THE EFFECT OF EDIBLE COATINGS AND ZnO NANOPARTICLES BASED ON MORINGA EXTRACTS ON THE POSTHARVEST QUALITY OF AVOCADO (*Persea americana* Mill.) FRUIT

Sibonelo Ngubane

217031227

Submitted in Partial Fulfilment of the Requirements for

MASTER OF SCIENCE IN AGRICULTURE

(HORTICULTURAL SCIENCE)

in the

Discipline of Horticultural Science

School of Agricultural, Earth and Environmental Sciences

College of Agriculture, Engineering and Science

Pietermaritzburg campus

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

December 2023

SUPERVISOR'S APPROVAL

As the candidate's supervisors, I have approved this review and proposal for submission.

Supervisor: … Date: 13/6/24

Professor SZ Tesfay

Co-supervisor: … Date: …..

Professor A Mditshwa

Co-supervisor: … Date: …..

Professor LS Magwaza:

DECLARATIONS

I, **Sibonelo Ngubane,** declare that:

1. The research reported in this thesis is my original research, except where otherwise indicated.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs, or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
   a. their words have been re-written, but the general information attributed to them have been referenced, and
   b. where their exact words have been used, then their writing has been placed in italics and inside quotation marks and referenced.
5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed: [redacted] Date: 23 November 2023

Sibonelo Ngubane

ACKNOWLEDGEMENTS

First and foremost, I would like to thank God the Almighty for being with me throughout this journey. I would also like to send my heartfelt gratitude and appreciation to the following people and parties for their immeasurable support and contribution to the success of this project:

- Professor Samson Tesfay - for his supervision, advice, encouragement, and academic and personal support throughout this research period.
- Professor Asanda Mditshwa and Professor Lembe Magwaza – Co-supervisors, for their guidance, support, and valuable input on this project.
- Mr Sipho Sekhune – Manager Pack Production, Westfalia Technological Service, Howick, South Africa, for providing the avocado fruit used in this research.
- Mr Thokozani Nkosi, Senior laboratory technician, for organizing all the laboratory chemicals I needed for this project and assisting me with laboratory instrument operations.
- My family - for their prayers, support, and believing in me.
- Nomzamo Dyantyi - for helping me conduct my second experiment.
- Nqobile Bhengu - for the invaluable support and encouragement.
- Special thanks to all my friends, for their genuine support and availability throughout my academic life.
- My fraternal friends (Senzo, Yozi, Hlonti) – for always being available whenever I needed their support.
- National Research Foundation (NRF) – for financial support.

DEDICATION

I would like to dedicate this dissertation to ***MANZIMASE,*** my mother.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

CA	controlled atmosphere
CMC	carboxymethyl cellulose
CO_2	carbon dioxide
DM	dry matter
ETH	ethanol
MAP	modified atmosphere packaging
MCP	methylcyclopropene
MLE	moringa leaf extract
NO	nitric oxide
NPs	nanoparticles
O_2	oxygen
O_3	ozone
PDA	potato dextrose agar
RH	relative humidity
UV	ultraviolet
UV-A	ultraviolet A
UV-B	ultraviolet B
UV-C	ultraviolet C
ZnO	zinc oxide

GENERAL ABSTRACT

The production and consumption of avocado has been steadily increasing for many years in most countries, including South Africa, due to its various health benefits to humans. Similar to other climacteric fruits, avocados continue with metabolic changes even at postharvest, resulting in fruit deterioration during storage. These changes compromise the shelf life and create challenges for the market, especially the export market, considering the transportation period. Avocado is also susceptible to several postharvest diseases, such as skin rot anthracnose (*Colletotrichum gloeosporioides*), as the fruit ripens. Control has depended almost entirely upon prochloraz, especially for South African avocados. However, prochloraz has been banned in fruit destined for Europe, hence the primary target market for South Africa. Therefore, this necessitates continued research to develop or improve organic postharvest treatments. The present study was, therefore, driven by the following objectives: (1) To determine the effect of *Moringa oleifera* leaf extracts (MLE) incorporated with carboxymethyl cellulose (CMC) on the physical and biochemical properties of 'Hass' avocado harvested at different maturities (chapter 3); (2) To evaluate the efficacy of moringa-based zinc oxide(ZnO) nanoparticles (NPs) (ZnO-NPs) in suppressing the mycelium growth of *Colletotrichum gloeosprioides* (Anthracnose) in 'Hass' avocado fruit (chapter 4). In Chapter 3, different MLE concentrations (8 and 16%) in combination with 5% CMC preserved the quality and extended the shelf life of 'Hass' avocado. The treatments resulted in reduced mass loss and firmness loss, delayed colour change, and reduction in sugar content and antioxidant activity. Furthermore, different concentrations (0, 0.25, 0.5, 1, and 2%) of zinc oxide nanoparticles (ZnO-NPs) synthesized using moringa leaf extract significantly inhibited the growth of *Colletotrichum gloeosporioides* isolates in Petri dishes (chapter 4). The highest inhibition percentage (72%) was observed in 1% MLE-based ZnO-NPs treatment. Overall, this research demonstrated moringa-based edible coatings and nanoparticles as the best alternative -postharvest organic preservatives to be used compared to commercially used chemicals to preserve the quality of avocados during storage.

Key words: Climacteric fruit; shelf life, marketability; postharvest diseases; prochloraz; nanotechnology

Chapter 1

1.1 General introduction

1.1.1 Background

Avocado (*Persea americana* Mill.*)* is one of the most popular climacteric fruit grown in subtropical and tropical regions of different countries in the world. It is commercially produced in various parts of the world, including Mexico, Spain, South Africa, and Peru (Naamani, 2007). The production and consumption of avocado has been steadily increasing for the last decade in most countries. In South Africa, the total avocado plantings of 15 439 ha recorded in 2021 are still expected to increase by +/- 800 ha (SAAGA, 2022). This production mainly occurs in Limpopo, Mpumalanga, KwaZulu-Natal, Western Cape, and some parts of the Eastern Cape (Snijder et al., 2003). The global increase in the demand for avocado is due to its various health benefits to humans. This fruit is an excellent source of various antioxidants such as lutein and zeaxanthin, and it is also an essential source of folic acid, fibre, and mineral nutrients, such as iron, phosphorus, zinc, copper, and manganese (Araújo et al., 2018; Munhuweyi et al., 2020 Jimenez et al., 2021). Its mesocarp tissue is a great source of seven-carbon sugars, D-mannoheptulose and perseitol, and unsaturated fatty acids, which contribute positively to the human body diet (Tesfay and Magwaza, 2017). Due to its antioxidants, avocado consumption helps in preventing many chronic diseases such as cancer, diabetes, and cardiovascular diseases (Carvajal-Zarrabal et al., 2014).

The South African avocado industry is export-oriented; thus, it is highly critical to produce superior-quality fruit to retain the lucrative international markets, particularly the European Union (Sibulali, 2018). Avocado growers still face a huge challenge to maintain avocado quality throughout its supply chain, and this is due to the distance between South Africa and international markets. The quality of avocado is rated based on its size, firmness, absence of defects, and oil/dry matter content. The industry largely relies on postharvest treatments and optimized storage conditions to ensure the best quality throughout the marketing chain.

However, using chemicals to preserve the quality has become a major concern in recent years. This is due to health and environmental risks associated with some postharvest chemicals.

1.1.2 Problem statement and motivation of the study

Avocado fruit is highly perishable, it quickly loses quality when improperly handled after harvest. The loss of the avocado quality during storage is mainly due to its fast metabolic and respiration rate together with its high endogenous ethylene production, resulting in the short postharvest life of this fruit (Tesfay and Magwaza, 2017; Kubheka et al., 2020). The quality degradation of the fruit results in significant economic losses since spoiled fruit are not marketable. Furthermore, avocado fruits are mostly susceptible to various diseases caused by microorganisms such as *Colletotrichum gloeosprioides* (Penz.) Penz. & Sacc, which is responsible for the development of anthracnose, and *Diplodia natalensis* Pole-Evans, which causes stem end rot (Kader and Yahia, 2011). The control of these postharvest diseases is achieved by the use of prochloraz, which has long been demonstrated to suppress stem-end rot and anthracnose (Mavuso and Van Niekerk, 2010; Daneel et al., 2017). This non-systematic fungicide inhibits pathogen mycelial growth and delays the synthesis of fatty acid "ergosterol" which is a very significant fungal cell wall compound (Bill et al., 2014b; Obianom and Sivakumar, 2018). However, using fungicides has some disadvantages; the pathogens may develop resistance over time and may also have adverse effects on human health. In fact, prochloraz has recently been listed as a priority pollutant by the US Environmental Protection Agency (EPA) (Shimshoni et al., 2020). Similar concerns have been raised in Europe with the call to totally ban prochloraz as a postharvest treatment (Sivakumar et al., 2021). Thus, the future of prochloraz as a postharvest treatment is in limbo. Consumers demand fruit that are safe for consumption and free from defects and diseases. The substitute for prochloraz is, therefore, urgently needed to ensure the continued supply of the fruit to overseas markets.

The use of natural edible coatings has gained a lot of popularity worldwide. Recently, scientists have increasingly shifted their interests to edible coatings and films derived from natural plant parts to preserve the quality of horticultural commodities (Ncama et al., 2018). This approach is reliable, environmentally friendly, and affordable even for small-scale farmers (Germano et al., 2019; Kocira et al., 2021). Edible coatings based on proteins, polysaccharides, and lipids have been demonstrated to be very effective in reducing postharvest disease incidents and

extending the postharvest shelf life of most fruit, including avocado. These coatings carry antimicrobial and anti-browning agents, allowing them to delay biological activities that promote senescence in fruit (Bill et al., 2014a; Kocira et al., 2021). Currently, extracts from different parts of the moringa tree, such as leaves, roots, and seeds, are being used in edible coatings. These parts are a rich source of natural antioxidants (phenolics, ascorbic acid, and carotenoids). In addition, they are a great source of essential minerals (zinc, phosphorus, potassium, calcium, iron, and magnesium), vitamins (vitamin A, B1, B2, B3, and B-6), and amino acids (Liamngee et al., 2019). Furthermore, all the presented compounds contribute to the antimicrobial and antioxidant activity of such a coating (Abdull Razis et al., 2014). Although many countries have their own approved synthetic antimicrobial and antioxidant agents, there is still a burning issue about their environmental and health concerns (Liamngee et al., 2019).

Nanotechnology has currently drawn attention to many researchers due to its many applications including antimicrobial properties. Enriching nanoparticles in a coating solution has become one of the most effective tools in improving the work efficiency of coatings. Introducing nanotechnology to postharvest treatments could contribute to increased food production by lowering the percentage of postharvest losses and ensuring the best quality of the food items. It is becoming a norm to apply nanotechnology in postharvest preservation of fruit through different nano-systems, including nano-emulsions, nanoparticles, and nanocomposites (Zambrano-Zaragoza et al., 2018). Different inorganic particles consisting of metal or metal oxide, including but not limited to gold (Au), silver (Ag), iron oxide (Fe_3O_4), titanium oxide (TiO_2), and zinc oxide (ZnO) have been minimally used in postharvest treatments for their antimicrobial properties to prevent diseases on fruit and vegetables postharvest during storage (He and Hwang, 2016; Chandirika et al., 2018; Le et al., 2021; Saekow et al., 2019). More focus has been put on inorganic nanomaterials because of their high stability and antimicrobial activity when compared to organic nanomaterials; in addition, organic nanomaterials are heat-liable compounds (Saekow et al., 2019).

Many studies have been conducted on the postharvest treatments of avocado fruit (Daneel et al., 2017; Tesfay and Magwaza, 2017; Obianom and Sivakumar, 2018; Kubheka et al., 2020) but little information is available on the effect of moringa-based postharvest treatment in combination with nanotechnology on improving the postharvest quality of avocado,

particularly “Hass” cultivar. This study was, therefore, driven by the following aims and specific objectives.

1.2 Research aims and objectives

Aim

The aim of this research was to assess the effectiveness of moringa and carboxymethyl cellulose composite coating on the postharvest quality and shelf life of avocado fruit during storage and to synthesize moringa-based ZnO nanoparticles to control avocado postharvest diseases.

Objectives

The specific objectives of this study are to:

- determine the effect of *Moringa oleifera* extracts incorporated with carboxymethyl cellulose on the physical and biochemical properties of “Hass” avocado harvested at different maturity stages.
- characterize and determine the efficacy of moringa-based ZnO nanoparticles in suppressing the mycelium growth of *Colletotrichum gloeosprioides* (Anthracnose) in ‘Hass’ avocado fruit.

1.3 References

Abdull Razis, A. F., Ibrahim, M. D., and Kntayya, S. B. (2014). Health benefits of *Moringa oleifera*. *Asian Pacific Journal of Cancer Prevention* **15**, 8571-8576.

Araújo, R. G., Rodriguez-Jasso, R. M., Ruiz, H. A., Pintado, M. M. E., and Aguilar, C. N. (2018). Avocado by-products: Nutritional and functional properties. *Trends in Food Science and Technology* **80**, 51-60.

Bill, M., Sivakumar, D., Korsten, L., and Thompson, A. K. (2014a). The efficacy of combined application of edible coatings and thyme oil in inducing resistance components in avocado (*Persea americana Mill*.) against anthracnose during post-harvest storage. *Crop Protection* **64**, 159-167.

Bill, M., Sivakumar, D., Thompson, A. K., and Korsten, L. (2014b). Avocado fruit quality management during the postharvest supply chain. *Food Reviews International* **30**, 169-202.

Carvajal-Zarrabal, O., Nolasco-Hipolito, C., Aguilar-Uscanga, M. G., Melo-Santiesteban, G., Hayward-Jones, P. M., and Barradas-Dermitz, D. M. (2014). Avocado oil supplementation modifies cardiovascular risk profile markers in a rat model of sucrose-induced metabolic changes. *Disease Markers* **2014**, 2-9.

Chandirika, J. U., Selvi, S. T., and Annadurai, G. (2018). Synthesis and characterization of silver nanoparticle using Melia azedarach for vegetable coating and antibacterial activity. *Journal of Innovations in Pharmaceutical and Biological Sciences* **5**, 38-42.

Daneel, M., Sippel, A., de Beer, M., and Mlimi, J. (2017). Phrocloraz–A look into factors affecting the variability of residue levels on avocado fruit. *South African Avocado Growers' Association Yearbook* **40**, 72-77.

Germano, T. A., Aguiar, R. P., Bastos, M. S. R., Moreira, R. A., Ayala-Zavala, J. F., and de Miranda, M. R. A. (2019). Galactomannan-carnauba wax coating improves the antioxidant status and reduces chilling injury of 'Paluma'guava. *Postharvest Biology and Technology* **149**, 9-17.

He, X., and Hwang, H.-M. (2016). Nanotechnology in food science: Functionality, applicability, and safety assessment. J*ournal of Food and Drug Analysis* **24**, 671-681.

Jimenez, P., Garcia, P., Quitral, V., Vasquez, K., Parra-Ruiz, C., Reyes-Farias, M., Garcia-Diaz, D. F., Robert, P., Encina, C., and Soto-Covasich, J. (2021). Pulp, leaf, peel and

seed of avocado fruit: A review of bioactive compounds and healthy benefits. *Food Reviews International* **37**, 619-655.

Kader, A. A., and Yahia, E. (2011). Postharvest biology of tropical and subtropical fruits. *In Postharvest Biology and Technology of Tropical and Subtropical Fruits*, pp. 79-111. Woodhead Publishing..

Kocira, A., Kozłowicz, K., Panasiewicz, K., Staniak, M., Szpunar-Krok, E., and Hortyńska, P. (2021). Polysaccharides as edible films and coatings: Characteristics and influence on fruit and vegetable quality—A review. *Agronomy* **11**, 813.

Kubheka, S. F., Tesfay, S. Z., Mditshwa, A., and Magwaza, L. S. (2020). Evaluating the efficacy of edible coatings incorporated with moringa leaf extract on postharvest of 'Maluma'avocado fruit quality and its biofungicidal effect. *HortScience* **55**, 410-415.

Le, K. H., Nguyen, M. D.-B., Dai Tran, L., Thi, H. P. N., Van Tran, C., Van Tran, K., Thi, H. P. N., Thi, N. D., Yoon, Y. S., and Nguyen, D. D. (2021). A novel antimicrobial ZnO nanoparticles-added polysaccharide edible coating for the preservation of postharvest avocado under ambient conditions. *Progress in Organic Coatings* **158**, 106339.

Liamngee, K., Terna, A., and Ussuh, M. (2019). Effect of Moringa oleifera leaf extract on the postharvest quality of tomato fruits during storage. *Journal of Postharvest Technology* **7**, 45-55.

Mavuso, Z., and Van Niekerk, J. (2010). Development of a more effective post-harvest treatment for the control of post-harvest diseases of avocado fruit. *South African Avocado Growers' Association Yearbook* **33**, 53-55.

Munhuweyi, K., Mpai, S., and Sivakumar, D. (2020). Extension of avocado fruit postharvest quality using non-chemical treatments. *Agronomy* **10**, 212.

Naamani, G. (2007). Developments in the avocado world. *California Avocado Society Yearbook* **90**, 71-76.

Ncama, K., Magwaza, L. S., Mditshwa, A., and Tesfay, S. Z. (2018). Plant-based edible coatings for managing postharvest quality of fresh horticultural produce: A review. *Food Packaging and Shelf Life* **16**, 157-167.

Obianom, C., and Sivakumar, D. (2018). Differential response to combined prochloraz and thyme oil drench treatment in avocados against the control of anthracnose and stem-end rot. *Phytoparasitica* **46**, 273-281.

SAAGA, S. A. A. G. A. (2022). Overview of the South African avocado industry.

Saekow, M., Naradisorn, M., Tongdeesoontorn, W., and Hamauzu, Y. (2019). Effect of carboxymethyl cellulose coating containing ZnO-nanoparticles for prolonging shelf life of persimmon and tomato fruit. *Journal of Food Science and Agricultural Technology (JFAT)* **5**, 41-48.

Shimshoni, J. A., Bommuraj, V., Chen, Y., Sperling, R., Barel, S., Feygenberg, O., Maurer, D., and Alkan, N. (2020). Postharvest fungicide for avocado fruits: antifungal efficacy and peel to pulp distribution kinetics. *Foods* **9**, 124.

Sibulali, A. (2018). Analysing the competitive performance of the South African subtropical fruit industry (Doctoral dissertation, Stellenbosch: Stellenbosch University).

Sivakumar, D., Tuna Gunes, N., and Romanazzi, G. (2021). A comprehensive review on the impact of edible coatings, essential oils, and their nano formulations on postharvest decay anthracnose of avocados, mangoes, and papayas. *Frontiers in Microbiology* **12**, 711092.

Snijder, B., Mathumbu, J., and Kruger, F. (2003). Development of fruit maturity and mineral content norms for export avocado cultivars from different South African avocado growing regions. *In* "Actas V Congreso Mundial Del Aguacate", pp. 641-645. Citeseer.

Tesfay, S. Z., and Magwaza, L. S. (2017). Evaluating the efficacy of moringa leaf extract, chitosan and carboxymethyl cellulose as edible coatings for enhancing quality and extending postharvest life of avocado (*Persea americana* Mill.) fruit. *Food Packaging and Shelf Life* **11**, 40-48.

Zambrano-Zaragoza, M. L., González-Reza, R., Mendoza-Muñoz, N., Miranda-Linares, V., Bernal-Couoh, T. F., Mendoza-Elvira, S., and Quintanar-Guerrero, D. (2018). Nanosystems in edible coatings: A novel strategy for food preservation. *International Journal of Molecular Sciences* **19**, 705.

Chapter 2

Postharvest treatments of climacteric fruit: A review

2.1 Abstract

Climacteric fruits produce high ethylene, a ripening hormone, and have high respiration rate during storage. This leads to excessive ripening, fruit deterioration, and reduced postharvest life. Different technologies have been developed for postharvest handling to minimize the incidences of fruit deterioration. Postharvest treatments have a significant role in maintaining the quality and extending the shelf life of produce during handling chain, distribution, and transport. The widely used treatments include physical (coatings, heat treatments, irradiation), gaseous (low oxygen storages and controlled atmosphere), chemical (fungicides, ethylene inhibitors), and other biological techniques. However, there are reported drawbacks to the use of some synthetic chemical treatments. For instance, there is an imposed ban on prochloraz, a commercially acceptable fungicide, due to its contribution to environmental pollution, and other treatments are not very effective when applied as single treatments. The loss of fresh fruits continues to increase regardless of the available technologies for preserving quality. This, therefore, requires a close look at the status of currently used postharvest treatments to introduce new alternatives where necessary, such as adopting nanotechnology. Modifying available treatments with these new technologies could be remarkably beneficial in dealing with the food security crisis posed mainly by postharvest losses. For this reason, this paper reviews the literature on the effect of existing postharvest treatments and emerging technologies, particularly nanotechnology, on climacteric fruit quality. This review explored the use of nanoparticles in extending the shelf life of climacteric fruits and it was found that inorganic nanoparticles with greater stability have a positive effect on extending the shelf life of various fruits. There is ample evidence that edible coating in combination with nanoparticles have the positive effect in extending fruit quality.

