetables (Adetunji *et al.,* 2013, Ghosh *et al.,* 2015, Yousef *et al.,* 2015). In recent years, such approaches have received considerable attention due to their advantages, the approaches include edible packaging materials over synthetic films (Misir *et al.,* 2014). Edible coatings are thin layers of edible material applied on the surface of the product as a replacement for natural protective waxy coatings (Misir *et al.,* 2014). Several strategies, such as dipping, spraying and brushing directly are used to apply edible coatings on the food surface to provide a barrier to moisture, oxygen and solute movement in the food (Misir *et al.,* 2014). Edible coatings are effective for the protection of perishable food products from deterioration by retarding dehydration, suppressing respiration, improving textural quality, retain volatile flavour compounds and

reducing microbial growth (Yousef *et al.*, 2015). The coatings have the ability to create a modified atmosphere and to reduce weight loss during transportation and storage of fruits and vegetables (Misir *et al.,* 2014). Edible coatings or biodegradable films are prepared from polymers such as polysaccharides, lipids, proteins or a blend of these compounds (Dashipour *et al.,* 2014; Vyas *et al.,* 2014).

Chitosan is a cationic polysaccharide that is found in exoskeletons of the shellfish and is obtained by the alkaline deacetylation of chitin (Chien *et al.,* 2013). Chitosan is soluble in dilute organic acids and it has been used theoretically for coating fruits as a preservative (Chien *et al.,* 2013). Carboxymethylcellulose (CMC) is an anionic polysaccharide that is linear, long-chained and water-soluble (Adetunji *et al.,* 2013). It is one of the most common cellulose derivatives with good film forming property, can form transparent films and has high mechanical strength (Dashipour *et al.,* 2014; Qi *et al.,* 2016).

Moringa (*Moringa oleifera*) is a special food for the tropics and all parts of the plant are used for livestock as food and forage (Yousef *et al.,* 2015). Moringa is well known for its ability to protect perishable food products from deterioration when used as a coating agent (Yousef *et al.,* 2015). Moringa extracts applied in fruits and vegetables improve textural quality, retain volatile compounds, suppress respiration and reduce microbial growth (Yousef *et al.,* 2015). Although the benefits of edible coatings and botanical extracts have been documented, however, information on their utilization for coating to enhance shelf life and improve postharvest quality of fruits is limited. Natural products are known to have film-forming properties, antimicrobial actions, and biochemical properties. They are also known for their biodegradability and for being environmentally friendly. Therefore they can be used as an alternative preservative coatings for fruits and vegetables.

1.3. Research aim

The aim of the study was to assess the effect *Moringa oleifera* extract incorporated with edible coating in extending the shelf-life and monitoring decay levels of papaya fruits during different storage conditions.

1.4. Research objectives

- To assess the effect of *Moringa oleifera* extract incorporated with CMC and chitosan on quality of papaya fruit.
- To evaluate the effect of *Moringa oleifera* extracts on papaya fruit decay under *in-vitro* analysis.

1.5. Thesis outline

This thesis is divided into five chapters as follows;

- Chapter One: This chapter covers the general introduction and background on papaya fruit, its production levels, nutritional composition, physiology and understanding on edible coatings and benefits to fruit industry. Emphasis is given to botanical extracts (i.e. Moringa extracts) and their effectiveness in maintaining the quality of fruits. The rationale for the study, aim and objectives are also included in this chapter.
- Chapter Two: A review of the literature regarding the botanical description of papaya, factors that promote deterioration, postharvest pathology, quality parameters, and quality improvement using botanical extracts, CMC and chitosan are reviewed.
- Chapter Three: This chapter reports investigation of *Moringa oleifera* extracts incorporated with CMC and chitosan as potential preservatives of papaya fruits quality.
- Chapter Four: This chapter reports investigation of the effect of *Moringa oleifera* extracts incorporated with CMC and chitosan on the decay levels of papaya fruit.
- Chapter Five: This chapter contains the General Discussion, Conclusion and Recommendations of the study.

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CHAPTER TWO

LITERATURE REVIEW

2.1. Overview

In the tropical and subtropical regions, papaya is regarded as an important fruit for domestic and export markets (Barrera *et al*., 2015). However, due to its physiology and chemical composition, it is perishable after harvesting resulting to considerable postharvest problems and losses during handling and storage due to increased perishability. Its high perishability results in more wastage compared to consumption. Papaya fruits are also susceptible to pathogenic microorganisms. About 30- 50% of the harvested papaya has been reported to never reach the consumers due to postharvest spoilage (Sharmin *et al.*, 2015). This review will provide the insight on different cultivars of papaya fruits, utilization, post-harvest opportunities and challenges, and diseases associated with the fruit.

Keywords: Postharvest loss, *Carica papaya*, fruit preservation, plant extracts, fruit quality

2.2 Background

The existence of papaya tree was first reported in Europe in 1535 by the Spanish author, G.H. de Oviedo (De Oliveira and Vitória, 2011). It was discovered between the south of Mexico and the north of Nicaragua (De Oliveira and Vitória, 2011). It is believed that the first seeds were taken from this region to Panama, Santo Domingo, some of the Caribbean islands, and parts of South America before being distributed to different regions worldwide (De Oliveira and Vitória, 2011). The papaya tree spread widely throughout the tropics after the discovery of the New World, most particularly in Africa and Asia (De Oliveira and Vitória, 2011). In South Africa, it was introduced by Jan van Riebeeck in 1652 in seed form (Schulze and Maharaj, 2007). However, it was only grown commercially by a Captain Elphick in the Lowveld of Mpumalanga for the first time in the early 20th century (Schulze and Maharaj, 2007). Papaya is a species that is adapted to tropical and subtropical regions (De Oliveira and Vitória, 2011). It requires temperatures ranging between 21 and 33 °C (De Oliveira and Vitória, 2011). Moreover, it does not tolerate cold weather and prolonged dry periods (De Oliveira and Vitória, 2011).

2.3 Botanical description

Papaya is a member of *Caricaceae* family consisting of four genera and 31 species (FAO, 2003; Ali *et al.*, 2010, Anuar *et al.*, 2008). The genus *Carica* consists of 22 species and is the only genus that has edible fruit species in the family (De Villiers, 1999). *Carica papaya* is the species that is mostly consumed as fruit, while *C. chilensis*, *C. gouditiana*, *C. monoica* and *C. pubescence* species are mostly consumed as vegetables (De Villiers, 1999). *C. papaya* is a small, sparingly branching, soft tree characterized by fast upright-growth which reaches a height of 3-10 m (De Villiers, 1999, Vij and Prashar, 2015). The tree has a fleshy, hollow stem, and a well-developed fibrous root system (De Villiers, 1999). Papaya tree is surmounted by a

terminal panache of leaves on long petioles with 5-7 lobes (Krishna *et al.*, 2008; Vij and Prashar, 2015). It is characterized by the growth habit of a palm where the stem is marked by scars where leaves have fallen off (Vij and Prashar, 2015).

All members of *Caricaceae* are deciduous but vary greatly amongst three species of *Carica* namely *C. monoica*, *C. pubescence* and *C. papaya* (De Villiers, 1999). The species *Carica papaya* compromises of three basic sex forms that includes male, female and hermaphrodite (De Villiers, 1999). Male and female aromatic flowers are born on separate individuals (De Villiers, 1999). The male flower is characterized by many-flowers, densely pubescent cymes at the tips of the pendulous and fistular rachis (Krishna *et al.*, 2008, Vij and Prashar, 2015). The female flowers are large, solitary with a few flowered racemes and different types of enzymes (Krishna *et al.*, 2008, Vij and Prashar, 2015). The female flowers are usually short-stalked without stamens and are born in the leaf axils on the upper part of the trunk (De Villiers, 1999). The flowers are stalkless, predominantly staminate without pistil in the male plant and are borne in clusters on long hanging compound spikes (De Villiers, 1999).

The papaya fruits are usually separate but sometimes appear in small clusters and are axillary borne on the main stem (Yogiraj *et al.*, 2014). Papaya fruit is an elongated berry of different sizes with a smooth thin skin (De Oliveira and Vitória, 2011). Papaya fruits are green, taking yellow or red colour when ripe and have a weight ranging from 0.23 to 9.07 kg (Rathod and Chavan, 2012; Yogiraj *et al.*, 2014). The papaya fruits are big oval in shape and resemble melon by having a central seed cavity, hence called pepo-like berries (Yogiraj *et al.*, 2014). The seed cavity in the fruit can be star-shape to round (Paull *et al.*, 1997). The fruits are fleshy and juicy, and the flesh of the fruit at maturity varies from yellow-orange to salmon (pinkish-orange). The fruit produces a pleasant, sweet and mellow flavour with high amounts of water, sugar, vitamin A and C, protein and ash (Rathod and Chavan, 2012).

2.3.1 Papaya Cultivars

Fruits have great variability in size, colour, shape and eating quality as a result of a great number of different varieties that have been improved (Bautista-Banos *et al*., 2013). The growing area can be used to as indication of the preference for certain papaya cultivars (Bautista-Banos *et al*., 2013). There are many papaya cultivars on the market and are usually named according to their location and preference (i.e. 'Subang', 'Sitiawan', 'Batu Arang', 'Koko', 'Sunrise', 'Maradol', 'Solo', 'Eksotika' and 'Taiwan'. Cultivars 'Solo' and 'Eksotika' are the fruits that have been recently introduced and they have small size and pyriform or round-shaped characteristics (Bautista-Banos *et al*., 2013). In South Africa, the important cultivars are Af-1, Sunrise Solo (a papino), Tainung no. 1 and 2, FI-2 and Honey Gold (Schulze and Maharaj, 2007). South African Sunrise Solo (Figure 2-1C), Baixinho and Af-1 cultivars have small size (300-500 g) which make them suitable for export (Department of Agriculture, Forestry and Fisheries (DAFF) and Agricultural Research Council (ARC), 2002). In contrast, Tainung (Figure 2-1B) and Hortus Gold (such as FI-2) varieties produce larger fruits and they are mostly preferred by fresh produce markets and farm stalls (Department of Agriculture, Forestry and Fisheries (DAFF) and Agricultural Research Council (ARC), 2002). Hortus Gold papaya fruit has a round-oval shape and golden-yellow colour (Figure 2-1A), it is known for its early maturing characteristics (Jain and Priyadarshan, 2008). Its characteristics are similar to that of Honey Gold, however, Honey Gold has improved sugar content and disease resistance (anthracnose is the reported disease) (Jain and Priyadarshan, 2008).

Figure 2-1: Fruits of different Carica papaya cultivars displaying different features. A- Hortus Gold (Anem, 2015), B- Tainung (Known you seed, 2018) and C- Sunrise Solo (papino) (Neofresh, 2015). Accessed on 07 July 2018.

Hawaiian varieties such as 'Solo' and 'Sunrise' have a great acceptance around the world due to their sensory qualities and size (Bautista-Banos *et al*., 2013). Cultivar 'Solo' is characterized by a pear-shaped or oval appearance and the fruit mass ranges from 400 and 600 g (De Oliveira and Vitoria, 2011). Unlike Solo papaya, Taiwan papaya consist of large size mass ranging between 800 and 2000 g (De Oliveira and Vitoria, 2011). Taiwan varieties produce a pear oblong-shaped fruit with higher sugar levels and increased resistance during transportation (De Oliveira and Vitoria, 2011). Cultivar 'Maradol' is regarded as an important cultivar around the world originating from Cuba and highly cultivated in Mexico, which has become the world's leading papaya exporter (De Oliveira and Vitoria, 2011; Bautista-Banos *et al*., 2013). Cultivar 'Eksotika' is a high yielding and good quality papaya hybrid that has gained popularity in domestic and export markets due to its characteristics (Ali *et al*., 2011).

2.4 Utilization of Papaya fruits

In Asian countries, the unripe or green fruit, and leaves of papaya are widely used in salads and cooking (Ikram *et al.*, 2015). Traditionally, the ripe papaya fruit is consumed like a melon (Saran *et al.*, 2015). The fruit is peeled, seeds removed, cut into pieces and served fresh (Saran *et al.*, 2015). Ripe papaya is used in processed products such as jam, jelly, marmalade and other products containing added sugar (Saran *et al.*, 2015). Moreover, puree or wine, nectar, juice,

frozen slices or chucks, mixed beverages, papaya powder, baby food, concentrated and candied items are processed from papaya fruit (Saran *et al.*, 2015).

2.5 Factors influencing deterioration of papaya fruit quality and shelf life

2.5.1 Cultivation practices and environmental factors

Papaya has a long harvest period, which allows it to be available throughout the year (Department of Agriculture, Forestry and Fisheries (DAFF) and Agricultural Research Council (ARC), 2002). The papaya tree requires to be cultivated under suitable temperature range of 21-33 °C and in mildly acid soil with pH range of 6.0-6.5 (Macalood *et al*., 2014). It is preferable to use seeds that were generated from controlled crosses (female × bisexual or bisexual-selfed) to grow papaya successfully (FAO, 2003). The seeds from bisexual trees are known to have a higher degree of self-pollination under field conditions and are favourable for cultivation (FAO, 2003). Papaya fruit tree require good drainage soil and is known to lose their vigour due to flood conditions (FAO, 2003). Inconsistent water supply may cause growth retardation, flower abortion, and dropping of young fruits (FAO, 2003). Appropriate irrigation should be applied during dry spells (FAO, 2003). Previous reports indicated that environmental factors that affect fruit quality include climate (temperature, wind, rainfall), air quality, as well as positional effects both within a planting and within the tree (Arpaia, 1994). Other environmental factors effects can result in fruit scarring, which will lead to a direct loss of the fruit from the postharvest chain caused by environmental factors such as wind, heavy precipitation, and frost (Arpaia, 1994). High rainfall during flowering might also result in increased incidence of plant pathogen i.e. diseases, such as anthracnose and loss of fruit related to freeze damage. Fruit quality can also be influenced by temperature during fruit growth and maturation, hastening and delaying horticultural maturity. Temperature and light intensity also have a strong influence on the nutritional quality of fruits and vegetables (Kader, 2002). The light intensity and quality are important for optimum plant productivity, harvest index and their

effects are either direct or indirect (Benkeblia and Tennant, 2011). The photosynthetic photon flux on the rate of electron flow can be affected directly, whereas leaf photosynthetic capacity can be indirectly affected (Benkeblia and Tennant, 2011). Generally, lower light intensity during plant growth results in lower content of ascorbic acid in plant tissues (Weston and Barth, 1996). Hence, the fruit exposed to the sun and sides of the fruit that receive higher amount of sunlight during growth have higher levels of ascorbic acid than shaded fruit (Magwaza *et al*., 2017). Generally, ascorbic acid concentration increases with increased exposure to light (Magwaza *et al*., 2017). Moreover, light has been shown to be required for the formation of β-carotene in tomatoes (Weston and Barth, 1996). The atmospheric conditions, such as carbon dioxide concentration, relative humidity, and temperature, are often unmanageable in the field, but have strong implications for crop quality (Weston and Barth, 1996). Mostly profound effects on the growth and development of produce quality is the initiation of the reproductive cycle in higher plants (Weston and Barth, 1996). Flower initiation is often temperature dependent and variations between day and night temperatures regulate stem elongation and flower stalk initiation (Weston and Barth, 1996). The effects of temperature on fruit growth are at the sink level, i.e. fruit demand and growth rate. In some circumstances, the effects changes the fruit shape and size (Benkeblia and Tennant, 2011).

2.5.2 Pre-harvest practices

Pre-harvest management practices are well documented and have effects on postharvest quality (Blakey, 2011). These include factors such as environment and cultural practices which closely influence postharvest quality of horticultural crops (Wang, 1997). Quality pattern of the fruit is related to many factors, such as cultivation practices, abiotic factors (soil humidity, temperature, relative humidity and availability of mineral nutrients), genetic variability and cultivar traits (Martins and De Resende, 2013). Moreover, seasonal growing light conditions, amount of rainfall and irrigation, pest management, and maturity at harvest can affect

postharvest quality, storage life, and susceptibility of crops to disorder and diseases (Wang, 1997). Quality does not improve after harvest in many horticultural crops, therefore, the best quality of the crop is achieved at the time of harvest (Weston and Barth, 1996; Wang, 1997).

2.5.3 Genetics and cultivar selection

Genetics and cultivar selection are major factors involved in postharvest quality outcomes for fruits and vegetables (Benkeblia and Tennant, 2011). Cultivars vary in their genetic factors which makes the traits such as size, colour, flavour, texture, nutrition, pest resistance, processing ability, eating quality, and yield to differ prominently (Weston and Barth, 1996). Magwaza *et al*. (2017) indicated that the chemical and nutritional attributes such as carotenoids and ascorbic acid content are largely determined by multigenic inheritance in citrus fruit. Most climacteric fruits, including papaya fruit, have a short shelf life, hence the choice and combination of genes controlling the traits are considered as a pre-harvest factor which influence fruit quality (Benkeblia and Tennant, 2011). Fruit quality attributes and the products of physiological processes during the ripening period are determined by some characteristics, such as textural quality it is determined by firmness, succulence, and sensory qualities (Benkeblia and Tennant, 2011). Each quality attribute is the result of highly regulated, multiple processes inherent in the individual fruit (Benkeblia and Tennant, 2011). In every crop, the range of genotypic variation differences affecting composition, quality, and postharvest life potential has resulted in a tremendous number of species and cultivars with different quality attributes (Benkeblia and Tennant, 2011). Cultivars are developed for improved disease resistance, environmental adaptability, high quality harvested fruit and vegetable products, however, nutritional quality vary greatly with cultivar (Weston and Barth, 1996). Previous reports indicated that traditional plant breeding based on selection of desirable variety and development of inbred lines offers potential to reduce susceptibility to environmentally-induced decay and improve postharvest quality (Weston and Barth, 1996).

2.5.4 Postharvest factors and ripening process

After harvest, fresh fruits undergo vigorous biochemical reactions and their respiration accelerates the natural loss of fruit tissue (Niazmand *et al*., 2009). Thus, fruits tend to lose water at room temperature (Niazmand *et al*., 2009). The fruit appearance, texture and quality change might result in reduction of commercial value. The quality and nutritional content of fleshy fruits is affected in different ways during ripening (Fabi *et al.*, 2007). The changes take place quickly in climacteric fruits, such as tomatoes, bananas, pears, mangos, and papayas, compared to non-climacteric fruits (Fabi *et al.*, 2007). The ripe fruits soften rapidly, are easily infected by diseases and are prone to other negative postharvest changes, such as postharvest deterioration, scratches and punctures when in contact with rough or sharp surface and, chilling injury following exposure to low temperature (Fabi *et al*., 2009; Vyas *et al*., 2014; Workneh *et al*., 2012). These postharvest losses can result from fast ripening caused by the ripening trigger chemical substance, which could also come from any climacteric ripe fruit stored in the same environment with any green fruit (Fabi *et al.*, 2007). Therefore, fruits must be stored away from ethylene sources to minimize the effects (Fabi *et al.*, 2007). Other options could include reduction in the hormone levels in the atmosphere with oxidation of potassium permanganate or ultraviolet light, although such commercial application approaches are limited (Fabi *et al.*, 2007).

2.5.5 Transpiration and respiration

Transpiration is water evaporation from plant tissues (Workneh *et al.*, 2012). Food products may contain several liquid and solid components, oils, flavour components, nutrients and water (Embuscado and Huber, 2009). These components will migrate throughout the product if there is concentration difference acting as a driving force (Embuscado and Huber, 2009). Deterioration of the product will happen due to transpiration process which leads to severe

consequences including loss of marketable weight and adversely affect the appearance due to wilting and shrivelling (Workneh *et al.,* 2012). The use of various packaging materials such as polythene, tissue paper, newspaper, paddy straw and shrink film can be used to reduce weight loss (Singh *et al.*, 2012a). Respiration is a major metabolic process that takes place in harvested produce or in any living plant product (Workneh *et al.,* 2012). Respiration can be either aerobic or anaerobic depending on oxygen level, and during this process oxygen and carbon dioxide are used up and/or released (Embuscado and Huber, 2009). Generally, anaerobic respiration starts replacing the Krebs cycle when oxygen drops below 3%, resulting in glycolytic pathway releasing unacceptable flavours and causing other problems, such as changes in colour and texture (Embuscado and Huber, 2009). The process results in stored organic materials; carbohydrates, proteins, fats and other organic materials being broken down into a simple end products, with release of energy (Workneh *et al.*, 2012).

2.5.6 Storage temperature and postharvest handling

The fruit respiration is normally affected by the higher temperature which leads to faster the respiration rate (Misir *et al*., 2014). As the temperature around the fruit rises, respiration increases which leads to an increase of the temperature inside the fruit (Misir *et al*., 2014). Chilling injury is amongst the known cause of postharvest losses as it damages fruits exposed to low temperatures (Vyas *et al.*, 2014). The Ministry of Fisheries, Crop and Livestock, South America (2003) reported that, if papaya fruits are held at temperature below 10 °C it is possible to be susceptible to chilling injury (CI). Usually, CI symptoms include development of sunken lesions on the fruit surface (pitting), discolouration of the peel and the flesh, incomplete ripening, skin scald, hard lumps in the pulp around vascular bundles, and water soaking of flesh (The Ministry of Fisheries, Crop and Livestock, South America, 2003; Zhou *et al*., 2014).

There has been vibrant export trade of papaya fruit resulting from high yield, with ideal size (400-800 g) and superior quality of the fruit (Ali *et al.*, 2010). "However, the problems of postharvest handling and storage are inherent in the trade of papaya fruit" (Ali *et al.*, 2010). Postharvest activities such as harvesting, handling, storing, processing, packaging, transporting and marketing can result in postharvest losses (Kasso and Bekele, 2016). Fruit surfaces get easily bruised and cut, hence the requirement of proper postharvest handling practices to be followed (Sivakumar and Bautista-Banos, 2014). This includes not packing fruits that show signs of postharvest disease symptoms with healthy fruits. Earlier reports indicate that fruits should be removed from the cartons if symptoms are initially detected (Sivakumar and Bautista-Banos, 2014). In general, farmers should implement good orchard sanitation procedures as postharvest decay control is initiated in the field.

2.6 Postharvest pathology

Papaya fruit is susceptible to numerous diseases, physical disorders and over-ripeness/ faster ripening (Ali *et al.*, 2010, Gonzalez-Aguilar *et al.*, 2003; Perez-carrillo and Yahia, 2004). Spoilage in papaya can be referred to as rot or decay and major postharvest diseases include anthracnose caused by *Colletotrichum gloeosporioides*, stem-end rot caused by *Lasiodiplodia theobromae* and *Phomopsis* rot caused by *Phomopsis caricae-papayae* resulting in estimated damage of 45% (Awoite *et al*., 2013; Abeywickrama *et al*., 2012). Fruit affected by rot or decay can be characterized by excess softening, mycelia growth, loss of moisture, unpleasant odour, shrinkage and loss of water (Awoite *et al*., 2013). After harvest, the infection process is greatly aided by mechanical injuries to the skin of the produce such as fingernail scratches, abrasions, insect punctures and cut (Rahman *et al*., 2008). This may also lead to deterioration in fruit quality and leads to extensive postharvest losses during handling and storage (Gonzalez-Aguilar *et al.*, 2003; Awoite *et al*., 2013). There are several pathogens that affect papaya fruit in postharvest, however, only the most common will be discussed in this section.

2.6.1 Anthracnose

Anthracnose caused by *Colletotrichum spp*. is a devastating disease for most of the tropical fruits and vegetables (Zahid *et al*., 2012). This fungal spp. is responsible for anthracnose of different tropical fruits, including banana (*Colletotrichum musae*), papaya and dragon fruits (*Colletotrichum gloeosporioides*) (Zahid *et al*., 2012). Anthracnose caused by *Colletotrichum gloeosporioides* Penz. Sacc. is the most important fungal disease that affects papaya fruit (Gonzalez-Aguilar *et al.*, 2003; Li *et al.*, 2013; Sivakumar *et al.*, 2002). *Colletotrichum gloeosporioides* inoculums come from dying infected petioles of the lower leaves in the form of conidia (Ali *et al.*, 2010, Sivakumar *et al.*, 2002). Rain splash releases conidia into the atmosphere, which are then carried to developing fruits by air currents (Sivakumar *et al.*, 2002). The conidia will then develop appressoria in the presence of favourable conditions from which infection peg penetrate the skin of fruits and remain dormant until the fruit ripens (Ali *et al.*, 2010, Sivakumar *et al.*, 2002). Anthracnose can be identified by symptoms such as round, water-soaked spots on the surface of ripening fruit, which then enlarge and turn light brown (Hasan *et al*., 2012). A lesion may become as large as 5 cm in diameter and its centre can be covered by pinkish-orange areas that are formed by conidial masses (Hasan *et al*., 2012). These pinkish-orange areas that are formed by conidial masses are often produced in a concentric ring pattern (Hasan *et al*., 2012). Anthracnose symptoms only become apparent after ripening, resulting from its latency in the early ontogeny of the fruits (Ali *et al.*, 2010; Sivakumar *et al.*, 2002).

2.6.2 Black rot

Black rot is caused by the fungus *Mycosphaerella caricae* and its appearance can be in many different ways (Ministry of Fisheries, Crop and Livestock, South America, 2003). The early symptoms of this disease are small wrinkles that appear on the surface of the fruit, or slight browning of the peduncle (Alvarez and Nishijima, 1987). The disease will later shows sunken

circular lesions on the surface of the fruit, which enlarge up to a diameter of 4 cm (Ministry of Fisheries, Crop and Livestock, South America, 2003). The margin of the lesions becomes light brown and translucent (Ministry of Fisheries, Crop and Livestock, South America, 2003; Alvarez and Nishijima, 1987). As the infection advances the infected tissue becomes black, wrinkled and dry (Ministry of Fisheries, Crop and Livestock, South America, 2003; Alvarez and Nishijima, 1987). At an advanced stage of infection, white mycelium develop at the stem end or at the point of infection (Ministry of Fisheries, Crop and Livestock, South America, 2003; Alvarez and Nishijima, 1987).

2.6.3 Watery soft rot

Watery soft rot is rarely seen in the field and is an important disease during fruit storage and transit (Ministry of Fisheries, Crop and Livestock, South America, 2003). It is caused by the fungus *Rhizopus stolonifer* and is characterized by a soft and watery rot that collapse the entire fruit leaving the cuticle intact (Ministry of Fisheries, Crop and Livestock, South America 2003). The infected fruit can be identified by mass of coarse grey mycelia with black macroscopic sporangia, and the fruit quickly becomes colonized by yeasts and bacteria, and have sour odour (Ministry of Fisheries, Crop and Livestock, South America, 2003; Alvarez and Nishijima, 1987). The fungus can grow through any break in the cuticle spreading rapidly to adjacent fruits. It is required that wounding should be avoided during harvesting, transporting, and at postharvest handling as the fungus only enter the fruit through wounds (Ministry of Fisheries, Crop and Livestock, South America, 2003).

2.6.4 Wet fruit rot

Wet fruit rot is caused by the fungus *Phomopsis* and occurs most frequently as a stem-end rot (Ministry of Fisheries, Crop and Livestock, South America, 2003). Wet fruit rot can be recognized by a discolouration of the tissue around the stem end, which breaks down and become colonized by a whitish-grey mould (Ministry of Fisheries, Crop and Livestock, South

America, 2003). The infected area in the surface of the fruit become soft and translucent and formation of black pycnidia may occur at the centre of the lesion (Alvarez and Nishijima, 1987). Wet fruit rot resembles Rhizopus watery soft rot in its early stages (Ministry of Fisheries, Crop and Livestock, South America, 2003). However, this disease does not usually cause release of liquid as happens in tissues affected by Rhizopus watery soft rot, but the cuticle remains intact over the infected area and develops delicate, soft, mushy and wet (Ministry of Fisheries, Crop and Livestock, South America, 2003). A wet fruit rot advances rapidly, resulting in lesions to expand very quickly and extend into the cavity of the fruit (Alvarez and Nishijima, 1987). This disease is rarely seen on green fruits in the field and its symptoms usually appear on fully ripened fruits (Ministry of Fisheries, Crop and Livestock, South America, 2003). It is required that wounding should be avoided during harvesting, transporting, or postharvest handling as the fungus requires wounding of the fruit for infection (Ministry of Fisheries, Crop and Livestock, South America, 2003).

2.6.5 Stemphylium and Phytophthora fruit spot

Stemphylium fruit spot symptoms can be recognized by the development of small, round, dark brown surface lesions (Ministry of Fisheries, Crop and Livestock, South America, 2003; Alvarez and Nishijima, 1987). The lesions tend to be sunken and develop reddish brown to purple margins as they enlarge. A velvety, dark green spore mass can be recognized in the centre of the lesion (Alvarez and Nishijima, 1987). At advanced stage, the lesion becomes covered with white to grey fungal growth (Alvarez and Nishijima, 1987). The internal of the fruit becomes discoloured from reddish brown to dark brown and dry at the point of infection, and may develop small air pockets (Ministry of Fisheries, Crop and Livestock, South America, 2003). The symptoms of stemphylium fungus are similar to those of the fruit rot caused by the fungus *Phythophthora palmivora*.

Phythophthora palmivora fungus causes circular translucent lesions on the skin of infected mature fruits and become covered with a whitish to grey fungal growth (Vawdrey *et al*., 2015). The mycelium produces masses of sporangia which contain zoospores, which are dispersed by wind-blown rain (Vawdrey *et al*., 2015).