Keywords:

Climacteric; postharvest loses; fruit postharvest treatments; nanotechnology; fruit quality; shelf life

2.2 Introduction

Fresh fruits play a significant role in the human diet and are strongly recommended for daily consumption because of their high nutritional quality (Zhang et al., 2017; Kocira et al., 2021). Most fruits are rich in nutritional compounds such as vitamins, minerals, and fibers (Jafarzadeh et al., 2021; Kocira et al., 2021). The countless benefits offered by fruit consumption make them a high priority to consumers (Jafarzadeh et al., 2021). The observed increase in fruit consumption as a result of increased global population and improved standard of living has, therefore, demanded an increase in their production. It has, however, been reported that a significant amount (20 – 30%) of fresh fruit and vegetables never reach consumers due to postharvest losses (Lufu et al., 2020). Meanwhile, the global population is exponentially increasing and is expected to reach 9.1 billion by 2050, which will further require a significant increase in food availability (Rezaei and Liu, 2017). The postharvest losses have serious implications for the environment, economy, and food security (Mditshwa et al., 2023). This loss also limits human access to high-quality goods at the correct time and at a fair price, which makes a living more challenging. Postharvest losses are more serious, especially in developing countries that rely more on exporting their produce to other developing and developed countries (Yahia Kazuz, 2011). These fruits are, therefore, transported for a long distance to market, resulting in a high percentage of fruit losses, especially those of a climacteric nature (Yahia Kazuz, 2011).

Climacteric fruits are typically harvested when they are fully matured at a minimum threshold and continue with their ripening process during storage. The most common climacteric fruits include bananas (*Musa* spp.), avocados (*Persea americana* Mill), apples (*Malus domestica* (Borkh.)), peaches (*Prunus persica* (L.) Batsch), tomatoes (*Solanum lycopersicum* L), and plums (*Prunus domestica*). Climacteric fruits have a unique ripening mechanism; thus, preserving their quality is challenging (Jafarzadeh et al., 2021). In comparison to their non-climacteric counterparts, climacteric fruits produce more ethylene, a gas that initiates and promotes the ripening process (Payasi and Sanwal, 2010). By allowing the fruit to continue ripening even after harvest, this gas promotes quick perishability and makes the fruit more susceptible to pathogenic organisms (Sandhya, 2010; Zhang et al., 2017). Maintaining climacteric fruit quality postharvest is highly prioritized and requires emergence measures.

Different postharvest techniques, including physical, chemical, biological, and organic treatments, have been demonstrated to reduce postharvest fruit deterioration. However,

understanding the major causes of fruit deterioration is a crucial step that aids in reducing the losses and selecting or developing appropriate and most affordable technical procedures to extend the shelf life and preserve the quality of produce (Wu, 2010). Postharvest treatments remain the best solution to reduce the losses of climacteric fruits during their storage period. The status of such treatments needs to be reviewed from time to time, especially in this changing climate. Some treatments have long lost their effectiveness due to various limitations, such as developing disease resistance and environmental polluting.

Although extensive research has been carried out on novel postharvest technologies and treatments, research gaps still exist. For instance, most of the published work focuses on specific fruits or treatment(s). For example, Ncama et al. (2018) only focused on plant-based edible coatings and their effect on postharvest. Similarly, Garcia and Davidov-Pardo (2021) reviewed the "recent advances in the use of edible coatings for the preservation of avocados." Recently, Satekge and Magwaza (2022) focused on the factors affecting the efficacy of 1-Methylcyclopropene (1-MCP) on climacteric fruits postharvest. There is currently no recent review on postharvest treatments for climacteric fruits. Thus, this paper will review the status of available postharvest treatments and emerging technologies, particularly nanotechnology, which can potentially reduce losses of climacteric fruits. This work will be a handy reference for postharvest physiologists and technologists for minimizing postharvest losses experienced by the horticultural industry, especially on climacteric fruits.

2.3 Postharvest losses of climacteric fruits - An overview

2.3.1 Postharvest fruit biological processes and their implications on quality

Fresh fruits are living tissues that perform various metabolic processes, even postharvest, resulting in continuous changes that may result in fruit spoilage (Brizzolara et al., 2020). All the fruit biological processes are interrelated and impact quality and shelf life (Zheng and Wolff, 2000; Pech et al., 2013; Aindongo et al., 2014). Fruit biological factors such as respiration, transpiration, and ethylene production occurring in fruit during storage can promote fruit deterioration (Yahia Kazuz, 2011). However, the rate at which these factors occur differs for each fruit depending on its ripening pattern (Silva, 2008). The loss of fruit quality during storage occurs more rapidly in climacteric than non-climacteric fruits since climacteric fruits

have maturation and ripening processes associated with increased respiration and ethylene production, reducing the shelf life (Pech et al., 2013). The postharvest loss of fruits varies and may reach up to 50% depending on the fruit (Ahmad et al., 2015; Perumal et al., 2021). Several postharvest studies have mainly focused on reducing the rate of these processes to extend the shelf life of fruits and vegetables. Most of the available postharvest technologies and their effectiveness are discussed in this paper.

2.3.2 Postharvest fruit losses due to biological factors (diseases)

Plant diseases account for a more significant percentage of losses of climacteric fruits during storage. The most common organisms that produce a microbial effect in many fruits and vegetables are fungi, moulds, yeast, and bacteria (Kahramanoğlu, 2017). However, the most observed fruit deterioration symptoms during storage result from diseases instigated by fungi and bacteria, though fungi tend to dominate more than bacteria (Yahaya and Mardiyya, 2019; Yahia Kazuz, 2011). Diseases can attack fruit in either pre- or postharvest stages. Different postharvest pathogens causing decay in climacteric fruit include *Fusarium* spp., *Botrytis* spp., *Penicillium* spp., *Diplodia* spp., *Rhizhopus* spp., and *Colletotrichum* spp. (Coates and Johnson, 1997; Sommer et al., 2002; Kahramanoğlu, 2017; Yahaya and Mardiyya, 2019). Amongst the postharvest decay-causing organisms, *Colletotrichum gloeosprioides* (causing anthracnose) (Figure 2.1 A) and *Diplodia natalensis* (causing stem end rot) (Figure 2.1 B) have a profound effect on the quality of most climacteric fruit, including papaya (*Carica papaya* L), mango (*Mangifera indica* L), banana (*Musa* sp), and avocado (*Persea americana* Mill) (Yahia Kazuz, 2011). The exposure of fruit to any stress may decrease its resistance to pathogens. The development of postharvest decay-causing organisms is aggravated by high relative humidity and temperature during storage (Peters et al., 2016). The presence of pathogens in fruit can cause severe outbreaks of foodborne diseases since fresh fruits are most occasionally consumed raw or minimally processed (Warriner et al., 2009). Overripened and senescencing fruit tend to be more susceptible to pathogens attacks (Sommer et al., 2002). During the fruit growth stage, the infection caused by pathogens remains quiescent, as biotrophs, and only show symptoms once developed as necrotrophs during fruit ripening (Prusky and Lichter, 2007). The fruit's physiological changes during ripening parallel the difunctional mechanisms that protect the fruit from fungal attacks (Prusky and Lichter, 2007; Yahia Kazuz, 2011).

Figure 0.1: Symptoms of anthracnose (A) and stem end rot (B) in avocado fruit during storage (Madhupani and Adikaram, 2017; Dissanayake et al., 2021).

2.3.3 Postharvest fruit loss due to mechanical damage

Fruit mechanical damage (blemish, cracking, and bruising) resulting from improper handling during harvesting, transportation, packaging, and poor storage facilities is one of the leading causes of climacteric fruit postharvest losses (Kahramanoğlu, 2017). Besides being the critical entry point for diseases, mechanical damages also have a significant economic effect, mainly because of adverse changes in organoleptic attributes and internal breakdown reactions (Martinez-Romero et al., 2004). The visual defects in fresh produce resulting from mechanical damage reduce the product's quality to the consumer and its market value, thus reducing the retailers and producers' profit (Van Zeebroeck et al., 2007; Opara and Pathare, 2014). Furthermore, bruised fruits have been associated with decreased vitamin C (Lee and Kader, 2000), increased ethylene production, mass loss, respiration, and total soluble solids (Al-Dairi et al., 2022; Pathare and Al-Dairi, 2022). All these changes have an enormous effect on fruit decay. Therefore, minimizing damage impact during handling and proper storage management can contribute positively to postharvest fruit quality during storage.

2.3.4 Postharvest loses due to physiological disorders

Climacteric fruits are most vulnerable to postharvest physiological disorders during production, handling, and storage (Wongs-Aree and Noichinda, 2014). Various physiological disorders result from the produce response to different physical and environmental stresses. The disorders are usually induced by storage gaseous composition, relative humidity, light intensity, and temperature (Lobo et al., 2013). In most cases, these disorders are mainly caused by storage of improper gaseous composition (oxygen, ethylene, and carbon dioxide), pathogens, and extreme temperatures (Mditshwa et al., 2017). Generally, the stress, especially induced by high temperature, promotes ripening, making fruit more prone to biotic diseases. Currently, the extension of fruit shelf life mainly relies on cold storage; however, extended exposure of fruits to cold or extreme low temperatures can promote the development of fruit physiological disorders such as chilling injuries (Cis), stem-end flesh browning, and flesh browning (Mitcham et al., 2004; Pareek et al., 2014; Sutanto et al., 2019; Brizzolara et al., 2020). However, some disorders may develop on fruit pre-harvest, such as papaya skin freckles, and have an impact on the final quality of the fruit (de Oliveira Bianchi et al., 2022).

2.4 Postharvest treatment methods for preserving the quality and extending the shelf life of fruit

Various postharvest treatments have been developed for treating fruit during their storage period, aiming to maintain their high quality and extend their shelf life while ensuring the safety of consumers and the environment (Mahajan et al., 2014). Different postharvest treatments have demonstrated outstanding potential in maintaining the quality of fresh and minimally processed fruits and vegetables. However, there are drawbacks associated with some of the treatments. This section reviews some of these treatments.

2.4.1 Edible coating

An edible coating is a thin layer of any edible material that has the potential to preserve fruit quality when applied over its surface. This film creates a modified atmosphere around the fruit by acting as a partial water and air barrier, thereby reducing the rate of fruit transpiration and respiration (Olivas et al., 2008). Edible coatings also serve as antioxidants, nutrients, and

antimicrobial carriers, significantly impacting fruit quality (Olivas and Barbosa-Cánovas, 2005). Some other benefits associated with the application of edible coatings to fruit surface include reduced enzymatic oxidation, maintenance of fruit volatile compounds and structural integrity, as well as reduced loss of aroma and colour (Olivas and Barbosa-Cánovas, 2005; Mahajan et al., 2014). Edible coatings are classified into three groups according to the nature of their compositional materials, namely, hydrophobic (waxes and lipids), hydrocolloids, also termed hydrophilic (proteins and polysaccharides), and composite coatings, which is the integration of the two groups (Olivas et al., 2008). The integration of edible coatings with different characteristics is mainly for improving the work efficiency of the coating (Gol et al., 2013).

Applying edible coatings to fresh commodities before storage has been an effective tool for preserving quality and prolonging their shelf life. In a study conducted by Duan et al. (2011), the use of various food-grade coating materials showed a positive effect on the postharvest quality of fresh blueberries (*Vaccinium corymbosum* L.) (cv. Duke and Elliott). The authors reported that both acid and water-soluble chitosan coatings significantly reduced the decay rate on both evaluated cultivars. The semperfresh^{TM} and calcium caseinate coatings resulted in reduced mass and firmness loss, delayed the rate of fruit ripening, and improved the quality of 'Duke' and 'Elliot' cultivars during storage at ambient temperature. Similar results were also reported by Mannozzi et al. (2017) on blueberries treated with alginate/pectin coating. The composite of shellac (60%) and gelatin (40%) reduced the firmness and mass loss of bananas stored for 30 days at 25 ℃ (Soradech et al., 2017). Furthermore, Kubheka et al. (2019) reported the effect of gum arabic and carboxymethylcellulose incorporated with moringa leaf extract on the quality of avocado (cv. 'Maluma') and incidence of *C. gloeosporioides* during 21 days of cold storage at 5.5 ℃ and 7 days shelf life. The results showed the combination of 15% gum arabic + moringa, 10% gum arabic + moringa, and 1% Carboxymethylcellulose + moringa as the most effective treatments in suppressing the growth of *C. gloeosporiodes*, reducing both mass and firmness loss, as well as delaying color changes.

Daisy et al. (2020) extended the shelf life and maintained the quality of mango fruit 'apple variety' stored at ambient temperature using gum arabic edible coating. The coating possessed both gas and water vapour barrier properties. The gum arabic-treated mango had a delayed increase in total soluble solids, maintained ascorbic acid, increased total acidity, and delayed ripening compared to untreated fruit. Table 0.1 shows some of the presented work by various authors on the impact of edible coatings on climacteric fruits.

Table 0.1: The effect of the application of edible coatings on postharvest quality of climacteric fruits.

Treatment	Fruit examined	Postharvest results	Reference
Chitosan/ Carboxymethyl cellulose and moringa leaf extract	Avocado 'Fuerte' and 'Hass'	Reduced fruit firmness and mass loss and improved overall fruit quality.	Tesfay and Magwaza (2017)
Pectin/ chitosan	Tomato 'Barbados'	Inhibited disease incidence and severity, maintained higher ascorbic acid and phenolic content.	Abebe et al. (2017)
Carboxymethyl cellulose	Guava	Maintained fruit quality and extended the storage life for up to 12 days at room temperature (24 ± 1 ℃).	Kumar et al. (2021)
Gum Arabic/ *Aloe vera gel* with ginger or garlic extract	Guava 'Gola'	Suppressed disease incidence and skin browning and maintained the sensory quality.	Anjum et al. (2020)
Cassava starch/ chitosan	Mango 'Tommy Atkins'	Delayed colour and physicochemical changes.	Oliveira et al. (2018)
Rice starch	Banana 'Cavendish'	Reduced chlorophyll degradation and retained fruit firmness.	Thakur et al. (2019)
Whey protein with lemon/lemongrass essential oil	Pear 'Conference'	Colour and fruit firmness were preserved by soy protein isolate without essential oil.	Galus et al. (2021)
Maize starch/agar	Apple	Agar coating effectively delayed browning, while maize starch retained fruit colour	Kusnadi et al. (2023)

Treatment	Fruit examined	Postharvest results	Reference
Gum arabic/ lemongrass oil/ cinnamon oil	Banana 'Pisang Berangan' Papaya 'Eksotika II'	Inhibited the growth of *C. musae* and *C. gloeosporioides* and delayed overall fruit ripening	Maqbool et al. (2011)
Basil seed gum with oregano essential oil	Apricot 'Gheisi'	Reduced microbial population and enhanced antioxidant activity	Hashemi et al. (2017)

2.4.2 Nitric oxide (NO)

Nitric oxide (NO) acts as a signaling molecule associated with the regulation of an impressive spectrum of plants and animals' cellular functions (Wendehenne et al., 2001). It has a considerable role in plants' physiological processes, but its effect may be positive or negative depending on its location and concentration in plant cells (Siddiqui et al., 2016). This gas is known to control different plant pathophysiological and developmental processes (Lamattina et al., 2003). Many studies have demonstrated the potential of NO to maintain various fruits, vegetables, and flowers' quality after harvest; however, it must be applied at low concentrations as a short-term fumigation treatment to be most effective (Siddiqui et al., 2016). The fruit can also be dipped into the treatment.

The exogenous application of NO preserves climacteric fruit quality by delaying their ripening process through regulating fruit biological processes that promote the ripening (Siddiqui et al., 2016). This gas has been widely used to extend the shelf life of most climacteric fruit, including tomatoes, mangoes, peaches, papayas, bananas, and kiwifruits (Zaharah and Singh, 2011; Li et al., 2013). The application of NO in fruit suppresses respiration rate, ethylene production, disease development, and chilling injury (Manjunatha et al., 2010). Fumigating papaya fruit (cv. 'Sunrise') with 60 µL/L NO for 3 h resulted in suppression of ethylene accumulation, delayed peel colour change, reduced fruit respiration, and weight loss. Subsequently, it extended the overall shelf life of fruit evaluated for 20 days at 20 º C and 75 % relative humidity (Li et al., 2013). Similar results were presented by Flores et al. (2008) on peaches (cv. 'Rojo Rito') treated with 5 µL/L NO for 4 h and stored at 20 º C for 14 days; the treatment showed a positive impact on the fruit's antioxidant capacity. On the other hand, the application of NO (10, 20, and 40 µL/L) on the mango fruit (cv. 'Kensington Pride') postharvest was found to alleviate chilling injury while delaying the fruit colour change, softening, and ripening, thus preserving the fruit quality throughout two- and four-weeks cold storage (Zaharah and Singh, 2011). However, there are limitations to relying on the use of NO as a major technique to maintain and extend fruit shelf life postharvest, as this is a very advanced technique and requires the development of a sophisticated controlled NO release system or carrier (Mahajan et al., 2014). The other limiting factor is that NO is rapidly converted to NO_2 in the presence of oxygen; therefore, its exogenous application must occur in an atmosphere free

of oxygen (Wills et al., 2000). Normally, oxygen surrounding the produce is removed with nitrogen or argon before applying a low concentration of NO, which takes place for 2-24 hours, depending on the commodity, which is a costly process (Wills et al., 2000). Siddiqui et al. (2016) further pointed out the need to invent a new efficient, more reliable technique of applying NO in the atmosphere with the presence of O_2 without facing the complications of NO oxidation. This is important since depletion of O_2 may also result in undesired fruit metabolism.

2.4.3 Heat treatment

Heat treatment is a promising and environmentally friendly physical treatment to substitute or reduce harmful chemicals (Akbudak et al., 2007; Mari et al., 2007). This technique is applied in different forms, such as hot-dry air, vapour heat, and hot water dips. Amongst the prescribed heat treatments, hot water is mainly preferred and has been extensively used in commercials since water is the best heat transfer medium compared to air. The overall aim of applying heat to fresh fruit is to delay their ripening process, reduce chilling injury and disease incidence caused by various pathogens (Akbudak et al., 2007). The exposure of fresh products to high temperatures after harvests has been identified to play a huge role in preserving the product quality for some time in storage. It has been noticed that exposing the fruit to high temperatures retards some of the processes during ripening while also enhancing others. Generally, hot dry air is applied to control insects and fungal developments, while the vapour heat treatment is solely used for insect control.

Some climacteric fruit, including plums (*Prunus domestica*), avocados, tomatoes, and pears (*Pyrus communis* L.), tends to soften very slowly when treated with heat at temperatures between 30 and 40 ℃. Mari et al. (2007) reported that brown rot caused by *Monilinia* spp. on nectarines (*Prunus persica var. nucipersica*) (cv. Star Red Gold') and peaches (cv. 'Sweet Fire') could be reduced by dipping the fruit in hot water at 40 ℃ for 2 minutes. On the other hand, Li et al. (2013), found that dipping papaya fruit (cv. 'Sunrise') in hot water at 54 ℃ for 4 minutes slightly promotes the rate of fruit colouring and delays fruit softening while preventing the fruit peel from carrying *Colletotrichum gloeosporioides (C. gloeosporioides)* which consequently inhibits the anthracnose and stem-end rot incidence. Similar results were produced by Liu et al. (2012) on peaches treated with 40 ℃ hot water for 5 and 10 minutes; the treatment controlled the brown rot. These results

align with the one reported by Akbudak et al. (2007) on cherry tomatoes (cvs. “Naomi” and “Alona”). It was found that dipping cherry tomatoes in hot water (54 ℃) for 5 minutes delayed fruit ripening and preserved the fruit quality for 28 days. In combination with low O_2 and high CO_2, hot water treatment reduced disease occurrence during cold storage. Exposing the peach fruit (cv. Xiahui 5) to hot air at 38 ℃ for 3 hours and hot water at 48 ℃ for 10 minutes maintained the fruit quality and enhanced the antioxidant activity of the fruit during storage at 4 ℃ (Huan et al., 2017). In addition, hot water outperformed hot air in alleviating internal browning symptoms in fruit. This, therefore, shows that hot water treatment can be used to preserve the quality of climacteric fruit instead of chemicals. However, hot water can damage the fruit when exposed at higher than recommended temperatures for a very long time.

2.4.4 Chemical treatments

Different chemical treatments are used to maintain the quality and extend the shelf life of different climacteric fruit. However, some of these treatments are less effective in controlling fruit microbial growth during storage. Studies conducted by different authors have shown a significant effect of various treatments on improving fruit quality postharvest during storage. These treatments include but are not limited to 1-methylcyclopropene (1-MCP), calcium chloride, prochloraz, and fludioxonil. Among these treatments, prochloraz is the only registered fungicide used, especially in fruits designated to Israel and Europe (Shimshoni et al., 2020). However, due to health and environmental concerns, this fungicide will no longer be used as a postharvest treatment (Shimshoni et al., 2020). Furthermore, this has necessitated more postharvest treatment studies. Various authors have reported the effect of different chemical treatments for climacteric fruits. For instance, Oz and Ulukanli (2014) evaluated the effect of treating mulberries (*Morus alba. L.)* with 312.5 ppb of 1-MCP, $CaCl_2$ (1% w/v), and $CaCl_2$ (1% w/v) + 1-MCP (312.5 ppb) before storage. Their study identified $CaCl_2$ as the best treatment for maintaining the fruit colour, and ascorbic acid content, and slowing down the rate of fruit browning and microbial growth around the peel. Similarly, 1-MCP treatment retained the fruit quality by delaying fruit colour change and browning. However, this treatment was ineffective in preventing the loss of fruit sensory quality, ascorbic acid content, and reducing the respiration rate. Zheng et al. (2014) found rare earth,

generally used as a fertilizer to enhance plant biomass yield, to be very effective in preserving cut 'Fuji' apple quality by maintaining their surface colour and titratable acidity; however, this treatment was not effective as 1-MCP for reducing fruit firmness and mass loss. Furthermore, combining rare earth, calcium, and 1-MCP, was recommended for colour and firmness retention, and longer shelf life (Zheng et al., 2014). It was later reported that 1-MCP and CA increased late-harvested 'Empire' apple flesh browning, indicating that such treatment's performance still needs to be optimized (Jung and Choi, 2020). Table 0.2 presents some of the commonly used chemical treatments.