2.7 Harvesting

2.7.1 Maturity stage

Identification of optimum harvest maturity is the critical point in papaya fruit to ensure adequate fruit ripening to good eating quality and marketing (Saran *et al.*, 2015). Maturity of fruit at harvest influences fruit quality, storage behaviour, and also can be used to estimate the shelf life and selection of processing operations for value adding (Ngnambala, 2013; Saran *et al.*, 2015). Physiological maturity stage is when development of the fruit is complete and growing has ceased (Manrique and Lajolo, 2004). However, maturity is defined based on purposes for which it is harvested. According to plant physiologists, maturity is defined as the fruit stage where the fruit will ripen properly after harvest (Ngnambala, 2013). Whereas, postharvest technologists define maturity as a sufficient stage of development where after harvesting and postharvest handling, the fruits will possess at least the minimum acceptable quality for the ultimate consumer (Ngnambala, 2013). In the horticultural industry, maturity is defined as a stage of development at which a plant part possesses the prerequisites for consumption (Ngnambala, 2013).

Papaya fruits should be harvested at the yellow break stage, when the first streak of yellow colour has appeared (De Villiers, 1999; Paull *et al*., 1997; Teixeira da Silva *et al*., 2007). Fruits at this stage are normally physiologically mature and they will continue to ripen normally after harvest (De Villiers, 1999, Saran *et al.*, 2015). However, destructive indices, including the use

of internal pulp colour and percentage soluble solids (sugar content), can be used to determine harvest maturity (Ministry of Fisheries, Crops and Livestock, South America, 2003). The internal pulp colour of mature papaya fruit changes from cream to yellow-orange. The soluble solids content can be determined by placing several drops of juice on a hand-held refractometer and should at least be 11.5% (Ministry of Fisheries, Crops and Livestock, South America, 2003). A combination of external and internal maturity indices can be used to determine harvest date and time.

2.7.2 Harvesting time and method

Harvest time is fundamental to obtain a high-quality fruit with storage potential and has an effect on fruit sensorial quality (Bron and Jacomino, 2006). Fruits are harvested after they have reached physiological maturity stage (Manrique and Lajolo, 2004). After this maturity stage, postharvest ripening process will commence, and fruits acquire the organoleptic characteristics marketed for distribution and consumption. The sugar content in papaya fruits do not increase after harvest, hence, it is important to pick them at the proper maturity stage (Jayasheela *et al*., 2015). Fruits picked too early or too late become more susceptible to postharvest physiological disorders than fruits picked at the proper maturity stage (Ngnambala, 2013; Saran *et al.*, 2015). It is suggested that harvesting of papaya fruits should be done when its cooler, morning hours are recommended compared to afternoon hours (Ministry of Fisheries, Crops and Livestock, South America, 2003). This is because the temperature of the fruit rises in the afternoon due to the heat that occurs during the day, resulting in susceptibility to bruising injury (Ministry of Fisheries, Crops and Livestock, South America, 2003).

Papaya fruits are normally harvested by hands (Paull *et al.*, 1997; FAO, 2003; Teixeira da Silva *et al.*, 2007). The fruit is either snapped off or cut off of the tree when harvesting by hand or with knives (Ministry of Fisheries, Crops and Livestock, South America, 2003). Fruits that are inaccessible by hand due to height of the tree needs specialized tools, such as long pole, small

circular hoop with small mesh bag attached to hold fruits, and a horizontal blade that will placed above the hoop (FAO, 2003). Fruits have to be collected in smooth surfaced plastic crates, clean collection bags and then transferred into large lug collection bins (Teixeira da Silva *et al.*, 2007; Paull *et al.*, 1997).

2.7.3 Pre-storage treatments

The major role of pre-storage treatment is to serve as preservatives for quality of fruits and vegetables, and to control the agents of postharvest diseases before fruits are stored for a desired period of time (Shezi, 2016). There are various pre-storage treatments to choose from, such as reduction of temperature and/or oxygen, use of modified atmosphere packaging, edible coatings and treatment with gamma-irradiation or high pressure (Niazmand *et al*., 2009; Sivakumar and Bautista-Banos, 2014). Also, the use of bio-control agents; food preservatives, such as sodium carbonates, sodium bicarbonates and potassium sorbate; ozone exposure; heat treatments; methyl jasmonate and salicylic acid can be used. These pre-storage treatments are selected based on their effectiveness in controlling fruit postharvest diseases, low toxicity to mammals and less environmental effects (Sivakumar and Bautista-Banos, 2014; Shezi, 2016). In this section the pre-storage treatments that will be discussed are the common ones that has been reported to be effective in maintaining fruit quality.

2.7.3.1 Heat and low temperature treatment

Heat treatment generally applied as hot water dips, steam, or hot-air treatments to control pests and fungal diseases in fruits and vegetables (Chavez-Sanchez *et al*., 2011). It is an alternative quarantine insect control method for perishable commodities as they have fungicidal and insecticidal action (Bautista-Banos *et al*., 2013; Chavez-Sanchez *et al*., 2011). It has been demonstrated in many tropical and subtropical fruits, including papaya, that it is usable for insect control (Bautista-Banos *et al*., 2013). Heat treatments is also known to maintain quality of fruits (Chavez-Sanchez *et al*., 2011). This convection-heating medium, overall, eliminates

incipient infections by acting directly on the viability of the spores on the surface or beneath, resulting in delay of conidia germination, growth and sporulation (Bautista-Banos *et al*., 2013). Stem-end rot and anthracnose disease of papaya have been successfully controlled by spray, hot water immersions and forced-air heat treatments (Bautista-Banos *et al*., 2013). Moreover, they alleviate some physiological disorders such as chilling injury (Chavez-Sanchez *et al*., 2011).

Low temperature is the most commonly applied technique to control ripening by slowing down enzymatic reactions involved in respiration and senescence (Ahmad *et al*., 2013). Previous reports have indicated that low temperature can minimize loss of fruit quality (Ahmad *et al*., 2013). However, challenges of chilling injury after prolonged periods of low temperature storage have been reported (Ahmad *et al*., 2013).

2.7.4 Edible coatings

Alleviating postharvest decay using non-chemical control methods is becoming increasingly important from both economic and environmental viewpoints (Hasan *et al*., 2012). In recent years, edible film coatings have received considerable attention due to their advantages, such as their use as edible packaging materials over synthetic films (Misir *et al*., 2014). Edible coatings may be composed of polysaccharides, proteins and lipids (Oluwaseun *et al*., 2013). Edible coatings provide a barrier to moisture, oxygen and solute movement in and out of the fruit (Misir *et al*., 2014). They also protect perishable food products from deterioration by retarding dehydration, suppressing respiration, improving textural quality, retaining volatile flavour compounds and reducing microbial growth (Vyas *et al*, 2014; Yousef *et al.*, 2015). Furthermore, they assist in maintaining firmness and provide gloss to coated fruit (Oluwaseun *et al*., 2013). Several postharvest studies have reported the significance of edible coating such as chitosan in maintaining the quality of fruits and vegetables, reducing respiration rates, ethylene production, and transpiration (Bautista-Banos *et al*., 2003).

Chitosan is a cationic polysaccharide that is obtained by the alkaline deacetylation of chitin extracted from an abundant source of shellfish exoskeletons (Hewajulige *et al*, 2009; Chien *et al.*, 2013). It is a natural polymer composed of β-(1, 4)-2 acetamido-2-deoxy-D-glucose and β-(1, 4)-amino-2-deoxy-Dglucose units (Hernandez-Lauzardo *et al*., 2010). Chitosan is the second most abundant, naturally available, easily degradable biopolymer after cellulose (Hewajulige *et al*, 2009). The biological properties of chitosan have been recently investigated in the post-harvest storage of fruits and serve as an alternative for controlling postharvest fungal rotting (Ali *et al*., 2010; Hernandez-Lauzardo *et al*., 2010). Chitosan is normally used as coating to control decay and act against pathogens in fruits and vegetables (Hewajulige *et al*, 2009; Ali *et al*., 2010). It forms a semi-permeable film that inhibits the entry of a number of pathogenic fungi and activates multiple antifungal biological responses in plants (Ali *et al*., 2010). Chitosan forms a barrier, which controls gas exchange, modifies the internal atmosphere of the fruit and reduces water loss resulting in tissue firmness (Gonzalez-Aguilar *et al*., 2008).

Carboxymethylcellulose (CMC) is one of the most widely applied cellulose derivatives and it has good film forming property, it can form transparent films and possesses high mechanical strength (Qi *et al*., 2016). Cellulose is a linear, high molecular weight polymer and a biodegradable material (Rachtanapun, 2009). It does not dissolve readily in common solvents due to its strong inter- and intra-molecular hydrogen bonds (Rachtanapun, 2009). Cellulose has to be converted into its derivatives in order to be utilized in the food industry (Rachtanapun, 2009). CMC is an anionic polysaccharide that is linear, long-chained and water-soluble (Adetunji *et al.*, 2013). Purified CMC is a powder that is white-to cream-coloured, tasteless, odourless, free-flowing and has many applications such as edible films and coating (Rachtanapun, 2009; Dashipour *et al*., 2014).

2.7.5 Ethylene inhibitor 1-methylcyclopropene (1-MCP)

The ethylene receptor inhibitor 1-methylcyclopropene (1-MCP) is a non-toxic gas that acts as a non-competitive inhibitor of ethylene action and was developed by Edward Sisler and Sylvia Blankenship (Manenoi *et al*., 2007). It is commonly used as a tool for extending the postharvest shelf-life and improving the quality of a number of fruits (Fabi *et al*., 2010). It also prevents the non-homogeneous ripening and softening the flesh of the fruit caused by the exposure to exogenous ethylene or poor postharvest handling (Huerta-Ocampo *et al*., 2012). According to Manenoi *et al*. (2007) 1-MCP has been recommended for extending the postharvest life papaya fruit (which is shorter 2- 3 weeks at 8- 10 °C) and is effective in slowing the ripening process of the whole papaya fruit.

2.7.6 Natural compounds (Plant extracts and active compounds)

Different organs such as seeds, leaves and flowers from varying plant species produce antimicrobial compounds (Bautista-Banos *et al*., 2013). Antimicrobial properties of plant extracts collected from various species have been proven to affect fungal development in-vitro and in-vivo (Bautista-Banos *et al*., 2003). Plant extracts can stimulate or inhibit spore formation, germination, mycelial growth and infection. Many plant species of different botanical families and their derivatives have demonstrated extending the fungicidal potential of papaya against fungal diseases (Bautista-Banos *et al*., 2013). The control includes pathogens such as *C. gloeosporioides*, *Rhizopus spp*., *Aspergillus spp*. and *Mucor spp*. The plant extracts were from the following botanical families; Sapotaceae (*Achras sapota*, *Chrysophyllum cainito* and *Pouteria sapota*), Caricaceae (*C. papaya*), Leguminosae (*Pachyrrizus eresus* and *Phythecellobium dulce*), Solanaceae (*Centrum nocturnum*) and Verbenaceae (*Lantana camara*).

Aloe vera plant with medicinal properties has been used to preserve the quality of papaya fruit (Brishti *et al*., 2013). Gel-based edible coating from *Aloe vera* material has been reported to

have antifungal activity against many fungi, including *Colletotrichum* spp and is regarded as safe, environmentally friendly which makes it an alternative to synthetic preservatives such as sulphur dioxide (Marpudi *et al*., 2011; Brishti *et al*., 2013). *Aloe vera* gel has tasteless, colourless and odourless characteristics (Brishti *et al*., 2013). The gel forms a protective layer against the oxygen, air moisture and inhibits microorganism's action that cause food borne (Brishti *et al*., 2013). The gel has the ability to prevent moisture loss, control of respiratory rate, maturity development, oxidative browning delay and microorganism proliferation reduction (Marpudi *et al*., 2011; Brishti *et al*., 2013).

2.7.7 Gamma irradiation

Food irradiation is a process of exposing packaged/bulk food to a controlled amount of ionizing radiation for a specific period of time (Bautista-Banos *et al*., 2013). Ultraviolet C (UV-C) and gamma rays exhibit fungicidal effects and can also induce resistance in fruits (Cia *et al*., 2007). Treatment with gamma and UV-C (254 nm) can be used for the control of postharvest diseases. The UV-C treatment is recommended as it has the ability to extend the postharvest life of the fruit by delaying ripening (optimum dose is 0.75 kGy) and senescence (Pimentel and Walder, 2004). Previous reports stated that low irradiation doses exhibit insecticidal effects on fruit flies which makes it effective at all stages of the life cycle and makes it ready to be used as an efficacious quarantine treatment method (Cia *et al*., 2007; Pimentel and Walder, 2004). The high cost and prejudice by consumers in relation to irradiated foods are the greatest challenges in the use of irradiation in postharvest treatment (Cia *et al*., 2007).

2.7.8 Calcium chloride

Senescence in fruits may be delayed when calcium is applied during pre and postharvest stage to eliminate any detrimental effect on consumer acceptability (Singh *et al*., 2012b). Calcium chloride is a safe and effective alternative to control postharvest decay in fruits and vegetables (Singh *et al*., 2012b). It has been extensively used as preservative and firming agent in fruits

and vegetables (Al Eryani-Raqeeb *et al*., 2008). Singh *et al.* (2012b) stated that exogenously applied calcium stabilizes the plant cell wall and protects it from degrading enzymes. Calcium ions has been widely reviewed as both an essential element and in maintaining postharvest quality of fruit and vegetable by contributing to the linkages between pectic substances within the cell-walls (Singh *et al*., 2012b). Increase in the cohesion of cell-walls has been observed in the presence of calcium ions (Singh *et al*., 2012b). Calcium complexes with cell wall and middle lamella polygalacturonic acid residues which improves structural integrity (Al Eryani-Raqeeb *et al*., 2008). The cell wall become less accessible to the enzyme that cause softening due to the complexity and provide reduction in the rate of senescence and fruit ripening benefits (Al Eryani-Raqeeb *et al*., 2008).

2.7.9 Essential oils

The essential oils (EOs) are natural antioxidants known for their antimicrobial and biodegradable properties; and they do not leave any residual effect on fresh produce (Sivakumar and Bautista-Banos, 2014). They are a mixture of volatile compounds produced by plants through secondary metabolism (Bautista-Banos *et al*., 2013). They provide effective control over fungal phyto-pathogens (Bautista-Banos *et al*., 2013). The volatile nature of EOs facilitates the use of small concentrations that are safe for consumption and widely used in general culinary practices, hence, consumer acceptance (Sivakumar and Bautista-Banos, 2014). The essential oils are environmentally friendly and known as ‘reduced risk’ pesticides (Sivakumar and Bautista-Banos, 2014). “The GRAS (Generally Recognized As Safe) compounds status of EOs approves their application as biopesticides to control pests and diseases to provide safe food” (Sivakumar and Bautista-Banos, 2014). Their application as bio fumigant has been recognised by their antifungal activity during the vapour phase and control the postharvest diseases in fruit, if not subjected to aqueous sanitation in the packing line (Sivakumar and Bautista-Banos, 2014).

2.7.10 Antagonist

Many bio control agents, such as bacteria and yeast, have been tested on numerous postharvest papaya fungi (Bautista-Banos *et al*., 2013). The technology has positive effects which may vary according to the antagonistic species applied and the control levels differ when conducting an *in situ* laboratory experiment (Bautista-Banos *et al*., 2013). The combination of antagonistic with other control measures may contribute to reducing papaya disease levels (Bautista-Banos *et al*., 2013).

2.8 Postharvest pathology control methods

Fungicide groups such as benzimidazole (Thiabendazole and Benomyl), Imidazole (Phrochloraz) and ethylene, bisdiothiocarbamate (EBDC) are commonly used in controlling papaya postharvest diseases (Bautista-Banos *et al*., 2013). However, postharvest decay control is initiated in the field, hence control is achieved with an application of pre-harvest fungicide treatment (Sivakumar and Bautista-Banos, 2014). It also requires that a postharvest dip or drench treatment is applied to the fruit after harvesting (Sivakumar and Bautista-Banos, 2014).

2.9 Storage conditions

Packaging and handling systems have been developed to move products from farm to consumer expeditiously for minimization of quality degradation (Azene *et al*., 2011). Packaging fruits is one of the most frequently used postharvest practices that put them into bulk (Azene *et al*., 2011). It makes them easy to handle while protecting them from hazards of transportation and storage (Azene *et al*., 2011). Modified atmosphere packaging (MAP) is when a product is enclosed in a sealed box or bag filled with various gases (such as oxygen, carbon dioxide, and others) at appropriate, optimal temperature (Embuscado and Huber, 2009). Modified atmosphere packaging of fruits and vegetables for storage and transportation is commonly achieved by packing them in plastic films (Azene *et al*., 2011). Types of modified atmosphere storage include storage in plastic films with different kinds of combinations of materials,

perforation, inclusions of chemicals and individual seal packaging. Packaging materials include polythene, tissue paper, newspaper, paddy straw, shrink film and others (Singh *et al*., 2012a). Controlled atmosphere (CA) technique generally involves storing the fruit in an atmosphere consisting of reduced concentration of oxygen (O_2) and elevated carbon dioxide (CO_2) (Singh *et al*., 2013). It usually mixed with nitrogen at optimum temperature and relative humidity (Singh *et al*., 2013). Storage atmosphere modification consist of low oxygen (O_2) and high carbon dioxide (CO_2) which prolongs the storage potential of tropical fruits including papaya, and enhances the shelf-life of fruit and vegetables (Yahia, 2006; Waghmare and Annapure, 2013). An ideal papaya fruit storage atmospheres should range between 2-5 kPa for O_2 and 5-8 for kPa CO_2 (Yahia, 2006). Factors such as cultivar, fruit maturity and storage temperature determine the response of the fruit to controlled atmosphere (CA) or modified atmosphere (MA), and some of these factors are shown in Figure 2-2 (Yahia, 2006). Modified atmosphere storage procedures include lowering temperature, maintaining optimal relative humidity, adding chemical preservatives, and maintaining an optimal gaseous environment as shown in Figure 2-2 (Azene *et al*., 2011). Temperature is reduced, and optimal gaseous environment is maintained to slow respiration and senescence (Azene *et al*., 2011). Maintenance of optimal relative humidity is done to reduce water loss without accelerating decay. To achieve reduced physiological and microbial deterioration, chemical preservative should be added in this system (Azene *et al*., 2011).

Polymeric film wraps and waxing of papaya have successfully retarded colour development and water loss (Gonzalez-Aguilar *et al*., 2003). Individual shrink film wrapping is used to enhance the storage life and maintain the postharvest freshness of fruits and vegetables (Singh *et al*., 2012a). Modified atmosphere created inside the package as well as the reduction in water loss explain the beneficial effects of MAP (Gonzalez-Aguilar *et al*., 2003). CA/MA is associated with benefits such as inhibition of fruit ripening and reduction in papaya decay

(Yahia, 2006). MA/CA control decay, because they delay ripening and senescence of the commodity, which result in maintenance of resistance to pathogen attack (Yahia, 2006). Reduction in respiration rate, minimizing metabolic activity, delaying enzymatic browning and retaining visual appearance of fruits and vegetables are some of the advantages of the system (Waghmare and Annapure, 2013). Temperature is critical and must be maintained at constant level to avoid in-pack condensation which could lead to decay (Embuscado and Huber, 2009). Also, optimum gas composition for different products is variable depending on factors including type of product, physiological age, temperature and duration of treatment (Yahia, 2006). Certain physiological disorders, irregular ripening, increased susceptibility to decay and development of off-flavours can be intensified by the exposure to O_2 and CO_2 levels above their optimum tolerable range (Yahia, 2006).

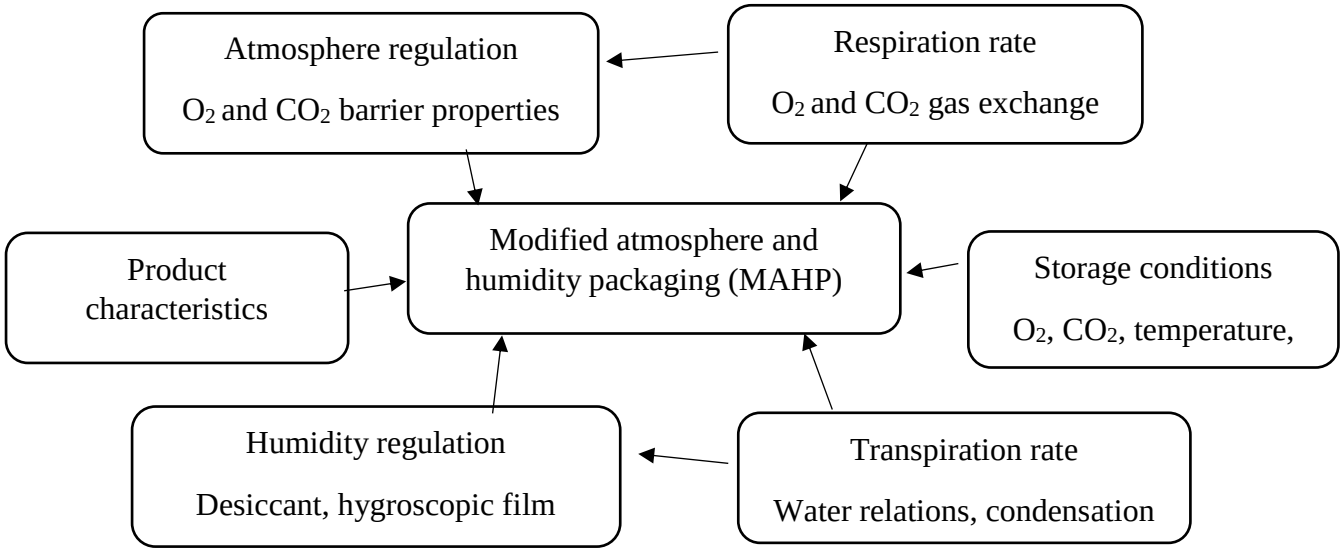

Figure 2-2: Factors to be considered when designing modified atmosphere and humidity packaging (MAHP) for fresh fruits and vegetables (Mahajan *et al.*, 2014).

2.10 Assessment of fruit quality

Fruit quality is defined differently in the postharvest chain (consumers, producers and handlers. Consumers define fruit quality according to shape, size, colour, aroma, and the absence of

defects such as cuts, bruises or decay (Sivakumar and Bautista-Banos, 2014). Producers and handlers define quality based on textural quality, appearance and long postharvest life (Kader, 2002). Enzymatic and non-enzymatic browning are the main changes that occur in fruits and vegetables, which results in the reduction of consumer acceptance (Niazmand *et al*., 2009). Appearance, colour, texture, flavour and nutritional value are fresh produce attributes that have been tradition quality criteria (Lin and Zhao, 2007; Mahajan *et al*., 2014). Safety (microbial, toxicological and chemical) and traceability are increasingly important for all the role players along the supply chain, from the farm to consumers. The quality attributes are determined by factors such as plant variety, stage of maturity/ripening, and the pre- and postharvest conditions (Lin and Zhao, 2007).

2.10.1 Physical properties

2.10.1.1 Firmness

The texture of fruits and vegetables is often interpreted in terms of firmness, crispness, juiciness, and toughness, where firm and crispy tissues are normally desired. (Lin and Zhao, 2007). Texture of fresh fruit and vegetables is a critical quality attribute in consumer acceptability (Misir *et al*., 2014). Factors such as shelf life, transport capability and disease resistance are texture dependent (Manrique and Lajolo, 2004). Texture is considered an important quality indicator for eating and cooking, and a factor in withstanding shipping stresses (Lin and Zhao, 2007). During storage, the rate and delay of firmness loss are the main factors that determine quality of the fruit and postharvest shelf life (Misir *et al*., 2014). Fruits softening occurs due to degradation of the middle lamella of cell wall (Misir *et al*., 2014). Enzymes hydrolases joint actions cause changes in cell wall structure and composition (Misir *et al*., 2014). Such enzymes include polygalacturonase (PG), pectinestarage (PE), β-Galactosidase (β-Gal), pectate lyase (PL) and cellulose (Cel).

2.10.1.2 Peel and pulp colour

Colour is amongst important visual attributes of fruits and the judgement is as the result of change in fruit skin colour and harvest index standard (Saran *et al.*, 2015; Jayasheela *et al*., 2015). The skin colour of papaya fruits will change from green to yellow or orange as the fruit matures and consumers mostly use colour of fruits to assess the quality (Jayasheela *et al*., 2015; Ngnambala, 2013). Visual assessment is the first impression and a key feature in the choice of fruits, hence peel and pulp colours are important in postharvest selection criteria (Ngnambala, 2013).

2.10.2 Chemical properties

2.10.2.1 Total soluble solids (TSS)

Fruits comprise many compounds such as sugars, acids, vitamin C and amino acids which are soluble in water (Ngnambala, 2013). Sugar are the main component when fruits ripen (Ngnambala, 2013). The sugar level of fruits is often the determinant of the required ripeness for marketing (Lin and Zhao, 2007). It is usually related to sucrose, glucose and fructose contents, which are often used as an index of ripening (Gómez *et al.*, 2002). The amount of TSS usually increases as the fruit mature and ripen, hence the soluble solids content of the fruit can be a useful index of maturity or stage of ripeness (Ngnambala, 2013). The total soluble solids content of fruits is measured using the refractometer and sugars are measured using high performance liquid chromatography (HPLC).

2.10.2.2 Total titratable acidity (TTA) and pH

The titratable acidity and pH of fruits are assessed to estimate consumption quality and hidden attributes (Ngnambala, 2013). Fruit juice pH values give a measure of the acidity or alkalinity of the product (Ngnambala, 2013). Titratable acidity gives a measure of the amount of acid present in a certain product (Ngnambala, 2013). Titratable acidity and pH could be considered as indicators of fruit maturity or ripeness (Lin and Zhao, 2007). Acid level is critical for

balance of flavour of certain fruits, including citrus species and grapes (Lin and Zhao, 2007). The acid level normally decreases during ripening and postharvest storage (Lin and Zhao, 2007). The total acid content of papaya consists of citric, malic, alpha-ketoglutaric and ascorbic acids, resulting in low acidity content for papaya (Martins and De Resende, 2013). Taste is mainly a balance between the sugar and acid contents, hence, acids make an important contribution to the postharvest quality of the fruit (Ngnambala, 2013).

2.10.2.3 Nutritional quality

Fresh fruits and vegetables are important source of nutrients, such as vitamins (B6, C, thiamine, niacin), minerals, dietary fibre, and significant amounts of phytochemicals that play important roles in human health (Lin and Zhao, 2007). Substantial postharvest losses in nutritional quality can be experienced, particularly for vitamin C content and other phytochemicals (Lin and Zhao, 2007). Ascorbic acid represents a major portion of the total acid content and papaya fruit contains about 85% (Martins and De Resende, 2013). The antioxidant capacity of fruits is vital for short postharvest shelf life (Zuhair *et al.*, 2013). The antioxidant capacity differs in fruits based on their genetic properties, time of harvest, season of harvest, postharvest and processing elements.

2.10.3 Physiological properties

2.10.3.1 Weight loss

Weight loss is the major determinant of the storage life and quality of papaya fruit (Espitia *et al*., 2012). Weight loss in fruits is principally due to high storage temperature, skin removal and cutting that exposes the interior tissues and drastically increases the water evaporation rate (Espitia *et al*., 2012). Fruit weight loss mostly occurs due to respiration and transpiration (Misir *et al*., 2014). During respiration, the fruit loss carbon reserves, and loss water during transpiration. The total weight of papaya fruit constitutes of about 90% of water and the major pathway for water loss is through the peel (Espitia *et al*., 2012). The water pressure gradient

between the fruit tissue and the surrounding atmosphere determines the rate of water loss (Misir *et al*., 2014).

2.10.3.2 Shelf-life

Shelf-life is defined as a period of time whereby a product is safe to eat, has acceptable taste, texture and appearance after being removed from the mother plant (Embuscado and Huber, 2009). Fruit shelf-life can be affected by factors such as respiration, biological structure, ethylene production, sensitivity, transpiration, developmental processes and physiological breakdown (Saran *et al.*, 2015). The shelf-life of Papaya fruit can be extended for up to 14 days if stored under controlled atmospheric conditions such as 2% oxygen and 5% carbon dioxide at a temperature of 16 °C (Saran *et al.*, 2015).

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CHAPTER 3

EFFECT OF EDIBLE COATINGS AND MORINGA EXTRACTS ON QUALITY PARAMETERS OF PAPAYA FRUITS

ABSTRACT

Papaya is a climacteric fruit that is perishable after harvesting, resulting in to up to 30-50% postharvest spoilage. A large amount of wastage and spoilage of the fruits occur due to poor keeping quality, difficulties in long distance transportation and due to poor preservation facilities. Due to that papaya fruit is very perishable after harvest, there is a need to find effective preservatives. However, due to that chemical preservatives tend to have negative effects, it is urgent to find alternative approaches. Edible coatings incorporated into botanical extracts are a promising alternative to chemical preservatives for improving fruit quality. The study evaluated with the effect chitosan (CH), carboxymethyl cellulose (CMC) incorporated with moringa leaf extract (MLE) and moringa seed extract (MSE) on the quality of mature green papaya fruits. The quality parameters assessed included pH, total titratable acidity, total soluble acids, weight loss, firmness and peel colour recorded at five days interval for 10 and 25 days under ambient and cold storage, respectively. Phytochemical profile, vitamin C and soluble sugars were also assessed. The results indicated that storage temperature affected shelf-life of the fruits. Treatment combinations of MSE+CMC and MLE+CMC maintained papaya quality, reduced weight loss, maintained firmness and delayed ripening of fruits as compared to control fruits.

Key words: Edible coatings; *Moringa oleifera*; fruit quality; postharvest storage

3.1 INTRODUCTION

The demand for high quality food and extended shelf life is increasing worldwide, as well as pressure to reduce the use of chemical preservatives (Misir *et al*., 2014). In tropical and subtropical regions, papaya is an important fruit for domestic and export markets (Barrera *et al*., 2015). Papaya fruits are naturally fragile and huge losses occur during storage. The losses are due to the fact that papaya fruit is highly perishable and susceptible to attack by pathogenic microorganisms (Barrera *et al*., 2015). Anthracnose causes the largest amount (up to 30-50%) of deterioration and spoilage in fruits during postharvest storage (Barrera *et al*., 2015). Synthetic chemical fungicides used to control diseases during storage have caused resistance in microorganisms and toxicity to humans (Barrera *et al*., 2015). Hence, the need for development of alternative treatments of non-chemical preservation approaches.

Previous reports indicated that preserved papaya can be maintained for a maximum period of 2 to 4 weeks at postharvest stage at 10 °C (Barrera *et al*., 2015). The controlled low temperature extended shelf life and reduced fungal susceptibility, however, papaya cannot tolerate low temperatures during storage (Pimentel and Walder, 2004). Several preservation methods, such as thermal treatments, storage in modified atmospheres, treatments with watery plant extracts, sodium bicarbonate and edible coatings have been evaluated for their efficacy in prolonging the shelf-life of papaya fruits (Barrera *et al*., 2015). Previous studies have evaluated efficacy of edible coatings to reduce the perishability of papaya (Ali *et al*., 2010; Bill *et al*., 2014; Aloui *et al*., 2014). Edible coatings have been used in several fruits including strawberry (Gol *et al*., 2013), carrot (Ojaghiam *et al*., 2014), avocados (Bill *et al*., 2014) and banana (Maqbool *et al*., 2010). Edible coatings have been reported to maintain fruit firmness, delayed ripening process and maintained quality in banana for up to 33 days (Maqbool *et al*., 2010). The edible coating chitosan has been used in the preservation of papaya fruits (Barrera *et al*., 2015). The chitosan

has the ability to form semi-permeable films that regulate the gaseous exchange, reduce water loss, reduce the production of ethylene and retard maturation (Barrera *et al*., 2015).

Biologically active natural products have the potential to replace synthetic preservatives and fungicides by controlling decay and prolonging the storage life of fruits (Barrera *et al*., 2015). *Moringa oleifera* extracts have properties such as retarding dehydration, suppressing respiration, improving textural quality, helping retain volatile flavour compounds and reducing microbial growth to protect perishable food products from deterioration (Yousef *et al.*, 2015). Carboxy methyl cellulose (CMC) is one of the most common cellulose derivatives and has good film forming property, which can form transparent films with mechanical strength (Dashipour *et al*., 2014; Qi *et al*., 2016). The application of edible coatings incorporated with moringa extracts has the potential to prolong storage life and retain fruit quality. The aim of this study was to evaluate the effect of edible coatings incorporated with moringa extracts on the postharvest quality parameters (weight loss, pH, total soluble solid, titratable acidity, firmness and colour), and secondary metabolites of papaya fruits. The effect of storage conditions (cold and ambient storage) on papaya fruits treated with edible coatings incorporated into moringa extracts was also evaluated in this study.

3.2 Methods and materials

3.2.1 Fruit samples

Papaya fruits (locally known as Papinos) were purchased at the Mkhondeni fresh produce market and were selected based on colour (green). The fruits (156) were transported to the Horticultural laboratory at the University of KwaZulu-Natal, Pietermaritzburg, to conduct the experiment. Fruits were washed under tap water and dried under room temperature. The fruits were labelled with a permanent marker using numbers for experimental purposes.

3.2.2 Experimental design

The fruits were grouped according to the storage conditions treatments (cold and ambient storage). The experiment for each set of temperature conditions was laid out with the following five treatments;

- Control (without any treatment applied)
- Moringa leaf extract incorporated with chitosan (MLE+CH)
- Moringa seed extract incorporated with chitosan (MSE+CH)
- Combination of moringa leaf extract and CMC (MLE+CMC)
- Combination of moringa seed extract and CMC (MSE+CMC)

The treatments were replicated three times and the experiment had six fruits in each replicate. Fruits were coated with specific treatment and were left to dry at room temperature. The fruits were later assigned to different storage conditions. The temperature fruits were stored in the laboratory at 21 °C, which represented ambient storage conditions. The other set of fruits with the same experiment layout, were stored at 10 °C, which represented cold storage conditions.

3.2.3 Physical quality measurements and pH

Quality assessment started from day zero and physical quality parameters were assessed in every treatment and storage condition. One fruit was sampled and replicated three times. The

fruits were peeled, 10 g of the pulp was weighed and mixed with 40 ml of distilled, a stirrer (ULTRATURRAX, IKA® T25 digital, Staufen, Germany) to homogenise the mixture. The mixture was then centrifuged at 6000 rpm for one minute and the supernatant was recovered through filtering with glass wool. Ten millilitres of the filtrates were added into scintillation vials. The pH was measured using a pH metre and values were observed at 5 day intervals for 10 and 25 days under ambient and cold storage, respectively.

3.2.4 Total titratable acidity (TTA)

The fruits were peeled, 10 g of the pulp for each treatment was weighed and homogenised with 40 ml of distilled water using a stirrer (ULTRATURRAX, IKA® T25 digital, Staufen, Germany). The mixture was then centrifuged at 6000 rpm for one minute and the supernatant was recovered through filtering with glass wool. Ten millilitres of the filtrates were added into conical flasks, separately. Two drops of phenolphthalein indicator were titrated against 0.1 N sodium hydroxide (NaOH) until a pink colour was observed. The volume of NaOH titrated was recorded in three replicates at 5 day intervals. Total titratable acidity (TTA) was calculated using the following formula:

$$\% \text{ malic acid} = \frac{v \times N \times equivalent\ factor}{ml}$$

where *v* represent volume titrated, *N* indicate NaOH normality and *ml* represent millilitres of juice. Equivalent factor of the predominant malic acid was 0.067.

3.2.5 Total soluble solids (TSS)

The fruits were squeezed and the juice was tested for TSS using a digital refractometer with a thermodynamic control system (RFM340+ refractometer, Bellingham and Stanley Ltd, Basingstoke, Hants, UK). Few drops were placed on the prism of the refractometer to allow for reading measurements. Total soluble solids of the fruits were expressed in °Brix.

3.2.6 Weight loss

The weight loss was evaluated by using separate samples in three replicates of each treatment and measured using a Mettler Toledo digital balance (+/- 0.00 g). The fruits weight was measured at the beginning of the experiment (i.e. 0 day) and at the end of each storage interval (at day 10 for ambient and day 25 for cold storage). The weight loss was determined by the following formula:

Weight loss (%) = $\frac{initial\ weight - final\ weight}{initial\ weight} \times 100$

3.2.7 Firmness

Fruit firmness was determined using a hand-held firmness tester (Bareiss, Germany) after every 5 days during storage for each treatment. Three readings were taken at the equatorial region of the fruit in a scale of 100 to 0, where 100 represented hard and unripe fruit and, 0 represented soft and overripe fruit. The decrease in scale from 100 showed loss of firmness as fruit ripened.

3.2.8 Peel colour

The colour of the fruits was analysed using a Minolta colorimeter which uses the Munsell colour system specified for three dimensions such as lightness, hue angle and chromaticity. The colour value L* indicates (0 = black and 100 = white), a* represent redness and b* indicates yellowness of the fruit. The hue angle (h^o) or hue is equivalent to (arctan (b*/a*). It represents the fruit colour changes, which ranges from red (0^o), yellow (90^o), and green-blue (180^o) to blue (270^o). Chroma (C*) levels describe the degree of saturation or the intensity of colour. Three readings were taken at the central region of three fruits used per treatment (5 treatments).

3.2.9 Vitamin C

Ascorbic acid concentration was determined according to Bohm *et al*. (2006) with slight modifications. An amount of 0.1 g of the sample was mixed with 0.5 mL of 0.56 M of metaphosphoric acid and then vigorously shaken. The mixture was centrifuged at 2988 g and the supernatant was transferred into a volumetric flask. This procedure was repeated twice, the

extracts were combined made to a final volume of 20 mL using 0.56 M metaphosphoric acid. Accurately, 0.200 µL of the extract was mixed with 0.300 µL of 0.3 M of trichloroacetic acid and the mixture was centrifuged at 17212 g for 10 minutes. About 300 µL of aliquots were mixed with 100 µL of 2, 4-dinitrophenylhydrazine reagent (0.013 M in 30 % perchloric acid), heated to 60 °C for 1 hour and cooled for 5 min in an ice bath. Then, 400 µL 15.75 M sulphuric acid was added to the sample and the absorbance read at 520 nm after 20 min using a UV-Vis spectrophotometer (UV-1800 Shimadzu Corporation, Kyoto, Japan). The ascorbic acid concentration was calculated by comparison of the values obtained with an L-ascorbic acid standard curve.

3.2.10 Determination of Flavonoid content

The total flavonoid content (TFC) was determined by the colorimetric method described by Abu Bakar *et al*. (2009) with slight modifications. A total of 0.5 mL of the extract of freeze-dried and powdered samples was mixed with 2.25 mL of distilled water in a test tube. An extract was replaced with water in one of the test tubes to serve as a control (blank). An amount of 0.15 mL (5% $NaNO_2$ solution) was added followed by 0.3 mL (10% $AlCl_3 \cdot 6H_2O$ solution) added after 6 minutes. The reaction was allowed to proceed for another 5 minutes before 1.0 mL of 1 M NaOH was added. The mixture was vortexed, and the absorbance was measured immediately at 510 nm using a UV-Vis spectrophotometer (UV-1800 Shimadzu Corporation, Kyoto, Japan). The results were expressed as grams of quercetin equivalents (QE) per 100 g of fresh sample (mg QE/100 g of DW).

3.2.11 Determination of Total phenolic content

Two grams of freeze-dried and powdered samples were extracted with 10 ml of 80% v/v of methanol in H_2O. The mixture was homogenised using a stirrer (ULTRATURRAX, IKA® T25 digital, Staufen, Germany), transferred into a glass tube and sealed with aluminium foil. The mixture was incubated at 45 °C for 1 hour in a shaking water bath. The extracts were filtered

through glass wool and kept at -60 °C for total phenolic content analysis. A sample of 0.1 mL of crude extract solution was placed in a test tube. Water served as a control (blank) and a sample of 0.1 mL of distilled was placed in the test tube. Afterwards, 0.5 ml of undiluted Folin–Ciocalteau reagent was added to the mixture. A sample of 1.5 mL of saturated sodium carbonate was added to the mixture after 30 seconds and lest to stand for 8 minutes. A sample of 0.9 mL of water was added to the solutions to give a final volume of 10 mL. The mixture was then vortexed and incubated at 40 °C for 2 hours. The absorption of total phenolics was determined at 765 nm using a UV-Vis spectrophotometer (UV-1800 Shimadzu Corporation, Kyoto, Japan). Total phenolic content was determined against the standard gallic acid calibration curve and the absorbance value was converted to gallic acid equivalents (GAE) per gram of fresh weight (mg GAE g^{-1} DW).

3.2.12 Antioxidant activity

3.2.12.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Based on the method of Musa *et al*. (2011), the antioxidant activity was assessed using the DPPH radical scavenging activity assay. The stock solution was obtained by dissolving 40 mg DPPH in 100 mL methanol and stored at -20 °C until analysis. Approximately 350 mL stock solution was mixed with 350 mL methanol to obtain the absorbance of 0.70±0.01 at 516 nm using a UV-Vis spectrophotometer (UV-1800 Shimadzu Corporation, Kyoto, Japan). In the dark, approximately 100 µL of papaya extracts with 1 mL of prepared methanolic DPPH solution was stored overnight for scavenging reaction. The percentage of DPPH scavenging activity was determined based on the following equation:

DPPH scavenging activity (%) = $\frac{A\ blank - A\ sample}{A\ blank} \times 100$

Where: A blank is the absorbance for control.

A sample is the absorbance for the test sample.

3.2.12.2 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay

The ABTS radical cation was generated by the interaction of ABTS (250 μM) and $K_2S_2O_8$ (40 μM). After the addition of 990 μL of ABTS solution to 10 mL of fruit extract, the absorbance was measured at 734 nm using UV-Vis spectrophotometer (UV-1800 Shimadzu Corporation, Kyoto, Japan). The percentage decrease of the absorbance was calculated.

Percentage of reduction power = $\frac{A\ blank - A\ sample}{A\ blank} \times 100$

Where: A blank is the absorbance for control.

A sample is the absorbance for the test sample.

3.2.13 Determination of soluble sugars

An amount of 0.1 g of freeze-dried material was weighed and 10 mL 80% v/v of ethanol in water was added and homogenised for 1 minute using a stirrer (ULTRATURRAX, IKA® T25 digital, Staufen, Germany). The solution was incubated for 1 hour in a shaking water bath set at 80 °C. The solution was stored in a refrigerator at 4 °C for 24 hours. The solution was centrifuged at 10 000 rpm (11953 g) for 15 minutes in refrigerated centrifuge at 4 °C and was then filtered through glass wool. The solution was dried overnight in Savant Vacuum drier (Genvac) and the dried extracts were diluted with 2 mL of Ultra-pure water. The supernatant was centrifuged at 10 000 rpm for 5 minutes and filtered through glass wool and filtered through 0.45 μm nylon filters and analysed using an HPLC-RID (high-performance liquid chromatography–refractive index detector) system (liquid chromatography (LC-20AT), Shimadzu Corporation, Kyoto, Japan) equipped with a refractive index detector (refractive index detector [RID-10A], Shimadzu Corporation, Kyoto, Japan) and a Rezex Monosaccharide column (300×7.8 mm) (8-micron pore size; Phenomenex®, Torrance, California, USA). The total soluble sugar content were separated into individual hexoses, alcohols, mannoheptulose, perseitol and compared with authentic sugar standards.

3.2.14 Statistical analysis

Data was subjected to analysis of variance (ANOVA) using GenStat 18th Edition (VSN International) under 5% levels of significance. The Duncan's multiple range tests was used to present significant difference between treatment means.

3.3 Results and Discussion

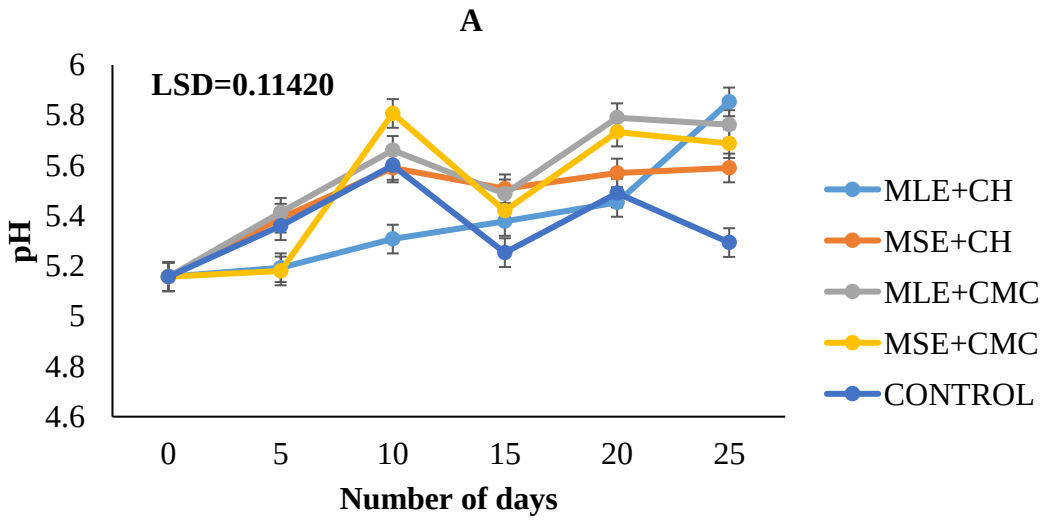
3.3.1 Effect of edible coatings on pH

Edible coatings and the duration of storage had a significant effect ($p<0.05$) on pH of papaya fruits (Figure 3-1 A and B). The pH values increased in all treatments with storage time for both cold and ambient storage conditions. Treatments MLE+CMC and MSE+CMC had the highest pH values compared to other treatments under both storage conditions. On day 10, treatment MLE+CMC exhibited higher pH values for cold and ambient storage (5.66 and 5.85, respectively). Treatment MLE+CMC also showed higher pH (5.79) at day 20 of cold storage conditions. MSE+CMC had pH values of 5.80 and 5.82 on day 10 of cold and ambient storage, respectively. Previous reports have indicated a significant increase in pH values in both coated and uncoated papaya fruits during storage time (Brishti *et al*. 2013; Singh *et al*. 2012b; Al Eryani-Raqeeb *et al*. 2008). Oluwaseun *et al*. (2013) reported a gradual increase in pH of cucumber during storage. The pH values ranged between 5.02 and 5.85 in the current study and are comparable to the values (5.0-5.8) reported by Azene *et al*. (2014) in papaya fruits that were stored in the evaporative cooler.

On day 15 of cold storage, control showed lower pH values of 5.25, a lower pH value of 5.29 was also recorded on day 25. On day 10 under ambient storage conditions, the pH value of 5.63 was recorded. The higher pH values observed for treated fruits could be associated with reduced respiration rate than in control fruits, and the finding is consistent with the report by Oluwaseun *et al*. (2013). The higher pH value in treated fruits can be attributed to modification of internal atmosphere such as the endogenous CO_2 and O_2 concentration of the fruit (Al Eryani-Raqeeb *et al*., 2008; Oluwaseun *et al*., 2013).

In this study, the pH values were higher under ambient storage conditions at day 10 compared to those obtained under cold storage conditions. The results contradict with earlier findings which reported that pH values decreased in papaya fruits that were stored under ambient

storage conditions (Nunes *et al*., 2006). High storage temperature leads to faster respiration rate which is responsible for acid production in papaya fruits (Azene *et al*., 2014). Azene *et al*. (2014) also reported increased production of acids from catabolism of sugar in papaya fruits at faster rate under ambient storage than in the evaporative cooler condition. Therefore, low temperature is crucial in papaya fruits to prevent increased respiration rate, which can lead to high production of acids (Azene *et al*., 2014). Lowering the storage temperature can also delay the senescence of papaya fruits. In this study, papaya fruits stored under ambient storage conditions lasted for up to 10 days compared to 25 days under cold storage conditions. Treatment MSE+CMC showed significant effect throughout the storage conditions (ambient and cold storage conditions). MLE+CMC showed significant effect from day 15 up to day 25 of the cold storage conditions, whereas it showed significant effect throughout the ambient storage conditions. Therefore, MSE+CMC can be used to preserve papaya quality for up to 25 days under cold storage and for up to 10 days under ambient storage. MLE+CMC can be used to preserve papaya fruit quality for up to 10 days under ambient storage conditions.



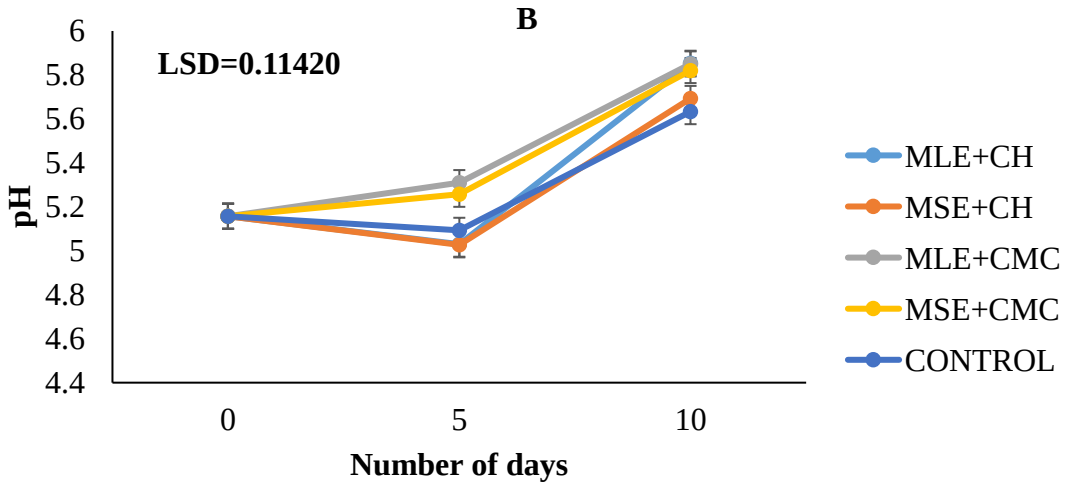


Figure 3-1: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with carboxymethylcellulose (MSE+CMC) and control on pH values of papaya fruits stored under (A) cold and (B) ambient storage conditions.

3.3.2 The effect of edible coatings on titratable acidity

Edible coatings and storage had a significant effect ($p<0.05$) on titratable acidity of papaya fruits. Fruits under cold storage had lower TTA values compared to fruits kept under ambient storage conditions. The TTA values in fruits stored at cold storage ranged from 0.09 and 0.29 (% malic acid), whereas TTA values under ambient storage ranged from 0.12 and 0.31 (Figure 3-2 A and B). All treatments were significant different from control on day 5 and day 25 of cold storage. On day 10 under cold storage conditions, all treatments showed significant difference from control, except for MLE+CMC. On day 5 under ambient storage conditions, all treatments were significant different from control, except for MLE+CMC. On day 10 under ambient storage conditions, All treatments, except for MSE+CH, were significant different from control. On day 20 under cold storage, treatment MSE+CMC recorded a TTA value of

0.09 which was relatively low compared to other treatments. Under cold storage, control had relatively higher TTA values compared to other treatments. Generally, titratable acidity of papaya fruits in all treatments showed an increasing trend under both cold and ambient storage conditions, followed by a decreasing trend, with exception of MLE+CMC under ambient storage (Figure 3-2 A & B). The results of this study agree with earlier findings as it was reported that TTA amount in papaya fruits in all treatments increased, then decreased (Azene *et al.,* 2014; Singh *et al.,* 2012b; Al Eryani-Raqeeb *et al.,* 2008). Singh *et al.* (2012b) reported decreased amounts in acidity of the fruits during storage in fruits wrapped with paddy straw. The results of this study are in agreement with Marpudi et al. (2011) findings that titratable acidity decreased in both treated and control fruits. The malic acid content decreases during ripening (Bron and Jacomino 2009; Othman, 2009). Nunes *et al.* (2006) reported a decrease in TTA content during handling irrespective of the temperature regime.

Storage conditions had a significant effect ($p<0.05$) on TTA content of papaya fruits. The lower TTA values in coated fruits under cold storage could be attributed to the reduced rate of respiration, which results in slow production of acids due to carbohydrate catabolism (Azene *et al.,* 2014). The lower TTA values for control under ambient storage conditions could be associated with the depletion of organic acids due to relatively faster respiration and ripening rate of fruits (Azene *et al.,* 2014). The decrease in TTA amount in treated papaya fruits might be attributed to the delay of respiration by edible coatings. Edible coatings delay respiration by modifying the atmosphere in and out side of the fruit, resulting in retardation of consumption of respiration substrates such as organic acids and sugars (Azene *et al.,* 2014). As the fruit respires, the level of O_2 decreases and the CO_2 level increases consequently as the atmosphere is modified (Azene *et al.,* 2014). High acidity in papaya fruits contributes in part to the flavour retention of ripened fruit. Under modified atmosphere, the respiration rate of the fruit decreases, which results in reduction of acidity.

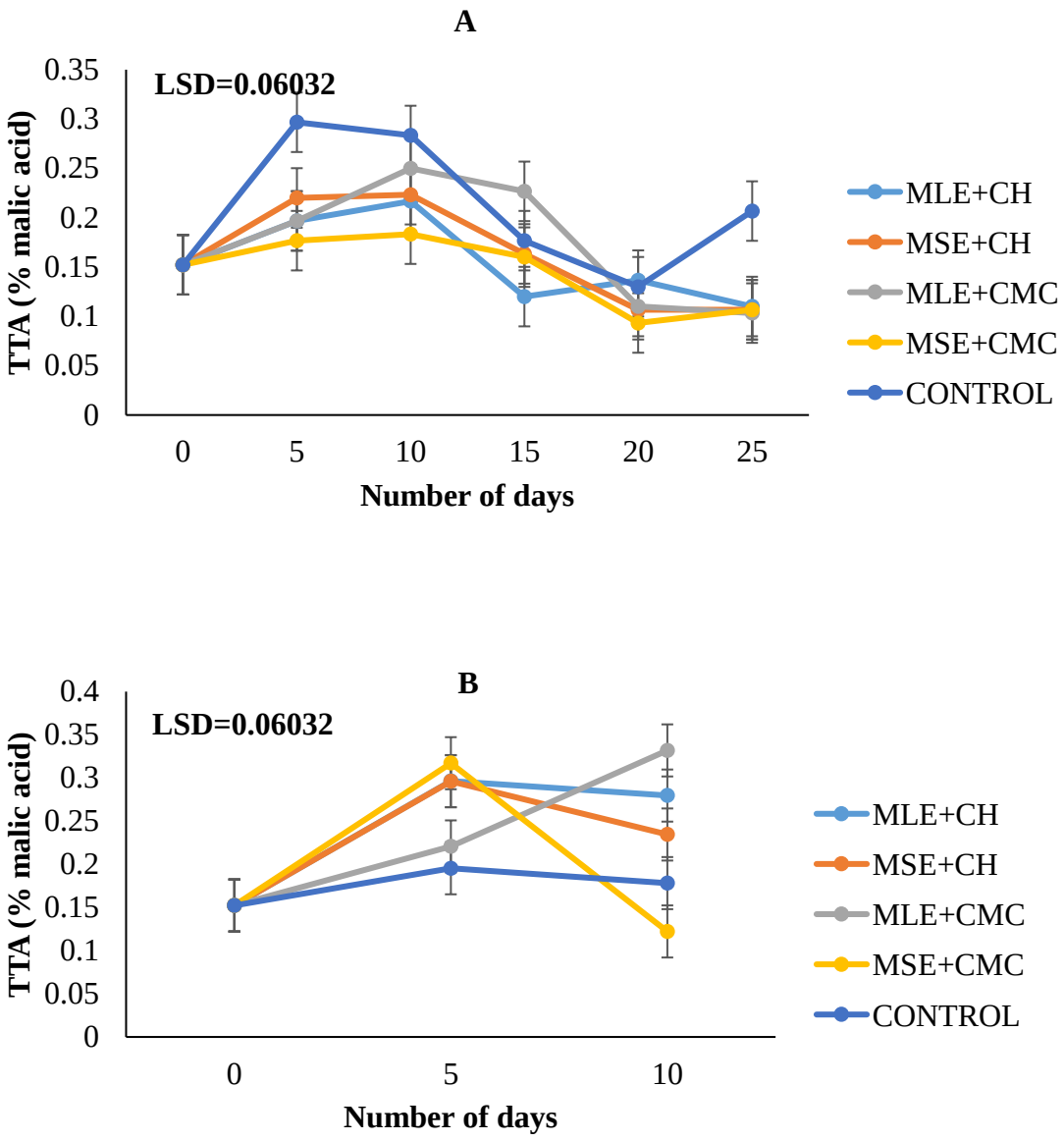


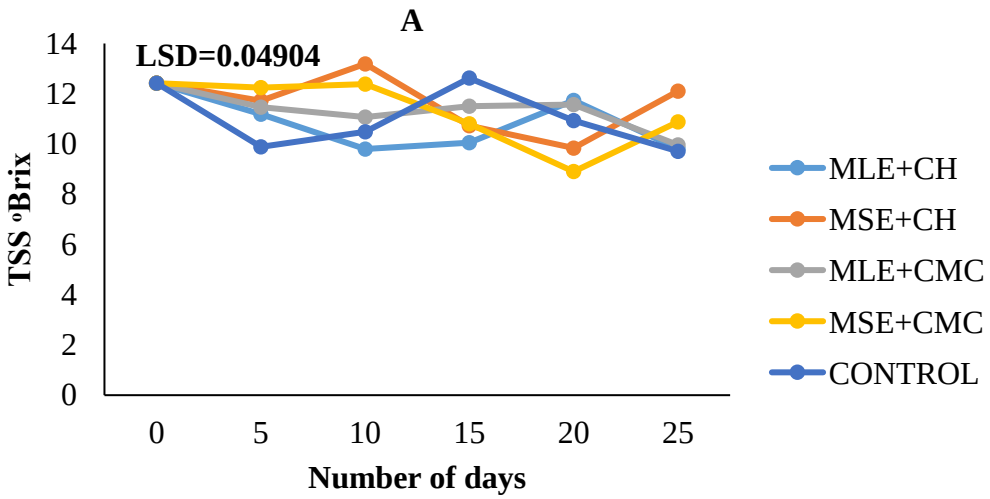
Figure 3-2: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with carboxymethylcellulose (MSE+CMC) and control on total titratable acidity (TTA) of papaya fruits stored under (A) cold and (B) ambient storage conditions.