Table 0.2: The effect of chemical treatments on postharvest quality of climacteric fruits.

Treatment	Fruit examined	Postharvest results	Reference
1% (w/v) $CaCl_2$ + 1% (w/v) ascorbic acid + 0.5% (w/v) cysteine	Banana 'Grand Nain'	Delayed fruit browning and softening for 6 days at 5 ℃.	Vilas-Boas and Kader (2006)
Ascorbic acid, calcium lactate, and cysteine	Pear 'Bartlett'	Reduced cut surface browning. Extended shelf-life.	Gorny et al. (2002)
$CaCl_2$, Ca $(NO_3)_2$ and KCl	Sapota 'PKM 1'	Reduced mass loss, spoilage, and increased shelf life.	Sudha et al. (2007)
Salicylic acid	Tomato 'Hisar-Arun'	Delayed the ripening and extended the fruit shelf life up to 4 to 6 days at room temperature (25 ± 1).	Kumar et al. (2018)
Citric acid	Peach 'Hujingmilu'	The citric acid (10g/L) effectively inhibited fruit decay. Prevented the reduction in fruit's malic acid and citric acid.	Yang et al. (2019)
1-MCP with cold storage (0.5 ℃)	Apple 'Empire'	Simulated polyphenol oxidase activities while elevating the flesh electrical conductivity percentage and peroxidase activity.	Jung and Choi (2020)

Treatment	Fruit examined	Postharvest results	Reference
Salicylic acid / $KMnO_4$	Kiwi 'Hayward'	Longer shelf-life	Bal and Çelik (2010)
Fludioxonil/ Prochloraz	Avocado 'Pinkerton', 'Ettinger', and 'Reed'	Both treatments had similar effects at a concentration range (75–300 µg/L); avocado postharvest decay was inhibited.	Shimshoni et al. (2020)

2.4.5 Ultraviolet (UV) treatments

There is an increasing popularity of ultraviolet treatment in postharvest fruit quality preservation studies. Generally, UV light is the electromagnetic spectrum band between the X-ray (200 nm) and the visible light (400 nm). The light between 200-280 is a short wave (UV-C), whereas medium wave (UV-B) exists between 280-320 nm (UV-B), and the long wave (UV-A) is between 320-400 nm (Bintsis et al., 2000; Urban et al., 2016). UV treatments are recommended as a safe and environmentally friendly sterilization method to prevent fungal infections (Andrade-Cuvi et al., 2017; Zhang and Jiang, 2019). UV-B and UV-C are mostly used to preserve most horticultural commodities' postharvest quality. Generally, this treatment involves exposing the fruit to radiation ranging between 200 and 300 nm for a pronounced period (George et al., 2016). The stress induced by irradiation influences the gene expression and level of enzyme activities, thereby modifying fruit metabolism (Civello et al., 2014).

The effect of UV as postharvest treatment has been evaluated by many authors, particularly the use of UV-C. For example, George et al. (2016) reported that UV-C treatment at doses 1.764 J m^{-2}, 3.525 J m^{-2}, and 7.056 J m^{-2} irradiated for 15, 30 and 60 minutes, respectively, preserved the quality and extended the shelf life of fresh cut mango fruit (cv. 'Chokana') by 15 days. Similarly, the shelf life of 'Kensington Pride' mango fruit was extended by up to 12 days following a short-term UV-C radiation at 4.0 kJ^{-2} and storage at 20 °C in 0.1 µL L^{-1} ethylene (Pristijono et al., 2018). Besides the extension of fruit shelf life, most research has also demonstrated the effect of UV treatments on the antioxidant status of most fruits. For example, Razali et al. (2021) demonstrated an increased total polyphenol, flavonoids, and ascorbic acid contents on cherry tomatoes treated with mucilage coating and irradiated at fluorescent germicidal lamps for 8 minutes at 15 cm away from the lamp. Pristijono et al. (2017) also reported an increased content of total phenolics on 'Neang Pich' tomato fruit treated with 0.5 µL L^{-1} 1-MCP and 13.6 kJ m^{-2} UV-C and stored at 20 °C in air containing 0.1 µL L^{-1} ethylene.

UV-C has also been found to display antifungal effects against major postharvest diseases. The combination of UV-C and chitosan enriched with essential oils was described to be very effective in inhibiting the growth of *Colletotrichum gloeosporioides* (Penz.) and *Rhizopus stolonifer* (Ehrenb.) inoculated in papaya (cv. Maradol) (Vázquez-Ovando et al., 2018). Like most

treatments, there are still some limitations on the use of UV radiation. Exposing fruit to UV radiation for extended periods can negatively affect fruit chlorophyll and carotenoids (Jaiswal et al., 2023).

2.4.6 Ozone (O_3) treatments

Ozone has been commended as the best replacement for the current traditional sanitizing agents due to the oxidizing properties possessed by this natural gas (Abrol et al., 2021). Ozone has a high oxidant capacity and is more effective over various microorganisms than chlorine and hypochlorous acid (Horvitz and Cantalejo, 2014). In fruits, ozone is used to preserve quality, extend shelf life, and eliminate undesirable flavour induced by bacteria during storage (Zhang et al., 2005). For instance, Liang et al. (2018) reported that the quality of tomato fruit was better maintained by treating the fruit with 17.14 mg/m^3 ozone gas for 60 minutes and storing the fruit at 0℃. Bambalele et al. (2023) maintained the quality and extended the shelf life of 'Keitt' mango fruit by 14 days by treating the fruit with O_3 (0.25 mg/L) for 36 hours. The fruits were evaluated for 21 days at cold storage (10 ℃) and 7 days shelf life at 25 ℃, and the fruit mass loss, total soluble solids (TSS) accumulation, and decline of total acidity (TA) were found to be delayed by this treatment. Another study by Minas et al. (2012) reported an increase in antioxidants and delayed ripening in 'Hayward' kiwifruit following the treatment with O_3 at 0.3 µL/L for 60 minutes and storage of 3 or 5 months at 0 ℃ and 95% RH and 12 days of shelf life at 20 ℃. The continuous application of O_3 (0.1 ± 0.08 µL L^{-1}) in cold storage at 2 ℃ and 90% RH resulted in a high rate of ethylene production and increased sugar content on apple fruit ('Granny Smith' and 'Cripps Pink') stored for 90 and 120 days at 0 ± 2 ℃ and 90 ± 5% RH (Tokala et al., 2021). Similarly, Ong et al. (2014) reported an increased ethylene concentration and damaged fruit tissue on 'Sekaki' papaya fruit exposed to ozone at a concentration greater than 3.5 µL/L for 96 hours. It can be concluded that applying ozone at higher concentrations or exposing the fruit for a more extended period results in physical injuries and oxidative stress in the fruit. Some adverse health effects of high ozone concentrations are also reported (Horvitz and Cantalejo, 2014).

2.4.7 Controlled atmosphere storage and modified atmospheric packaging

Modified atmosphere packaging (MAP) and controlled atmosphere (CA) storage have long been used to increase the shelf-life of various fruit and vegetables. These techniques are primarily used to prevent or retard fruit senescence and related biochemical and physiological changes (Singla et al., 2022). A modified atmosphere is achieved by amending the gaseous environment produced through respiration or by adding and removing gases as required from a food package to control the levels of both oxygen and carbon dioxide (Selcuk and Erkan, 2015). The elevation of carbon dioxide and/or depletion of oxygen levels can result in delayed ripening, reduced respiration, decreased ethylene accumulation, delayed fruit softening, and slowed ripening process, resulting in fruit having an extended shelf life. It has been reported that lowering the oxygen concentrations to levels below 8% and/or increasing carbon dioxide concentrations to above 1% delays the fruit ripening. However, further decreasing oxygens level to 2% may result in anaerobic respiration, thereby promoting off-odours and off-flavours (Erkan and Wang, 2006).

Majidi et al. (2014) reported that CA and MAP can retard the ripening process of 'super jeff' tomato fruit compared to cold storage. Their study further illustrated controlled atmosphere storage as the best method for maintaining the texture and colour of tomatoes, followed by the modified atmosphere and the least being cold storage. Another study was conducted by Daş et al. (2006) to analyze the behavior of *Salmonella enteritidis* inoculated at the spot or injected on the stem of cherry tomatoes and exposed to passive modified atmosphere packaging (MAP), controlled atmosphere (CA), and air storage at 7 and 22 °C. The results demonstrated a complete death of *S. enteritidis* cells on days 6 and 8 on tomatoes stored at 7 and 22 °C, respectively. Another study conducted by Arias et al. (2008) recommended the use of an atmosphere with a high concentration of carbon dioxide, such as the combinations of 21% O_2 + 10% CO_2 and 2% O_2 + 10% CO_2 as the most suitable to preserve the quality of different varieties of pears (Williams, Conference, Passacrassana) at low temperatures. It was also reported that MAP with oxygen levels at 12% and 4% carbon dioxide could reduce chilling injury in banana (cv. Kluai Khai) peel stored at 10 °C (Nguyen et al., 2004). Table 0.3 summarizes the published work on the use of modified and controlled atmosphere on climacteric fruits postharvest.

Table 0.3: The effect of controlled and modified atmosphere packaging on postharvest quality of climacteric fruits.

Treatment/ method	Fruit examined	Atmospheric composition (O_2 / CO_2)	Postharvest result	Reference
CA	Fig 'Ottomanit'	5 kPa/ 5 kPa	Maintained fruit firmness and integrity. Delayed fruit decay.	Bahar and Lichter (2018)
MAP	Apricots 'Shahroudi'	40 % / 20 %	Longer shelf life. Increased vitamin C content and carotenoid.	Dorostkar et al. (2022)
1-MCP + CA	Peach 'Tardibelle'	3 kPa / 10 kPa	Inhibited the enzyme activities for the production of volatile esters from fatty acids	Ortiz Catalán et al. (2010)
PVC + MAP	Plum 'Friar'	10 % / 4 %	Suppressed fruit colour change, chilling injury, and improved overall fruit quality	Wang et al. (2021)
MAP	Guava 'Hisar Safeda'	-	Delayed fruit ripening, firmness loss. Extended the shelf-life.	Rana et al. (2018)
CA	Banana 'Cavendish'	2 % / 4, 6, 8 %	Retained firmness which restricted mechanical injuries and disease occurrence	Ahmad et al. (2006)
CA	Mango 'Delta R2E2'	6 % / 3 %	Reduced respiration rate and delayed colour change	Lalel et al. (2005)

Treatment/ method	Fruit examined	Atmospheric composition (O_2 / CO_2)	Postharvest result	Reference
MAP	Avocado 'Hass'	4 % / 5 %	Inhibited fruit ripening. Reduced chilling injury	Meir et al. (1997)
CA	Avocado 'Hass'	5 kPa / 10 kPa	Reduced chilling injury. Maintained fruit colour and firmness	Alamar et al. (2017)

Nanotechnology as an emerging innovation for climacteric fruit protection postharvest

Nanotechnology is a new promising technology with great potential for various applications, including the agricultural industry. Bhattacharyya et al. (2009) defined nanotechnology as the integration of science, engineering, and technology at the nanoscale ranging from about 1 to 100 nanometers. Nanoparticles are generally classified as organic or inorganic based on their composition, which also determines their potential toxicity and gastrointestinal fate (McClements and Xiao, 2017). Organic nanoparticles are mainly solid particles synthesized from organic compounds, commonly lipids or polymerics. There is an increasing interest in the use of inorganic nanoparticles consisting of metals or metal oxides such as iron oxide (Fe_3O_4), zinc oxide (ZnO), titanium oxide (TiO_2), silver (Ag), gold (Au), aluminium oxides, and other carbon-based materials (Peters et al., 2014; He et al., 2016; Odetayo et al., 2022b). Nanoparticles have more interesting unique features than large materials owing to their high surface-to-volume ratio and smaller size (Elnashaie et al., 2015). Other interesting characteristics include their high stability, unique biological behaviors, a wide range of physical multifunctionality, and tunable compositions (Elnashaie et al., 2015). Various techniques are being used to synthesize nanomaterials, including chemical reduction, photochemical reduction, electrochemical reduction, and heat evaporation (Salisu et al., 2014). However, interest is currently in synthesizing the nanoparticles using a greener approach. This method uses plant extracts, yeast, fungi, and bacteria as reducing agents to obtain nanoparticles (Khan et al., 2017). Moreover, the green method is cheaper, less toxic, and eco-friendly. (Figure 0.2) demonstrates the preparation and incorporation of nanoparticles into coatings, especially synthesized using a greener method.

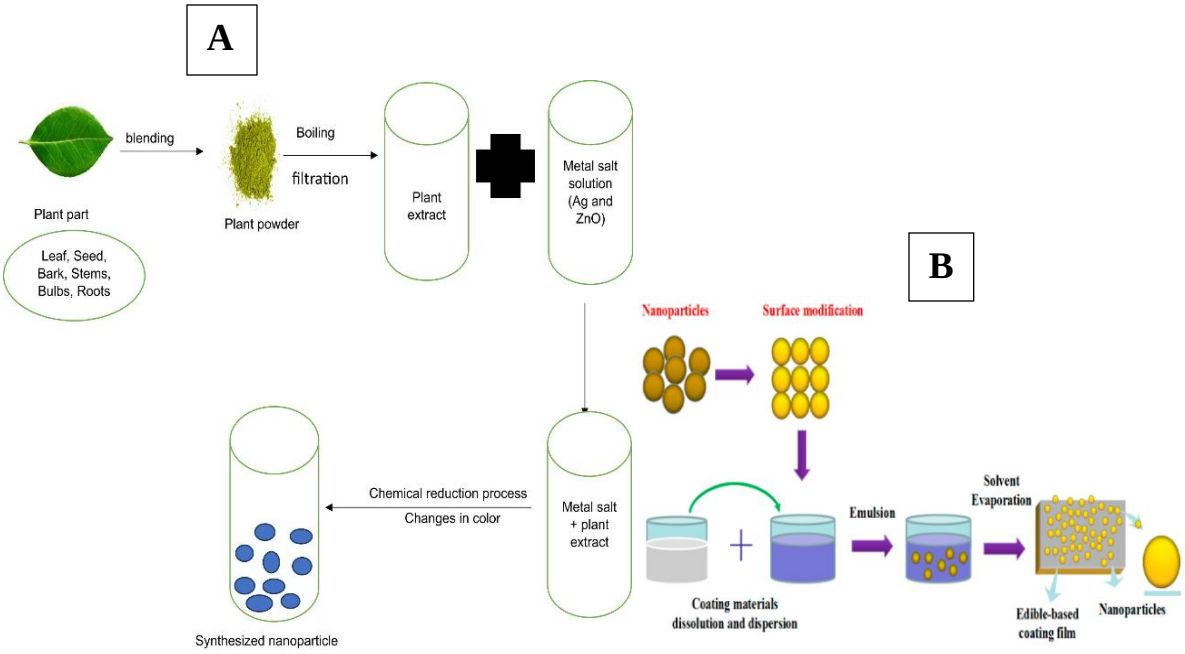

Figure 0.2: Development of nanoparticles enriched edible coating: adapted from (Xing et al., 2019; Odetayo et al., 2022b).

Various nanosystems, including nanoparticles, nano-emulsions, and nanocomposites, are currently used to improve the coating material for preserving fruit quality and extending shelf-life (Zambrano-Zaragoza et al., 2018). Typically, coatings are used as mediums to carry antimicrobial nanoparticles. Recently, more scientists have focused on using nanoscale materials to improve the quality of most horticultural produce. Some published research on adopting nanotechnology in edible coatings to preserve climacteric fruit quality postharvest is presented in Table 0.4.

Table 0.4: Published work on the postharvest application of nanoparticles enriched coatings on climacteric fruits

Fruit studied	Coating or film matrix	Nanoparticles	Treatment effect	Reference
Guavas	Alginate and chitosan	ZnO	ZnO-NPS displayed antimicrobial action against rot in guavas.	Jafarzadeh et al. (2021)
Banana	chitosan/gum arabic	ZnO	Enhanced the fruit quality and extended the shelf life at a storage temperature of 35℃ and 54 % relative humidity.	La et al. (2021)
Banana	Tara Gum	Ag	Integrating silver nanoparticles (5%) into Tara Gum preserved fruits colour, firmness, mass, and soluble solids. Extended the shelf-life of fruits.	de Oliveira Bianchi et al. (2022)
Pear	Chitosan	Cellulose	Reduced ethylene production, delayed ripening and deterioration throughout 3 weeks storage period.	Deng et al. (2017)
Apricot	Glycerol	Ag	AgNPs showed antibacterial and antifungal activities against gram-negative bacteria (*E coli* and *S. Typhimurium*) and A. *flavus* and *A. niger*, respectively.	Shahat et al. (2020)
Tomato	Chitosan-PVA Hydrogels	Cu	Reduced mass loss. Preserved physicochemical quality and increased bioactive compounds contents.	Hernández-Fuentes et al. (2023)
Tomato	Rosemary extract	Cu	Copper nanoparticles (0.25 %) inhibited microbial decay during the 10 days storage period	Bikdeloo et al. (2021)

Fruit studied	Coating or film matrix	Nanoparticles	Treatment effect	Reference
Mango	Chitosan	TiO_2	Chitosan/TiO_2 coating preserved the quality of mango fruits during storage.	Xing et al. (2020)

Most authors' have reported the best improvement on coatings when mixed with nanomaterials. For instance, An et al. (2008) reported the effect of silver nanoparticles-PVP-based coating on the quality of asparagus (*Asparagus officinalis*) (cv. 'UC800'). The coating was effective in hindering the growth of microorganisms while ensuring the best fruit quality throughout the 25 days storage period at 2 ℃. Another study by Shahat et al. (2020) showed that 'canino' apricots quality could be preserved for 24 days at 6 ℃ and for eight days at room temperature (25 °C) by coating the fruit with starch based AgNPs. The quality of the apples (cv. 'Anna') was preserved, and the shelf life was extended to 42 days by coating the fruit with gelatin/chitosan combined with Ag / ZnO nanoparticles (Bakhy et al., 2018).

Odetayo et al. (2022a) reported better preservation of Cavendish banana quality and extension of shelf life when using edible coating enriched with chitosan nanoparticles. It was observed that adding chitosan nanoparticles to the moringa or *aloe vera* (L.) Burm coating significantly reduced respiration rate, improved phenolic content, and retained firmness for 30 days of storage at 18 ℃. The results obtained from the study were much promising compared to previous results obtained by the same authors when using the coating alone, without the addition of nanoparticles. Similar results were reported by La et al. (2021) on bananas coated with chitosan/gum arabic mixed with ZnO nanoparticles synthesized through the hydrothermal method. The resulting edible coating significantly improved the quality of bananas throughout the storage period. It was reported that the coated fruits had an extended shelf life of more than 17 days when stored at 35 ℃ and relative humidity of 54 %, compared to uncoated fruits with less than 13 days of shelf life under similar conditions. Furthermore, incorporating ZnO nanoparticles into the coating solution showed a significant effect against *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*.

Recently, Guo et al. (2020) developed a coating to improve the quality of cherry tomatoes by incorporating plant essential oil cinnamaldehyde (CIN) and zinc oxide nanoparticles (ZnONPs) into carboxymethylcellulose (CMC) coating. The resulting CMC-based composite film had an improved antifungal property, physicomechanical, and barrier as an influence of ZnONPs and CIN. This was described to have been caused by adding CIM, which added the antifungal property to the film, whereas the addition of ZnONPs to the CIN/CMC film was reported to have improved the flexibility and strength of the film. Moreover, the film was good water, light, and oxygen barrier due to ZnO-NPs' addition.

The quality of pear (cv. D'Anjou and Bartlett) fruit was preserved after applying chitosan coating incorporated with cellulose nanocrystals (Deng et al., 2017). The coating delayed fruit deterioration by slowing the rate of ripening and improved the storability of pears postharvest stored in ambient and cold storage. Based on the results presented by (Hernández-Fuentes et al., 2017), the quality of 'Huno F1' tomato fruit can be maintained during storage at ambient temperature by spraying the fruit with copper nanoparticle-based coating. Sprayed fruits had a great content of total antioxidants and phenolic compounds. It was indicated that copper nanoparticles could control grey mould disease, known to be caused by *Botrytis cinerea* (Hashim et al., 2019). Applying copper nanoparticles at a concentration of 15 mmol/l completely suppressed the growth of botrytis in culture medium.

2.5 Conclusion and Prospects

Food insecurity continues to be a concern in many developing countries, and postharvest fruit losses make the situation even worse. The current postharvest techniques have many drawbacks, some of which have been pointed out in this paper. With the imposed ban on some important fungicides, particularly prochloraz, and the introduction of new international markets for some climacteric fruits, new eco-friendly treatments need to be developed urgently. The literature exposed the great potential of nanotechnology, especially for the particles synthesized using a green method. The edible coatings functionalized with different organic nanoparticles could be the best solution to prevent the quality loss of most fruits after they have been harvested. Further evaluation of the potential of the combination of nanotechnology with other promising technologies, such as O_3 and UV radiation technology, in preserving the quality, decreasing postharvest diseases, and extending the shelf life of climacteric fruits is still required. However, more reports are still needed that examines the concentration of nanoparticles that can be present in fruit edible portion to adhere to the standard and safety of both humans and the environment.

2.6 References

Abebe, Z., Tola, Y. B., and Mohammed, A. (2017). Effects of edible coating materials and stages of maturity at harvest on storage life and quality of tomato (*Lycopersicon Esculentum* Mill.) fruits. *African Journal of Agricultural Research* **12**, 550-565.

Abrol, G. S., Singh, A. K., Pal, R., and Sharma, G. (2021). Innovation in Postharvest Technology for the Maintenance of Quality of Fruits and Vegetables. *Recent Advances in Processing of Fruits and Vegetables, RLBCAU Publication, Jhansi*, 6-19.