3.2.3 Effect of edible coatings on total soluble solids (TSS)

Edible coatings, storage duration and storage conditions (ambient and cold) had a significant effect ($p<0.05$) on total soluble solids of papaya fruits. The TSS content decreased in all treatments in all storage conditions. Treatment MSE+CH recorded significantly high TSS content (13.19 °Brix) after 10 days under cold storage conditions. The control treatment showed lower TSS content (9.89 °Brix) after 5 days under cold storage compared to other treatments. The TSS content showed an increasing trend, then decreased (Figure 3-3 A and B). The TSS content ranged from 9.71 to 13.19 °Brix and control showed significantly lower TSS content (9.71 °Brix) at day 25 under cold storage conditions. Whereas, MSE+CH recorded significantly high TSS content (13.19 °Brix) at day 10 under cold storage. Treatments MSE+CH and MSE+CMC maintained higher TSS content from day 0 to 10 days under cold storage and decreased considerably at ripening. It was significant that after fruits were transferred to ambient conditions, they exhibited higher TSS content (Figure 3-3 B). At day 5 under ambient storage conditions, control had significantly higher TSS content (12.82 °Brix) compared to other treatments. Treatment MLE+CMC showed significantly lower TSS content under ambient storage conditions with a content of 9.87 and 9.28 °Brix at day 5 and day 10, respectively (Figure 3-3 A and B). Under ambient storage condition, coated fruits had lower TSS content compared to control on day 5 (Figure 3-3 B). This could be attributed to higher temperature and free access to O_2 which increases transpiration rate that accelerates ripening, resulting in faster conversion of starch to soluble sugars (Azene *et al*., 2014). An increase in TSS for control treatments fruits due to progressive boost in free sugars of fruit during storage periods has been reported (Brishti *et al*., 2013). The coatings are known to retard TSS development through decrease in respiration (Brishti *et al*., 2013; Sharmin *et al*., 2015).

Generally, TSS content was fluctuating with a notable decrease during storage . Oluwaseun *et al*. (2013) reported that loss of soluble solids during storage is as natural as sugars which are

the primary constituent of the soluble solids content of a product, consumed by respiration and used for the metabolic activities of the fruit. A decrease in total soluble solids content after 30 days of storage under ambient storage conditions has been reported (Martins and Resende, 2012). Nunes *et al*. (2006) reported that irrespective of the temperature regime, TSS content decreased during handling. In contrast, previous reports showed increased TSS in treatments with smaller increase in concentration of a bio-preservative compared to control (Brishti *et al*., 2013; Sharmin *et al*., 2015). The results of this study indicate that Treatments (MLE+CH, MSE+CH, MLE+CMC and MSE+CMC) had significant effect throughout storage conditions (ambient and cold storage), therefore, they can be used to preserve papaya fruit quality for up to 25 days under cold storage and up to 10 days under ambient storage.



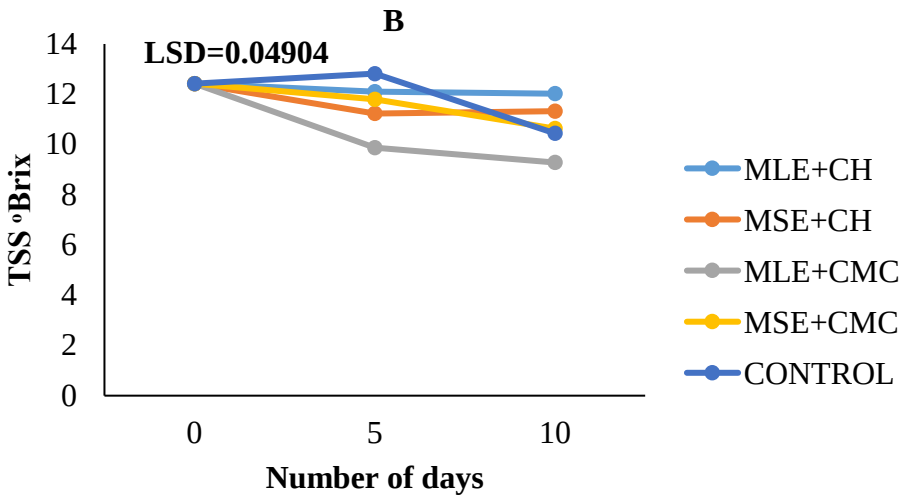


Figure 3-3: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with carboxymethylcellulose (MSE+CMC) and control on total soluble solids (°Brix) of papaya fruits stored under (A) cold and (B) ambient storage conditions.

3.2.4 Effect of edible coatings on weight loss in papaya fruits at postharvest

Edible coatings had a significant effect ($p<0.05$) on weight loss of papaya fruits. There was a progressive weight loss in papaya fruits during ripening. A sharp increase in weight loss was observed when fruits were transferred to ambient storage conditions. All coated fruits had lower weight loss compared to the controls, and fruits coated with moringa extracts incorporated with CMC had the lowest weight loss compared to fruits coated with moringa extracts incorporated with CH. The highest loss (15.14%) was observed on day 25 for the control. The lowest weight loss was observed for treatment MSE+CMC (1.09%) on day 5. An increase in weight loss that was greater in untreated than treated fruits has been reported (Sogvar *et al.*, 2016). The previous report indicated up to 21.3% loss in untreated fruits

compared to 18.1% in aloe vera, and 12.6 % in aloe vera combined with ascorbic acid-treated fruits (Sogvar *et al*., 2016).

Weight loss under the combined treatments (chitosan and calcium) was consistently lower throughout the storage period (Al Eryani-Raqeeb *et al*., 2008). Plainsirichai *et al*. (2014) reported that chitosan maintained the weight of rose apples under water loss and increased resistance to water vapour transmission. The dense structure of chitosan films makes them very effective gas barriers (Plainsirichai *et al*., 2014). Weight loss of fruits under cold storage conditions ranged from 1.09 to 15.14%, and 2.83 to 6.96% under ambient storage conditions. The percentage weight loss was delayed by 5 days when fruits were stored under cold storage compared to those stored under ambient storage conditions. High temperature stimulated transpiration and respiration processes, which are amongst factors that cause water loss from fruit resulting in loss of weight in fruits (Al Eryani-Raqeeb *et al*., 2008). The weight loss of papaya fruits is the result of fruit dehydration that occurs due to changes in surface transfer resistance to water vapour and changes in respiration rate (Al Eryani-Raqeeb *et al*., 2008). The occurrence of small fissures connecting the internal and external atmospheres also result in weight loss (Al Eryani-Raqeeb *et al*., 2008). The water loss might happen through the stem scar, the stomata and cuticle, and the amount of water lost depends on cuticle thickness (Ong *et al*., 2013). However, the cuticle thickness also depends on cultivar and fruit maturity at harvest (Ong *et al*., 2013). An increase in weight loss as the fruit ripens during storage can be attributed to the cuticle changes when fruits turns from green to half yellow (Ong *et al*., 2013). Previous reports attributed water lost to water pressure gradient between the fruit tissue and the surrounding atmosphere. Moringa extracts incorporated with CMC and CH showed a significant effect, therefore, they can be used to prevent weight loss in papaya fruit. Edible coatings were effective in providing barrier to moisture loss, hence, the observed retarding dehydration and shrivelling of the fruit (Al Eryani-Raqeeb *et al*., 2008). A report by Sogvar *et*

al. (2016) indicated that the benefits of edible coatings depend on their hygroscopic properties to enable formation of a water barrier between the fruit and the environment (Sogvar *et al*., 2016).

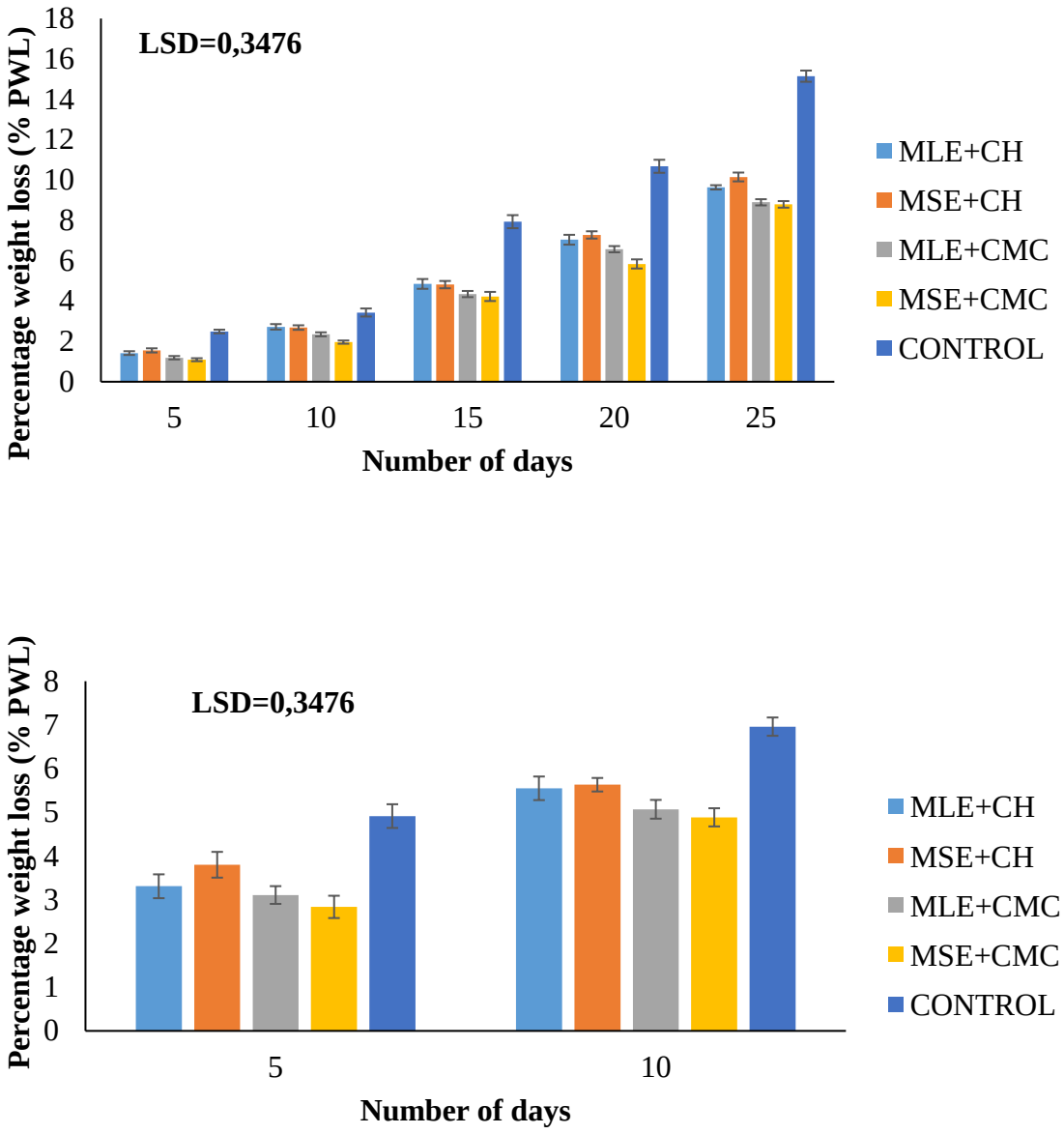


Figure 3-4: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with

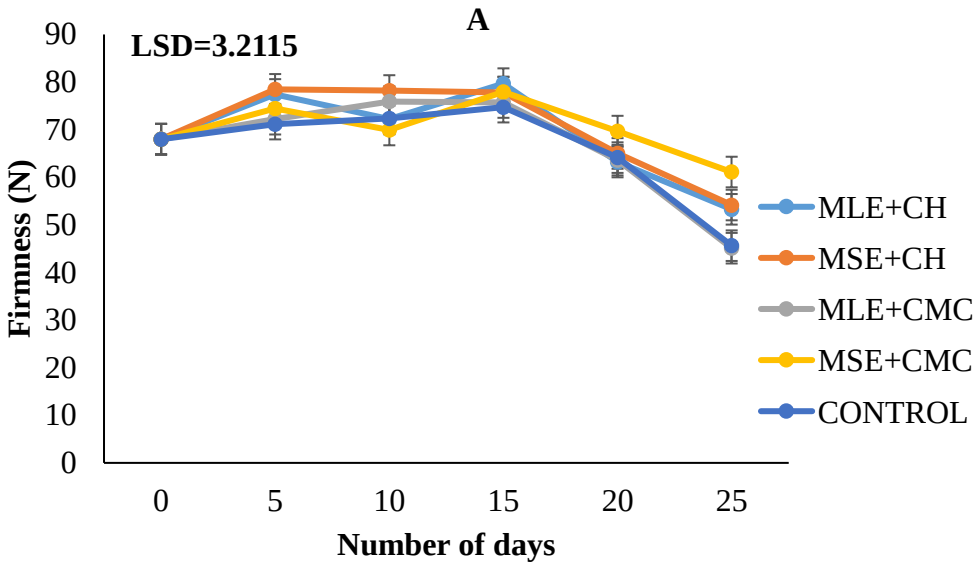
carboxymethylcellulose (MSE+CMC) and control on weight loss of papaya fruits stored under (A) cold and (B) ambient storage conditions.

3.2.5 Effect of edible coatings on papaya fruit firmness

Edible coatings had significant effect ($p<0.05$) on firmness of papaya fruits. Firmness decreased under all storage conditions and treatments (Figure 3-5 A and B). Fruits coated with MSE+CMC had significantly higher fruit firmness (ranging from 77.93 to 61.1N) compared to other treatments from day 20 to 25 under cold storage conditions (Figure 3-5 A). Treatment MSE+CH had the highest firmness from day 0 to 15 under cold storage (Figure 3-5 A). On day 5 under ambient storage conditions, treatments MLE+CMC and MSE+CMC showed higher firmness (55.17N and 54N, respectively), and also showed higher firmness on day 10 (MLE+CMC had 47.5N and MSE+CMC had 49.23N). Oluwaseun *et al*. (2013) reported that corn starch (CS) and CMC had an effect on the reduction of cell wall degrading-enzymes responsible for softening. The results of this study shows that treatments had no significant effect compared to control under ambient storage conditions. Under cold storage, MSE+CH was significantly different from control at day 5 and day 25. Treatments MSE+CMC and MLE+CH showed significant effect on day 25 of cold storage.

Loss of firmness is one of the main parameters that limit quality and postharvest shelf-life of fruits and vegetables (Al Eryani-Raqeeb *et al*., 2008; Oluwaseun *et al*., 2013). Generally, as the storage time progressed, the fruits soften, mainly due to degradation of pectins, cellulose and hemicellulose polysaccharides takes place during ripening (Azene *et al*., 2014). Fruit softening mainly occurs due to degradation of the middle lamella of the cell wall of cortical parenchyma cells, which considerably occurs during ripening due to enzyme activity on carbohydrate polymers (Al Eryani-Raqeeb *et al*., 2008; Azene *et al*., 2014). The differences in respiration rate that affect solubility and depolymerisation of pectins during ripening justifies

the differences in decreased firmness of papaya fruits in different treatments. Cell wall strength, cell to cell contact and cellular turgor are other characteristics that influence fruit firmness. The higher firmness of fruits under cold storage could be attributed to the presence of higher relative humidity and lower temperature which retard the transpiration and respiration rate of the fruits (Azene *et al*., 2014). Under ambient storage conditions, rapid loss of firmness of papaya is associated with increase in activity of polygalacturonase, pectin methyl esterase, β-galactosidase as well as with depolymerisation of cell wall pectins (Azene *et al*., 2014; Oluwaseun *et al*., 2013). Under ambient storage, treatments had no significant effect.



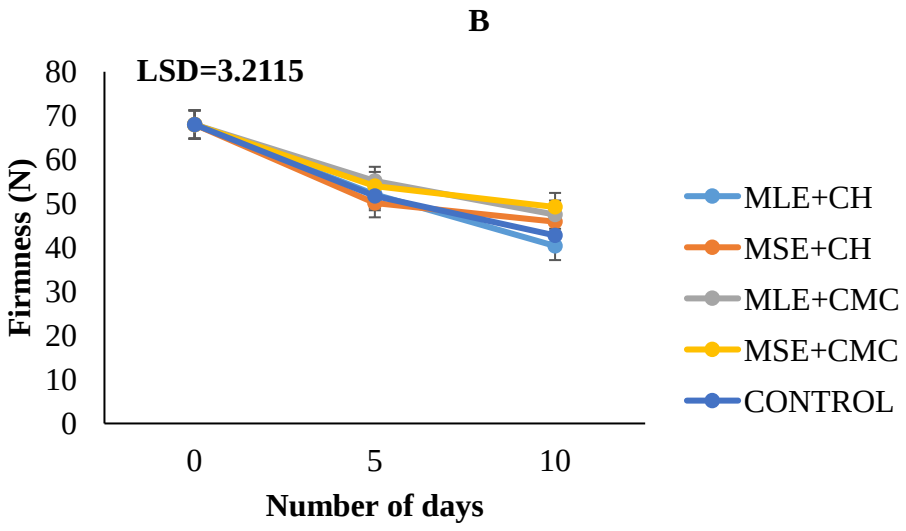


Figure 3-5: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with carboxymethylcellulose (MSE+CMC) and control on firmness of papaya fruits stored under (A) cold and (B) ambient storage conditions.

3.2.6 Effect of edible coatings on peel colour parameters

Edible coatings had no significant effect ($p<0.05$) on luminosity (Hunter L*) of papaya fruits. The Hunter L* values increased in all treatments under both storage conditions (ambient and cold) (Figure 3-6 A and B). The treatment and storage interaction show significant difference ($p<0.05$) on luminosity of papaya fruits. The luminosity values of papaya fruit ranged from Hunter L* 61.25 to 74.62 under ambient storage, while values ranged from Hunter L* 56.53 to 71.10 under cold storage (Figure 3-6 A and B). On day 5, treatment MSE+CMC showed the highest Hunter L* value of 62.51, while the control exhibited the lowest luminosity, Hunter L* value of 56.53. On day 10, treatment MLE+CMC showed the highest Hunter L* value of 68.57, while treatment MSE+CH showed lowest Hunter L* value of 63.44. On day 15, the treatment MLE+CMC showed significant effect and recorded highest Hunter L* value, while treatment MSE+CH showed the lowest (Hunter L* 70.94 and 59.36, respectively). On day 20, Treatment MLE+CH exhibited the highest Hunter L* value, with traetment MSE+CMC showed the lowest value (Hunter L* 71.10 and 61.95, respectively). The results showed Hunter L* values of 70.89 and 65.31 on day 25 for treatments MLE+CH and MSE+CMC, respectively.

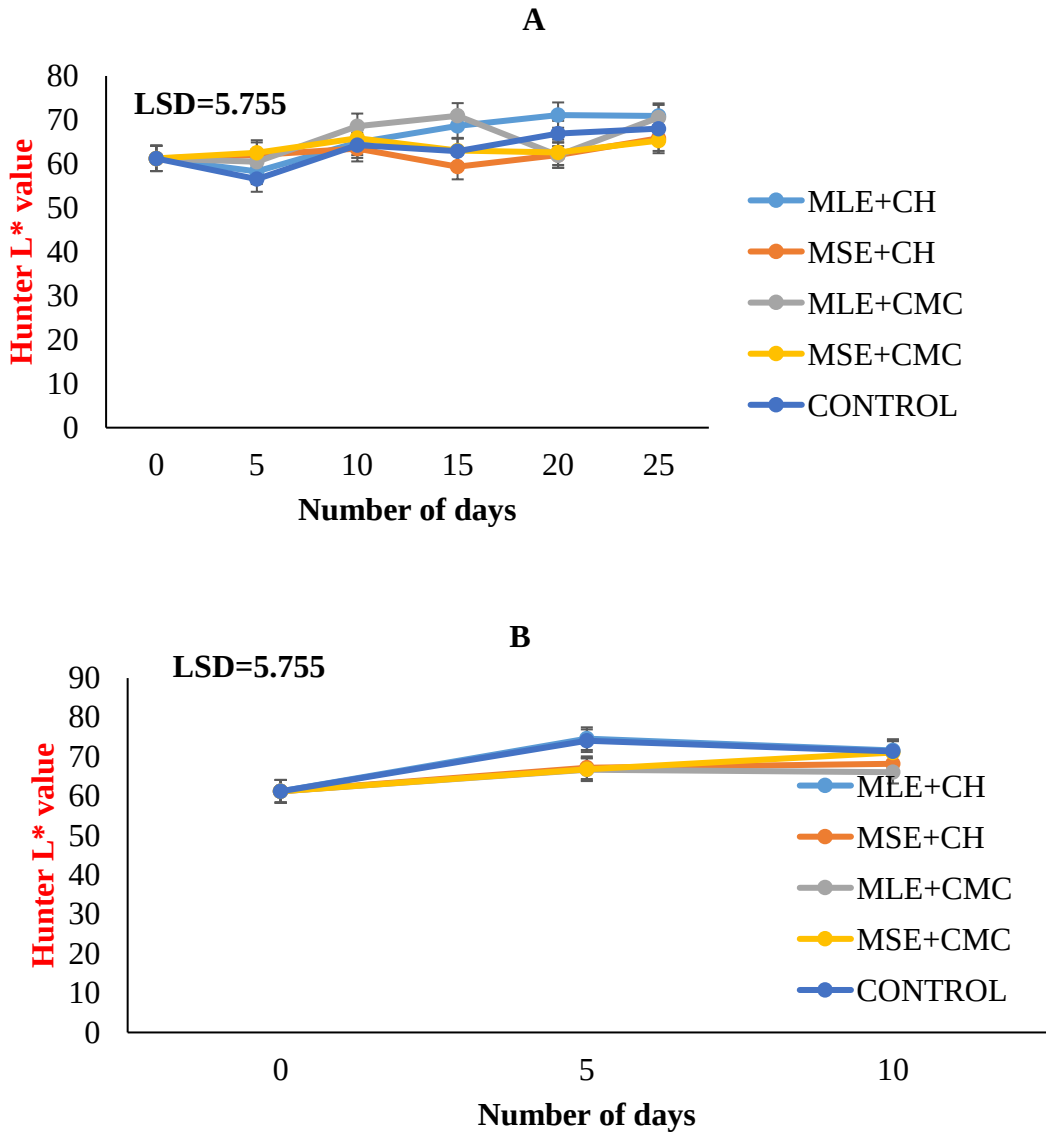


Figure 3-6: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with carboxymethylcellulose (MSE+CMC) and the control on Hunter L* values of papaya fruits stored under (A) cold and (B) ambient storage conditions.

On day 5 under ambient storage conditions, treatment MLE+CH and the control showed no significant difference in Hunter L* values 74.62 and 74.09, respectively. They also showed no significant difference on day 10 as MLE+CH exhibited Hunter L* 71.60 and control exhibited

Hunter L* 71.41. The treatment MLE+CMC reported the lowest Hunter L* values at 66.72 and 66.11 on day 5 and 10, respectively. The green colour of the fruit skin gradually decreased with advancing storage period and turned to yellow as the values of Hunter L*, b* and C* increased. Pereira *et al*. (2009) reported that Hunter L* values increased with maturation and the average values were above 50. The results showed that MLE+CMC, MSE+CMC and MSE+CH were significantly different from control on day 5 of ambient storage. Under cold storage, MLE+CMC was significantly different from control on day 15. Therefore, the results indicate that MLE+CMC, MSE+CMC and MSE+CH were effective for 5 days in delaying papaya fruits from turning yellow.

Edible coatings, duration and condition of storage had a significant effect ($p<0.05$) on Hunter a* values of papaya fruits. The Hunter a* values increased in all treatments under both cold and ambient storage conditions (Figure 3-7 A and B). The results exhibited negative Hunter a* values throughout cold storage. Under cold storage conditions, the Hunter a* values in treatments MLE+CMC, MLE+CH and MSE+CH and the control increased steadily reaching zero after day 25 of storage. Whereas, the treatments and the control at ambient storage reached zero after day 5 of storage. The results of this study are in agreement with Basulto *et al*. (2009) findings that showed negative Hunter a* values throughout the 15 days of storage. The negative Hunter a* values depicts the green colour in green fruits, and positive Hunter a* values depicts the red colour which indicates the initiation of ripening (Basulto *et al*., 2009). The Hunter a* values under ambient temperature ranged from -10.76 to 12.31, and -10.76 to 7.73 under cold storage. Treatments MSE+CH and MSE+CMC maintained fruits greenness from day 15 to 20 under cold storage (Figure 3-7 A). On day 15, MSE+CH and MSE+CMC recorded Hunter a* values of 13.45 and -11.70, respectively. On day 20, MSE+CH and MSE+CMC recorded Hunter a* values of -11.59 and -12.01, respectively. Under cold storage conditions, treatment

MSE+CMC maintained (a* value of -5.36) fruit greenness compared to other treatments at day 25. Treatment MSE+CMC showed significant effect on day 20 and day 25 of cold storage, and on day 10 of ambient storage. Treatment MLE+CH showed significant effect on day 5 of cold storage, whereas MLE+CMC showed significant effect at day 25 of cold storage.

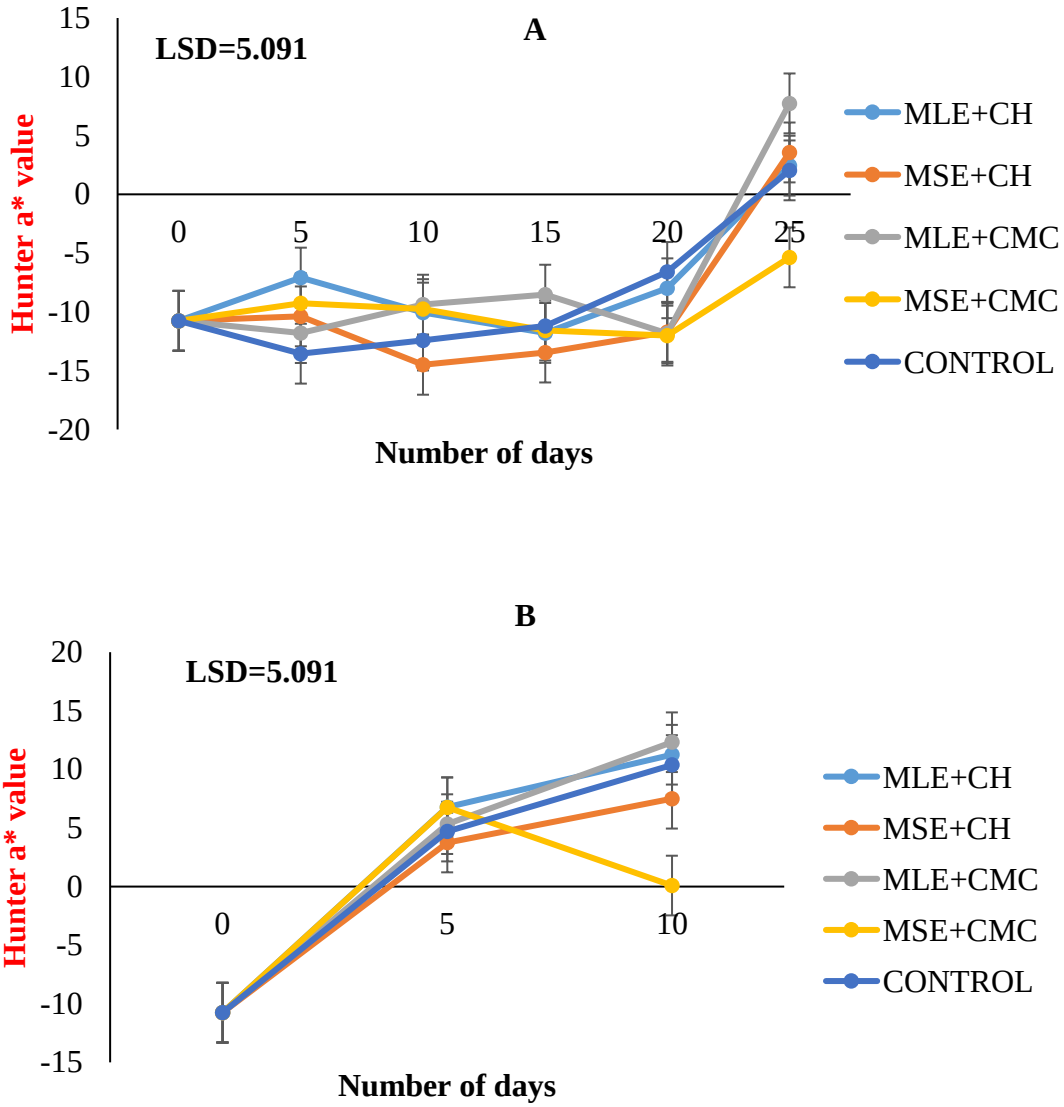
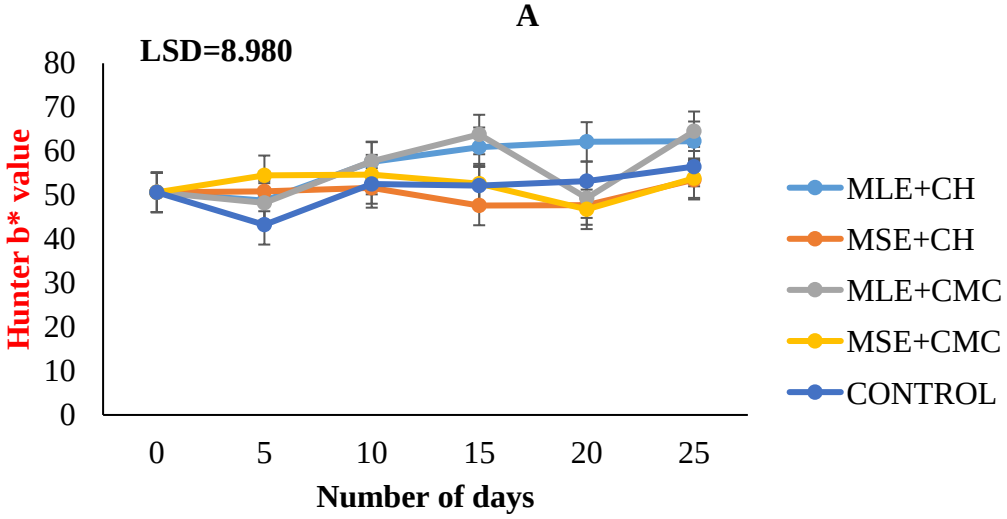


Figure 3-7: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with

carboxymethylcellulose (MSE+CMC) and control on Hunter a* values of papaya fruits stored under (A) cold and (B) ambient storage conditions.