Ahmad, M. S., Siddiqui, M. W., Ahmad, M. S., and Siddiqui, M. W. (2015). Factors affecting postharvest quality of fresh fruits. *Postharvest quality assurance of fruits: Practical Approaches for Developing Countries*, 7-32.

Ahmad, S., Perviez, M., Thompson, A., and Ullah, H. (2006). Effects of storage of banana in controlled atmosphere before ethylene treatments on its ripening and quality. *Journal of. Agricultural. Research* **44**, 219-229.

Aindongo, W. V., Caleb, O. J., Mahajan, P. V., Manley, M., and Opara, U. L. (2014). Effects of storage conditions on transpiration rate of pomegranate aril-sacs and arils. *South African Journal of Plant and Soil* **31**, 7-11.

Akbudak, B., Akbudak, N., Seniz, V., and Eris, A. (2007). Sequential treatments of hot water and modified atmosphere packaging in cherry tomatoes. *Journal of Food Quality* **30**, 896-910.

Al-Dairi, M., Pathare, P. B., Al-Yahyai, R., and Opara, U. L. (2022). Mechanical damage of fresh produce in postharvest transportation: Current status and future prospects. *Trends in Food Science and Technology* **124**, 195-207.

Alamar, M. C., Collings, E., Cools, K., and Terry, L. A. (2017). Impact of controlled atmosphere scheduling on strawberry and imported avocado fruit. *Postharvest Biology and Technology* **134**, 76-86.

An, J., Zhang, M., Wang, S., and Tang, J. (2008). Physical, chemical and microbiological changes in stored green asparagus spears as affected by coating of silver nanoparticles-PVP. *LWT-Food Science and Technology* **41**, 1100-1107.

Andrade-Cuvi, M. J., Moreno, C., Zaro, M. J., Vicente, A. R., and Concellón, A. (2017). Improvement of the antioxidant properties and postharvest life of three exotic Andean fruits by UV-C treatment. *Journal of Food Quality* **2017**.

Anjum, M. A., Akram, H., Zaidi, M., and Ali, S. (2020). Effect of gum arabic and Aloe vera gel based edible coatings in combination with plant extracts on postharvest quality and storability of 'Gola'guava fruits. *Scientia Horticulturae* **271**, 109506.

Arias, E., González, J., López-Buesa, P., and Oria, R. (2008). Optimization of processing of fresh-cut pear. *Journal of the Science of Food and Agriculture* **88**, 1755-1763.

Bahar, A., and Lichter, A. (2018). Effect of controlled atmosphere on the storage potential of Ottomanit fig fruit. *Scientia Horticulturae* **227**, 196-201.

Bakhy, E. A., Zidan, N. S., and Aboul-Anean, H. E. D. (2018). The effect of nano materials on edible coating and films' improvement. *International Journal of Pharmaceutical Research and Allied Sciences* **7**, 20-41.

Bal, E., and Çelik, S. (2010). The effects of postharvest treatments of salicylic acid and potassium permanganate on the storage of kiwifruit. *Bulgarian Journal of Agricultural Science* **16**, 576-584.

Bambalele, N. L., Mditshwa, A., Magwaza, L. S., and Tesfay, S. Z. (2023). Postharvest effect of gaseous ozone on physicochemical quality, carotenoid content and shelf-life of mango fruit. *Cogent Food and Agriculture* **9**, 2247678.

Bhattacharyya, D., Singh, S., Satnalika, N., Khandelwal, A., and Jeon, S.-H. (2009). Nanotechnology, big things from a tiny world: a review. *International Journal of u-and e-Service, Science and Technology* **2**, 29-38.

Bikdeloo, M., Ahsani Irvani, M., Roosta, H. R., and Ghanbari, D. (2021). Green synthesis of Copper Nanoparticles Using Rosemary Extract to Reduce Postharvest Decays Caused by *Botrytis Cinerea* in Tomato. *Journal of Nanostructures* **11**, 834-841.

Bintsis, T., Litopoulou-Tzanetaki, E., and Robinson, R. K. (2000). Existing and potential applications of ultraviolet light in the food industry–a critical review. *Journal of the Science of Food and Agriculture* **80**, 637-645.

Brizzolara, S., Manganaris, G. A., Fotopoulos, V., Watkins, C. B., and Tonutti, P. (2020). Primary metabolism in fresh fruits during storage. *Frontiers in Plant Science* **11**, 80.

Civello, P. M., Villarreal, N. M., Gómez Lobato, M. E., and Martínez, G. A. (2014). Physiological effects of postharvest UV treatments: recent progress. *Stewart Postharvest Review*, 3-8.

Coates, L., and Johnson, G. (1997). Postharvest diseases of fruit and vegetables. *Plant Pathogens and Plant Diseases*, 533-548.

Daisy, L. L., Nduko, J. M., Joseph, W. M., and Richard, S. M. (2020). Effect of edible gum Arabic coating on the shelf life and quality of mangoes (*Mangifera indica*) during storage. *Journal of Food Science and Technology* **57**, 79-85.

Daş, E., Gürakan, G. C., and Bayındırlı, A. (2006). Effect of controlled atmosphere storage, modified atmosphere packaging and gaseous ozone treatment on the survival of *Salmonella Enteritidis* on cherry tomatoes. *Food Microbiology* **23**, 430-438.

de Oliveira Bianchi, J. R., de Souza, S. M., and Santos, I. J. B. (2022). Post-Harvest application of tara gum coating incorporated with silver nanoparticles for preservation of banana. *Bionterface Research in Research Chemistry* **13**, 1-15.

Deng, Z., Jung, J., Simonsen, J., Wang, Y., and Zhao, Y. (2017). Cellulose nanocrystal reinforced chitosan coatings for improving the storability of postharvest pears under both ambient and cold storages. *Journal of Food Science* **82**, 453-462.

Dissanayake, D., Adikaram, N., Yakandawala, D., and Jayasinghe, L. (2021). Molecular phylogeny-based identification of *Colletotrichum endophytica* and *C. siamense* as causal agents of avocado anthracnose in Sri Lanka. *Ceylon Journal of Science* **50**, 449-458.

Dorostkar, M., Moradinezhad, F., and Ansarifar, E. (2022). Influence of active modified atmosphere packaging pre-treatment on shelf life and quality attributes of cold stored apricot fruit. *International Journal of Fruit Science* **22**, 402-413.

Duan, J., Wu, R., Strik, B. C., and Zhao, Y. (2011). Effect of edible coatings on the quality of fresh blueberries (Duke and Elliott) under commercial storage conditions. *Postharvest Biology and Technology* **59**, 71-79.

Elnashaie, S. S., Danafar, F., and Rafsanjani, H. H. (2015). "Nanotechnology for chemical engineers," Springer Singapore.

Erkan, M., and Wang, C. Y. (2006). Modified and controlled atmosphere storage of subtropical crops. *Stewart Postharvest Review* **5**, 1-8.

Flores, F. B., Sánchez-Bel, P., Valdenegro, M., Romojaro, F., Martínez-Madrid, M. C., and Egea, M. I. (2008). Effects of a pretreatment with nitric oxide on peach (*Prunus persica* L.) storage at room temperature. *European Food Research and Technology* **227**, 1599-1611.

Galus, S., Mikus, M., Ciurzyńska, A., Domian, E., Kowalska, J., Marzec, A., and Kowalska, H. (2021). The effect of whey protein-based edible coatings incorporated with lemon and lemongrass essential oils on the quality attributes of fresh-cut pears during storage. *Coatings* **11**, 745.

Garcia, F., and Davidov-Pardo, G. (2021). Recent advances in the use of edible coatings for preservation of avocados: A review. *Journal of Food Science* **86**, 6-15.

George, D. S., Razali, Z., Santhirasegaram, V., and Somasundram, C. (2016). Effect of postharvest ultraviolet-C treatment on the proteome changes in fresh cut mango (*Mangifera indica* L. cv. Chokanan). *Journal of the Science of Food and Agriculture* **96**, 2851-2860.

Gol, N. B., Patel, P. R., and Rao, T. R. (2013). Improvement of quality and shelf-life of strawberries with edible coatings enriched with chitosan. *Postharvest Biology and Technology* **85**, 185-195.

Gorny, J. R., Hess-Pierce, B., Cifuentes, R. A., and Kader, A. A. (2002). Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. *Postharvest Biology and Technology* **24**, 271-278.

Guo, X., Chen, B., Wu, X., Li, J., and Sun, Q. (2020). Utilization of cinnamaldehyde and zinc oxide nanoparticles in a carboxymethylcellulose-based composite coating to improve the postharvest quality of cherry tomatoes. *International Journal of Biological Macromolecules* **160**, 175-182.

Hashemi, S. M. B., Khaneghah, A. M., Ghahfarrokhi, M. G., and Eş, I. (2017). Basil-seed gum containing Origanum vulgare subsp. viride essential oil as edible coating for fresh cut apricots. *Postharvest Biology and Technology* **125**, 26-34.

Hashim, A. F., Youssef, K., and Abd-Elsalam, K. A. (2019). Ecofriendly nanomaterials for controlling gray mold of table grapes and maintaining postharvest quality. *European Journal of Plant Pathology* **154**, 377-388.

He, X., Sanders, S., Aker, W. G., Lin, Y., Douglas, J., and Hwang, H.-m. (2016). Assessing the effects of surface-bound humic acid on the phototoxicity of anatase and rutile TiO2 nanoparticles in vitro. *Journal of Environmental Sciences* **42**, 50-60.

Hernández-Fuentes, A. D., Arroyo-Aguilar, J. E., Gutiérrez-Tlahque, J., Santiago-Saenz, Y. O., Quintero-Lira, A., Reyes-Fuentes, M., and López-Palestina, C. U. (2023). Application of Cu Nanoparticles in Chitosan-PVA Hydrogels in a Native Tomato Genotype: Evaluation of the Postharvest Behavior of the Physicochemical and Bioactive Components of the Fruits. *Food and Bioprocess Technology*, 1-10.

Hernández-Fuentes, A. D., López-Vargas, E. R., Pinedo-Espinoza, J. M., Campos-Montiel, R. G., Valdés-Reyna, J., and Juárez-Maldonado, A. (2017). Postharvest behavior of bioactive compounds in tomato fruits treated with Cu nanoparticles and NaCl stress. *Applied Sciences* **7**, 980.

Horvitz, S., and Cantalejo, M. (2014). Application of ozone for the postharvest treatment of fruits and vegetables. *Critical Reviews in Food Science and Nutrition* **54**, 312-339.

Huan, C., Han, S., Jiang, L., An, X., Yu, M., Xu, Y., Ma, R., and Yu, Z. (2017). Postharvest hot air and hot water treatments affect the antioxidant system in peach fruit during refrigerated storage. *Postharvest Biology and Technology* **126**, 1-14.

Jafarzadeh, S., Nafchi, A. M., Salehabadi, A., Oladzad-Abbasabadi, N., and Jafari, S. M. (2021). Application of bio-nanocomposite films and edible coatings for extending the

shelf life of fresh fruits and vegetables. *Advances in Colloid and Interface Science* **291**, 102405.

Jaiswal, D., Pandey, A., Agrawal, M., and Agrawal, S. B. (2023). Photosynthetic, biochemical and secondary metabolite changes in a medicinal plant chlorophytum borivillianum (Safed musli) against low and high doses of UV-B radiation. *Photochemistry and Photobiology* **99**, 45-56.

Jung, S.-K., and Choi, H.-S. (2020). Browning of early and late-harvested 'Empire'apples affected by cold storage and 1-MCP. *Agronomy* **10**, 1050.

Kahramanoğlu, İ. (2017). Introductory chapter: Postharvest physiology and technology of horticultural crops. *Postharvest handling*, **13**, 1-5.

Khan, A., Shaheen, A., Mahmood, T., and Rehman, W. (2017). Yaqoob (2017) Novel synthesis and characterization of silver nanoparticles from leaf aqueous extract of Aloe vera and their antimicrobial activity. *Jounal of Nanoscience and Nano-Technol*ogy **1**, 103.

Kingwascharapong, P., Tanaka, F., and Tanaka, F. (2021). Effect of edible coatings developed from chitosan incorporated with tea seed oil on Japanese pear. *Scientia Horticulturae* **288**, 110314.

Kocira, A., Kozłowicz, K., Panasiewicz, K., Staniak, M., Szpunar-Krok, E., and Hortyńska, P. (2021). Polysaccharides as edible films and coatings: Characteristics and influence on fruit and vegetable quality—A review. *Agronomy* **11**, 813.

Kubheka, S. F., Tesfay, S., Mditshwa, A., and Magwaza, L. (2019). Efficacy of carboxymethyl cellulose and gum arabic edible coatings in combination with moringa leaf extract in improving postharvest quality of'Maluma'avocado fruit. *In* "II International Symposium on Moringa 1306", pp. 293-300.

Kumar, N., Tokas, J., Kumar, P., Singal, H., and Jayanti Tokas, C. (2018). Effect of salicylic acid on post-harvest quality of tomato (*Solanum lycopersicum* L.) fruit. *International Journal of Chemical Studies* **6**, 1744-1747.

Kumar, S., Baswal, A. K., Ramezanian, A., Gill, K., and Mirza, A. A. (2021). Impact of carboxymethyl cellulose based edible coating on storage life and quality of guava fruit

cv.‘Allahabad Safeda’under ambient storage conditions. *Journal of Food Measurement and Characterization* **15**, 4805-4812.

Kusnadi, J., Mahatmanto, T., Marsheli, N., Fawzia, N., Rahmawani, D. E., and Alexander, K. (2023). Development of low-cost edible coatings based on polysaccharides with active lactic acid bacteria for the protection of fresh produce modeled using fresh cut apples. *Food Science and Technology International* **29**, 287-298.

La, D. D., Nguyen-Tri, P., Le, K. H., Nguyen, P. T., Nguyen, M. D.-B., Vo, A. T., Nguyen, M. T., Chang, S. W., Tran, L. D., and Chung, W. J. (2021). Effects of antibacterial ZnO nanoparticles on the performance of a chitosan/gum arabic edible coating for post-harvest banana preservation. *Progress in Organic Coatings* **151**, 106057.

Lalel, H., Singh, Z., and Tan, S. (2005). Controlled atmosphere storage affects fruit ripening and quality of ‘Delta R2E2’mango. *The Journal of Horticultural Science and Biotechnology* **80**, 551-556.

Lamattina, L., García-Mata, C., Graziano, M., and Pagnussat, G. (2003). Nitric oxide: the versatility of an extensive signal molecule. *Annual Review of Plant Biology* **54**, 109-136.

Lee, S. K., and Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology* **20**, 207-220.

Li, X., Zhu, X., Zhao, N., Fu, D., Li, J., Chen, W., and Chen, W. (2013). Effects of hot water treatment on anthracnose disease in papaya fruit and its possible mechanism. *Postharvest Biology and Technology* **86**, 437-446.

Liang, Y., Ji, L., Chen, C., Dong, C., and Wang, C. (2018). Effects of ozone treatment on the storage quality of post-harvest tomato. *International Journal of Food Engineering* **14**, 20180012.

Liu, J., Sui, Y., Wisniewski, M., Droby, S., Tian, S., Norelli, J., and Hershkovitz, V. (2012). Effect of heat treatment on inhibition of *Monilinia fructicola* and induction of disease resistance in peach fruit. *Postharvest Biology and Technology* **65**, 61-68.

Lobo, M. G., Yahia, E. M., and Kader, A. A. (2013). Biology and postharvest physiology of date fruit. *Dates: Postharvest Science, Processing Technology and Health Benefits*, 57-80.

Lufu, R., Ambaw, A., and Opara, U. L. (2020). Water loss of fresh fruit: Influencing pre-harvest, harvest and postharvest factors. *Scientia Horticulturae* **272**, 109519.

Madhupani, Y., and Adikaram, N. (2017). Delayed incidence of stem-end rot and enhanced defences in *Aureobasidium pullulans*-treated avocado (*Persea americana* Mill.) fruit. *Journal of Plant Diseases and Protection* **124**, 227-234.

Mahajan, P. V., Caleb, O. J., Singh, Z., Watkins, C. B., and Geyer, M. (2014). Postharvest treatments of fresh produce. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* **372**, 20130309.

Majidi, H., Minaei, S., Almassi, M., and Mostofi, Y. (2014). Tomato quality in controlled atmosphere storage, modified atmosphere packaging and cold storage. *Journal of food science and technology* **51**, 2155-2161.

Manjunatha, G., Lokesh, V., and Neelwarne, B. (2010). Nitric oxide in fruit ripening: trends and opportunities. *Biotechnology Advances* **28**, 489-499.

Mannozzi, C., Cecchini, J. P., Tylewicz, U., Siroli, L., Patrignani, F., Lanciotti, R., Rocculi, P., Dalla Rosa, M., and Romani, S. (2017). Study on the efficacy of edible coatings on quality of blueberry fruits during shelf-life. *LWT-Food Science and Technology* **85**, 440-444.

Maqbool, M., Ali, A., Alderson, P. G., Mohamed, M. T. M., Siddiqui, Y., and Zahid, N. (2011). Postharvest application of gum arabic and essential oils for controlling anthracnose and quality of banana and papaya during cold storage. *Postharvest Biology and Technology* **62**, 71-76.

Mari, M., Torres, R., Casalini, L., Lamarca, N., Mandrin, J. F., Lichou, J., Larena, I., De Cal, M. A., Melgarejo, P., and Usall, J. (2007). Control of post-harvest brown rot on nectarine by *Epicoccum nigrum* and physico-chemical treatments. *Journal of the Science of Food and Agriculture* **87**, 1271-1277.

Martinez-Romero, D., Serrano, M., Carbonell, A., Castillo, S., Riquelme, F., and Valero, D. (2004). Mechanical damage during fruit post-harvest handling: Technical and physiological implications. *Production practices and quality assessment of food Crops: Quality handling and evaluation*, 233-252.

McClements, D. J., and Xiao, H. (2017). Is nano safe in foods? Establishing the factors impacting the gastrointestinal fate and toxicity of organic and inorganic food-grade nanoparticles. *npj Science of Food* **1**, 6.

Mditshwa, A., Khaliq, G., Hussein, Z., and Ejaz, S. (2023). Sustainable postharvest management practices for fresh produce. *Frontiers in Sustainable Food Systems* **7**, 1143759.

Mditshwa, A., Magwaza, L. S., Tesfay, S. Z., and Opara, U. L. (2017). Postharvest factors affecting vitamin C content of citrus fruits: A review. *Scientia Horticulturae* **218**, 95-104.

Meir, S., Naiman, D., Akerman, M., Hyman, J. Y., Zauberman, G., and Fuchs, Y. (1997). Prolonged storage ofHass' avocado fruit using modified atmosphere packaging. *Postharvest Biology and Technology* **12**, 51-60.

Minas, I. S., Tanou, G., Belghazi, M., Job, D., Manganaris, G. A., Molassiotis, A., and Vasilakakis, M. (2012). Physiological and proteomic approaches to address the active role of ozone in kiwifruit post-harvest ripening. *Journal of Experimental Botany* **63**, 2449-2464.

Mitcham, E., Tanner, D., Tustin, S., Wilkinson, I., Zanella, A., Jobling, J., and Brown, G. (2004). Flesh browning of Pink Lady™ apples: why do symptoms occur? Results from an international collaborative study. *In* "V International Postharvest Symposium 682", pp. 851-858.

Ncama, K., Magwaza, L. S., Mditshwa, A., and Tesfay, S. Z. (2018). Plant-based edible coatings for managing postharvest quality of fresh horticultural produce: A review. *Food Packaging and Shelf Life* **16**, 157-167.

Nguyen, T. B. T., Ketsa, S., and van Doorn, W. G. (2004). Effect of modified atmosphere packaging on chilling-induced peel browning in banana. *Postharvest Biology and Technology* **31**, 313-317.

Odetayo, T., Sithole, L., Shezi, S., Nomngongo, P., Tesfay, S., and Ngobese, N. Z. (2022a). Effect of nanoparticle-enriched coatings on the shelf life of Cavendish bananas. *Scientia Horticulturae* **304**, 111312.

Odetayo, T., Tesfay, S., and Ngobese, N. Z. (2022b). Nanotechnology-enhanced edible coating application on climacteric fruits. *Food Science and Nutrition* **10**, 2149-2167.

Olivas, G., and Barbosa-Cánovas, G. (2005). Edible coatings for fresh-cut fruits. *Critical reviews in food science and nutrition* **45**, 657-670.

Olivas, G., Dávila-Aviña, J., Salas-Salazar, N., and Molina, F. (2008). Use of edible coatings to preserve the quality of fruits and vegetables during storage. *Stewart Postharvest Review* **3**, 1-10.

Oliveira, T., Paiva, C., Silva, A., Nascimento, L., Leite, R., and Aroucha, E. (2018). Postharvest quality of tommy atkins mangoes coated with cassava starch and chitosan-based coatings. *Journal of Agricultural Science* **10**, 11.

Ong, M. K., Ali, A., Alderson, P. G., and Forney, C. F. (2014). Effect of different concentrations of ozone on physiological changes associated to gas exchange, fruit ripening, fruit surface quality and defence-related enzymes levels in papaya fruit during ambient storage. *Scientia Horticulturae* **179**, 163-169.

Opara, U. L., and Pathare, P. B. (2014). Bruise damage measurement and analysis of fresh horticultural produce—A review. *Postharvest Biology and Technology* **91**, 9-24.