Edible coatings had no significant effect ($p<0.05$) on Hunter b* values of papaya fruits. The Hunter b* values increased in all treatments and under both storage conditions (Figure 3-8 A and B). The Hunter b* values ranged from 50.63 to 66.08 under ambient storage, while under cold storage the Hunter b* values ranged from 43.25 to 64.53. Storage duration had no significant effect ($p<0.05$) on the Hunter b* values of papaya fruits. On day 5 under cold storage, treatment MSE+CMC showed significant effect and recorded the highest Hunter b* value (54.52), while the control had the lowest Hunter b* value (43.25). On day 10, treatment MLE+CMC exhibited the highest Hunter b* value (57.68), while treatment MSE+CH showed the lowest Hunter b* value (51.64). On day 15, treatment MLE+CMC showed significant effect and recorded the highest Hunter b* value, while treatment MSE+CH showing the lowest Hunter b* value (63.79 and 47.64, respectively). On day 20, treatment MLE+CH showed the highest Hunter b* value, while treatment MSE+CMC had the lowest Hunter b* value (62.11 and 46.77, respectively). On day 25, treatment MLE+CMC exhibited the highest Hunter b* value (64.53), with treatment MSE+CH showing the lowest Hunter b* value (53.51).



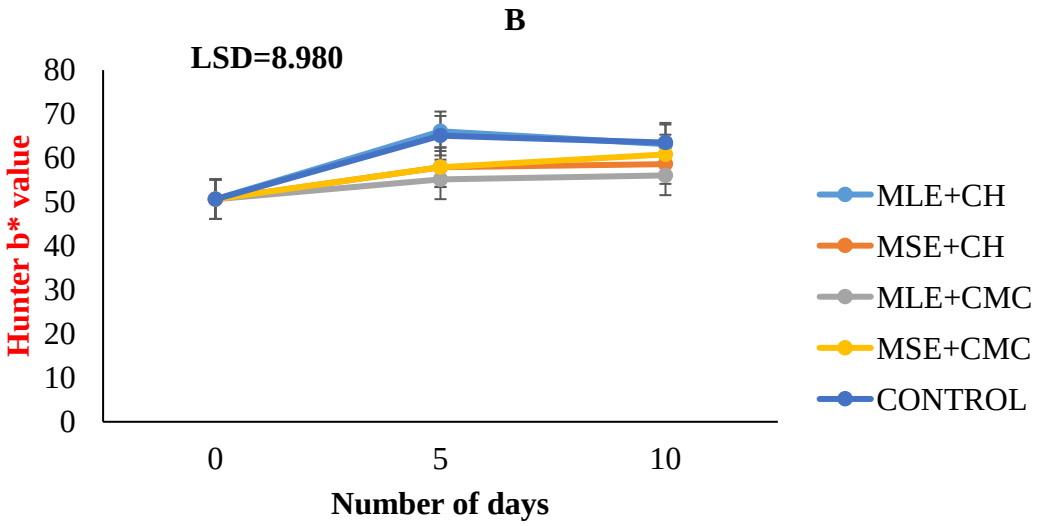


Figure 3-8: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with carboxymethylcellulose (MSE+CMC) and control on Hunter b* values of papaya fruits stored under (A) cold and (B) ambient storage conditions.

On day 5, treatment MLE+CMC showed significant effect and recorded the lowest Hunter b* value 55.13 under ambient storage conditions. The treatment also showed lowest Hunter b* value 56.04 on day 10. The reults showed no significant difference between treatment MLE+CH and the control. On day 5, the Hunter b* values were 66.08 and 65.08, and Hunter b* values were 63.13 and 63.50 on day 10, respectively. Generally, yellowness increased with increasing storage period, indicating the ripening ability of the fruit. Treatments MLE+CH, MLE+CMC and the control showed more yellow colour followed by treatments MSE+CH and MSE+CMC under cold storage conditions (Figure 3.8 A). Under ambient storage conditions, treatment MLE+CH and the control had higher yellow colour values compared to other

treatments. Treatment MSE+CMC maintained slow green to yellow colour evolution, which is evident in a relatively low decrease in hue angle values and increase in Hunter a* values.

Edible coatings had a significant effect ($p<0.05$) on Hue angle values of papaya fruits. The hue angle values decreased in all treatments under cold and ambient storage conditions (Figure 3-9 and Figure 3-10). Storage duration had a significant effect ($p<0.05$) on Hue angle values of papaya fruits. Hue angle values decreased, ranging from 103.45 to 77.83 under ambient storage conditions, while under cold storage values were between h° 107.52 initially to h° 83.10 after fruits were transferred to ambient storage conditions. On day 5, the control had relatively higher hue angle value and treatment MLE+CH recorded the lowest values compared to other treatments. Treatment MLE+CH and MSE+CMC showed significant effect on day 5 of cold storage. On day 10, treatment MSE+CH exhibited relatively higher hue angle value (h° 105.72), while treatment MLE+CMC showed the lowest hue angle value (h° 99.51). On day 15, treatment MSE+CH had the highest hue angle value, whereas treatment MLE+CMC recorded the lowest hue angle value. On day 20, treatment MSE+CH showed relatively higher hue angle value, while the control showed low h° value (104.70 and 97.04 respectively). On day 25 of cold storage, treatment MSE+CMC showed significant effect and had the highest hue angle value and treatment MLE+CMC recorded the lowest h° values (96.12 and 83.10, respectively).

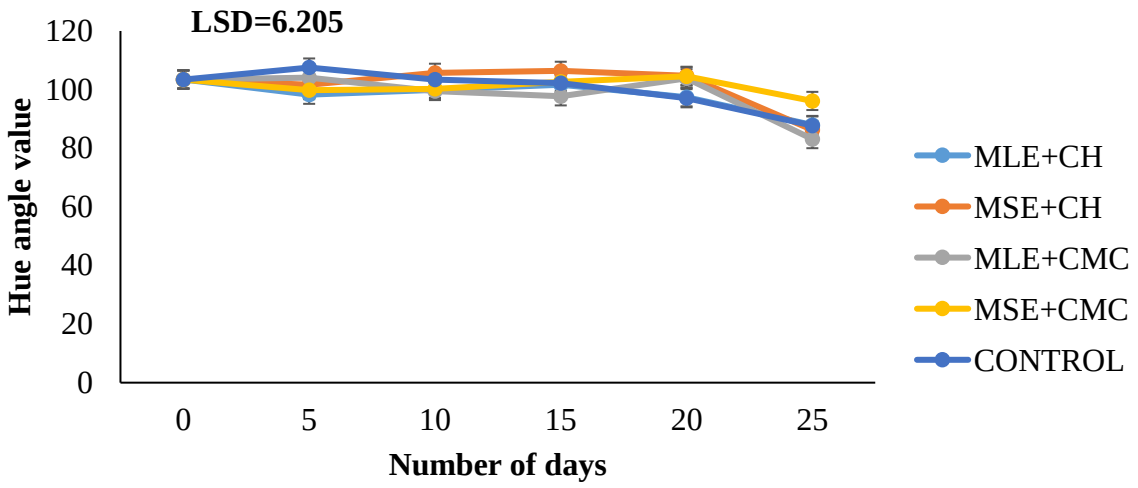


Figure 3-9: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with carboxymethylcellulose (MSE+CMC) and control on hue angle values of papaya fruits stored under cold storage condition.

The results showed that under ambient storage condition, treatment MSE+CMC had the highest hue angle values (h° 96.64 and h° 89.87 on day 5 and 10, respectively). Treatment MSE+CMC showed significant effect throughout the ambient storage. On the other hand, treatment MLE+CH showed the lowest hue angle values (h° 84.4 and h° 80.17 on day 5 and 10, respectively).

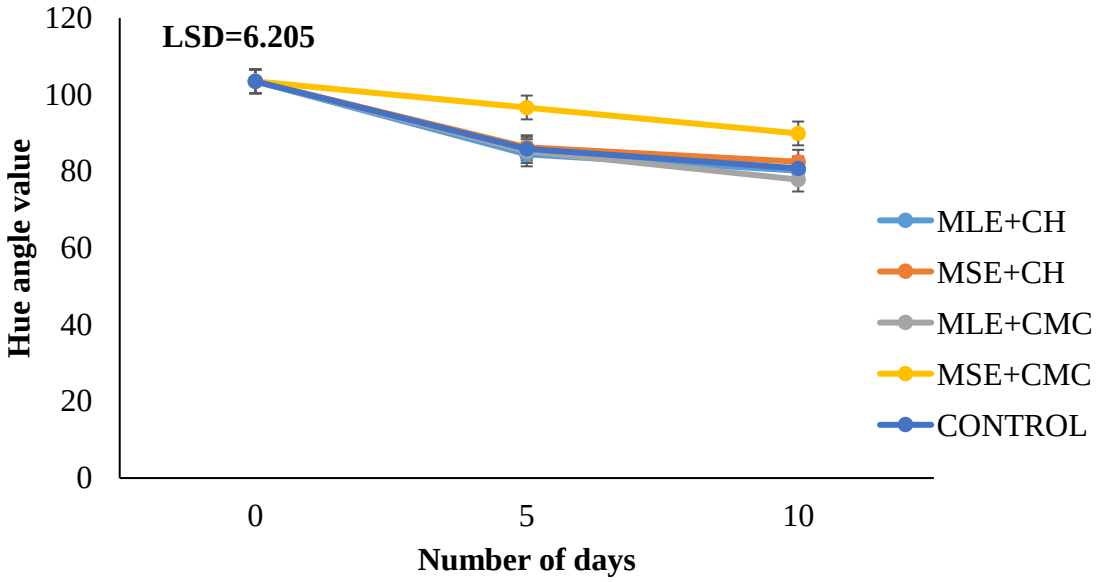
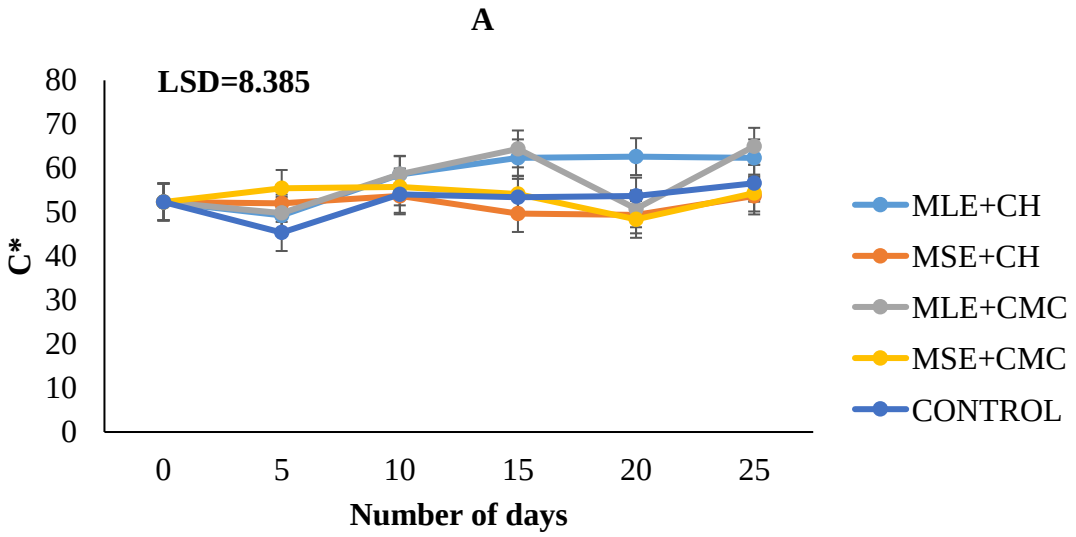


Figure 3-10: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with carboxymethylcellulose (MSE+CMC) and the control on hue angle values of papaya fruits stored under ambient storage condition.

The decrease in hue angle values is in agreement with findings that indicated that hue angle values of golden papaya decreased from h° 109.58 initially to h° 88.36 after fruits were transferred to from 20 °C to 10 °C (Caron *et al*., 2013). The results indicate that under cold storage, hue angle values ranged from h° 107.52 to h° 97.04 after 20 days of storage. The variation was previously reported to be normal for climacteric fruit which ripen faster at high temperature than non-climacteric fruits (Caron *et al*., 2013). The decrease in hue angle values indicates the evolution of green to yellow colour (Pereira *et al*., 2009). There was a slow evolution of colour under the cold storage as depicted by slow decrease in hue angle values from day 0 to 20. Evolution of green to yellow colour was significant after the fruits were transferred to ambient storage conditions. However, treatment MSE+CMC maintained the green colour as indicated by lower values of a* and higher hue angle values compared to other

treatments. MSE+CMC is also significant different from control throughout the ambient storage conditions, therefore, it can be used to delay papaya fruits from turning yellow.

Edible coatings had no significant effect ($p<0.05$) on chroma (C*) values of papaya fruits. The chroma values increased in all treatments under all storage conditions (Figure 3-11 A and B). Chroma values ranged from 52.34 to 66.55 under ambient temperature, while under cold storage the chroma values were between 45.37 and 65.00. The treatment MLE+CH showed higher C* values under both storage conditions (Figure 3-11 A and B). An increase in lightness (Hunter L* values) and chroma, and a reduction in hue angle values was observed when the fruits were fully ripened (Ong *et al*., 2013). Overall, it was observed that Hunter L*, b*, and C* values increased, and also decreased after 10 days of storage under ambient conditions and this can be attributed to senescence in papaya fruits. Under cold storage conditions, treatment MSE+CMC showed significant effect at day 5. Treatments MLE+CMC and MLE+CH showed significant effect at day 15 and day 20 of cold storage, respectively. Under ambient storage conditions, MLE+CMC showed significant effect at day 5.



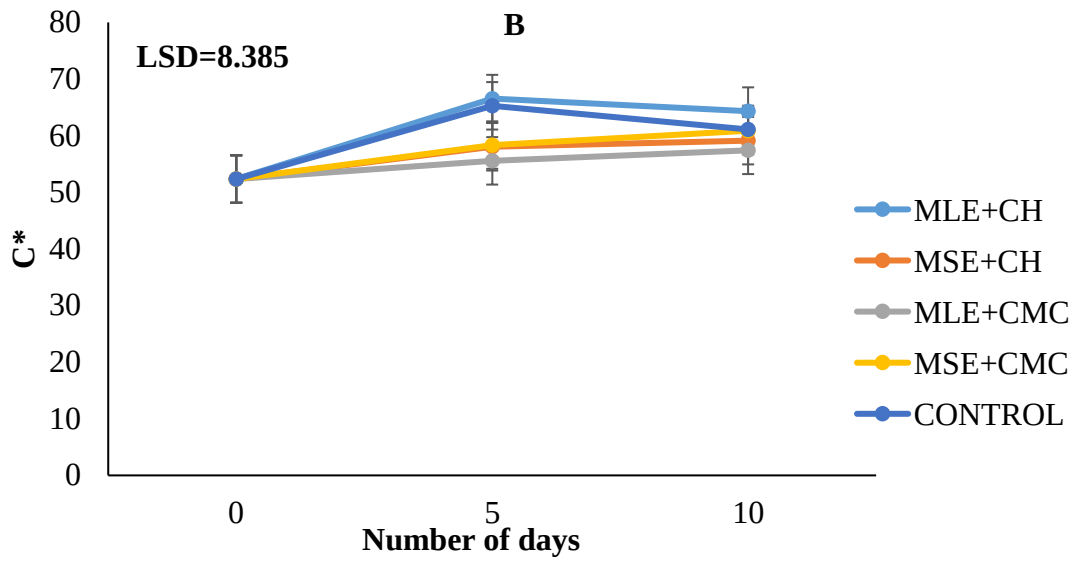
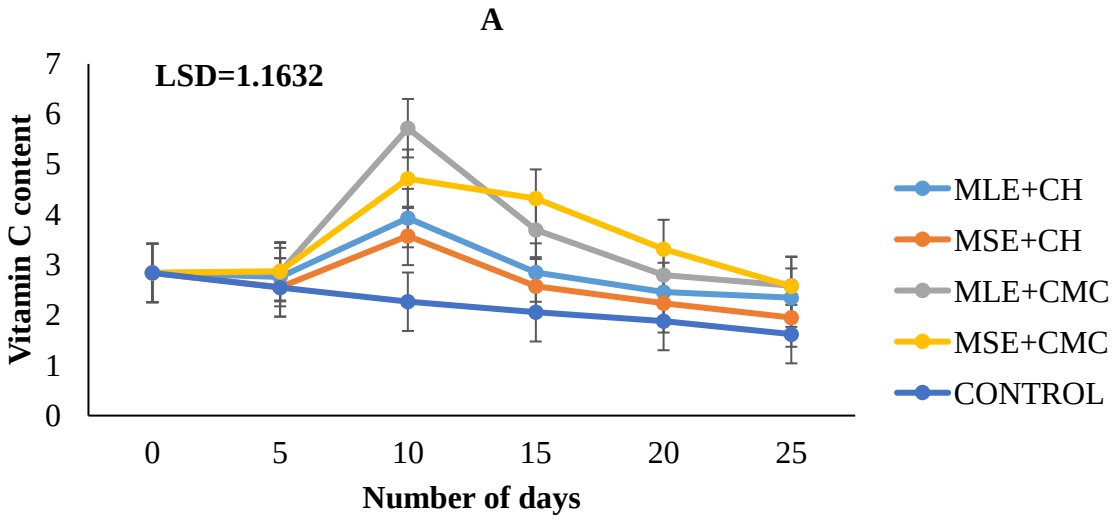


Figure 3-11: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with carboxymethylcellulose (MSE+CMC) and control on Chroma (C*) values of papaya fruits stored under (A) cold and (B) ambient storage conditions.

3.2.7 Effect of edible coatings on Vitamin C

Ascorbic acid is a water soluble and powerful antioxidant that acts to prevent the damage caused by reactive oxygen species (ROS) in fruit (Khaliq *et al.*, 2015). Ascorbic acid is considered the main ROS detoxifying compound scavenging and reducing H_2O_2 to water through ascorbate peroxidase reaction. Edible coatings had significant effect ($p<0.05$) on the vitamin C content of papaya fruits. The duration of storage had no significant effect ($p<0.05$) on the vitamin C content of papaya fruits. Vitamin C content increased with increasing cold storage period, except for the control. On day 5, the results showed that vitamin C content decreased under cold and ambient storage conditions (Figure 3-12 A and B). Vitamin C content ranged from 1.62 to 5.72 mg/mL under cold storage conditions (Figure 3-12 A), and ranged from 2.11 to 4.759 mg/mL under ambient storage conditions (Figure 3-12 B). Under both

storage conditions, treatments MLE+CMC and MSE+CMC showed higher vitamin C content than other treatments. On day 10, vitamin C content increased in coated papaya fruits under cold storage conditions. Treatments MLE+CMC showed the highest vitamin C content on day 10 (5.72 mg/mL) and the control showed the lowest vitamin C content (2.266 mg/mL) under cold storage conditions (Figure 3-12 A). The treatment MSE+CMC showed increased vitamin C content compared to other treatments from day 15 onwards. The results indicated that ascorbic acid (AA) content increased with the ripening stage and decreased once the fruit reached full ripe stage. Fruits are a natural source of ascorbic acid and losses occur during ripening (Khaliq *et al*., 2015). The loss in vitamin C content during storage is associated with autoxidation (Sogvar *et al*., 2016). Autoxidation occurs spontaneously when the ascorbic acid combines with oxygen in the air (Sogvar *et al*., 2016). Autoxidation of ascorbic acid decreases as coatings form a protective layer on the surface of the fruit and control the permeability of O_2 and CO_2 (Sogvar *et al*., 2016). Treatments MSE+CMC and MLE+CMC were reported to be more effective in reducing vitamin C than other treatments. These treatments showed that they are significant different from control on day 10 and 15 of cold storage, and on day 10 of ambient storage conditions. Treatments MSE+CH and MLE+CH showed significant effect on day 5 of cold storage.



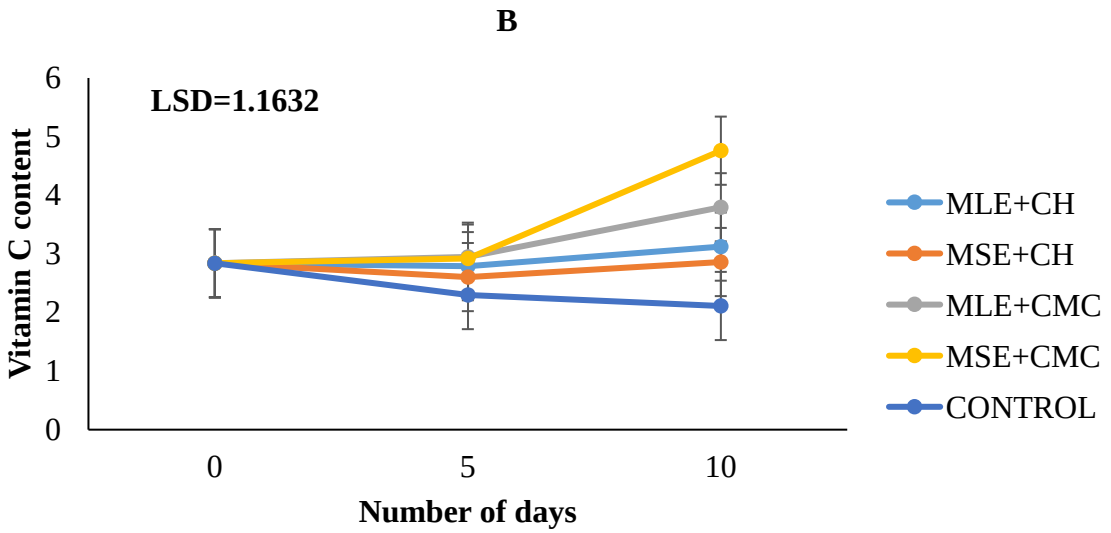
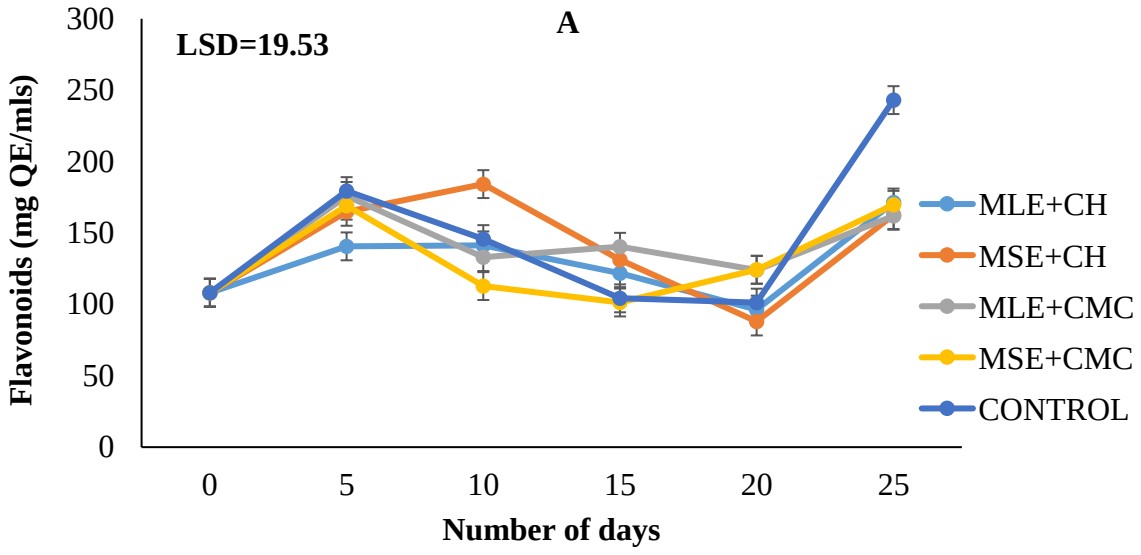


Figure 3-6: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with carboxymethylcellulose (MSE+CMC) and control on vitamin C content of papaya fruits stored under (A) cold and (B) ambient storage conditions.

3.7.8 Effect of edible coatings on flavonoids

Edible coatings had a significant effect ($p<0.05$) on flavonoids content of papaya fruits. The flavonoids content of papaya fruits was inconsistent; however, the observed content increased and followed a decreasing pattern until after day 20 under cold storage (Figure 3-13 A). After fruits were transferred to ambient storage, the increase in flavonoid content was observed and a rapid increase was recorded for the control. Under ambient storage, a similar trend was observed, with flavonoid content showing a gradual increase after 5 days of storage (Figure 3-13 B). Under cold and ambient storage conditions, treatment MSE+CMC showed the lowest flavonoid content compared to other treatments. The flavonoid content of fruits treated with MLE+CH remained relatively low (140.6 mg QE/g DW), but increased in other treatments at day 5 of cold storage. The control showed the highest flavonoid content (179.3 mg QE/g DW) compared to other treatments at day 5 of cold storage. On day 10 under cold and ambient

storage conditions, treatment MLE+CH did not vary significantly from the control. Treatment MSE+CH showed the highest flavonoid content (184.2 mg QE/g DW) at day 10 under cold storage conditions (Figure 3-13 A). Flavonoid content values ranged from 108.1 to 243 mg QE/g DW under cold storage conditions, and 108.1 to 194.9 mg QE/g DW under ambient storage conditions (Figure 3-13 A and B). Treatment MSE+CMC showed the lowest flavonoids content on day 10 and 15 (112.8 and 101.3 mg QE/g DW, respectively) under cold storage. An increased flavonoid content (243 mg QE/ g DW) was observed for the control after 25 days of storage. On day 5, treatment MLE+CMC had the highest flavonoid content (203 mg QE/g DW) under ambient storage condition, while treatment MLE+CH had the lowest flavonoids content (158.5 mg QE/g DW). Treatment MLE+CH showed significant effect at day 5 and day 25 of cold storage. Treatment MSE+CH showed significant effect at day 10, 15 and 25 of cold storage. Treatment MSE+CMC showed significant effect at day 10, 20 and 25 of cold storage, whereas MLE+CMC showed significant effect from day 15 up to day 25 of cold storage. Treatment MLE+CMC also showed significant effect throughout the ambient storage. Whereas, MLE+CH showed significant effect on day 5, and MSE+CMC showed significant effect on day 10 of cold storage.



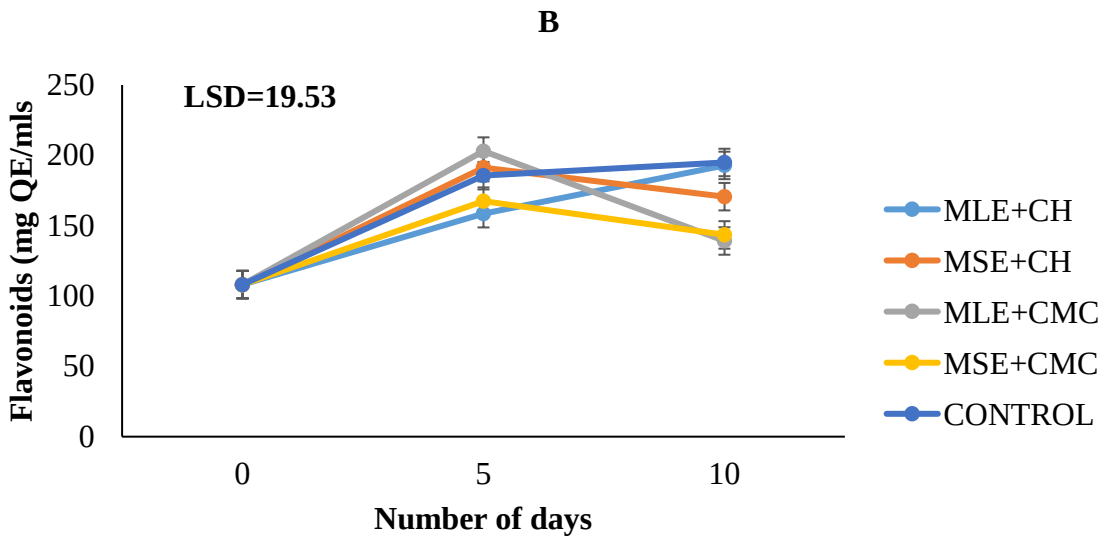
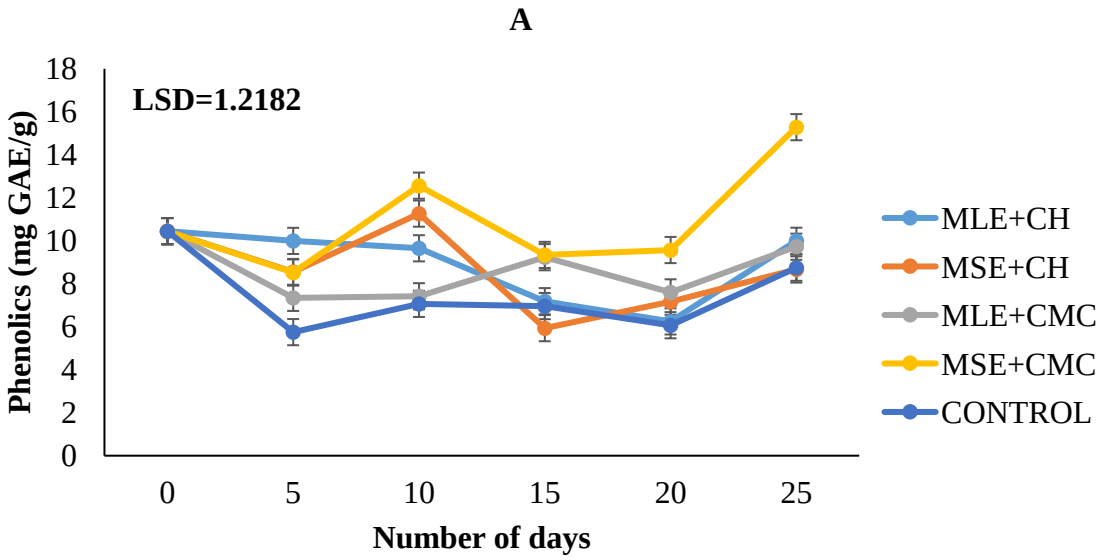


Figure 3-7: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with carboxymethylcellulose (MSE+CMC) and control on flavonoid content of papaya fruits stored under (A) cold and (B) ambient storage conditions.