Ortiz Catalán, A., Graell i Sarle, J., López Fructuoso, M., Echeverría Cortada, G., and Lara Ayala, I. (2010). Volatile ester-synthesising capacity in'Tardibelle'peach fruit in response to controlled atmosphere and 1-MCP treatment. *Food Chemistrey* **123**, 698-704

Oz, A. T., and Ulukanli, Z. (2014). The Effects of Calcium Chloride and 1-Methylcyclopropene (1-MCP) on the Shelf Life of Mulberries (M orus alba L.). *Journal of Food Processing and Preservation* **38**, 1279-1288.

Pareek, S., Benkeblia, N., Janick, J., Cao, S., and Yahia, E. M. (2014). Postharvest physiology and technology of loquat (Eriobotrya japonica Lindl.) fruit. *Journal of the Science of Food and Agriculture* **94**, 1495-1504.

Pathare, P. B., and Al-Dairi, M. (2022). Effect of mechanical damage on the quality characteristics of banana fruits during short-term storage. *Discover Food* **2**, 4.

Payasi, A., and Sanwal, G. (2010). Ripening of climacteric fruits and their control. *Journal of food Biochemistry* **34**, 679-710.

Pech, J.-C., Purgatto, E., Girardi, C. L., Rombaldi, C. V., and Latché, A. (2013). Current challenges in postharvest biology of fruit ripening. *Current Agricultural Science and Technology* **19**, pp. 1-18.

Perumal, A. B., Nambiar, R. B., Sellamuthu, P. S., and Emmanuel, R. S. (2021). Use of modified atmosphere packaging combined with essential oils for prolonging post-harvest shelf life of mango (cv. Banganapalli and cv. Totapuri). *Food Science and Technology* **148**, 111662.

Peters, J., Harper, G., Brierley, J., Lees, A., Wale, S., Hilton, A., Gladders, P., Boonham, N., and Cunnington, A. (2016). The effect of post-harvest storage conditions on the development of black dot (Colletotrichum coccodes) on potato in crops grown for different durations. *Plant Pathology* **65**, 1484-1491.

Peters, R. J., van Bemmel, G., Herrera-Rivera, Z., Helsper, H. P., Marvin, H. J., Weigel, S., Tromp, P. C., Oomen, A. G., Rietveld, A. G., and Bouwmeester, H. (2014). Characterization of titanium dioxide nanoparticles in food products: analytical methods to define nanoparticles. *Journal of Agricultural and Food Chemistry* **62**, 6285-6293.

Pristijono, P., Golding, J. B., and Bowyer, M. C. (2018). Postharvest UV-C treatment, followed by storage in a continuous low-level ethylene atmosphere, maintains the quality of 'Kensington pride'mango fruit stored at 20 C. *Horticulturae* **5**, 1.

Pristijono, P., Papoutsis, K., Scarlett, C. J., Bowyer, M. C., Vuong, Q. V., Stathopoulos, C. E., and Golding, J. B. (2017). Postharvest UV-C treatment combined with 1-methylcyclopropene (1-MCP), followed by storage in continuous low-level ethylene atmosphere, improves the quality of tomatoes. *The Journal of Horticultural Science and Biotechnology* **92**, 521-529.

Prusky, D., and Lichter, A. (2007). Activation of quiescent infections by postharvest pathogens during transition from the biotrophic to the necrotrophic stage. *FEMS Microbiology Letters* **268**, 1-8.

Rana, S., Siddiqui, S., and Gandhi, K. (2018). Effect of individual vacuum and modified atmosphere packaging on shelf life of guava. *International Journal of Chemical Studies* **6**, 966-972.

Razali, Z., Somasundram, C., Nurulain, S. Z., Kunasekaran, W., and Alias, M. R. (2021). Postharvest quality of cherry tomatoes coated with mucilage from dragon fruit and irradiated with UV-C. *Polymers* **13**, 2919.

Rezaei, M., and Liu, B. (2017). Food loss and waste in the food supply chain. *International Nut and Dried Fruit Council: Reus, Spain*, 26-27.

Salisu, I. B., Abubakar, A. S., and Abdullahi, M. (2014). A novel biosynthesis, characterization and antimicrobial activity of silver nanoparticles using leaves extract of Aloe vera plant. *International Journal of Science and Research* **3**, 311-314.

Sandhya (2010). Modified atmosphere packaging of fresh produce: Current status and future needs. *LWT-Food Science and Technology* **43**, 381-392.

Satekge, T. K., and Magwaza, L. S. (2022). Postharvest application of 1-Methylcyclopropene (1-MCP) on climacteric fruits: Factors affecting efficacy. *International Journal of Fruit Science* **22**, 595-607.

Selcuk, N., and Erkan, M. (2015). Changes in phenolic compounds and antioxidant activity of sour–sweet pomegranates cv.'Hicaznar'during long-term storage under modified atmosphere packaging. *Postharvest Biology and Technology* **109**, 30-39.

Shahat, M., Ibrahim, M., Osheba, A., and Taha, I. (2020). Preparation and characterization of silver nanoparticles and their use for improving the quality of apricot fruits. *Al-Azhar Journal of Agricultural Research* **45**, 38-55.

Shimshoni, J. A., Bommuraj, V., Chen, Y., Sperling, R., Barel, S., Feygenberg, O., Maurer, D., and Alkan, N. (2020). Postharvest fungicide for avocado fruits: antifungal efficacy and peel to pulp distribution kinetics. *Foods* **9**, 124.

Siddiqui, M. W., Zavala, J. F. A., and Hwang, C.-A. A. (2016). *Postharvest management approaches for maintaining quality of fresh produce*. Cham, Switzerland: Springer.

Silva, E. (2008). Respiration and ethylene and their relationship to postharvest handling In Wholesale success: a farmer's guide to selling, postharvest handling, and packing produce (Midwest edition).

Singla, R., Kaur, B., and Goel, R. (2022). Controlled atmosphere and modified atmosphere storage for horticulture commodities: A review. *The Pharma Innovation Journal*. **11**, 1581-1589

Sommer, N. F., Fortlage, R. J., and Edwards, D. C. (2002). The Pathogen. *Postharvest Technology of Horticultural Crops* **3311**, 197.

Soradech, S., Nunthanid, J., Limmatvapirat, S., and Luangtana-anan, M. (2017). Utilization of shellac and gelatin composite film for coating to extend the shelf life of banana. *Food Control* **73**, 1310-1317.

Sudha, R., Amutha, R., Muthulaksmi, S., Baby Rani, W., Indira, K., and Mareeswari, P. (2007). Influence of pre and post harvest chemical treatments on physical characteristics of sapota (*Achras sapota* L.) var. PKM 1. *Research Journal of Agriculture and Biological Sciences* **3**, 450-452.

Sutanto, G., Al Shoffe, Y., Gunes, N., Zhang, Y., and DeLong, J. (2019). Managing stem-end flesh browning, a physiological disorder of'Gala'apples. *Scientia Horticulturae,* **9,** 163-168.

Tesfay, S. Z., and Magwaza, L. S. (2017). Evaluating the efficacy of moringa leaf extract, chitosan and carboxymethyl cellulose as edible coatings for enhancing quality and

extending postharvest life of avocado (*Persea americana* Mill.) fruit. *Food Packaging and Shelf Life* **11**, 40-48.

Thakur, R., Pristijono, P., Bowyer, M., Singh, S. P., Scarlett, C. J., Stathopoulos, C. E., and Vuong, Q. V. (2019). A starch edible surface coating delays banana fruit ripening. *Lwt-Food Science and Technology* **100**, 341-347.

Thwin, Y. W., Chang, M. S., Hong, Y., and Lim, S. (2020). Effects of 1-MCP and calcium chloride treatments on quality maintenance of full-ripe cherry tomatoes. *Korean Journal of Food Preservation* **27**, 433-445.

Tokala, V. Y., Singh, Z., and Kyaw, P. N. (2021). Postharvest fruit quality of apple influenced by ethylene antagonist fumigation and ozonized cold storage. *Food Chemistry* **341**, 128293.

Urban, L., Charles, F., de Miranda, M. R. A., and Aarrouf, J. (2016). Understanding the physiological effects of UV-C light and exploiting its agronomic potential before and after harvest. *Plant Physiology and Biochemistry* **105**, 1-11.

Van Zeebroeck, M., Ramon, H., De Baerdemaeker, J., Nicolaï, B., and Tijskens, E. (2007). Impact damage of apples during transport and handling. *Postharvest Biology and Technology* **45**, 157-167.

Vázquez-Ovando, A., López-Hilerio, H., Salvador-Figueroa, M., Adriano-Anaya, L., Rosas-Quijano, R., and Gálvez-López, D. (2018). Combination of uv-c radiation and chitosan films enriched with essential oils for fungi control in papaya 'Maradol'. *Revista Brasileira de Fruticultura* **40**, e-688.

Vilas-Boas, E. V. d. B., and Kader, A. A. (2006). Effect of atmospheric modification, 1-MCP and chemicals on quality of fresh-cut banana. *Postharvest Biology and Technology* **39**, 155-162.

Wang, L., Hong, K., Xu, R., Zhao, Z., and Cao, J. (2021). The alleviation of cold-stimulated flesh reddening in ‘Friar’plum fruit by the elevated CO2 with polyvinyl chloride (PVC) packaging. *Scientia Horticulturae* **281**, 109997.

Warriner, K., Huber, A., Namvar, A., Fan, W., and Dunfield, K. (2009). Recent advances in the microbial safety of fresh fruits and vegetables. *Advances in Food and Nutrition Research* **57**, 155-208.

Wendehenne, D., Pugin, A., Klessig, D. F., and Durner, J. (2001). Nitric oxide: comparative synthesis and signaling in animal and plant cells. *Trends in Plant Science* **6**, 177-183.

Wills, R., Ku, V., and Leshem, Y. (2000). Fumigation with nitric oxide to extend the postharvest life of strawberries. *Postharvest Biology and Technology* **18**, 75-79.

Wongs-Aree, C., and Noichinda, S. (2014). Postharvest physiology and quality maintenance of tropical fruits. *In "Postharvest Handling"*, 275-312.

Wu, C.-T. (2010). An overview of postharvest biology and technology of fruits and vegetables. *In "Technology on Reducing Post-harvest Losses and Maintaining Quality of Fruits and Vegetables Proceedings of 2010 AARDO Workshop"*, **147**, 2-11.

Xing, Y., Li, W., Wang, Q., Li, X., Xu, Q., Guo, X., Bi, X., Liu, X., Shui, Y., and Lin, H. (2019). Antimicrobial nanoparticles incorporated in edible coatings and films for the preservation of fruits and vegetables. *Molecules* **24**, 1695.

Xing, Y., Yang, H., Guo, X., Bi, X., Liu, X., Xu, Q., Wang, Q., Li, W., Li, X., and Shui, Y. (2020). Effect of chitosan/Nano-TiO2 composite coatings on the postharvest quality and physicochemical characteristics of mango fruits. *Scientia Horticulturae* **263**, 109135.

Yahaya, S., and Mardiyya, A. (2019). Review of post-harvest losses of fruits and vegetables. *Biomed. J. Sci. Tech. Res* **13**, 10192-200.

Yahia Kazuz, E. M. (2011). "Postharvest biology and technology of tropical and subtropical fruits," Rep. No. 0857090909. Woodhead Publishing.

Yang, C., Chen, T., Shen, B., Sun, S., Song, H., Chen, D., and Xi, W. (2019). Citric acid treatment reduces decay and maintains the postharvest quality of peach (*Prunus persica* L.) fruit. *Food science & nutrition* **7**, 3635-3643.

Zaharah, S., and Singh, Z. (2011). Postharvest nitric oxide fumigation alleviates chilling injury, delays fruit ripening and maintains quality in cold-stored ‘Kensington Pride’mango. *Postharvest Biology and Technology* **60**, 202-210.

Zambrano-Zaragoza, M. L., González-Reza, R., Mendoza-Muñoz, N., Miranda-Linares, V., Bernal-Couoh, T. F., Mendoza-Elvira, S., and Quintanar-Guerrero, D. (2018). Nanosystems in edible coatings: A novel strategy for food preservation. *International Journal of Molecular Sciences* **19**, 705.

Zhang, J., Cheng, D., Wang, B., Khan, I., and Ni, Y. (2017). Ethylene control technologies in extending postharvest shelf life of climacteric fruit. *Journal of Agricultural and Food Chemistry* **65**, 7308-7319.

Zhang, L., Lu, Z., Yu, Z., and Gao, X. (2005). Preservation of fresh-cut celery by treatment of ozonated water. *Food control* **16**, 279-283.

Zhang, W., and Jiang, W. (2019). UV treatment improved the quality of postharvest fruits and vegetables by inducing resistance. *Trends in Food Science and Technology* **92**, 71-80.

Zheng, W.-W., Chun, I.-J., Hong, S.-B., and Zang, Y.-X. (2014). Quality characteristics of fresh-cut ‘Fuji’apple slices from 1-methylcyclopropene-, calcium chloride-, and rare earth-treated intact fruits. *Scientia Horticulturae* **173**, 100-105.

Zheng, X., and Wolff, D. (2000). Ethylene production, shelf-life and evidence of RFLP polymorphisms linked to ethylene genes in melon (*Cucumis melo* L.). *Theoretical and Applied Genetics* **101**, 613-624.

Chapter 3

The Effect of Composite Edible Coating: Carboxymethyl Cellulose and Moringa Leaf Extract on the Postharvest Quality of 'Hass' Avocado Fruit treated at Different Harvest Maturity Stages

3.1 Abstract

Edible coatings play a critical role in reducing postharvest losses during storage and supply chain of horticultural commodities. The present study evaluated the efficacy of different concentrations of moringa leaf extract (MLE) combined with carboxymethyl cellulose (CMC) edible coating in preserving the quality and extending the shelf life of 'Hass' avocado. Fruit were harvested at different stages of maturity and evaluated by dry matter content. Different concentrations of moringa (8 and 16%) extracted with either chilled ethanol (100%) or non-chilled ethanol (50%) and functionalized with CMC (5%), were used to treat the fruit. Treated fruit were then stored at 5.5℃ and 90% RH for 28 days plus an additional seven days at 23℃. The changes in physicochemical and biochemical fruit attributes were evaluated at weekly intervals. The application of moringa and CMC-based edible coatings maintained the quality of 'Hass' avocado by preserving phenolics, flavonoids, and antioxidant activity. The treatments significantly ($P < 0.001$) reduced the loss of mass and firmness. Furthermore, treated fruits were found to have a delayed colour change and reduction in sugar concentration, particularly mannoheptulose, compared to the control treatment. Therefore, edible coatings prepared by combining CMC and MLE could be the best alternative for substituting the currently used health-compromising synthetic chemicals.

Keywords: Postharvest losses; fruit quality, edible coatings; shelf life; nutritional compounds

3.2 Introduction

Avocado (*Persea americana* Mill.) is one of the most economically essential fruits from the *Lauraceae* family. This fruit is mainly produced in tropical and subtropical regions (Eça et al., 2014). The consumption of avocado is steadily increasing because of the health-related benefits it has, which makes it considered as a "superfruit" (Sivakumar et al., 2021). Its mesocarp tissue consists of bioactive phytochemicals, such as sterols, vitamin E, and carotenoids, that provide antioxidants and radical scavenging activities (Bill et al., 2014). A recent report by Sivakumar et al. (2021) highlighted that the primary target for the world avocado market is to reach a market value of about US$21.56 billion by 2026. However, the rate of postharvest losses remains a serious threat and could make this target unreachable.

Most avocado production in countries such as Spain, Chile, Israel, and South Africa is exported to distant overseas markets, mainly Europe (Kassim et al., 2013). Usually, it takes about 21 or more days to transport the fruits from South Africa to the target market overseas. Generally, fruit maturity at harvest significantly influences its postharvest storage life and quality, impacting the marketing, handling, and transporting decisions (Kader, 1997). Therefore, the harvesting decision must accommodate the transporting period and make the marketing flexible (Magwaza and Tesfay, 2015). Most commercials use dry matter content, moisture content, and mesocarp oil content to determine the maturity of avocados at harvest (Magwaza and Tesfay, 2015; Rivera et al., 2017). Amongst these maturity indices, dry matter is preferable in commercials due to its cost-effectiveness and less time required, making this technique even more convenient (Blakey et al., 2012).

Due to the climacteric nature of avocados, it produces more ethylene and continue to ripen during storage. This compromises the shelf life and makes it difficult to market this fruit, especially to international markets with a long transporting period. The avocado industry highly depends on various synthetic edible films and coatings after harvesting and before storage to maintain the quality and extend the shelf life of this fruit (Liu et al., 2020). Most authors have highlighted the need to develop eco-friendly treatments to replace synthetic fungicides. This is due to health-related concerns caused by the application of chemical-based treatments. Besides their environmental unfriendliness and high residues in the fruit's edible portion, most pathogens have also developed resistance against some of these fungicides (Sivakumar and Bautista-Baños, 2014; Romanazzi et al., 2018; Sivakumar et al., 2021).

Polysaccharide coating materials have gained popularity for their application in fresh produce because of their characteristics, such as exceptionally high stability and solubility (Panahirad et al., 2021). Coatings from polysaccharides are the most convenient ones due to their easy accessibility, non-toxicity, and cost effectiveness (Singh et al., 2019). Amongst cellulose derivatives, hydropropyl cellulose (HPC), methylcellulose (MC), hydropropylmethyl cellulose (HPMC), and carboxymethyl cellulose (CMC) have been extensively used for coating most fruit and vegetables (Maftoonazad and Ramaswamy, 2005; Malmiri et al., 2011). However, CMC is the commonly used commercial derivative, with greater production and applications in the food sector (Dhall, 2016). This is due to its easily accessibility because of its reasonable price and it is a nontoxic polysaccharide, thus, safe for human consumption.

Moringa oleifera Lam has recently drawn more research attention for its use in postharvest quality preservation. This owes to the exceptional performance of edible coatings containing moringa extract in suppressing fruit postharvest diseases, thereby preserving the fruit quality and extending its shelf life (Tesfay and Magwaza, 2017; Tesfay et al., 2017). Most developed countries have opted to use fresh organic products in food preservatives, which necessitates continued research aiming to develop or improve organic postharvest treatments. This study, therefore, evaluated the effect of moringa leaf extract and carboxymethyl cellulose edible coating on the quality and shelf life of 'Hass' avocados.

3.3 Materials and Methods

3.3.1 Preparation of moringa leaf extracts

Fresh moringa leaf powder was obtained from the Agricultural Research Council (ARC)**,** located in Pretoria, South Africa. Moringa extracts were prepared following a modified method previously described by Addo et al. (2022), using chilled 100% ethanol (ETH 1), which was firstly refrigerated at -20 ℃ overnight before use, and non-chilled 50% ethanol (ETH 2). Different moringa extracts were prepared, 8% (g/v) and 16% (g/v). Briefly, 160 and 80 g of moringa leaf powder were separately extracted with 1 L of ethanol for 2 hours with constant agitation to extract the free polyphenols. The extract was passed through a 150 µm sieve. After filtering the extract, 1 L of 50% acidified ethanol was added to the crude, followed by heating

at 90 ºC in a water bath for 1 hour to extract the membrane-bound polyphenols. The extract was collected and stored at ambient temperature to prepare the coating solutions.

3.3.2 Preparation of coating solution

To prepare the coating solution, 50 g of CMC powder was dissolved in 1 L of the prepared moringa solution to obtain 5% CMC. The solution was heated to 51 º C with constant stirring until the powder was dissolved. The resulting solution was used to treat avocado fruit.

3.3.3 Application of treatments and storage

The 'Hass' avocados used in this study were supplied by Westfalia Fruit (Pty) LTD commercial farm located in Howick, South Africa. The fruit were harvested at different maturity stages, determined by dry matter content (DM), which was found to be 25, 27, and 30% for fruit harvested in July, maturity1 (M1); August (M2), and October (M3), respectively, in the year of 2022. From each maturity stage, a total of 250 fresh avocado fruit, free from mechanical damage and diseases, were assigned into five treatments: Control (T1), 5 % CMC + 8% MLE/ETH 1 (T2), 5% CMC + 16% MLE/ETH 1 (T3), 5% CMC + 8% MLE/ETH 2 (T4), and 5% CMC + 16% MLE/ETH 2 (T5). Each treatment was assigned 50 fruits and replicated five times, with each replicate having 10 fruits. Just before cold storage, a sum of five fruits was sampled to assess the fruit status at harvest and as a reference. Before the application of treatments, all fruits were first washed with distilled water to avoid any potential contamination. The fruits were dipped into their assigned treatment for one minute, whereas the control was only washed with distilled water; no treatment was applied. Following treatments, fruits were allowed to dry at room temperature, placed in labelled open boxes, and kept at 5.5 º C and 90% relative humidity (RH) for 28 days. After 28 days of cold storage, the fruits were transferred to room temperature (23 ℃) at the laboratory shelf-life benches for seven days. The changes in fruit quality were observed at weekly intervals throughout the 35-day storage period.

3.4 Evaluation of postharvest fruit quality

3.4.1 Fruit firmness

Fruit firmness was measured using a whole-fruit compression analysis described by (Jeong and Huber, 2004). In this analysis, firmness was measured on unpeeled fruit using a Texture Analyzer (Instron3345 Universal Testing machine, Buck, United Kingdom) fitted with a probe of 5 cm in diameter and 100 N load cell. The probe was allowed to establish zero-force contact with the equatorial region of the fruit before it was driven with a crosshead speed of 5 mm/sec. The deformation force was recorded at 10 mm deformation depth on three opposite sides of the equatorial region of each fruit. The data was automatically loaded on the Easy-Match-QC software, and the firmness was recorded in newtons (N) as the maximum force required for mesocarp tissue failure.