3.7.9 Effect of edible coatings on total phenolic content

Edible coatings had a significant effect ($p<0.05$) on phenolic content for papaya fruits. The phenolic content of papaya decreased with storage period (Figure 3-14 A and B). Phenolic content ranged from 5.74 to 15.283 mg GAE/g DW, and from 7.74 to 13.40 mg GAE/g DW under cold and ambient storage conditions, respectively (Figure 3-14 A and B). On day 10, treatment MSE+CMC showed higher phenolic content under cold storage conditions (12.56 mg GAE/g DW) compared to other treatments. Whereas, the control showed the lowest phenolic content (7.063 mg GAE/g DW). The phenolic content increased after 5 days of storage under ambient condition and this could be attributed to an increase in temperature. Treatment MSE+CMC showed the highest phenolic content from day 10 under cold storage (Figure 3-14 A). Under ambient storage, the phenolic content decreased as indicated in Figure 3-14 B, however, an increase in phenolic content was observed for treatment MLE+CH under cold

storage (Figure 3-14 A). At day 10 under ambient storage condition, treatment MLE+CH recorded the highest phenolic content (13.40 mg GAE/g DW), while fruits treated with MSE+CMC showed the lowest phenolic content (7.74 mg GAE/g DW). Generally, phenolic content was highest during fruit growth and decreased with ripening and storage time under normal ripening conditions (Khaliq *et al*., 2015). Treatment MSE+CMC showed significant effect throughout cold storage conditions, whereas MLE+CMC showed significant effect at day 5, 15 and 20. Treatments MLE+CH and MSE+CH showed significant effect at day 5 and day 10 of cold storage. Therefore, the applied treatments MSE+CMC and MLE+CMC delayed fruit ripening under cold storage conditions. Treatments MLE+CH and MSE+CH showed significant effect on day 5 of ambient storage. All treatments were significantly different from control on day 10 of ambient storage. Treatment MLE+CH delayed fruit ripening under ambient storage conditions.



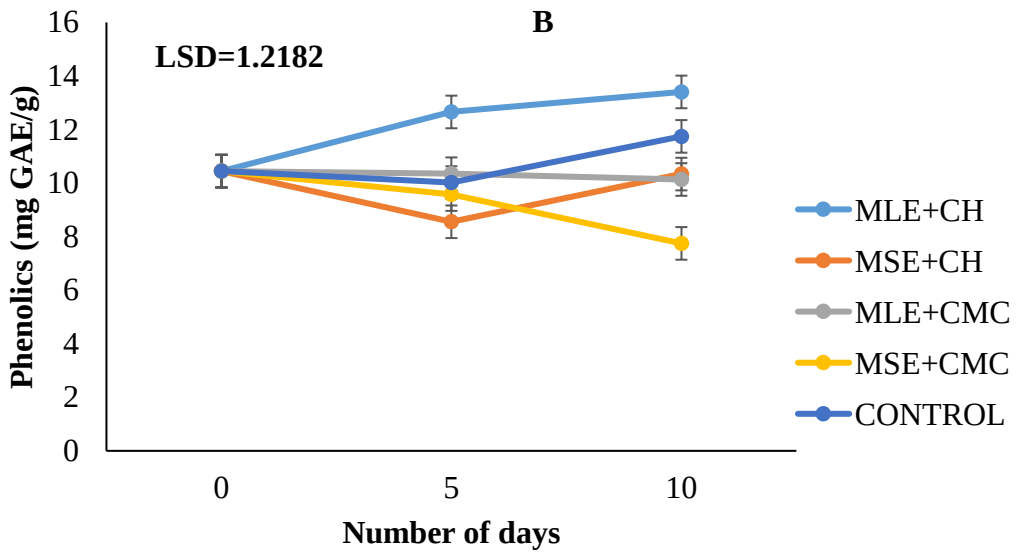


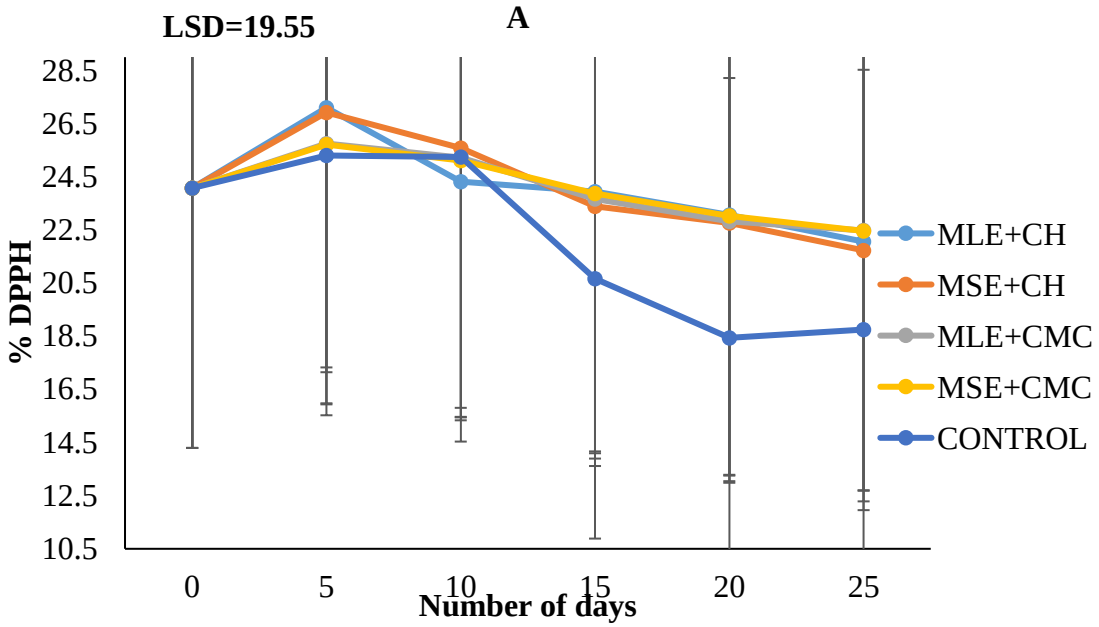
Figure 3-8: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with carboxymethylcellulose (MSE+CMC) and the control on phenolic content of papaya fruits stored under (A) cold and (B) ambient storage conditions.

3.7.10 Effect of edible coatings on papaya antioxidant activity

3.7.10.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity

The antioxidant capacity of bioactive compounds present in plant tissue has been evaluated using antioxidant activity (Khaliq *et al*., 2015). The DPPH radical scavenging activity is used to measure the non-enzymatic antioxidant capacity. The antioxidant potential of methanol extracts of papaya tissues was evaluated on their ability to scavenge stable free DPPH radicals. All treatments under both storage conditions (cold and ambient storage) showed no significant effect compared to control. Under cold storage conditions, DPPH scavenging activity of coated papaya fruits increased (Figure 3-15 A). The control showed the low percentage of DPPH scavenging activity. The DPPH scavenging activity percentage ranged from 18.44 to 27.10%. On day 5 under cold storage, treatment MLE+CH had lower scavenging activity (27.10%). Under ambient temperature, it was observed that the DPPH radical scavenging activity of

papaya fruits increased for uncoated fruits (Figure 3-15 B). The control was observed to have high DPPH radical scavenging activity (23.12 %), while treatment MLE+CMC showed low radical scavenging activity (25.54%) on day 5 under ambient storage (Figure 3-15 B). After 10 days under ambient storage, treatment MSE+CMC recorded 25.43% of DPPH, which is relatively low scavenging activity compared to other treatments. The control showed higher DPPH radical scavenging activity (23.11%) compared to other treatments under ambient storage. Addai *et al*. (2013) reported that papaya fruits treated with 10% gum arabic showed a delayed increase in antioxidant activity compared to control and fruits coated with low concentration (5%) gum arabic. The results of the current study are in agreement with findings by Nair *et al.* (2017) who indicated that chitosan incorporated with pomegranate peel extract (CHE) and CH samples had significantly low DPPH scavenging activity than the control. These results were associated with delayed biochemical and physiological changes that occur during cold storage on treated fruits (Addai *et al*., 2013). Hence, edible coatings tend to modify the internal atmosphere which slows the metabolism in fresh produce and also minimize the synthesis of phenolics and flavonoids (Nair *et al.*, 2017).



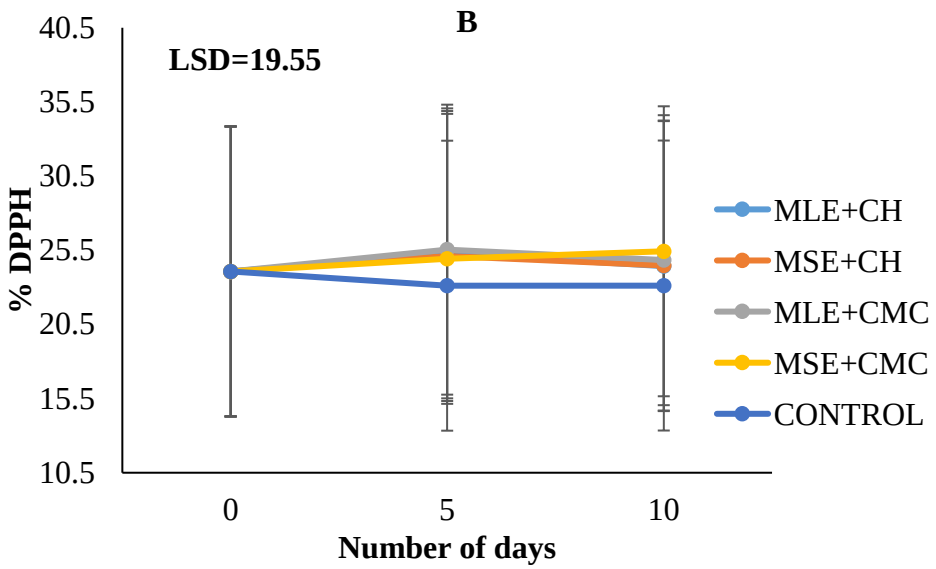
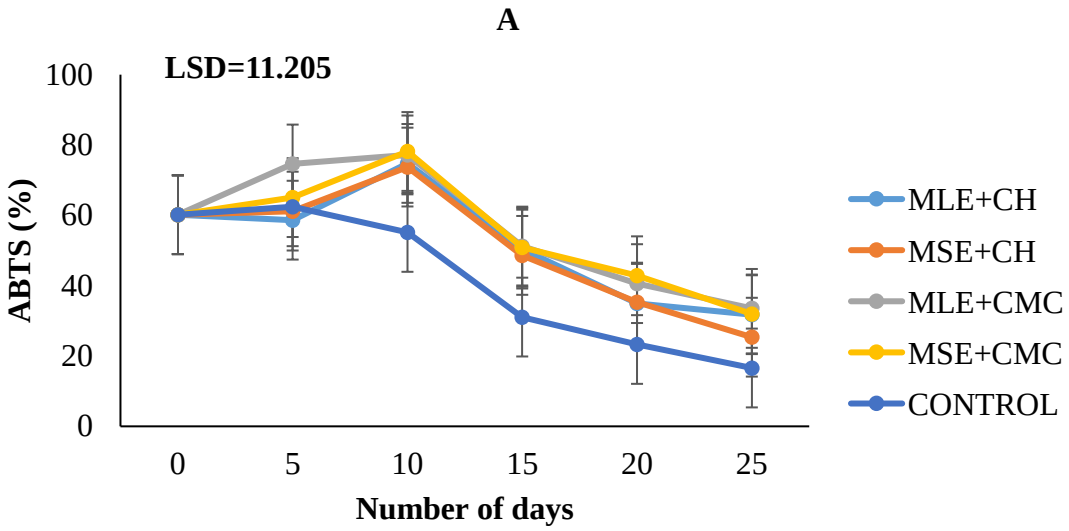


Figure 3-9: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with carboxymethylcellulose (MSE+CMC) and control on DPPH of papaya fruits stored under (A) cold and (B) ambient storage conditions.

3.7.10.2 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation

Edible coatings showed no significant effect ($p>0.05$) on ABTS activity of papaya fruits. The mean percentage of ABTS increased under cold storage (Figure 3-16 A). A percentage ABTS ranged from 16.59 to 78.15%. Applied edible coatings showed no significant effect from the control under cold storage conditions. Whereas, treatment MLE+CMC showed significant effect under ambient storage. The coated fruits maintained higher ABTS until day 10, while ABTS percentage of the control decreased after 5 days of storage. Treatments MLE+CMC and MSE+CMC maintained higher antioxidant activity (ABTS) throughout the cold storage (Figure 3-16 A). Antioxidant activity in fruits coated with MLE+CMC was the highest on day 5 under cold storage (74.59 %), while treatment MSE+CMC showed highest ABTS on day 10 (78.15%) compared to other treatments. Under ambient storage conditions, the ABTS percentage ranged from 33.83 to 64.49%. Treatment MLE+CMC had a significantly higher ABTS activity (64.49

%) compared to other treatments. The control fruits had lower ABTS activity (33.83%) on day 10 under ambient storage (Figure 3-16 A). The ABTS activity results confirmed findings that attributed the senescence and decay to the decline in antioxidant activity (Sogvar *et al*., 2016). The ascorbic acid (AA) capacity has the ability to retain fruit quality attributes, decrease rate of decay and the inhibition enzyme activity (Sogvar *et al.*, 2016). The increase in antioxidant activity of treated fruit is linked to the existence of natural antioxidants, which in turn is ascribed to their hydrogen donating ability (Khaliq *et al*., 2015). In general, the ascorbic acid and phenolics contributed to the antioxidant activity of papaya fruits. Treatments MLE+CMC and MSE+CMC were effective in increasing ABTS activity and DPPH radical scavenging activity. Therefore, the treatments were effective in increasing the resistance of tissues to decay by enhancing their antioxidant system (Sogvar *et al*., 2016).



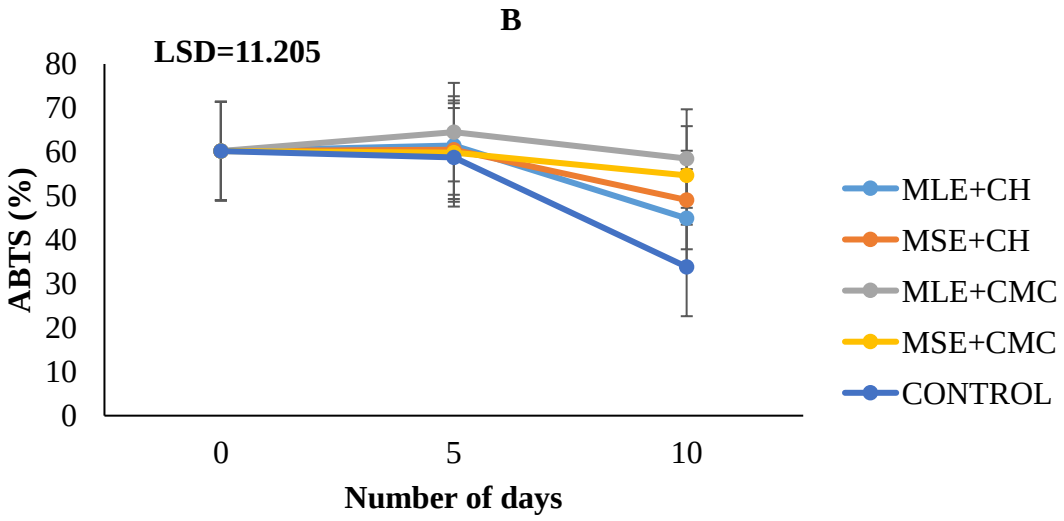


Figure 3-10: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with carboxymethylcellulose (MSE+CMC) and control on ABTS of papaya fruits stored under (A) cold and (B) ambient storage conditions.

3.7.11 Effect of edible coatings on total sugar

There are basic criteria to evaluate fruit ripening, and the amount of total sugar in fruit is considered as one important factor (Vyas *et al*., 2014). Fructose, glucose and sucrose are considered as major soluble sugars produced during fruit ripening (Li *et al*., 2014). Edible coatings had no significant effect ($p>0.05$) on sucrose content of papaya fruits. Glucose and fructose content increased in all treatments (Figure 3-17 and 3-18), while sucrose was only detected at 0 of storage (Table 3-1). The observed sucrose content in papaya fruits was 2.71 mg/mL at day 0, where three fruits were used for measurements and all the treatments were considered to have the same amount of sucrose content, hence, they were at the same stage of development. Sucrose content became undetectable thereafter until the end of storage period. Edible coatings had a significant effect ($p<0.05$) on glucose and fructose content of papaya

fruits. Glucose content ranged from 2.203 to 8.913 mg/mL, while fructose content ranged from 1.94 to 6.76 mg/mL. The results indicated inconsistent glucose and fructose values for papaya fruits.

Table 3-1: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with carboxymethylcellulose (MSE+CMC) and control on sucrose content of papaya fruits at day 0.

Treatments at day 0	Sucrose content (mg/mL)
MLE+CH	2.71^{b}
MSE+CH	2.71^{b}
MLE+CMC	2.71^{b}
MSE+CMC	2.71^{b}
Control	2.71^{b}
p	> 0.05
LSD	0.09902
CV%	9.8

Treatment MLE+CMC showed significant effect at day 5 and day 15 of cold storage, whereas MSE+CMC showed significant effect at day 5, 10 and 25. Treatment MLE+CH showed significant effect on day 5 of cold storage. Whereas, MSE+CH showed significant effect at day 10, 15 and 25 of cold storage. Under ambient storage conditions, treatment MSE+CMC showed significant effect at day 5, while MLE+CMC showed significant effect at day 10.

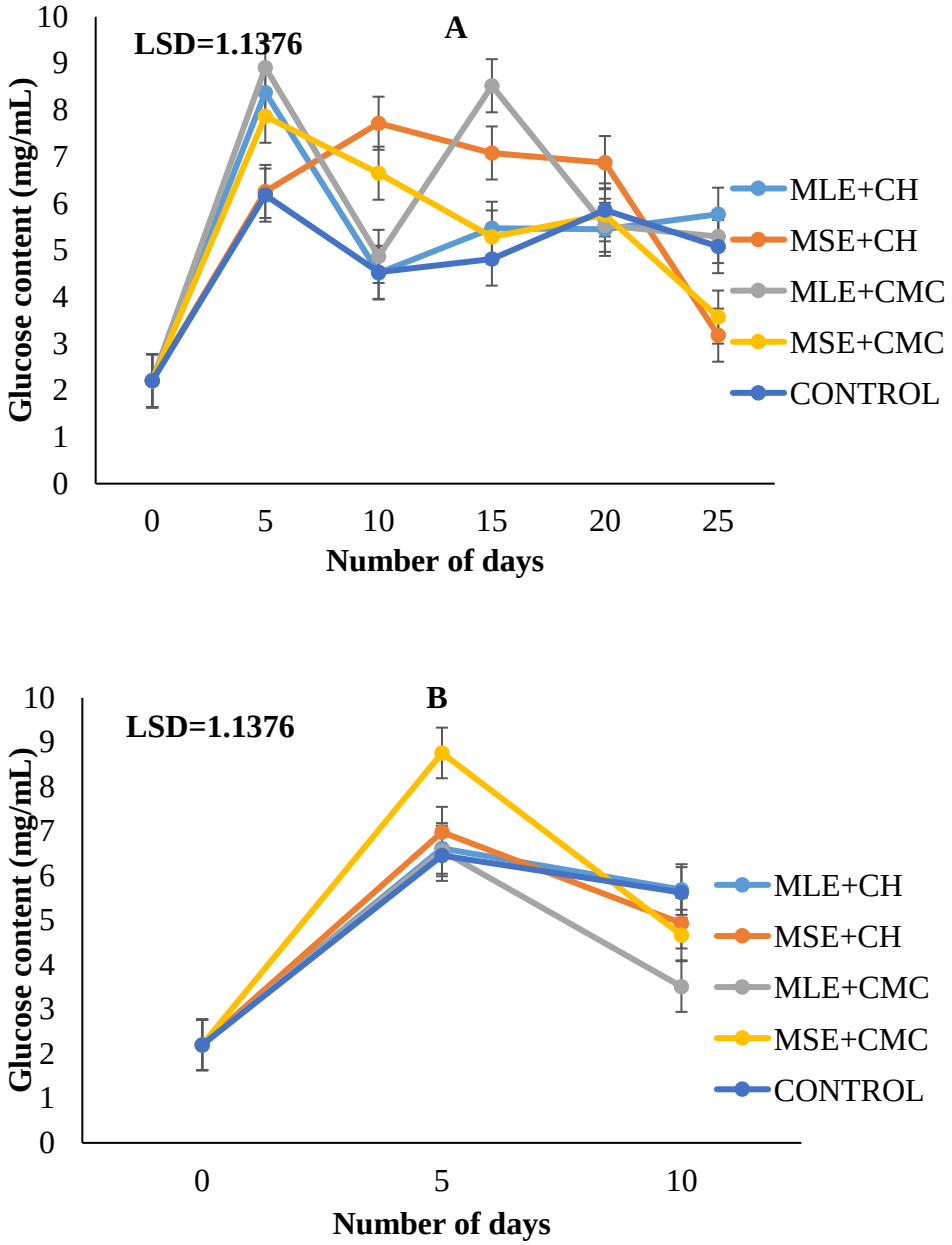
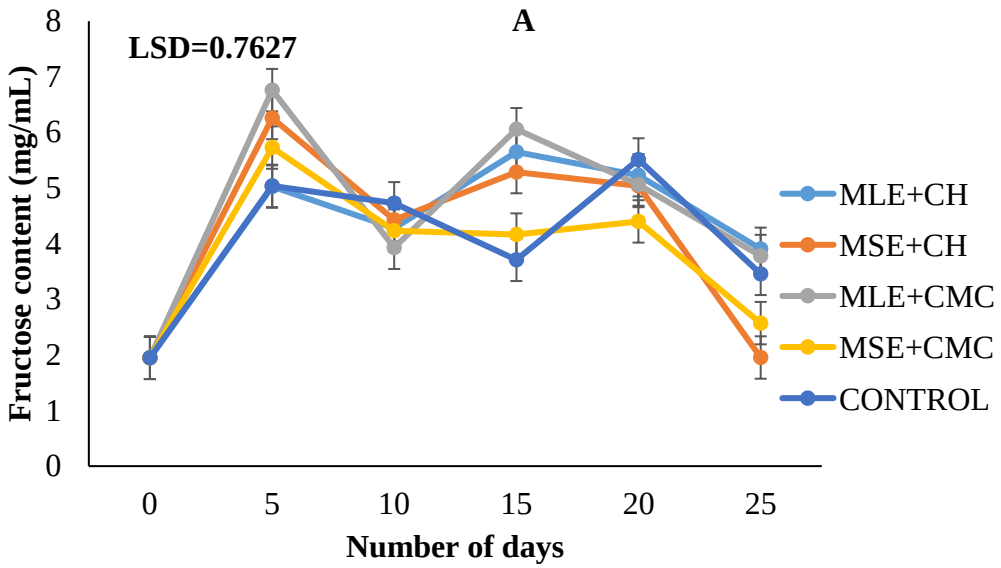


Figure 3-11: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with carboxymethylcellulose (MSE+CMC) and control on glucose content of papaya fruits stored under (A) cold and (B) ambient storage conditions.

Under ambient storage, the fructose and glucose content increased after 5 days of storage (Figure 3-17 B and 3-18 B). Treatment MSE+CMC showed the highest glucose and fructose content on day 5 of under ambient storage, 8.76 and 6.44 mg/mL, respectively. Fruits treated

with MLE+CMC showed the lowest glucose and fructose content after 10 days under ambient storage, 3.51 and 3.25 mg/mL, respectively. This could be for the fact that edible coatings delay loss of firmness resulting in retardation of activity of polygalacturonase, pectin methyl esterase, β-galactosidase as well as depolymerisation of cell wall pectins (Azene *et al*., 2014; Oluwaseun *et al*., 2013). They also delay fruit softening, resulting in delay of mastication and liberation of the sugars (Azene *et al*., 2014; Gomez *et al*., 2002). Glucose and fructose content decreased after 10 days of storage in all treatments. Treatment MLE+CMC showed significant effect on glucose content at day 5 and day 15 of cold storage, whereas MSE+CMC showed significant effect at day 5, 10 and 25. Treatment MLE+CH showed significant effect on glucose content at day 5 of cold storage. Whereas, MSE+CH showed significant effect at day 10, 15 and 25 of cold storage. Under ambient storage conditions, treatment MSE+CMC showed significant effect on glucose content at day 5, while MLE+CMC showed significant effect at day 10.



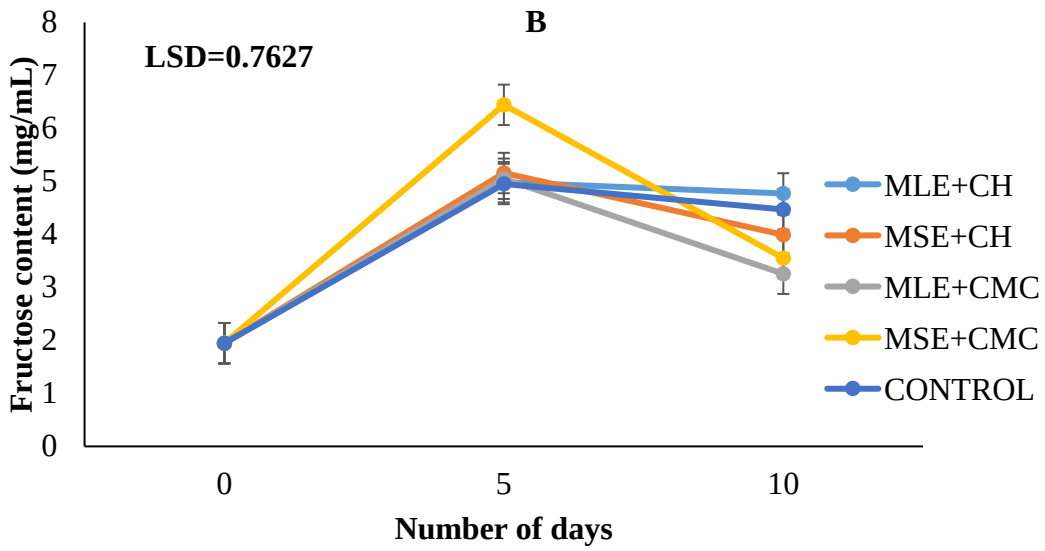


Figure 3-12: The effect of moringa leaf extract incorporated with chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC), moringa seed extract incorporated with carboxymethylcellulose (MSE+CMC) and control on fructose content of papaya fruits stored under (A) cold and (B) ambient storage conditions.

The results are in agreement with the findings of Othman (2009) that indicated reduced sugars and total sugars content of the papaya fruits increased during the ripening process while sucrose content decreased during this period. The results further agree with Vyas *et al*. (2014) who reported that total sugar reached peak and drastically declined. The decline is associated with breakdown of sugar during the respiration process and fermentation during prolonged storage (Vyas *et al*., 2014). At ripening stage, sucrose will convert into glucose and fructose, hence the reducing sugar level is normally higher than non-reducing sugar during fruit ripening (Vyas *et al*., 2014). The increase in reducing sugars (fructose and glucose) might be attributed to enzymatic conversion of starch to reducing sugar (Vyas *et al*., 2014). Also, the increase in reducing sugars might be attributed to conversion of some non-reducing sugar (sucrose) to reducing sugar (fructose and glucose) through the process of inversion (Vyas *et al*., 2014). Parallel changes in reducing sugars, total sugars and sucrose, and a decrease with time at each

storage conditions has been reported (Padmanaban *et al*., 2014). Rate of decrease of sugars declined due to a decrease in respiration rate (Padmanaban *et al*., 2014). Treatment MLE+CMC showed significant effect on fructose content at day 5 and day 15 of cold storage, whereas MSE+CMC showed significant effect at day 20 and day 25. Treatment MLE+CH showed significant effect on day 15 of cold storage. Whereas, MSE+CH showed significant effect at day 5, 15 and 25 of cold storage. Treatment MSE+CMC showed significant effect throughout ambient storage, while MLE+CMC showed significant effect at day 10.

In general, the applied treatments retarded biochemical processes and reduced infection of diseases on papaya fruits. On day 25 after fruits were transferred to ambient temperature conditions from cold storage treatment, the control and MLE+CH showed disease symptoms (Figure 3-19), while other treatments delayed the appearance of disease symptoms. The results indicate that as much as MLE+CH improved fruit quality, it did not prevent papaya fruits from postharvest diseases. Therefore, the treatment can be improved by testing different concentrations of moringa extracts incorporated into chitosan.

Figure 3-13: Pictures showing presence and absence of papaya diseases on fruit after 25 days of cold storage. Where A-E present control, moringa leaf extract incorporated with

chitosan (MLE+CH), moringa seed extract incorporated with chitosan (MSE+CH), moringa leaf extract incorporated with carboxymethylcellulose (MLE+CMC) and moringa seed extract incorporated with carboxymethylcellulose (MSE+CMC).

3.8 Conclusions

According to observations of the study, the treatments applied maintained papaya fruit quality compared to control treatments under cold and ambient storage conditions. Storage temperature affected shelf-life of the fruits, fruits were stored for up to 25 days under cold storage and 10 days under ambient storage. Treatments MSE+CMC and MLE+CMC significantly increased pH of papaya fruits. MSE+CMC, MLE+CMC, MLE+CH and MSE+CH significantly reduced weight loss and increased vitamin C and phenolic content. Treatment MSE+CH easily showed high flavonoids content from day 10 to 20 under cold storage. Treatments MSE+CMC, MSE+CH and MLE+CH maintained firmness of the fruits, and MSE+CMC, MLE+CMC and MSE+CH delayed change of colour of the papaya pulp, from green to yellow as compared to control fruits. The botanical extracts reduced papaya disease incidence. The study gives implication that *Moringa oleifera* incorporated with edible coatings can be used as an alternative for fruit quality preservation approach.

3.9 References

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CHAPTER 4

EFFECT OF MORINGA EXTRACTS AT DIFFERENT CONCENTRATIONS ON THE DECAY OF PAPAYA FRUITS

ABSTRACT

Fruit industry experiences huge losses that account for about 50% due to poor storage conditions, which lead to postharvest diseases. Postharvest diseases are normally controlled by synthetic fungicides, and their excessive use has led to negative effect on human health and the environment, accompanied with high costs, residues in plants, and development of resistance. Plant extracts are a promising alternative to fungicides for managing postharvest diseases of fruits. Pathogenicity test and in-vitro tests were done, data was recorded after 7 days of incubation. Isolates were subjected to light microscope for identification through morphological structures. After isolates were identified, in-vitro assay was performed, and isolates were inoculated onto PDA agar amended with 10, 20 and 30% of moringa leaf aqueous extract (MLWE), moringa seed aqueous extract (MSWE), moringa leaf ethanolic extract (MLEE), moringa seed ethanolic extract (MSEE). The mycelia growth was measured and the effect of moringa extracts were later evaluated by viewing samples under scanning electron microscope (SEM). Moringa extracts inhibited growth of pathogens by breaking, shrinking hyphae of pathogens and reducing number of spores. Treatments MLEE and MSEE inhibited 100% of the pathogens, and MLWE inhibited pathogens compared to control. *Moringa oleifera* incorporated with edible coatings can be used as an alternative for fruit quality preservation approach to reduce synthetic chemicals. Moreover, it can be used as a readily available fungicide and as environmental-friendly means of controlling fungal pathogens.

Keywords: Papaya decay, moringa extracts, scanning electron microscope, antifungal activity

4.1 Introduction

Postharvest diseases are posing a major problem to the agriculture industry, accounting to about 50% losses in fruits stored in poor storage conditions (Arowora and Adetunji, 2014). These losses significantly affect farmers' and traders' income and food security (Gwa and Nwankiti, 2017). Traditionally, the postharvest diseases are controlled by synthetic fungicides such as thiabendazole, imazalil and sodium ortho-phenyl phonate (Arowora and Adetunji, 2014). However, their excessive use has left a negative effect on human health and the environment, accompanied with high costs, residues in plants, and development of resistance (Arowora and Adetunji, 2014). Consumers dislike chemically preserved food, and some chemicals (such as copper based fungicides) are associated with public health risk (Arowora and Adetunji, 2014; Nkya *et al*., 2014). Other countries have restricted the use of chemical treatment, such as postharvest fungicide, to avoid dangerous chemical compounds in food for human consumption (Chávez-Sánchez *et al.*, 2013; Nkya *et al*., 2014). Fungicides' excessive and improper use in field application presents a danger to the human health, animals and environment (El-Mohamedy and Abdalla, 2014) and they are not locally available to smallholder producers (Mvumi *et al*, 2017). Hence, there is a need for development of alternative treatments such non-chemical approaches (Yousef *et al.*, 2015). It has been observed that development of new natural preservatives and antimicrobials has increased as alternatives (Misir *et al*., 2014). Scientists are working towards replacing these chemical fungicides due to their disadvantages, such as that they are very expensive and cause serious environmental pollution (Nkya *et al*., 2014). The scientists replace these chemical fungicides by environmentally friendly natural products (El-Mohamedy and Abdalla, 2014).

Botanicals are currently emerging as safer and more compatible approach to control phytopathogens (El-Mohamedy and Abdalla, 2014). In a wide range of crops, plant extracts are a promising alternative to fungicides for managing postharvest diseases of fruits (Arowora

and Adetunji, 2014). The plant world is also known to be a rich source of natural chemicals that could be exploited as pesticides (El-Mohamedy and Abdalla, 2014). Higher plants are acknowledged to have fungitoxicity against spore germination and mycelial growth of phytopathogenic fungi (El-Mohamedy and Abdalla, 2014). Many plant products (plant extracts, essential oils, gums, resins etc.) were shown to exert biological activity in-vitro and in-vivo and used as bio-fungicidal compounds (El-Mohamedy and Abdalla, 2014). They are eco-friendly, accessible to rural dwellers, cost effective and no or less phytotoxic reports which is an advantage for their use compared to chemical fungicides (Arowora and Adetunji, 2014).

Moringa oleifera Lam., from *Moringaceae* family, is a plant distributed in many countries and is highly valued (Sahab and Nawar, 2015). It has multiple uses and benefits to agriculture and industry (El-Mohamedy and Abdalla, 2014). *Moringa oleifera* is widely cultivated African countries, South America and South-east Asia (Busani *et al*., 2012). The plant is drought tolerant, and it thrives best under tropical climate and tolerates different soil types (Busani *et al*., 2012). The plant is distributed in Limpopo, KwaZulu-Natal and Mpumalanga provinces in South Africa (Busani *et al*., 2012). Almost every part of the tree can be used for food and for therapeutic purposes. Hence, the plant is highly valued and considered as one of the most useful trees in the world (Busani *et al*., 2012; Arowora and Adetunji, 2014). Different parts of this plant are a good source of protein, vitamins, carotene, amino acids and different phenolics (Sahab and Nawar, 2015). Moringa is recognized for properties such as antispasmodic, anti-inflammatory, diuretic, obortificient, emmenagogue and ecbolic (Nkya *et al*., 2014). It is also useful in treatment of many diseases, including fungal diseases (Nkya *et al*., 2014).

Many investigators have recorded the fungicidal effect of moringa extracts on some soil-borne fungi such as *Rhizoctonia*, *Pythium* and *Fusarium* (El-Mohamedy and Abdalla, 2014). Previous reports indicated that 75% (v/v) *Moringa oleifera* extracts of leaves, bark and seeds showed significant inhibition of the mycelial growth of *Fusarium solani* and *Fusarium oxysporum* f.

sp. *Lycopersici* (Dwivedi and Enespa, 2012). *Moringa oleifera* provides a rich and rare combination of zeatin, quercetin, b-sitsterol, caffeolyquininc acid and kaempferol which are reported to have antifungal and antibacterial activities (El-Mohamedy and Abdalla, 2014). It is an excellent crop growth enhancer as the leaves are rich in zeatin which is a cytokinin. In addition, the leaves contain other growth enhancing compounds like ascorbates, phenolic compounds and minerals like Ca, K, and Fe (El-Mohamedy and Abdalla, 2014). The plant is also used as an insect repellent and fungicide (El-Mohamedy and Abdalla, 2014). Although previous reports have indicated the potential use of moringa as a natural compound, eco-friendly agent and a promising approach to fungicides for managing postharvest diseases of fruits, there is still limited information on the improvement of fruit quality and reduced diseases under storage facilities. This study evaluated the antifungal activity of *Moringa oliefera* plant extracts against papaya fungal pathogens.

4.2 Materials and methods

4.2.1 Samples collection

Six (6) papaya fruits showing disease symptoms were collected from Spar at Hayfields in Pietermaritzburg, South Africa. The papaya fruits were stored for 5 days prior to isolation.

4.2.2 Media preparation and pathogen isolation

The pathogen isolates were obtained from symptomatic fruits of papaya. The potato dextrose agar (PDA) was prepared by mixing 39 g PDA with 1 L of water. The PDA was autoclaved for 15 minutes at 121 ^{o}C and was cooled in a water bath at 40 ^{o}C. Small portions of symptomatic tissue from the fruit was cut and inoculated on petri dishes containing PDA and the dishes were incubated for 7 days at 25 ^{o}C. Isolated colonies were sub-cultured on fresh PDA plates until pure cultures were obtained. The pure cultures were maintained on fresh PDA plates until analysis.

4.2.3 Identification of pathogen isolates

The morphological structures were viewed under the light microscope (Zeiss Scope .A1 with AxiocCam ERc5s camera, Carl Zeiss, Germany) at 40x and 100x magnification. The isolates were identified based on the shape of their spores and the orientation of their hyphae.

4.2.4 Pathogenicity assay

The maintained pure cultures of the isolates were used for pathogenicity test. Symptomless fruits were obtained from Spar at Hayfields in Pietermaritzburg. The fruits were taken to Horticultural laboratory and were surface sterilized by washing with 70% ethanol. The fungal mycelium from pure cultures was cut into small pieces with a sterilized scalpel and the pieces were inoculated on artificial injured healthy fruits. The inoculated wounds were covered with a sterilized cotton wool and sealed with a tape. The fruits were stored at room temperature for 7 days. After seven days of inoculation, disease incidence was measured and expressed as the

percentage of fruit showing the disease on the inoculated hole out of the total number of holes in each treatment. Disease incidence was assessed visually on a scale 0 to 100%. 0= no disease symptoms, 50%= disease symptoms in one hole of inoculation, and 100%= disease symptoms in two holes of inoculation. The treatments were arranged in a completely randomized design with three different isolates (replicated five times) and the control was used. The control fruits were not wounded or inoculated with the mycelium.

4.2.5 Moringa extracts preparation for in-vitro assay

Moringa leaves and seeds were ground to fine powder using a blender. About 100 g fine powder of moringa (both leaf and seed) was extracted, separately, using water and ethanol as follows:

4.2.5.1 Water extraction

An amount of 100 g of moringa seeds and leaves powder was extracted with 500 mL of water for 24 hours. The extracts were centrifuged at 10 000 rpm at 4 ^{o}C. The supernatant was filtered through glass wool to get a clear liquid.

4.2.5.2 Ethanol extraction

An amount of 100 g of moringa seeds and leaves powder was extracted with 500 mL of 90% v/v ethanol for 24 hours. The extracts were centrifuged at 10 000 rpm at 4 ^{o}C. The supernatant was filtered through glass wool to get a clear liquid. The sealable glass bottles were labelled as moringa leaf aqueous extract (MLWE), moringa seed aqueous extract (MSWE), moringa leaf ethanolic extract (MLEE) and moringa seed ethanolic extract (MSEE) The extracts were then transferred into bottles and kept in a refrigerator at 4 ^{o}C until analysis.

4.2.6 In-vitro assay

The antifungal activity of moringa extracts was evaluated against three different isolates, namely *Colletotrichum gloeosporioides*, *Rhizopus stolonifer* and *Phytophthora palmivora*. The PDA was prepared as described earlier. The moringa extracts were prepared at different

concentrations, 10%, 20% and 30% of aqueous and ethanol. The PDA agar was then amended with these moringa extracts, separately. The control plates contained PDA only. After PDA had solidified, petri plates were inoculated with three different isolates, separately. The treatments were arranged in a completely randomized design (replicated three times) and petri plates were incubated for 7 days at 25 ^{o}C. After 7 days of incubation, the diameter of the colony was measured to evaluate the mycelial growth. The PDA plates containing the sample isolates were kept in an incubator for scanning electron microscope viewing to observe the effect of moringa extracts on the morphology of the three different isolates.

4.2.7 Scanning electron microscopy

Fungal isolates which were on the treated PDA were prepared for scanning electron microscope (SEM) analysis and viewed using the SEM (Zeiss EVO LS15). Fungal samples were fixated in 3% glutaraldehyde and washed with cacodylate buffer for 1 hour. Fungal samples were dehydrated using ascending ethanol concentrations (10, 30, 50, 70, 90 and 100%) for 10 minutes at each concentration. To complete the dehydration, they were immersed three times in absolute ethanol for 10 minutes each. The same tissues were dried using a critical point drier Quorum K850. The dried samples were mounted on SEM stubs and were sputter coated with gold using Quorum Q150R ES coater. Thereafter, the samples were viewed in five replicates.

4.2.8 Statistical analysis

Data was subjected to analysis of variance (ANOVA) using GenStat 18th Edition (VSN International) at 5% levels of significance. The Duncan's multiple range tests was used to analyse for significant differences between treatment means.

4.3 Results and Discussion

4.3.1 Identification of fungal isolates

Rhizopus stolonifer (Figure 4-1 A) was identified on the basis of its branching mycelia, broad hyphae, sporangiophores, rounded, unicellular and brown in colour sporangiospores at the tip of sporangiophores. The conidia in Figure 4-1 B were single celled and cylindrical with obtuse ends, and the fungus was identified as *Colletotrichum gloeosporioides*. In Figure 4-1 C, the hyphae were lateral and irregular, and oospores were observed, therefore, the isolate was identified as *Phytophthora palmivora*.

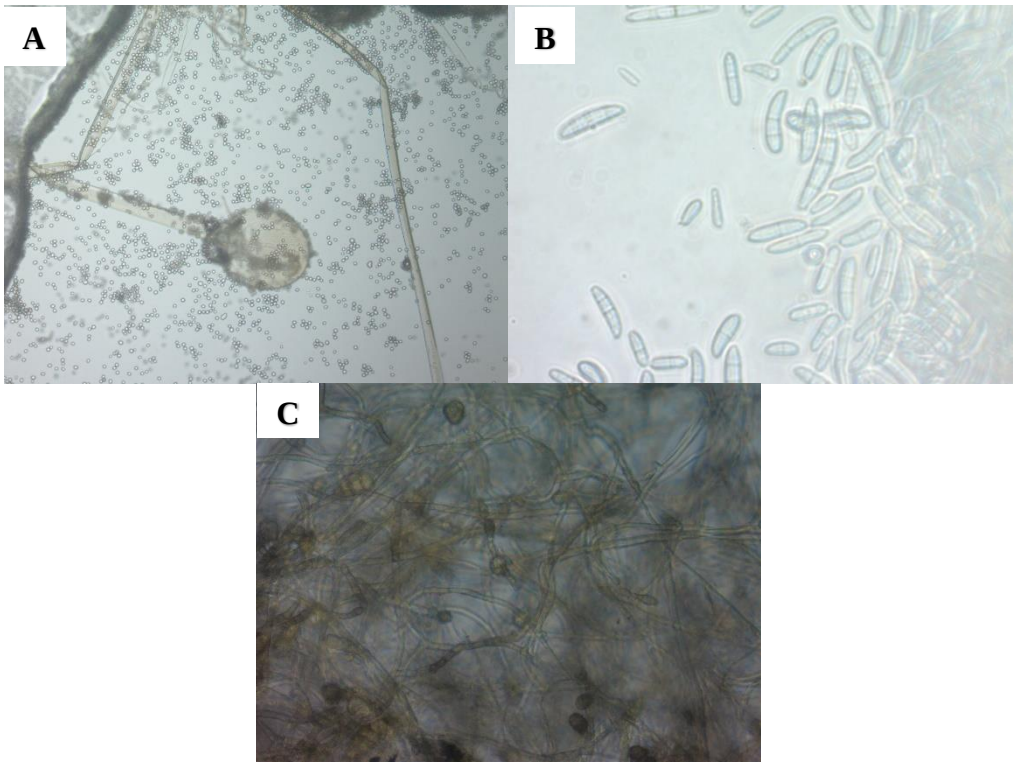


Figure 4-1: Light microscope images of morphology of fungal isolates after 7 days of inoculation A, B and C represent *R. stolonifer*, *C. gloeosporioides* and *P. palmivora*, respectively.

4.3.2 Pathogenicity assay results

A disease incidence was measured using a visual scale of 0= no disease symptoms, 50%= disease symptoms in one hole that was inoculated, and 100%= disease symptoms in two holes

that were inoculated. There was a statistically significant difference ($p<0.001$) in the disease incidence between the fruits inoculated with three different fungal isolates. The *C. gloeosporioide*s and *R. stolonifer* were the most pathogenic isolates from all inoculated fruits and showed disease symptoms after seven days. The *P. palmivora* was less pathogenic compared to *C. gloeosporioides* and *R. stolonifer* and only 80% of the inoculated fruits showed symptoms. The results showed no variations between *C. gloeosporioides* and *R. stolonifer*.

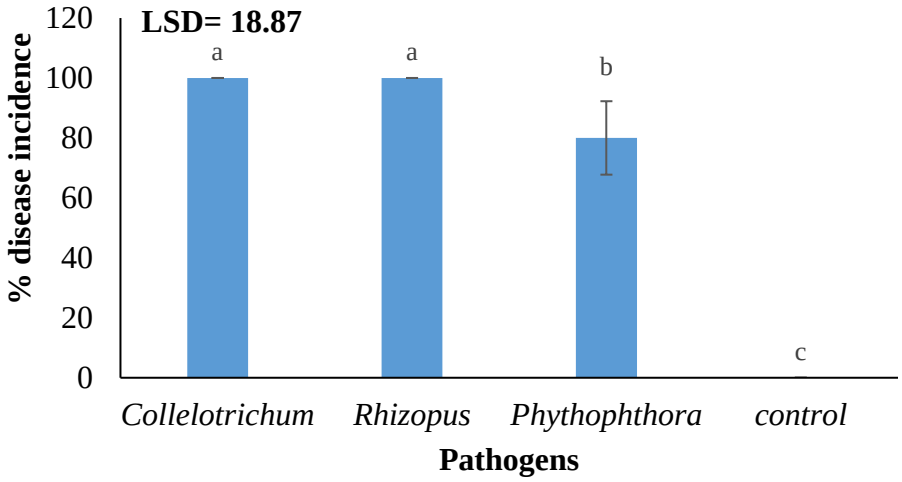


Figure 4-2: Percentage disease incidence of pathogenicity test of control, *Phytophthora palmivora, Colletotrichum gloeosporioides* and *Rhizopus stolonifer.*

4.3.2.1 Symptoms

The pathogenicity test was performed on healthy fruits and disease symptoms were observed. The fruits collapsed quickly due to soft and watery rot. The grey, hairy mycelia were observed (Ministry of Fisheries, Crop and Livestock, South America, 2003; Alvarez and Nishijima, 1987), and the pathogen was identified as *R. stolonifer* (Figure 4-3 A). Round, water-soaked spots symptoms on the surface of the papaya were observed. The spots then enlarge and turn brown, the sunken spots developed on the surface (Hasan *et al*., 2012), and the pathogen was

identified as *C. gloeosporioides* (Figure 4-3 B). Water-soaked spot appeared on the surface of the fruit and was then covered with whitish mycelia (Vawdrey *et al.*, 2015). The isolate was then identified as *P. palmivora* (Figure 4-3 C). The control did not show any symptoms or mycelial growth in the holes that were made (Figure 4-3 D).

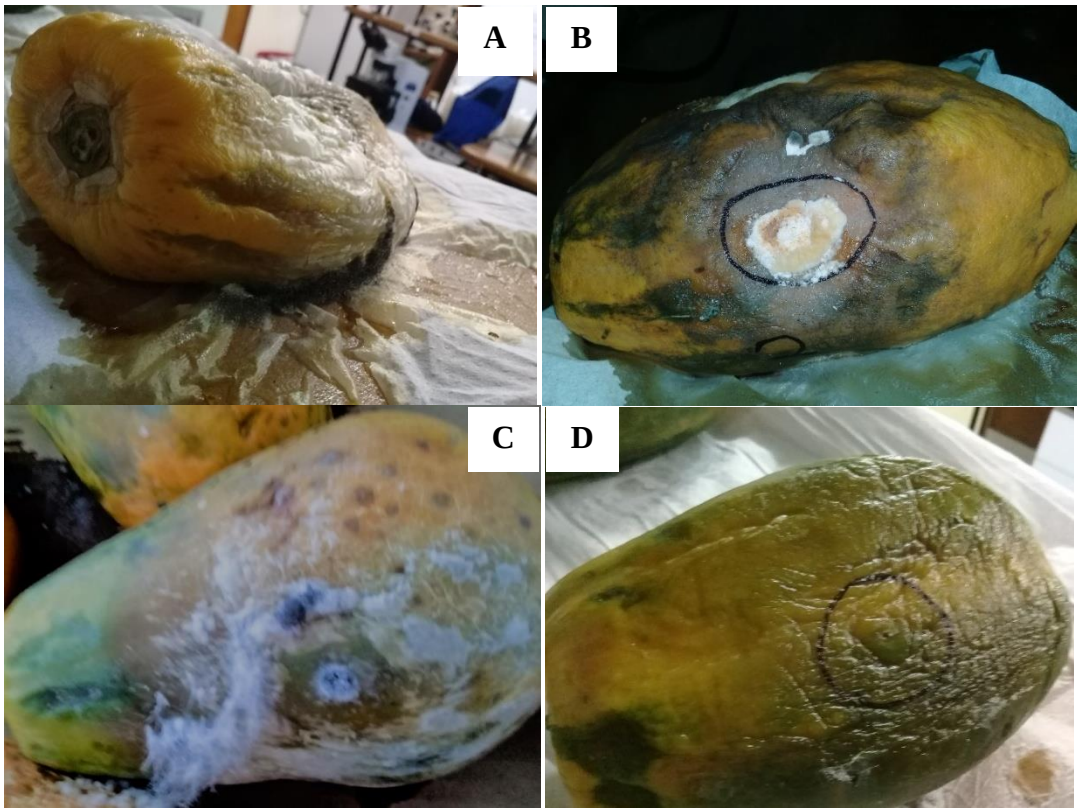


Figure 4-3: The pictures presenting the pathogenicity test of isolates, A, B, C and D are *R. stolonifer, C. gloeosporioides, P. palmivora* and control, respectively.

4.3.3 Results of in-vitro analysis

Plant derived products tend to have low toxicity to humans, less environmental effects and wide public acceptance, their use as diseases control agents have been investigated by Ademe *et al* (2014). There was a statistically significant difference ($p<0.001$) in percentage inhibition between treatments at different concentrations and the control. Both aqueous and ethanolic extracts (leaf and seed) showed a significant effect on the growth rate of the fungal isolates compared to the control groups. The formulations containing ethanolic leaf and seed extracts

completely inhibited growth of microorganisms. For *in-vitro* analysis, there was 100% mycelial growth inhibition in PDA amended with moringa extracted with ethanol. The aqueous extracts were more effective against *P. palmivora,* followed by *C. gloeosporioides*. The aqueous extracts were less effective in inhibiting the *R. stolonifer*. Aqueous effects on *C. gloeosporioides* showed a linear decrease with increasing concentration of extracts, leaf extracts were more effective compared to seed extracts (Figure 4-4). Treatment MLWE at a concentration of 30% showed superior inhibition of both *R. stolonifer* and *P. palmivora* compared to other MLWE concentrations (10 and 20%). Treatment MSWE at a concentration of 10% showed enhanced inhibition of both *C. gloeosporioides* and *P. palmivora* compared to other MLWE concentrations (20 and 30%). It was observed that a low concentration of aqueous leaf and seed extracts were more effective against *C. gloeosporioides*, while the low concentration of aqueous seed extract was more effective against *P. palmivora*. The results showed no significant difference between low and high concentration of aqueous leaf extract (Figure 4-4). However, inhibition of *R. stolonifer* at a higher concentration of aqueous leaf extract was higher. The results are in agreement with findings by Mvumi *et al*. (2017) who indicated that germination of *Alternaria solani* conidia decreased with an increased concentration of aqueous and chloroform leaf extracts. In the case of *C. gloeosporioides* and *P. palmivora*, however, the results showed a decrease in mycelial growth with a decrease in aqueous leaf extract concentration. All tested concentrations (10%, 20% and 30%) had relatively high inhibitory potential compared to the control. El-Mohamedy and Abdalla (2014) reported that all fungal mycelial growth gradually decreased with an increase in the concentration of moringa leaves, seed and pod extracts. Furthermore, Mvumi *et al.* (2017) reported that *R. solani* mycelial growth showed less sensitivity to moringa leaf extracts than *Fusarium oxysporum Alternaria Alternata.* In this study, *R. stolonifer* showed less sensitivity to aqueous leaf and seed extracts.

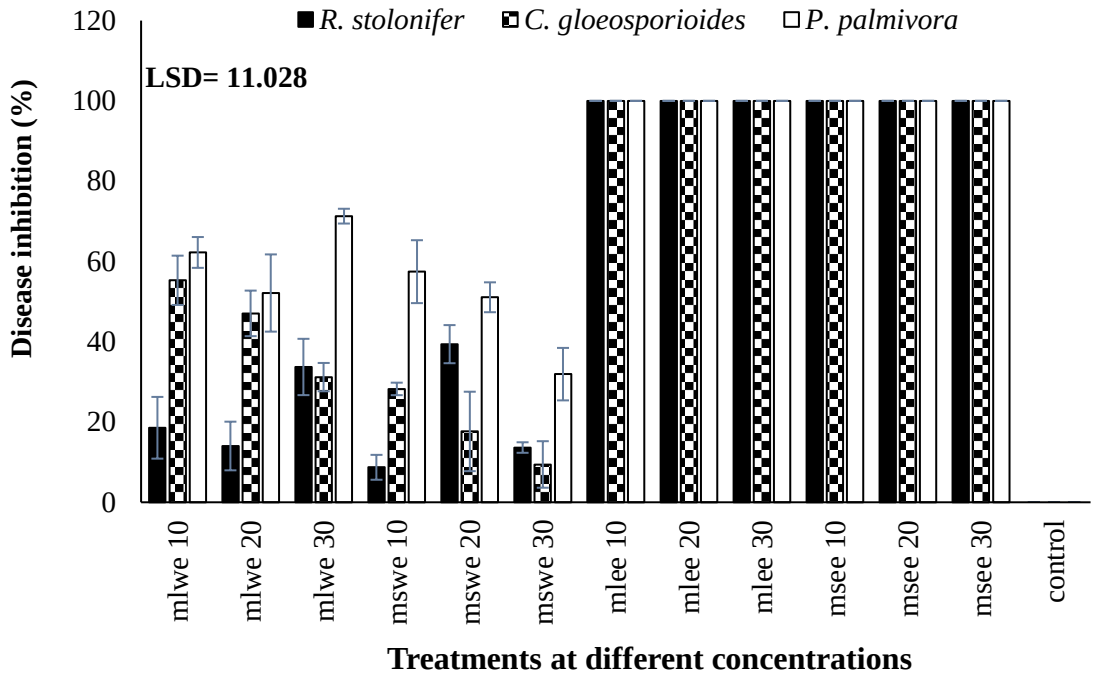


Figure 4-4: Graph representing *R. stolonifer*, *C. gloeosporioides and P. palmivora* isolates percentage inhibition by moringa aqueous and ethanolic leaf and seed extracts at different concentrations.

All the different concentrations of ethanolic moringa leaf and seed extracts (MLEE and MSEE) showed 100% inhibition of different fungal isolates. The percentage inhibition by moringa aqueous leaf extracts (MLWE) of *C. gloeosporioides* ranged from 31.18 to 55.3%, while the moringa aqueous seed extracts (MSWE) inhibition ranged from 9.42 to 28.24%. Inhibition by the moringa aqueous leaf extracts (MLWE) ranged from 52.13 and 71.28% for isolate *P. palmivora*, while inhibition by moringa aqueous seed extracts (MSWE) ranged from 31.92 to 57.45% (Table 4-1). Inhibition by the moringa aqueous leaf extracts (MLWE) ranged from 14.02 and 33.71% for isolate *R. stolonifer*, while inhibition by moringa aqueous seed extracts (MSWE) ranged from 8.71 to 39.39% (Table 4-1). The results showed that moringa ethanolic leaf and seed extracts (MLEE and MSEE) had higher antifungal activity compared to moringa aqueous leaf and seed extracts (MLWE and MSWE) (Table 4-1). The results also show that

moringa aqueous leaf extracts (MLWE) have high antifungal activity compared to moringa aqueous seed extracts (MSWE). The high antifungal activity of the leaf extracts is strongly associated with higher concentration of phenolic compounds in the tissue (Tesfay *et al*., 2017). Previous reports indicated that ethanolic leaf and seed extracts reduced *C. gloeosporioides* and *A. alternate* (Tesfay *et al*., 2017). After 10 days of incubation, ethanolic leaf extract was reported to be the most effective followed by ethanolic seed extract with 43.6 and 42.9% inhibition of *C. gloeosporioides* and *A. alternate* (Tesfay *et al*., 2017). A report by Nkya *et al*. (2014) showed maximum antifungal activity in leaves and stem bark extracts (ethyl acetate) against *Gibberella xylarioides* (0.38 mg/mL) at minimum inhibition concentration compared to the flower and seed extracts.

Table 4-1: Percentage inhibition of R. stolonifer, C. gloeosporioides and P. palmivora isolates by aqueous and ethanolic extracts (leaf and seed) at different concentrations.