3.4.2 Fruit mass loss percentage

The fruit mass was measured using a digital weighing scale (RADWAG Wagi Electronic Inc., Poland) and determined as mass loss percentage using Eq. 3.1 below:

$$\% \text{ Mass loss (ML)} = \frac{IM-FM}{IM} X100 \qquad (3.1)$$

Where ML = mass loss (%), IM = initial mass of fruit (g), FM = final mass of fruit (g)

3.4.3 Fruit colour

Avocado fruit colour was determined on five (5) fruits per treatment using a CR 400 Chromameter (Minolta Co. Ltd., Osaka, Japan). The readings were taken at the same portion of the fruit's pericarp throughout the experiment. First, the Chromameter was calibrated against a standard white tile. The values for L*, a*, and b* were recorded, where the L* value represented the lightness, the a* value represented the redness (positive) or greenness (negative), and the b* value represented the yellowness (positive) or blueness (negative). The value for Hue angle (H*) was also recorded.

3.4.4 Total Phenolic Content (TPC)

The determination of phenolic compounds was performed following a slightly modified Folin-Ciocalteau method previously described by Milbury et al. (2006). Briefly, 0.5 g of freeze-dried avocado mesocarp was extracted with 15 ml ethanol (70% v/v), followed by shaking the mixture at room temperature for 10 minutes. The solution was then centrifuged for 10 minutes at 10 000 rpm and 4 ℃. Thereafter, 0.5 mL of clear extract was pipetted into a test tube, followed by adding 2 mL of 7.5% sodium carbonate. The mixture was allowed to rest for 3 minutes before adding 2.5 mL of Folin-Ciocalteau reagent (0.2 N). The resulting solution was then heated at 45 ℃ in the ultrasonic water bath for 15 minutes. The solution was then allowed to cool in water before measuring the absorbance at 765 nm using a UV-1800 Spectrophotometer (Shimadzu Scientific Instruments INC., Columbia, USA) against ethanol as blank. The total phenolic content was determined and expressed as Gallic Acid Equivalent (GAE) mg/g DM.

3.4.5 Total Flavonoid Content (TFC)

The total flavonoid (TF) concentration was determined following the method previously described by Obeng et al. (2020), with slight modifications. Briefly, 60 µL of avocado extract aliquot was mixed with 2 mL of distilled water, then 150 µL of 5% (w/v) sodium nitrite ($NaNO_2$) was added. The solution was allowed to settle for 5 minutes before adding 0.8 mL of 10% (w/v) aluminium chloride. The mixture was then allowed to settle for another 5 minutes, and 2 mL of 1.0 M sodium hydroxide (NaOH) was added, followed by vortexing for 30 seconds. The absorbance was measured at 510 nm against the blank using a UV-1800 Spectrophotometer (Shimadzu Scientific Instruments INC., Columbia, USA), and the determined total flavonoids were expressed as Quercetin Equivalents (QTE) mg/g DM. The calibration curve was prepared by preparing quercetin solutions at concentrations of 10 to 100 µg/ml in ethanol.

3.4.6 2,2’ Diphenyl-1-picrylhydrazyl (DPPH) Antioxidant Assay

The DPPH assay was used to estimate avocado mesocarp tissue's free radical scavenging ability following the modified method previously described by Fan et al. (2022). Briefly, 260 µL of methanolic DPPH reagent (0.1 mM) was added into 40 µL of sample in a cuvette and incubated for 30 minutes in a dark at room temperature. The absorbance was read at 517 nm against the ethanol as blank using a UV-1800 Spectrophotometer (Shimadzu Scientific Instruments INC., Columbia, USA), and the DPPH scavenging capability was calculated using Eq. 2 below:

$$\text{Inhibition (\%)} = \frac{Ac-At}{Ac} x100 \quad (3.2)$$

Where Ac = absorbance of control; At = absorbance of the extract.

3.4.7 Determination of sugars (Mannoheptulose and Perseitol)

Determination and quantification of soluble sugars were based on the slightly modified method previously described by Tesfay and Magwaza (2017). Briefly, 0.1 g of freeze-dried mesocarp was added to 10 ml of 80% v/v ethanol/H_2O and homogenized for 1 minute using Ultraturrax. The resulting mixture was then incubated for 1 hour at 80 ℃ in an ultrasonic water bath, followed by storing the samples in a refrigerator at 4 ℃ overnight to facilitate the release of soluble sugars. The samples were then centrifuged at 10 000 rpm and 4 ℃ for 15 minutes and, thereafter, filtered through glass wool. The filtrates were dried overnight under a vacuum in a GenVac® concentrator (SP Scientific, Genevac LTD., Suffolk, UK). Dried samples were reconstituted with 2 mL of ultra-pure water and filtered through a 0.4 µL nylon syringe filter into high performance liquid chromatography (HPLC) vials. Thereafter, HPLC (Shimadzu, Kyoto, Japan) equipped with a refractive index detector was used to determine the sugars. Different sugars, mannoheptulose, and perseitol, were determined by co-elution with their standards and their concentrations calculated using a standard curve for each sugar.

3.5 Statistical analysis

The collected data were subjected to the analysis of variance (ANOVA) using GenStat statistical software (GenStat 20th Edition, VSN International Ltd, UK). The mean separation was performed using Duncan's Multiple Range Test (DMRT) at 5% significance level.

3.6 Results and Discussion

3.6.1 Fruit firmness

Fruit firmness is one of the key quality attributes influencing consumer purchase decision and determining the shelf life and market value of most fresh fruits. The firmness of fruit is mainly affected by different factors such as harvest maturity, relative humidity, and storage temperature. This study showed a significant change in fruit firmness for both the treated and untreated avocado fruit during the 28 days of cold storage (5 ℃) and 7 days of shelf life at room temperature (23 ℃) (Table 0.1). As expected, a significant firmness loss was observed after 28 days of storage when the fruits were transferred to 23 ℃. Generally, firmness loss occurs due to water loss, mainly regulated by the temperature (Paniagua et al., 2013). The results demonstrated a significant effect of the interaction between harvest time and treatments ($p < 0.05$) on fruit firmness loss during the storage period. As usual, all fruits showed firmness loss during the storage period regardless of the harvest time and treatments; however, the loss was severe in untreated fruits (Table 0.1). At the end of the storage period, the untreated fruits recorded lower firmness than all the treated fruits which were 6.59, 7.91, and 6.81 N for maturity 1, 2, and 3, respectively. According to the ripening standards described by Jeong and Huber (2004) for avocado fruits, based on the whole fruit compression analysis, the untreated fruits were over-ripe (<10 N) and no longer suitable for markets. Briefly, these standards classify fruits as ripened and ready for consumption when the whole fruit compression attains values ranging between 10 and 20 N. The firmness declines to below 10 N on over-ripened fruit (Jeong and Huber, 2004). Although no sensory evaluations were conducted, the results from this study are aligned with these classifications based on the observations and statistical analysis.

Different concentrations of MLE in combination with CMC delayed firmness loss depending on the harvest time; however, the chilled treatments were most effective than non-chilled. Given that all the untreated fruit had compression values of less than 10 N at the end of the

storage period, this indicates that MLE and CMC composite coating could delay the fruit ripening, thereby delaying the rate of fruit softening. Based on these results, the different concentrations (8 and 16%) of chilled MLE and CMC used in this study potentially delayed changes that take place in different components, such as cell wall structure weakening, hydrolysis of cellulose and hemicellulose, loss of membrane integrity, and depolymerization of pectin and starch, thereby delaying firmness loss (Yaman and Bayoındırlı, 2002). Combining 8% chilled MLE and CMC delayed firmness loss on fruit harvested at maturity 1 and 3, whereas increasing the concentration to 16% resulted in reduced firmness loss on fruit harvested at maturity 2 stage. The effectiveness of these treatments could be due to the presence of CMC. The carboxylic group in CMC's chemical structure results in hydrogen bonding inside the coating matrix and between the coating and the fruit peel, resulting in preserved firmness (Panahirad et al., 2019). This positive effect may also be attributed to reduced enzyme activities, including pectin-methylesterase, which contributed to delayed fruit ripening. Pectin methylesterase is a major enzyme that depolymerizes pectin substances (Payasi et al., 2009). This also implies that the coatings could serve as a gas barrier, as the enzymatic activities are reduced by low oxygen and high carbon dioxide concentrations, which ultimately retain the fruit firmness (Payasi et al., 2009). Similarly, Kubheka et al. (2019) reported a reduced firmness loss in 'Maluma' avocado treated with 1 % CMC and moringa leaf extract.

Table 0.1: The effect of CMC and different MLE concentrations on the firmness (N) of 'Hass' avocadoes harvested at different maturity stages during 28 days cold storage at ± 5 ℃ followed by seven days shelf life at ± 23 ℃.

		Storage period (days)					
Harvest time	**Treatment**	**0**	**7**	**14**	**21**	**28**	**35**
	Control	222.42 ± 23.82^{a}	198.96 ± 19.62^{bc}	174.26 ± 28.45^{bcd}	130.54 ± 40.28^{ab}	40.94 ± 22.73^{a}	6.59 ± 0.98^{a}
	CMC + 8% chilled MLE	222.42 ± 23.82^{a}	202.85 ± 13.62^{c}	181.55 ± 21.37^{cd}	125.99 ± 38.32^{a}	81.39 ± 22.27^{bc}	18.58 ± 2.80^{d}
Maturity 1	CMC + 16% chilled MLE	222.42 ± 23.82^{a}	192.85 ± 20.65^{bc}	186.64 ± 23.75^{d}	162.13 ± 19.06^{b}	39.45 ± 17.98^{a}	15.14 ± 2.71^{cd}
	CMC + 8% non-chilled MLE	222.42 ± 23.82^{a}	188.84 ± 18.14^{bc}	160.29 ± 21.53^{abc}	146.64 ± 13.10^{ab}	110.63 ± 18.27^{de}	14.32 ± 0.67^{cd}
	CMC + 16% non-chilled MLE	222.42 ± 23.82^{a}	190.50 ± 22.57^{bc}	167.99 ± 4.53^{abcd}	162.23 ± 3,40^{b}	58.82 ± 19.62^{ab}	16.84 ± 3.92^{d}
	Control	211.97 ± 24.02^{a}	191.29 ± 26.63^{bc}	153.30 ± 2.37^{ab}	133.30 ± 11.11^{ab}	122.34 ± 16.28^{de}	7.91 ± 0.79^{ab}
	CMC + 8% chilled MLE	211.97 ± 24.02^{a}	195.21 ± 18.84^{bc}	166.74 ± 7.03^{abcd}	152.91 ± 11.76^{ab}	96.56 ± 30.79^{cd}	16.3 ± 2.93^{d}
Maturity 2	CMC + 16% chilled MLE	211.97 ± 24.02^{a}	194.98 ± 13.80^{bc}	159.79 ± 5.21^{abc}	152.08 ± 3.50^{ab}	118.63 ± 5.74^{de}	18.17 ± 4.7^{d}

		Storage period (days)					
Harvest time	**Treatment**	**0**	**7**	**14**	**21**	**28**	**35**
	CMC + 8% non-chilled MLE	211.97 ± 24.02^{a}	186.27 ± 12.69^{bc}	159.15 ± 7.04^{abc}	157.12 ± 7.93^{ab}	119.89 ± 2.08^{de}	9.71 ± 0.98^{ab}
	CMC + 16% non-chilled MLE	211.97 ± 24.02^{a}	185.29 ± 7.18^{bc}	166.21 ± 9.26^{abcd}	143.66 ± 6.58^{ab}	81.69 ± 9.66^{bc}	14.45 ± 2.65^{cd}
	Control	194.41 ± 12.71^{a}	156.58 ± 7.80^{a}	144.99 ± 3.44^{a}	129.33 ± 3.73^{ab}	115.26 ± 6.38^{de}	6.81 ± 0.88^{a}
	CMC + 8% chilled MLE	194.41 ± 12.71^{a}	181.02 ± 8.92^{bc}	173.50 ± 7.33^{bcd}	163.28 ± 13.77^{b}	125.60 ± 9.42^{de}	11.89 ± 2.40^{bc}
Maturity 3	CMC + 16% chilled MLE	194.41 ± 12.71^{a}	187.54 ± 10.63^{bc}	165.64 ± 12.04^{abcd}	152.09 ± 16.20^{ab}	136.21 ± 8.06^{e}	9.56 ± 1.96^{ab}
	CMC + 8% non-chilled MLE	194.41 ± 12.71^{a}	174.64 ± 12.65^{ab}	160.91 ± 4.56^{abc}	140.44 ± 13.42^{ab}	129.40 ± 9.02^{e}	11.58 ± 1.34^{bc}
	CMC + 16% non-chilled MLE	194.41 ± 12.71^{a}	187.96 ± 3.05^{bc}	150.34 ± 7.61^{ab}	138.50 ± 12.36^{ab}	129.31 ± 6.25^{e}	11.55 ± 0.90^{bc}
l.s.d (H*T)		**28.98**	**20.78**	**20.49**	**28.94**	**26.60**	**4.041**
P-value							
Harvest time (H)		**< 0.001**	**0.002**	**0.004**	**0.879**	**< 0.001**	**< 0.001**
Treatment (T)		**1.000**	**0.268**	**0.033**	**0.071**	**0.004**	**< 0.001**

Harvest time * Treatment	**1.000**	**0.206**	**0.331**	**0.185**	**<0.001**	**0.021**

* The results were presented as mean ± standard deviation (SD). Mean values in the same column followed by the same letter(s) shows no significant difference according to Duncan's Multiple Range Test (DMRT) Test (P = 0.05); H, harvest time; T, treatment; CMC, carboxymethyl cellulose; MLE, moringa leaf extract.

3.6.2 Fruit mass loss (%)

Mass loss is mainly caused by the water loss during metabolic processes such as transpiration and respiration, and its rate depends on the storage environment (Abebe et al., 2017). This loss of water takes place through stomatal openings and skin cracks. The storage temperature and relative humidity impact the fruit mass loss due to the effect caused by the differences in vapour pressure between the fruit and the atmosphere (Wróblewska-Krepsztul et al., 2018). This was evident when the fruit from all the treatments showed the highest mass loss during the last week, from day 28 to 35, when the fruits were transferred to ambient conditions (23 ℃), compared to cold storage (Figure 0.1). The interaction between coatings, storage period, and harvest time significantly affected the fruit mass ($P < 0.05$). All fruits suffered a weight loss throughout the storage time; however, the untreated fruit suffered the most, especially after cold storage, at ambient temperature. All the evaluated edible coating treatments resulted in a lower mass loss percentage than the control for all harvests. The CMC and MLE treatments preserved the fresh mass of treated fruits throughout the evaluated 28 days of cold storage at 5 ℃ and 7 days of shelf life at 23 ℃. However, 16% chilled MLE was most effective than all other treatments. This can be attributed to the hydrophilic nature of these treatments. It can be argued that the treatments inhibited the transfer of water between the fruit and the atmosphere by forming a semipermeable layer that acted as a barrier between the fruit and the environment, covering the fruit surface and protecting it from mechanical injury and, therefore, reducing desiccation (Khorram et al., 2017). These results agree with those of Tesfay et al. (2017), who reported a reduced mass loss in avocado fruits treated with CMC combined with moringa leaf or seed extract. Similar results were also reported by Kubheka et al. (2019), where the CMC (1%) incorporated with moringa reduced the avocado mass loss throughout the 21 days of cold storage and 7 days of shelf life. Another study conducted by Zhang et al. (2019) reported that *Osmunda japonica*-CMC coatings significantly reduced the water loss in tomato fruit compared to untreated fruit. The fruit mass loss, caused by water loss, may also result in changes in the whole fruit texture and flavour (Ballesteros et al., 2022), and eventually, the fruit starts to decay as the loss gets severe, which was evident in this study.

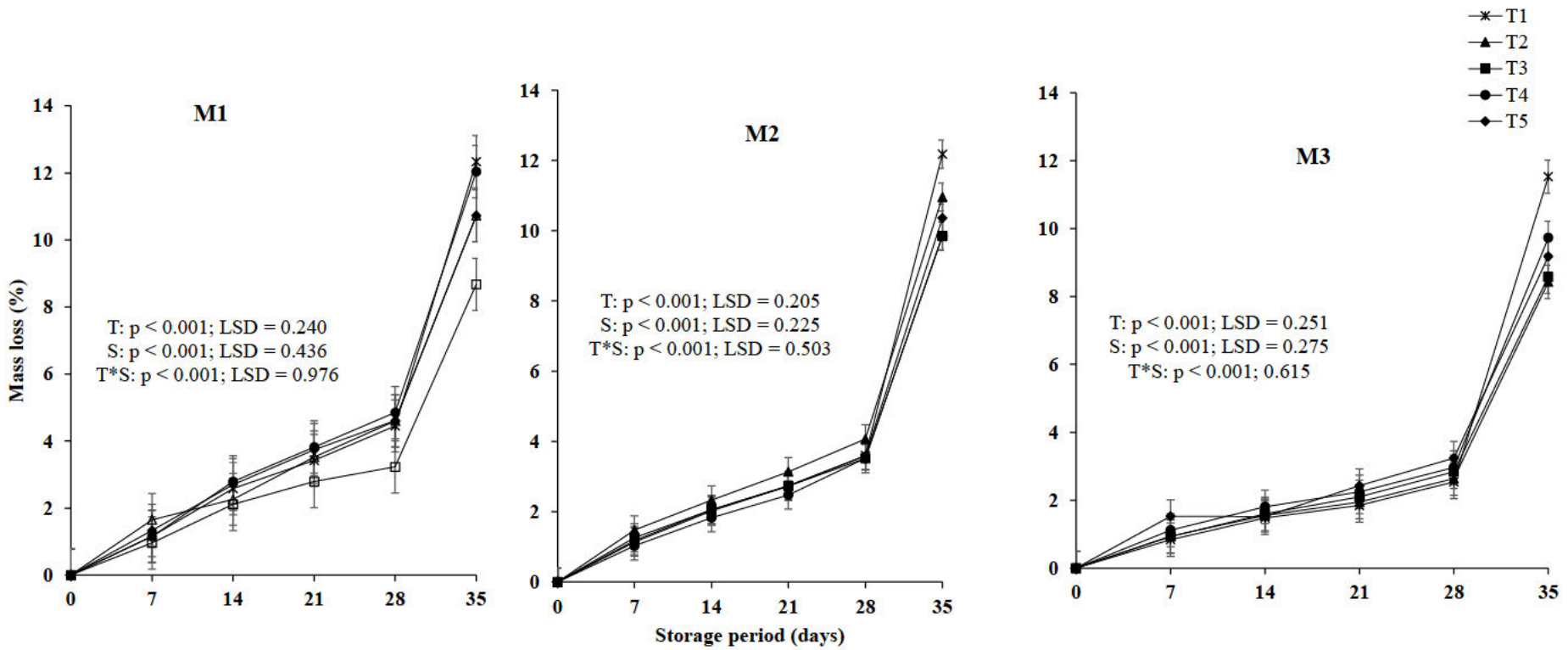

Figure 0.1: Mass loss of 'Hass' avocado fruit harvested at different maturity stages (M1, M2, and M3) as influenced by CMC and different MLE concentrations during 28 days of cold storage and seven days of shelf life. *The vertical bars represent standard error (SE) at n = 5; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

3.6.3 Fruit colour

Fruit colour is the best indicator for the ripening stage in avocadoes, particularly the 'Hass' cultivar. This cultivar is characterized by the ripening process that is accompanied by the colour change from green to purple or black. This study showed a decrease in yellowness (b*), lightness (L*), and hue angle (h^o) values (Figure 0.3, Figure 0.4, and Figure 0.5). In contrast, the greenness (a*) values increased during storage, as the fruit ripens regardless of the treatments and harvest time (Figure 0.2). There was a negligible colour change during the cold storage period, with significant changes observed between days 28 and 35. These observations agree with Mwelase et al. (2022), who also reported the influence of temperature on avocado fruit colour. Similarly, the higher temperature accelerated the avocado colour change compared to cold storage. There was a significant effect ($p < 0.001$) of treatments and storage time on the decrease in h^o and L* values. Inversely, no statistical difference ($p > 0.05$) existed for the increase in a* and decrease in b* values. This observed increase in a* value from negative to positive indicates colour reduction from greener to red with fruit ripening, which was clearly expected in this study. This could indicate that the composite edible coating of MLE and CMC potentially delay the transition of chloroplasts into chromoplasts that contain yellow and red pigments, thereby inhibiting colour change and enzymatic browning (Sharma et al., 2019).

The correlation between the a*, b*, L*, and h^o values in this study is in line with Handayani et al. (2018), who reported an inverse relationship between the a* and b* values on avocados treated with cassava peel edible coating. There was a highly significant effect ($p < 0.001$) of treatments and storage time on L* and h*; however, a sharp decline was observed between days 28 and 35 at room temperature. This delayed colour change observed in coated fruits could be linked to the effect of the coatings in modifying the fruit's atmosphere. Edible coatings slow the respiration rate and ethylene accumulation, the ripening hormone (Ali et al., 2011). The results for the L* values and visual judgments also indicated that the temperature, especially in cold storage, was suitable for storing avocado without causing chilling injury, which causes the darkening of fruit pulp (Careli-Gondim et al., 2020).

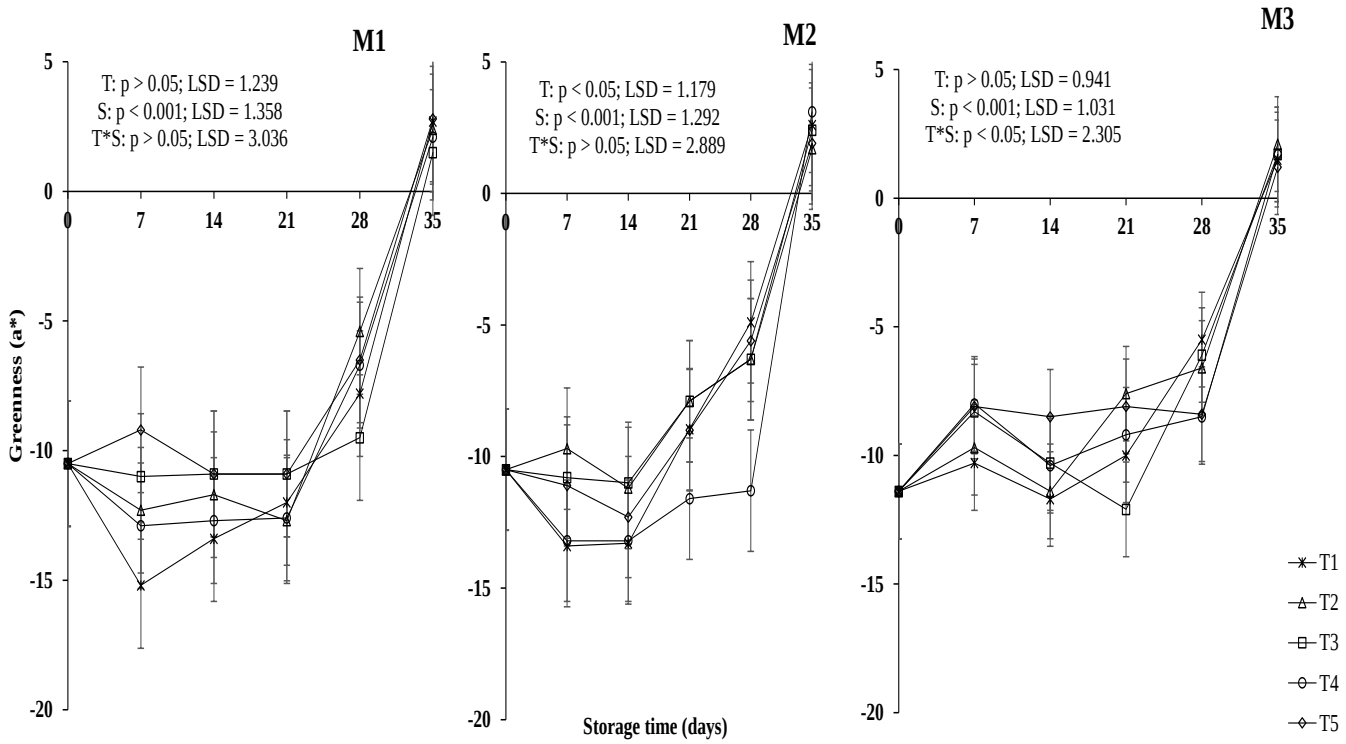

Figure 0.2: The effect of CMC and MLE composite coating on the exocarp colour (a*) of 'Hass' avocado fruit harvested at maturity M1, M2, and M3 during 28 days of cold storage and seven days shelf-life. *The vertical bars represent standard error (SE) at n = 5; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

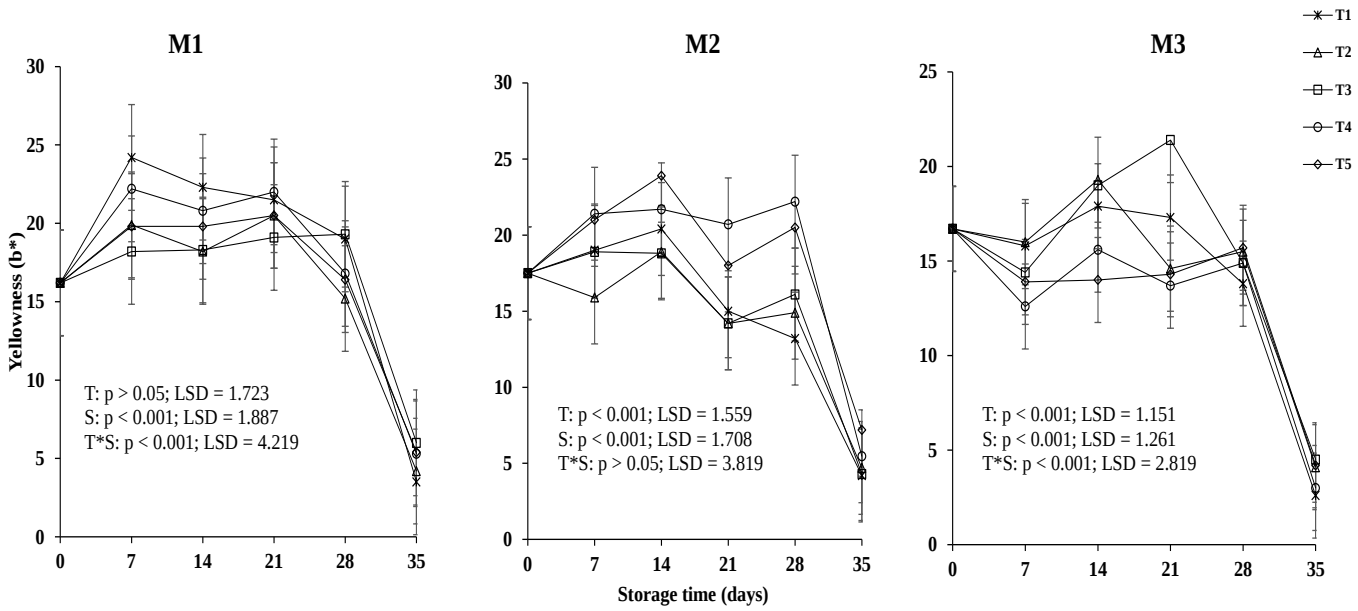

Figure 0.3: The effect of CMC and MLE composite coating on the exocarp colour (b*) of 'Hass' avocado fruit harvested at maturity M1, M2, and M3 during 28 days of cold storage and seven days shelf-life storage. *The vertical bars represent standard error (SE) at n = 5; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

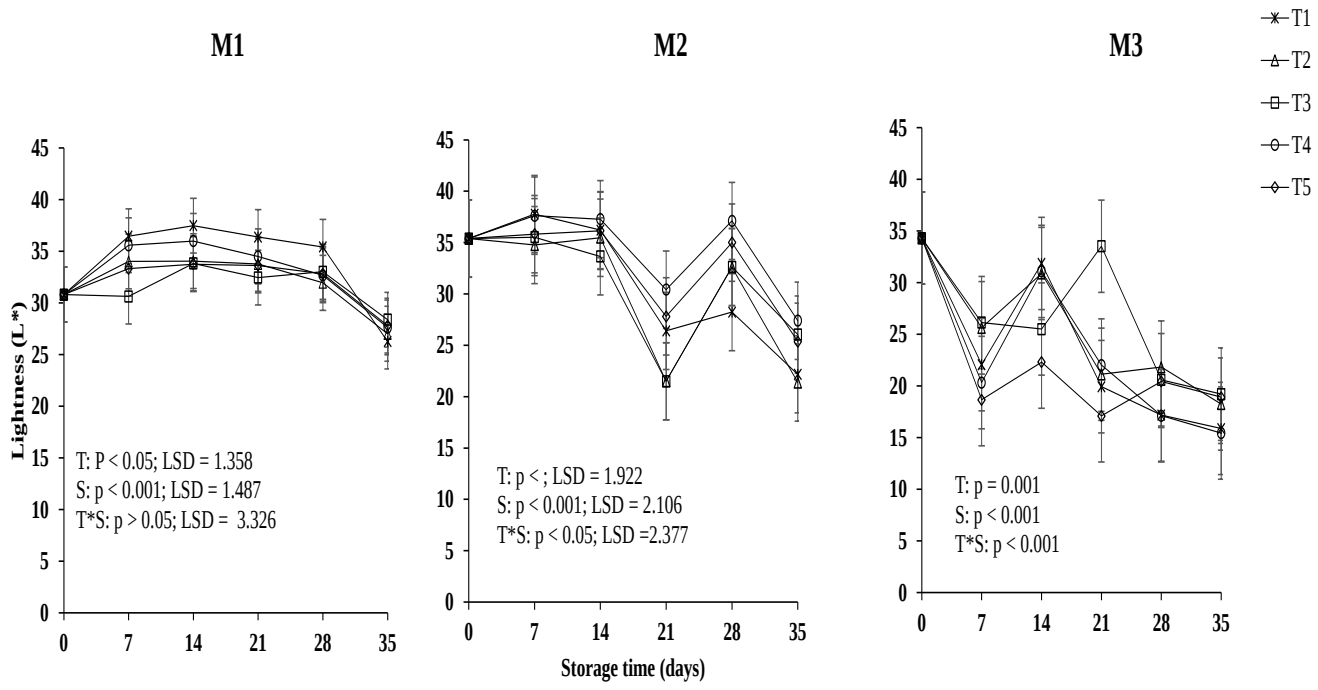

Figure 0.4: The effect of CMC and MLE composite coating on the exocarp colour (L*) of 'Hass' avocado fruit harvested at maturity M1, M2, and M3 during 28 days of cold storage and seven days of shelf life. *The vertical bars represent standard error (SE) at n = 5; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

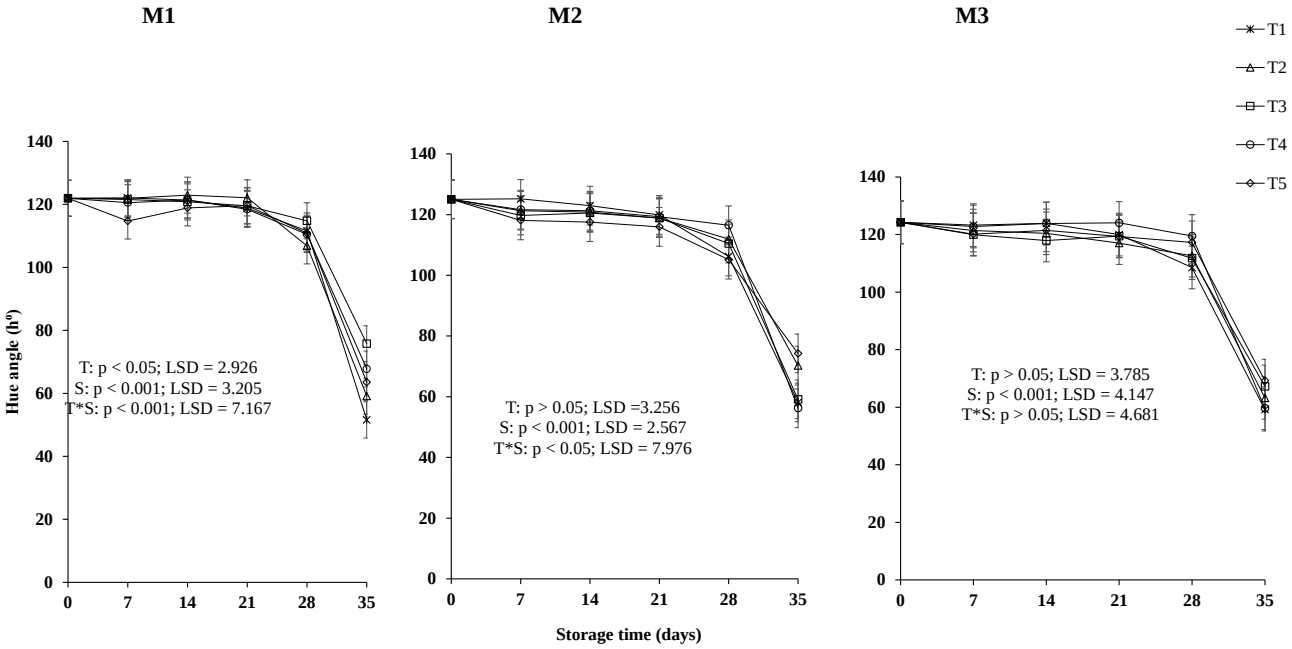

Figure 0.5: The effect of CMC and MLE composite coating on the exocarp colour (h°) of 'Hass' avocado fruit harvested at maturity M1, M2, and M3 during 28 days of cold storage and seven days of shelf-life. *The vertical bars represent standard error (SE) at n = 5; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

3.6.4 Total phenolics

Phenolics are produced in fruit tissues as secondary metabolites that activate antioxidants against oxidative stress (Peretto et al., 2017). These phytochemicals have a crucial role in the sensory and nutritional properties of the produce. The storage time, conditions, and stress

severity affect the secondary metabolites in fruit. While storage period and harvest time showed a significant effect ($p < 0.001$), no statistically significant difference ($p > 0.05$) existed between treatments on the total phenolic compounds. The changes in total phenolics followed the same trend for all harvest times (Figure 0.6). Early harvested fruit showed a decline in phenolic content for the first seven days of cold storage; after that, slight changes occurred depending on treatments. The decline in phenolics could be caused by the stress induced by the cold storage. These observed changes in phenolics may also result from applying edible coatings. Edible coatings have been previously reported to influence the production of phenolic compounds by modifying the produce metabolism, producing abiotic stress on produce (Dávila-Aviña et al., 2014). There were no remarkable differences in the fruit's phenolic content throughout the storage period between the treatments, especially in mid- and late-harvested fruits. This indicates that, beside the potential of CMC and MLE coating to extend the shelf life, they can also retain the fruit phenolic concentration. This corroborates with Maringgal et al. (2020), who demonstrated that edible coatings help preserve the phytonutrients in fruit. Moreover, these results validate that edible coatings modify the internal atmosphere by serving as selective barriers to O_2 and CO_2, reducing respiration rate and delaying phenolic changes (Awad et al., 2017). The findings from this study are consistent with those presented by Chiabrando and Giacalone (2015), where the decrease in phenolic content and antioxidant capacity in blueberries was delayed by applying polysaccharide (chitosan) coatings. It is, however, important to mention that CMC, in combination with 16% non-chilled MLE, resulted in a slightly increased phenolic content than all the other treatments at the end of the storage period, particularly in early and late-harvested fruits. At the same time, the combination of CMC and 8% chilled MLE showed a slight increase in mid-harvested fruits. This indicate that the coatings were able to delay fruit senescence which results to disrupted cell structure, thereby, resulting to reduced phenolics (Riaz et al., 2021). Consequently, the observed differences may have resulted from a higher respiration rate in untreated fruits associated with the breakdown of total phenols (Nair et al., 2018).

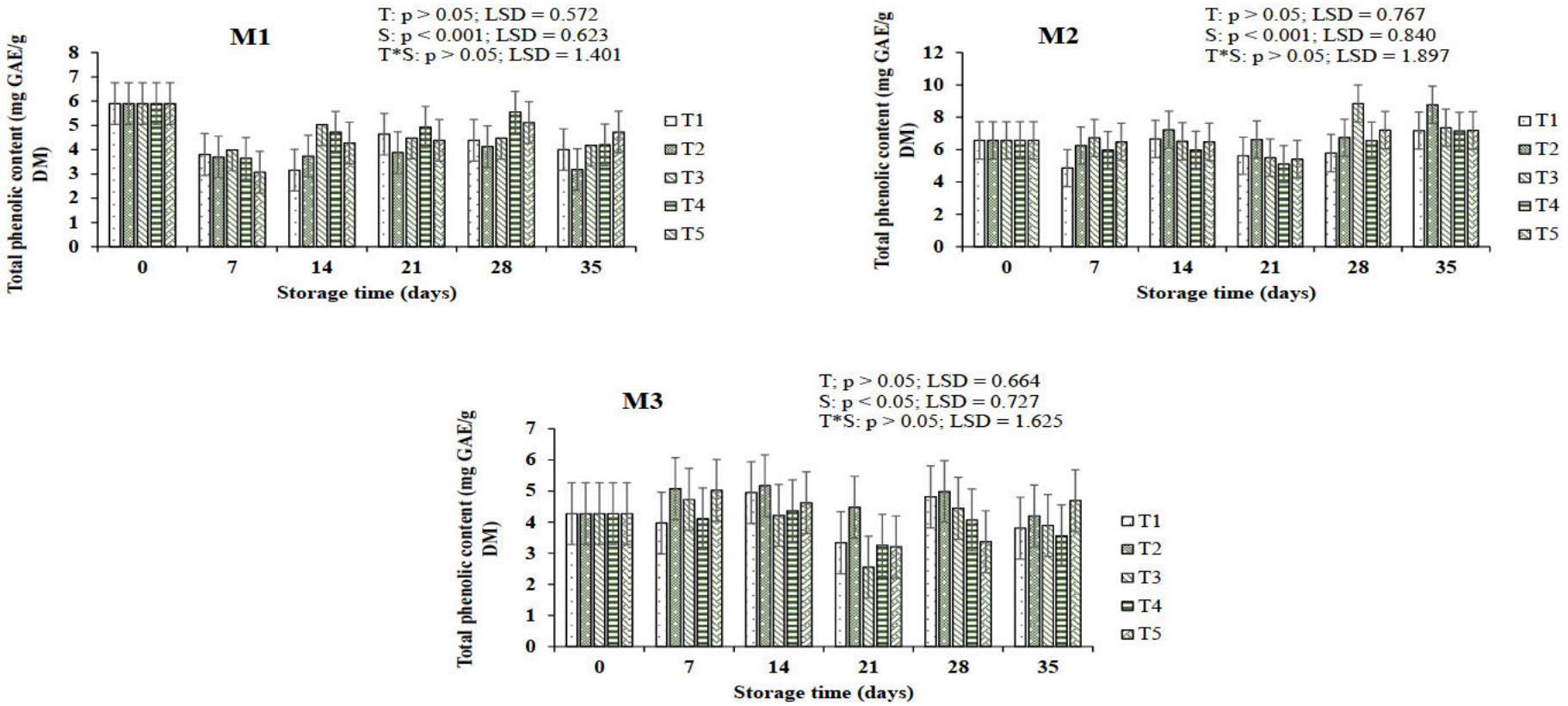

Figure 0.6: The effect of CMC and Moringa-based edible coatings on the changes in phenolic content of 'Hass' avocado fruit harvested at maturity M1, M2, and M3 during 28 days of cold storage and seven days of shelf-life. *The vertical bars represent standard error (SE) at n = 3; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

3.6.5 Total Flavonoids content

Flavonoids are secondary metabolites that resemble variable phenolic structures and are involved in colouring many fruits, vegetables, and flowers. This phytochemical also provides health benefits such as anti-cancer, anti-inflammatory, and antioxidant properties (Zahedi et al., 2019). In the present study, total flavonoids were significantly ($p < 0.001$) affected by the storage period and harvest time (Figure 0.7). However, the coating treatments did not affect flavonoid concentration ($p > 0.05$). Cordenunsi et al. (2005) reported that storage conditions influence the concentration of flavonoids. The total flavonoid concentration was similar for all the treatments and harvesting times; however, the untreated fruits from the early harvest showed an increased flavonoid content after 35 days of storage period. This was opposite for the fruit treated with CMC combined with 16% non-chilled MLE which resulted in fruits with lower flavonoids content than all other treatments, regardless of harvest time. These results are comparable to those presented by Panahirad et al. (2019), wherein plums treated with 0.5% CMC-based edible coating resulted in higher flavonoid content. In contrast, those treated with concentrations above 0.5% (1 and 1.5%) had less content than the untreated fruits. Langa (2018) also reported a rapid increase of flavonoids in untreated papaya fruit compared to those treated with CMC + moringa leaf or seed extract. Although the results presented in this study are inconsistent, a progressive decline in total flavonoids during the 28 days of cold storage was, however, observed. This was followed by a slight increase during the seven days of shelf life, especially in early and mid-harvested fruits. Similarly, Ballesteros et al. (2022) reported an increase in flavonoid content in goldenberries stored at 20 ℃ and 65% relative humidity for 12 days, irrespective of CMC-based coatings; this was, however, inverse for fruits stored for 28 days at 4 ℃ and 95% RH. These results show that storage conditions influence flavonoids. In addition, cold storage tends to decrease, while high temperatures increase the flavonoids.