Treatments	Concentration (%)	Isolates		
		R. stolonifer	*C. gloeosporioides*	*P. palmivora*
MLWE	10	18,56gh	55,3cd	62,24bc
MLWE	20	14,02h	47,06de	52,13cd
MLWE	30	33,71f	31,18f	71,28b
MSWE	10	8,71hi	28,24fg	57,45cd
MSWE	20	39,39ef	17,65gh	51,07cd
MSWE	30	13,64h	9,42hi	31,92f
MLEE	10	100a	100a	100a
MLEE	20	100a	100a	100a
MLEE	30	100a	100a	100a
MSEE	10	100a	100a	100a
MSEE	20	100a	100a	100a
MSEE	30	100a	100a	100a
CONTROL	0	0i	0i	0i

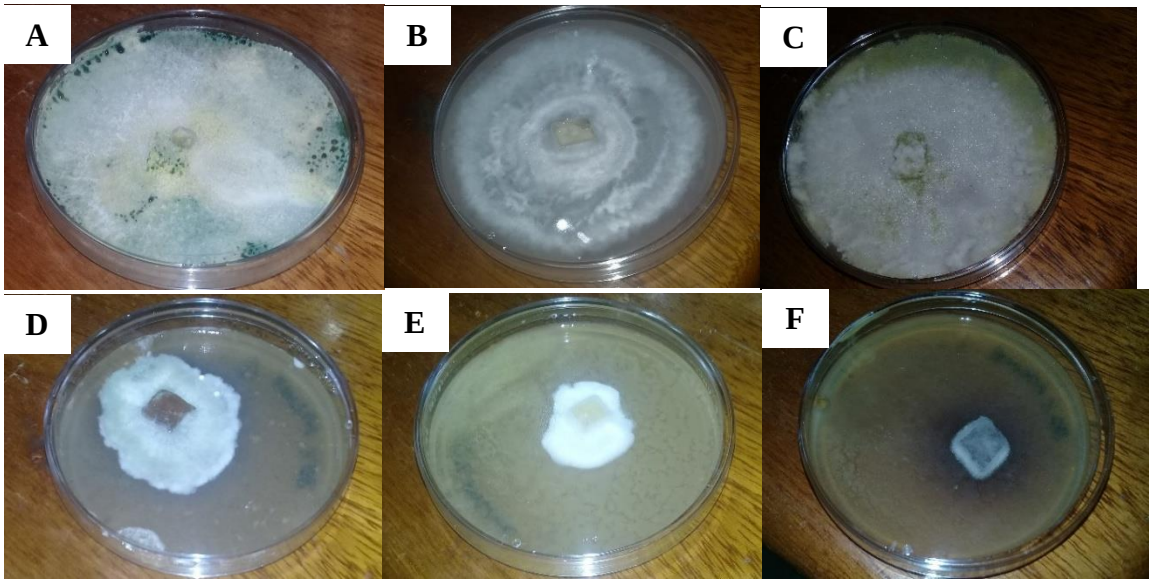
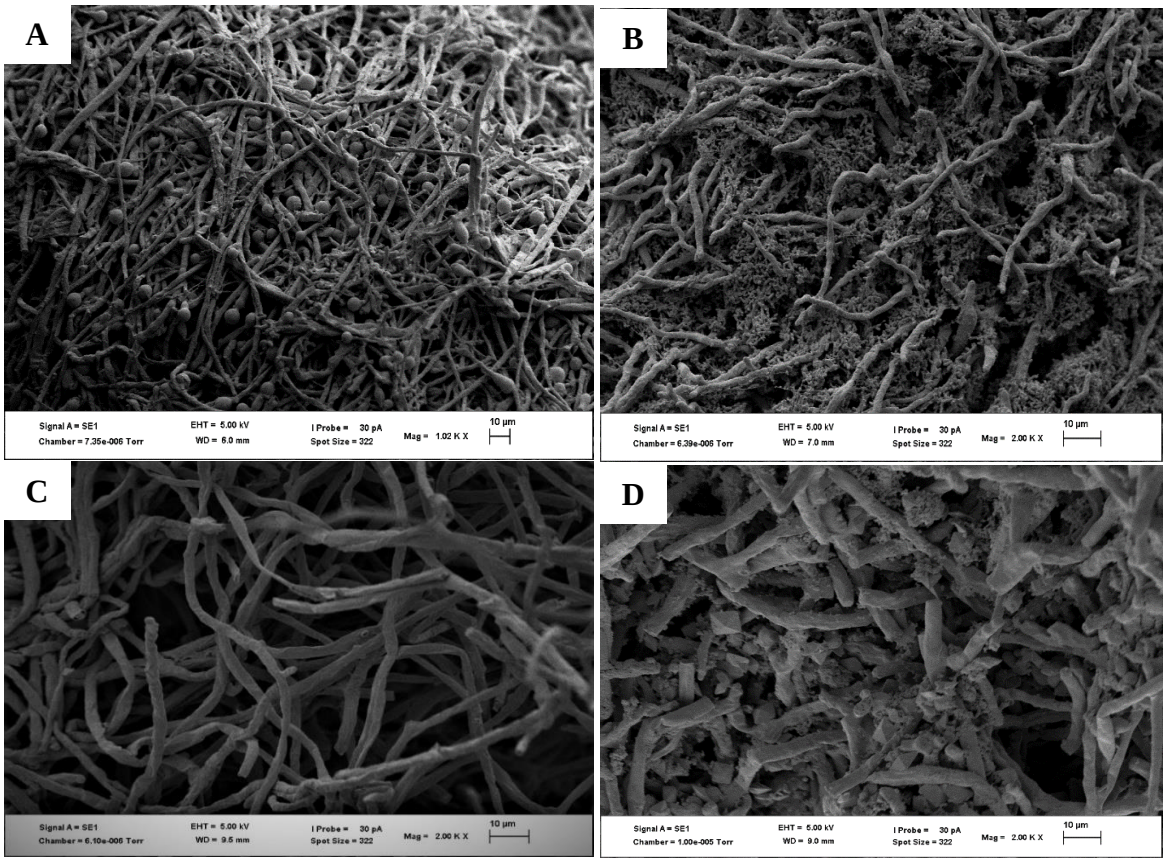


Figure 4-5: The pictures presenting the results of in-vitro test of fungal isolates. Letters A, B and C are control treatments for R. stolonifer, C. gloeosporioides and P. palmivora, respectively. Letters D, E and F represent R. stolonifer (30%), E is C. gloeosporioides (10%), F is P. palmivora (30%) isolate in PDA amended with MLWE.

4.3.4 Scanning electron microscopy results

Moringa extracts had an effect on hyphae of papaya pathogens as seen in the scanning electron microscopy images (Figure 4-6). Morphological changes were observed in treated samples, some hyphae were broken (Figure 4-6 D) under concentration of 30% of MLWE, reduction of hyphae and stacked together (Figure 4-6 F, G and H) under concentration of 30% of MSWE. Rhizopus spores had holes (Figure 4-6 G), reduced in numbers and others were separated (loose) from hyphae (Figure 4-6 B and G) under concentration of 30% of both MLWE and MSWE. The antifungal activity of the crude extract had an effect on the growth of filamentous fungi causing membrane permeabilization due to the presence of lipophilic compounds that bind within or internal to the cytoplasmic membrane (Zaffer *et al*., 2015). Small peptides found in moringa leaf extracts played an essential role in the plant's antimicrobial defence system (Zaffer *et al*., 2015). The proteins/peptides are associated to the defence mechanism against

phytopathogenic fungi by inhibiting the growth of micro-organisms through diverse molecular modes (Zaffer *et al*., 2015). The diverse molecular modes might include binding to increasing the permeability of the fungal membranes (Zaffer *et al*., 2015). Moringa provides zeatin, quercetin, b-sitsterol, caffeoylquinic acid and kaempferol rich in antifungal and antibacterial activities (El-Mohamedy and Abdalla, 2014). The results suggest that *Moringa oleifera* extracts can be used as a natural, environmental-friendly fungicide to control fungal pathogens, and the dependence on the expensive and toxic synthetic fungicides will be reduced. Further investigation is needed to test antifungal activities of moringa extracts against different fungal species that cause fruit diseases, using different solvents at different concentrations.



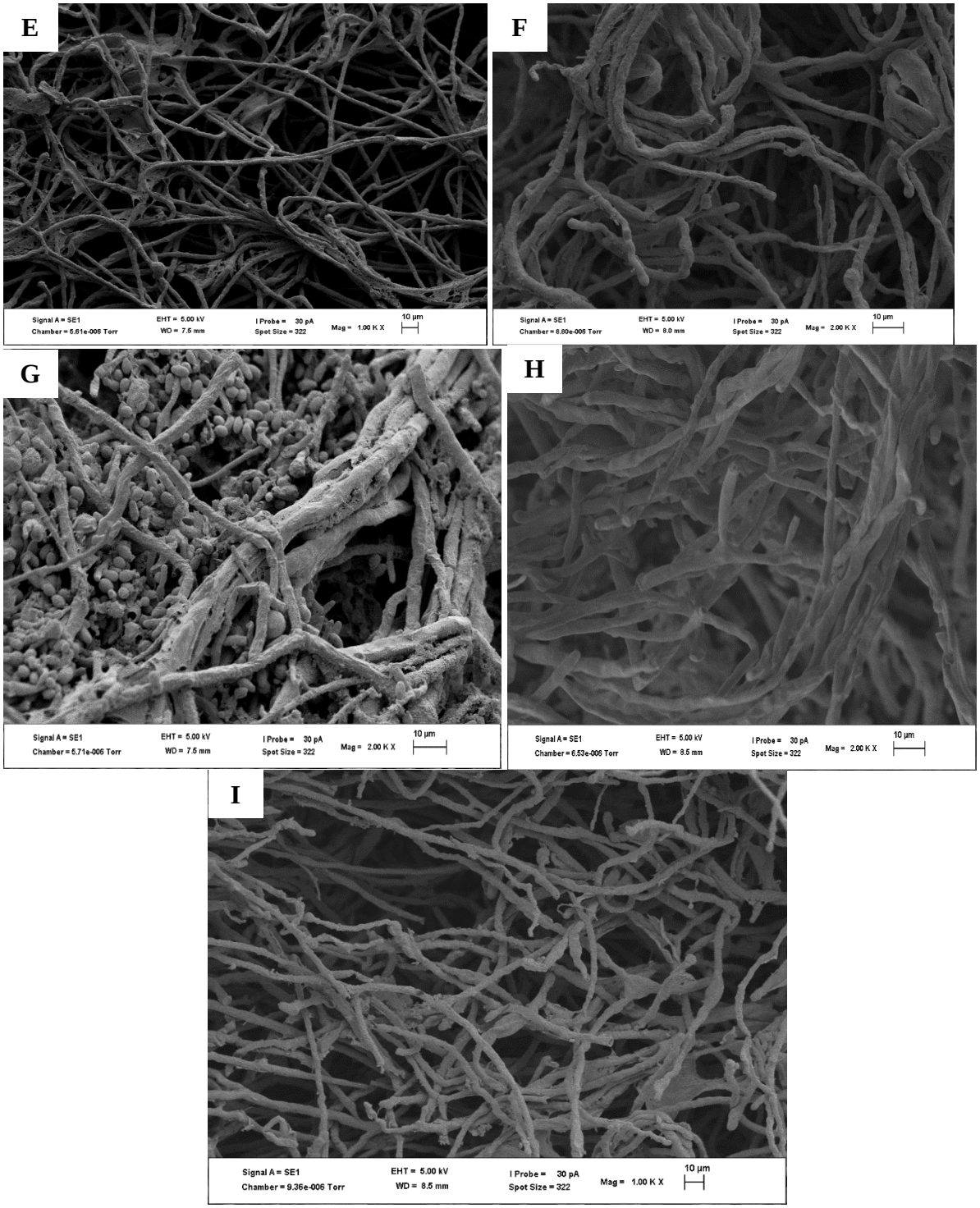


Figure 4-6: Scanning electron microscope images of isolates *R. stolonifer*, *C. gloeosporioides* and *P. palmivora* where A, C and E are control, respectively. Letters B, D and F are isolates treated with MLWE at a concentration of 30%, and G, H and I are isolates treated with MSWE at a concentration of 30%.

4.4 Conclusions

The study evaluated the antifungal activity of plant extracts against fungal pathogens that affect postharvest quality of papaya fruits. *Moringa oleifera* extracts, in the form of moringa aqueous and ethanolic leaf and seed extracts (MLWE, MSWE, MLEE and MSEE) were used as natural readily available fungicides and as environmental-friendly means of controlling fungal pathogens. *Rhizopus stolonifer, Colletotrichum gloeosporioides* and *Phytophthora palmivora* were identified in the present study. For pathogenicity test, *C. gloeosporioide*s and *R. stolonifer* were the most pathogenic isolates from all inoculated fruits.

Under *in-vitro* analysis, all tested concentrations of moringa leaf and seed extracts (10%, 20% and 30%) had relatively high inhibitory potential compared to the control. A 100% mycelial growth inhibition in PDA amended with moringa extracted with ethanol was observed. The aqueous extracts were more effective against *P. palmivora*, followed by *C. gloeosporioides*. Treatment MLWE at a concentration of 30% showed superior inhibition of both *R. stolonifer* and *P. palmivora* compared to other MLWE concentrations (10 and 20%). Treatment MSWE, at a concentration of 10%, showed enhanced inhibition of both *C. gloeosporioides* and *P. palmivora* compared to other MLWE concentrations (20 and 30%). When the inhibitory effects of extracts on identified pathogens were viewed under the scanning electron microscope, morphological changes were observed in treated samples. Some hyphae were broken, reduction of hyphae and stacked together. *Rhizopus* spores had holes, reduced in numbers and others were separated (loose) from hyphae. The results indicate that moringa seed and leaf extracts have the potential for use as an alternative means of controlling fungal diseases in papaya fruit and thereby reduce use of synthetic fungicides.

4.5 References

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CHAPTER 5

GENERAL DISCUSSION AND RECOMMENDATIONS

In tropical and subtropical regions, papaya is an important fruit for domestic and export markets (Barrera *et al*., 2015). However, its availability to the market can be limited due to the fact that papaya fruit has a thin skin, resulting in susceptibility to various postharvest injuries and mechanical damages (Pimentel and Walder, 2004). Papaya fruit has climacteric nature, it is very perishable after harvesting (Chien *et al.*, 2013, Li *et al.*, 2013, Pérez-Carrillo and Yahia, 2004, Waghmare and Annapure, 2013). High perishability makes it prone to postharvest problems during handling and storage, such as diseases, physical disorders and faster ripening (Ali *et al.*, 2010, Gonzalez-Aguilar *et al.*, 2003; Perez-carrillo and Yahia, 2004; Vyas *et al.*, 2014). About 30-50% estimated loses that occur due to postharvest spoilage, pathogens are believed to contribute more in fruit deterioration (Barrera *et al*., 2015; Sharmin *et al.*, 2015).

The study evaluated the effect of edible coatings on papaya fruit quality and safety and antifungal activity of moringa plant extracts against fungal pathogens that affect postharvest quality and safety of fruits. In the first section of the study, *Moringa oleifera* extracts (seed and leaf) incorporated with chitosan and CMC were used as an alternative for synthetic fungicides. The quality parameters (such as pH, total titratable acidity, total soluble acids, weight loss, firmness and peel colour) and secondary metabolites of papaya fruits were assessed to observe the effectiveness of treatments under cold and ambient storage conditions. Edible coatings had a significant effect ($p<0.05$) on quality and safety parameters of papaya fruits. Treatments applied (MLE+CH, MSE+CH, MLE+CMC and MSE+CMC) maintained papaya fruit quality compared to the control under both ambient and cold storage conditions. Treatment MSE+CMC, MSE+CH and MLE+CMC showed better fruit quality maintenance compared to other treatments. This was evident as the fruits treated with these coatings did not show any symptoms of pathogens. Treatments MSE+CMC, MSE+CH and MLE+CMC also exhibited

slow evolution of green colour to yellow. Under cold storage, the quality of papaya fruits was extended by fifteen days compared to 10 days that was achieved under ambient storage.

In the pathogenicity test of this study, *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* showed to be the most pathogenic isolates. These pathogens also showed resistance compared to *P. palmivora* under in-vitro analysis when were treated with moringa leaf and seed extracts, separately. However, all tested concentrations of moringa leaf and seed extracts exhibited high inhibitory potential compared to control. Their effects were indicated by the damage that was observed on hyphal strands and spores of the pathogens.

The study findings indicate that the moringa plant extracts can be used as natural readily available fungicides in controlling fungal pathogens and preserving papaya fruit quality. Moringa extracts combined with edible coatings can be used as an alternative approach to extend shelf life and to maintain fruit quality. Also, the plant extracts are less risky, affordable, accessible and they are less likely to develop resistance following prolonged usage. Based on the findings of this study, it is recommended that future studies use papaya fruits at different stages of development to assess different parameters such as shelf-life and firmness to improve quality. The study further recommend that future research incorporate papaya fruits collected from a farm level and avoid utilization of treated fruits since sterilising can have effects on the response to treatments. Different concentrations of moringa extracts with other edible coatings can be improved and maintained for use at a large scale. Antifungal activities and efficacy of moringa extracts can be assessed in response to different fungal fruit diseases under in-vitro setup using different solvents at different concentrations. The use of edible coatings and botanical extracts is essential as several active ingredients can be incorporated into the polymer matrix and consumed with the food, thus enhancing safety or even nutritional and sensory attributes.

5.1 References

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APPENDIX

APPENDIX A: Anova tables of measured variables

Analysis of variance

Variate: pH

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2		0.173847	0.086923	8.77	
Replicates.*Units* stratum						
Treatment	4		1.117787	0.279447	28.21	<.001
day	5		7.494906	1.498981	151.31	<.001
storage	1		0.000022	0.000022	0.00	0.962
Treatment.day	20		1.590665	0.079533	8.03	<.001
Treatment.storage	4		0.248603	0.062151	6.27	<.001
day.storage	2	(3)	0.438322	0.219161	22.12	<.001
Treatment.day.storage	8	(12)	0.312280	0.039035	3.94	<.001
Residual	88	(30)	0.871787	0.009907		
Total	134	(45)	9.763200			

Analysis of variance

Variate:_malic_acid

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2		0.002803	0.001402	0.51	
Replicates.*Units* stratum						
Treatment	4		0.019104	0.004776	1.73	0.151
day	5		0.379664	0.075933	27.47	<.001
storage	1		0.005193	0.005193	1.88	0.174
Treatment.day	20		0.123263	0.006163	2.23	0.006
Treatment.storage	4		0.080167	0.020042	7.25	<.001
day.storage	2	(3)	0.010074	0.005037	1.82	0.168
Treatment.day.storage	8	(12)	0.050613	0.006327	2.29	0.028
Residual	88	(30)	0.243245	0.002764		
Total	134	(45)	0.793617			

Analysis of variance

Variate: TSS

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replicates stratum	5		0.043481	0.008696	2.34	
Replicates.*Units* stratum						
Treatment	4		24.447519	6.111880	1646.78	<.001
day	5		159.704373	31.940875	8606.12	<.001

storage	1		3.311455	3.311455	892.24	<.001
Treatment.day	20		242.959762	12.147988	3273.14	<.001
Treatment.storage	4		43.626237	10.906559	2938.65	<.001
day.storage	2	(3)	16.265388	8.132694	2191.27	<.001
Treatment.day.storage	5	(15)	18.951108	3.790222	1021.23	<.001
Residual	205	(90)	0.760840	0.003711		
Total	251	(108)	316.136708			

Analysis of variance

Variate: PWL

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replicates stratum	8		28.2455	3.5307	12.59	
Replicates.*Units* stratum						
Treatment	4		759.7123	189.9281	677.06	<.001
day	4		4391.6177	1097.9044	3913.85	<.001
storage	1		482.4938	482.4938	1720.01	<.001
Treatment.day	16		202.4671	12.6542	45.11	<.001
Treatment.storage	4		0.8715	0.2179	0.78	0.541
day.storage	1	(3)	24.9385	24.9385	88.90	<.001
Treatment.day.storage	4	(12)	2.8151	0.7038	2.51	0.042
Residual	272	(120)	76.3008	0.2805		
Total	314	(135)	3156.8600			

Analysis of variance

Variate: Firmness

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2		509.37	254.69	8.13	
Replicates.*Units* stratum						
Treatment	4		559.43	139.86	4.46	0.002
day	5		12815.84	2563.17	81.79	<.001
storage	1		11925.87	11925.87	380.54	<.001
Treatment.day	20		1063.08	53.15	1.70	0.049
Treatment.storage	4		242.13	60.53	1.93	0.112
day.storage	2	(3)	3385.00	1692.50	54.01	<.001
Treatment.day.storage	8	(12)	128.36	16.04	0.51	0.844
Residual	88	(30)	2757.88	31.34		
Total	134	(45)	19987.09			

Analysis of variance

Variate: L*

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.

Replicates stratum	2		365.59	182.79	7.26	
Replicates.*Units* stratum						
Treatment	4		643.01	160.75	6.39	<.001
day	5		1378.61	275.72	10.96	<.001
storage	1		923.13	923.13	36.69	<.001
Treatment.day	20		811.66	40.58	1.61	0.067
Treatment.storage	4		302.52	75.63	3.01	0.022
day.storage	2	(3)	375.06	187.53	7.45	0.001
Treatment.day.storage	8	(12)	173.59	21.70	0.86	0.551
Residual	88	(30)	2214.17	25.16		
Total	134	(45)	5061.10			

Analysis of variance

Variate: a*

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2		557.40	278.70	14.16	
Replicates.*Units* stratum						
Treatment	4		983.88	245.97	12.49	<.001
day	5		5397.62	1079.52	54.83	<.001
storage	1		4975.61	4975.61	252.71	<.001
Treatment.day	20		824.04	41.20	2.09	0.010
Treatment.storage	4		479.93	119.98	6.09	<.001
day.storage	2	(3)	1495.33	747.67	37.97	<.001
Treatment.day.storage	8	(12)	144.67	18.08	0.92	0.505
Residual	88	(30)	1732.64	19.69		
Total	134	(45)	10548.69			

Analysis of variance

Variate: b*

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2		1417.23	708.61	11.57	
Replicates.*Units* stratum						
Treatment	4		1349.58	337.40	5.51	<.001
day	5		1852.13	370.43	6.05	<.001
storage	1		1312.99	1312.99	21.43	<.001
Treatment.day	20		1627.99	81.40	1.33	0.183
Treatment.storage	4		447.41	111.85	1.83	0.131
day.storage	2	(3)	481.54	240.77	3.93	0.023
Treatment.day.storage	8	(12)	254.51	31.81	0.52	0.839
Residual	88	(30)	5390.59	61.26		
Total	134	(45)	10710.87			

Analysis of variance

Variate: Hue angle

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2		1015.78	507.89	17.37	
Replicates.*Units* stratum						
Treatment	4		1079.73	269.93	9.23	<.001
day	5		6851.53	1370.31	46.85	<.001
storage	1		5395.86	5395.86	184.49	<.001
Treatment.day	20		1170.37	58.52	2.00	0.015
Treatment.storage	4		521.14	130.28	4.45	0.003
day.storage	2	(3)	1555.86	777.93	26.60	<.001
Treatment.day.storage	8	(12)	174.85	21.86	0.75	0.650
Residual	88	(30)	2573.77	29.25		
Total	134	(45)	13151.52			

Analysis of variance

Variate: C*

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2		1146.23	573.12	10.73	
Replicates.*Units* stratum						
Treatment	4		1300.04	325.01	6.08	<.001
day	5		1391.10	278.22	5.21	<.001
storage	1		1007.96	1007.96	18.87	<.001
Treatment.day	20		1453.10	72.66	1.36	0.165
Treatment.storage	4		343.58	85.89	1.61	0.179
day.storage	2	(3)	408.62	204.31	3.83	0.026
Treatment.day.storage	8	(12)	227.41	28.43	0.53	0.829
Residual	88	(30)	4700.36	53.41		
Total	134	(45)	9075.32			

Analysis of variance

Variate: Vitamin_C

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2		18.654	9.327	9.08	
Replicates.*Units* stratum						
Treatment	4		12.597	3.149	3.06	0.021
day	5		50.281	10.056	9.78	<.001
storage	1		1.028	1.028	1.00	0.320
Treatment.day	20		23.142	1.157	1.13	0.339
Treatment.storage	4		7.438	1.859	1.81	0.134
day.storage	2	(3)	2.223	1.111	1.08	0.344
Treatment.day.storage	8	(12)	7.583	0.948	0.92	0.502

Residual	88	(30)	90.443	1.028		
Total	134	(45)	188.598			

Analysis of variance

Variate: Flavonoids

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2		529.6	264.8	0.91	
Replicates.*Units* stratum						
Treatment	4		7519.5	1879.9	6.49	<.001
day	5		165437.2	33087.4	114.24	<.001
storage	1		7389.3	7389.3	25.51	<.001
Treatment.day	20		53168.4	2658.4	9.18	<.001
Treatment.storage	4		1645.5	411.4	1.42	0.234
day.storage	2	(3)	2344.8	1172.4	4.05	0.021
Treatment.day.storage	8	(12)	4750.8	593.8	2.05	0.049
Residual	88	(30)	25486.5	289.6		
Total	134	(45)	196122.3			

Analysis of variance

Variate: Phenolics

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2		2.095	1.048	0.93	
Replicates.*Units* stratum						
Treatment	4		69.920	17.480	15.51	<.001
day	5		237.817	47.563	42.19	<.001
storage	1		52.699	52.699	46.75	<.001
Treatment.day	20		181.349	9.067	8.04	<.001
Treatment.storage	4		109.240	27.310	24.22	<.001
day.storage	2	(3)	18.166	9.083	8.06	<.001
Treatment.day.storage	8	(12)	52.121	6.515	5.78	<.001
Residual	88	(30)	99.208	1.127		
Total	134	(45)	640.154			

Analysis of variance

Variate: % DPPH

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2		8703.2	4351.6	14.99	
Replicates.*Units* stratum						

Treatment	4		6286.2	1571.6	5.41	<.001
day	5		7456.6	1491.3	5.14	<.001
storage	1		20480.5	20480.5	70.56	<.001
Treatment.day	20		13424.2	671.2	2.31	0.004
Treatment.storage	4		5747.5	1436.9	4.95	0.001
day.storage	2	(3)	5353.0	2676.5	9.22	<.001
Treatment.day.storage	8	(12)	1863.2	232.9	0.80	0.602
Residual	88	(30)	25542.5	290.3		
Total	134	(45)	83922.6			

Analysis of variance

Variate: % ABTS

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2		5845.8	2922.9	7.66	
Replicates.*Units* stratum						
Treatment	4		3407.7	851.9	2.23	0.072
day	5		44725.5	8945.1	23.45	<.001
storage	1		2894.6	2894.6	7.59	0.007
Treatment.day	20		6011.4	300.6	0.79	0.721
Treatment.storage	4		2591.8	648.0	1.70	0.157
day.storage	2	(3)	1654.7	827.4	2.17	0.120
Treatment.day.storage	8	(12)	2679.7	335.0	0.88	0.538
Residual	88	(30)	33563.0	381.4		
Total	134	(45)	81840.4			

Analysis of variance

Variate: Sucrose

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2		0.249625	0.124813	16.76	
Replicates.*Units* stratum						
Treatment	4		0.000000	0.000000	0.00	1.000
day	5		182.213722	36.442744	4893.08	<.001
storage	1		0.023605	0.023605	3.17	0.078
Treatment.day	20		0.000000	0.000000	0.00	1.000
Treatment.storage	4		0.000000	0.000000	0.00	1.000
day.storage	2	(3)	0.023605	0.011803	1.58	0.211
Treatment.day.storage	8	(12)	0.000000	0.000000	0.00	1.000
Residual	88	(30)	0.655407	0.007448		
Total	134	(45)	172.626815			

Analysis of variance

Variate: Glucose

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2		1.5088	0.7544	0.77	
Replicates.*Units* stratum						
Treatment	4		2.0460	0.5115	0.52	0.721
day	5		450.9761	90.1952	91.76	<.001
storage	1		7.6866	7.6866	7.82	0.006
Treatment.day	20		116.5926	5.8296	5.93	<.001
Treatment.storage	4		26.1419	6.5355	6.65	<.001
day.storage	2	(3)	2.2447	1.1224	1.14	0.324
Treatment.day.storage	8	(12)	16.4949	2.0619	2.10	0.044
Residual	88	(30)	86.5034	0.9830		
Total	134	(45)	636.9957			

Analysis of variance

Variate: fructose

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replicates stratum	2		1.7398	0.8699	1.97	
Replicates.*Units* stratum						
Treatment	4		6.5407	1.6352	3.70	0.008
day	5		273.8928	54.7786	123.98	<.001
storage	1		2.9675	2.9675	6.72	0.011
Treatment.day	20		44.7745	2.2387	5.07	<.001
Treatment.storage	4		5.0650	1.2662	2.87	0.028
day.storage	2	(3)	0.7787	0.3893	0.88	0.418
Treatment.day.storage	8	(12)	4.2196	0.5274	1.19	0.312
Residual	88	(30)	38.8806	0.4418		
Total	134	(45)	322.2842			

Analysis of variance

Variate: Pathogenicity Test

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicates stratum	4	750.0	187.5	1.00	
replicates.*Units* stratum					
disease	3	34000.0	11333.3	60.44	<.001
Residual	12	2250.0	187.5		
Total	19	37000.0			

Analysis of variance

Variate: diameter_mm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replicates stratum	2	82.32	41.16	1.52	
replicates.*Units* stratum					
sample	2	9313.56	4656.78	171.66	<.001
treatment	4	74847.73	18711.93	689.78	<.001
concentration	3	54.50	18.17	0.67	0.573
sample.treatment	8	8821.93	1102.74	40.65	<.001
sample.concentration	6	439.44	73.24	2.70	0.020
treatment.concentration	5	946.09	189.22	6.98	<.001
sample.treatment.concentration	10	1409.07	140.91	5.19	<.001
Residual	76	2061.68	27.13		
Total	116	97976.32			