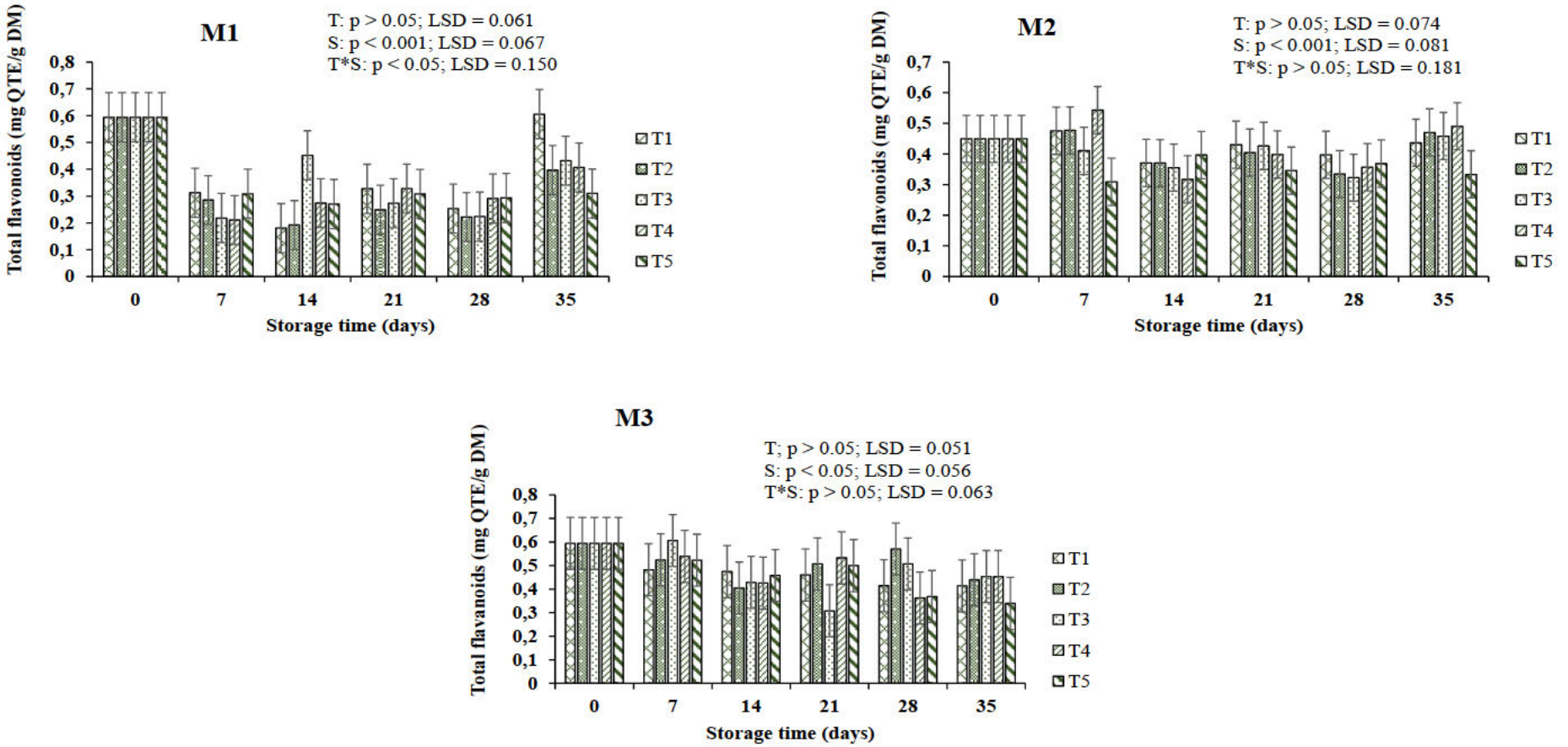

Figure 0.7: The effect of CMC and Moringa-based edible coatings on the changes in flavonoid content of 'Hass' avocado fruit harvested at maturity M1, M2, and M3 during 28 days of cold storage and seven days of shelf-life. *The vertical bars represent standard error (SE) at n = 3; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

3.6.6 2,2’ Diphenyl-1-picrylhydrazyl (DPPH) Antioxidant Assay

Antioxidant activity is a very important parameter that determines the health-related benefits and is usually determined using different methods, including the DPPH radical scavenging assay. This technique is one of the most popular methods to measure antioxidant activity due to its accuracy and convenience. The fruit’s antioxidant properties are greatly influenced by the presence of various secondary metabolites, including flavonoids and phenolics (Maringgal et al., 2020). This study showed a significant effect of the storage period ($p < 0.001$) on the antioxidant activity of avocados. Figure 3.8 shows an increasing trend in the DPPH radical scavenging activity over time during the cold storage period and a decrease at ambient temperature, regardless of the harvest time. Although there was no significant difference between treatments ($p > 0.05$), the reduction in antioxidants was more pronounced in uncoated fruit than in MLE and CMC-coated avocados, which may indicate the positive effect of these composite coatings. The high decline in antioxidants activity in untreated fruits could be attributed to the fast rate of ripening which is associated with fruit senescence and decay (Wang and Gao, 2013). The trend displayed by the antioxidant activity in this study contradicts the results by Kumar et al. (2021) on bell peppers treated with chitosan-pullulan composite coating and stored for 18 days at 4℃. These authors reported a decreasing trend in antioxidant activity. However, the present study aligns with Fernando et al. (2014), who reported an increase in the antioxidant activities as the banana ripens and declines with senescence. Another study by Thakur et al. (2018) revealed the same trend: the scavenging activity in uncoated plums declined with ripening. Zahedi et al. (2019) also reported that chitosan coated ‘Langra’ mango fruit had higher antioxidant activities than control after 24 days, at 15 ± 2 and 85 – 90% RH storage conditions. The authors further stated that this may result from edible coatings forming a protective barrier on the fruit surface, which reduces the decline in antioxidant activity, nutrient loss, and water evaporation. This could show the potential of the MLE and CMC used in this study in retaining the scavenging activity of avocado fruit at 5℃ and 23℃. Usually, the bioactive compounds in the fruit have an impact on its antioxidant activity (Maftoonazad and Ramaswamy, 2005). This was supported by the results of this study, where the trend between phenolics and flavonoids showed an inverse relationship with antioxidant capacity, which could be associated with changes in these compounds (Awad et al., 2017).

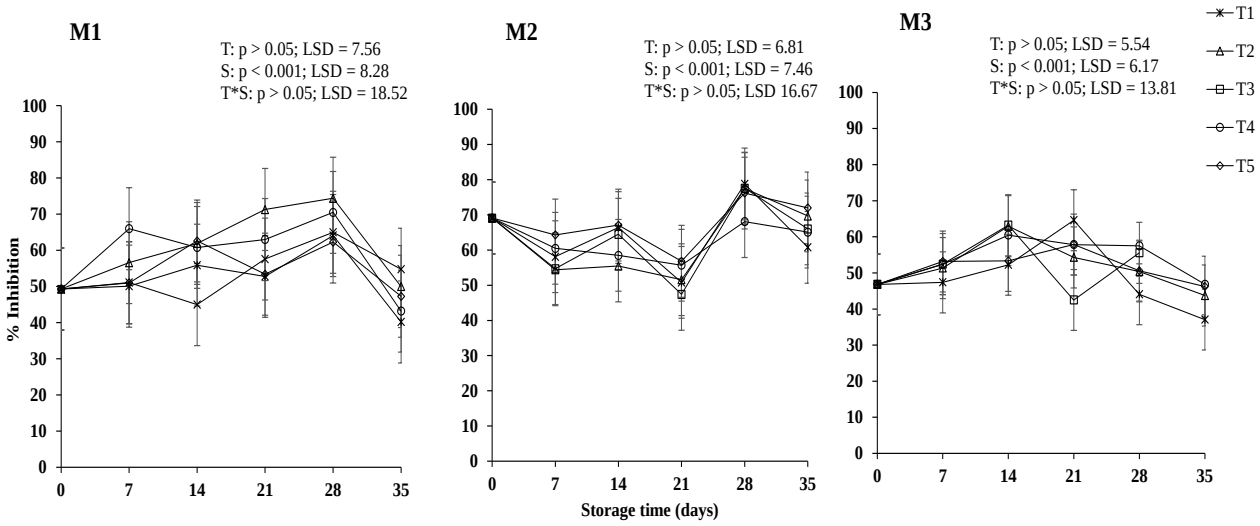

Figure 0.8: The effect of CMC and Moringa-based edible coatings on antioxidant activity of 'Hass' avocado fruit harvested at maturity M1, M2, and M3 during 28 days of cold storage and seven days of shelf-life. *The vertical bars represent standard error (SE) at n = 3; H; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

3.6.7 Sugars (mannoheptulose and perseitol)

Sugar content in avocado fruit is a critical quality indicator (Le et al., 2021). Amongst other fruits, avocado consists of unique sugars, such as perseitol, d-*manno*-heptulose (reducing sugar), and the seven-carbon sugar alcohol. Avocados produce D-mannoheptulose and perseitol in higher concentrations than other sugars, such as hexoses (Tesfay and Magwaza, 2017). Generally, the ripening of this fruit is associated with increased glucose and fructose and decreased D-mannoheptulose and perseitol concentrations. It is still in the best interest to evaluate the duration that fruit can be stored and still have optimal sugar concentrations without losing its quality. Figure 0.9 and Figure 0.10 show the changes in sugar content, mainly mannoheptulose and perseitol, respectively of CMC-moringa coated avocado fruit at 7-day intervals during the storage period. The initial sugar concentrations before treatment were determined to be 19.67, 34.04, and 47.34 mg/g DW for mannoheptulose and 15.37, 21.77, and 17.2 mg/g DW for perseitol and for early, mid, and late harvested fruits, respectively. The

results showed a significant effect ($p > 0.001$) of the storage period on the concentration of mannoheptulose and perseitol. The concentrations for these two sugars showed a progressive decline throughout the storage period. In the present study, the untreated fruit had less mannoheptulose concentrations of 4.85, 4.28, and 5.54 mg/g DW for the early, mid, and late harvested fruits, respectively, than fruits coated with different concentrations of moringa and 5% CMC. This indicates a 75.3, 87.4, and 88.3% reduction from the initial concentrations for the early, mid, and late-harvested fruits, respectively. Similar to mannoheptulose, the most reduction in perseitol concentrations was observed in untreated fruits for the early and mid-harvested fruit. The reduction in these C7 sugars is due to their high contribution to the total carbohydrate concentration than the 6-carbon (C6) sugars (sucrose, starch, and hexose), with perseitol being dominant (Liu et al., 2002). This reduction validates the assertion by Wolstenholme (2012) that the C7 sugars concentration depends on the ripening stage of the avocado, and its reduction can go above 80% and, in some cultivars, can be depleted. In addition, it was previously reported that the ripening and its associated physiological processes such as, increased ethylene production and respiration does not occur until the C7 sugars drops to below a threshold (20 mg/g DW) (Liu et al., 2002). This could indicate that the C7 sugars are metabolised during the ripening or are the main that control the ripening process (Landahl et al., 2009; Blakey et al., 2012). The trend observed in this study is similar to that reported by Shezi et al. (2020) for ‘Hass’ avocado fruit harvested inside and outside the canopy during storage. Moreover, these results are comparable to those reported by Tesfay and Magwaza (2017), who observed a decrease in soluble sugars in ‘Fuerte’ and ‘Hass’ avocados treated with CMC and chitosan-based on moringa extracts. Similarly, Kubheka et al. (2019) reported a higher D-mannoheptulose in ‘Maluma’ avocado fruit treated with 1% CMC and MLE. Although there was no significant difference among treatments based on their performance in the retention of sugars, 8% non-chilled MLE was consistent in the fruit at all harvests. Overall, based on these findings, it was clearly observed that treating fruit with CMC (5%) and moringa leaf extract is beneficial in minimizing the reduction in C7 sugars.

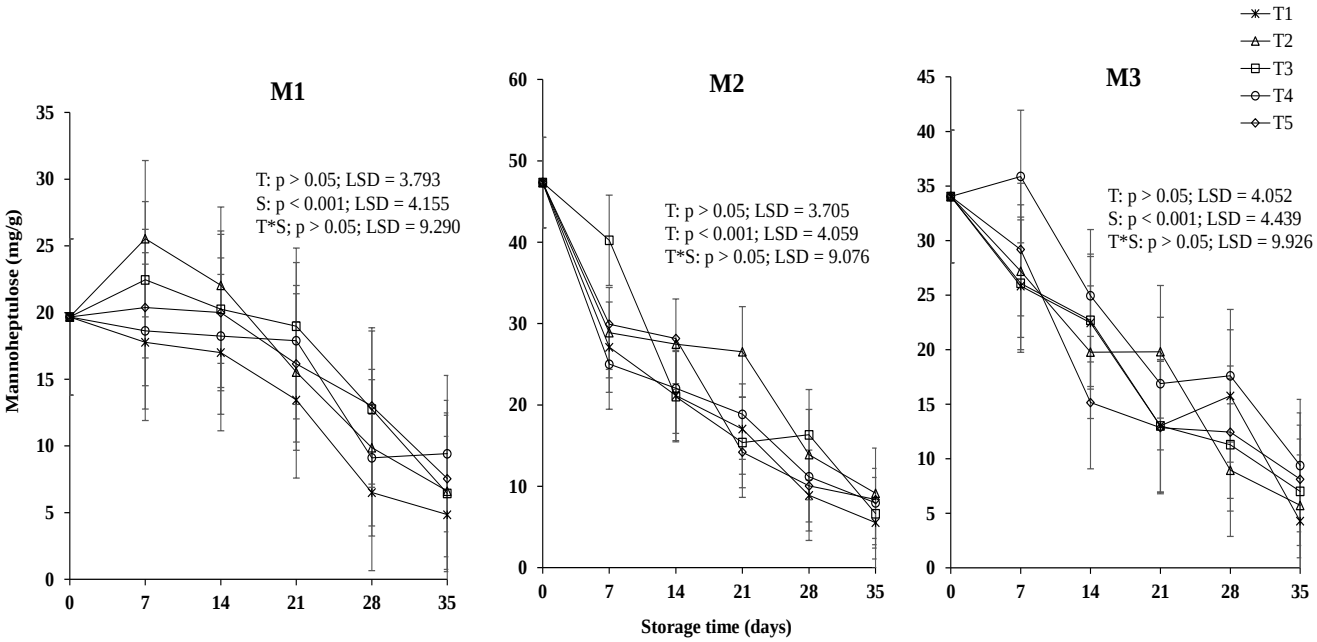

Figure 0.9: The effect of CMC and MLE-based edible coatings on mannoheptulose of 'Hass' avocado harvested at different maturities: maturity M1, M2, M3 during 28 days of cold storage and seven days of shelf-life. *The vertical bars represent standard error (SE) at n = 3; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

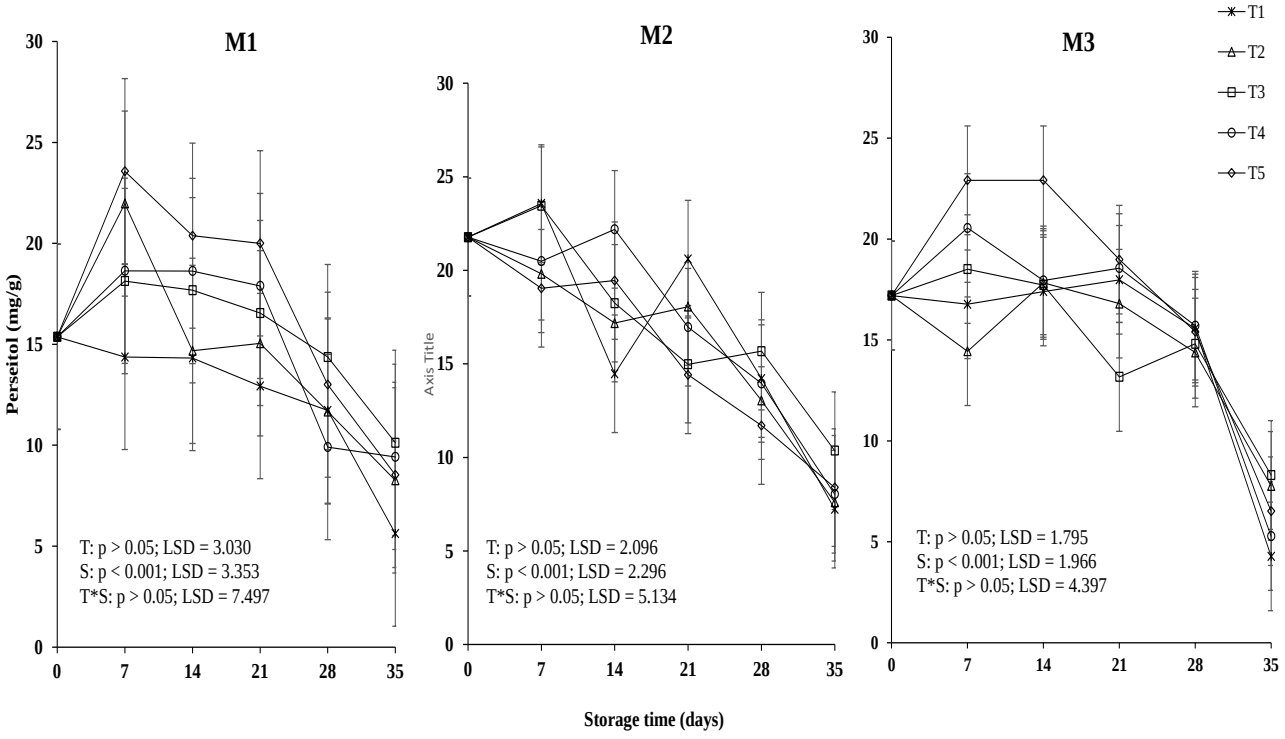

Figure 0.10: The effect of CMC and MLE-based edible coatings on perseitol of 'Hass' avocado harvested at maturity M1, M2, and M3 during 28 days of cold storage and seven days of shelf-life. *The vertical bars represent standard error (SE) at n = 3; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

3.7 Conclusion

Based on the results of the current study, it can be concluded that CMC and MLE were effective in conserving the postharvest quality of 'Hass' avocado fruit during a storage period of 35 days. Different moringa-based treatments successfully inhibited firmness and mass loss, consequently extending fruit shelf life. The coated fruit also had a reduced reduction in soluble sugars, especially mannoheptulose. This is the best indication that CMC and moringa-based edible coatings can be the best alternative for substituting the risky chemicals and costly avocado preservative techniques to extend the shelf life of this fruit. This research has also shown the ability of the used coatings to extend the shelf life of avocados without compromising the nutritional quality or the compounds of interest of this fruit. This is the most effective, environmentally friendly, and affordable technique that can be useful in commercials. The observed fluctuation in the concentrations of most biochemical parameters could be due to the fact that the fruit were not separated according to canopy positions. Previous research has reported the effect of canopy position on fruit ripening patterns and biochemical quality. Therefore, this must be considered for further investigation.

3.8 References

Abebe, Z., Tola, Y. B., and Mohammed, A. (2017). Effects of edible coating materials and stages of maturity at harvest on storage life and quality of tomato (*Lycopersicon Esculentum Mill*.) fruits. *African Journal of Agricultural Research* **12**, 550-565.

Addo, P. W., Sagili, S. U. K. R., Bilodeau, S. E., Gladu-Gallant, F.-A., MacKenzie, D. A., Bates, J., McRae, G., MacPherson, S., Paris, M., and Raghavan, V. (2022). Cold

Ethanol Extraction of Cannabinoids and Terpenes from Cannabis Using Response Surface Methodology: Optimization and Comparative Study. *Molecules* **27**, 8780.

Ali, A., Muhammad, M. T. M., Sijam, K., and Siddiqui, Y. (2011). Effect of chitosan coatings on the physicochemical characteristics of Eksotika II papaya (*Carica papaya L.*) fruit during cold storage. *Food Chemistry* **124**, 620-626.

Awad, M. A., Al-Qurashi, A. D., Mohamed, S. A., and El-Shishtawy, R. M. (2017). Quality and biochemical changes of 'Hindi-Besennara'mangoes during shelf life as affected by chitosan, gallic acid and chitosan gallate. *Journal of Food Science and Technology* **54**, 4139-4148.

Ballesteros, L. F., Teixeira, J. A., and Cerqueira, M. A. (2022). Active carboxymethyl cellulose-based edible coatings for the extension of fresh goldenberries shelf-life. *Horticulturae* **8**, 936.

Bill, M., Sivakumar, D., Thompson, A. K., and Korsten, L. (2014). Avocado fruit quality management during the postharvest supply chain. *Food Reviews International* **30**, 169-202.

Blakey, R., Tesfay, S., Bertling, I., and Bower, J. (2012). Changes in sugars, total protein, and oil in 'Hass' avocado (*Persea americana Mill.)* fruit during ripening. *The Journal of Horticultural Science and Biotechnology* **87**, 381-387.

Careli-Gondim, Í., Mesquita, T. C., Vilas Boas, E. V. d. B., Caliari, M., and Soares Júnior, M. S. (2020). The effect of active coating and refrigerated storage on the quality of avocado cultivar, Quintal. *Journal of Food Science and Technology* **57**, 143-151.

Chiabrando, V., and Giacalone, G. (2015). Anthocyanins, phenolics and antioxidant capacity after fresh storage of blueberry treated with edible coatings. *International Journal of Food Sciences and Nutrition* **66**, 248-253.

Cordenunsi, B. R., Genovese, M. I., do Nascimento, J. R. O., Hassimotto, N. M. A., dos Santos, R. J., and Lajolo, F. M. (2005). Effects of temperature on the chemical composition and antioxidant activity of three strawberry cultivars. *Food chemistry* **91**, 113-121.

Dávila-Aviña, J. E., Villa-Rodríguez, J. A., Villegas-Ochoa, M. A., Tortoledo-Ortiz, O., Olivas, G. I., Ayala-Zavala, J. F., and González-Aguilar, G. A. (2014). Effect of edible coatings on bioactive compounds and antioxidant capacity of tomatoes at different maturity stages. *Journal of Food Science and Technology* **51**, 2706-2712.

Dhall, R. K. (2016). Application of Edible Films and Coatings on Fruits and Vegetables. *In* "Edible Films and Coatings", pp. 381-408. CRC Press.

Eça, K. S., Sartori, T., and Menegalli, F. C. (2014). Films and edible coatings containing antioxidants-a review. *Brazilian Journal of Food Technology* **17**, 98-112.

Fan, S., Qi, Y., Shi, L., Giovani, M., Zaki, N. A. A., Guo, S., and Suleria, H. A. R. (2022). Screening of phenolic compounds in rejected avocado and determination of their antioxidant potential. *Processes* **10**, 1747.

Fernando, H., Srilaong, V., Pongprasert, N., Boonyaritthongchai, P., and Jitareerat, P. (2014). Changes in antioxidant properties and chemical composition during ripening in banana variety'Hom Thong'(AAA group) and'Khai'(AA group). *International Food Research Journal* **21**, 749.

Handayani, M., Karlina, S., Sugiarti, Y., and Cakrawati, D. (2018). Application of edible coating from cassava peel–bay leaf on avocado. *In* "Journal of Physics: Conference Series", Vol. 1013, pp. 012168. IOP Publishing.

Jeong, J., and Huber, D. J. (2004). Suppression of avocado (*Persea americana Mill.)* fruit softening and changes in cell wall matrix polysaccharides and enzyme activities: differential responses to 1-MCP and delayed ethylene application. *Journal of the American Society for Horticultural Science* **129**, 752-759.

Kader, A. A. (1997). Fruit maturity, ripening, and quality relationships. *In* "International Symposium Effect of Pre-& Postharvest factors in Fruit Storage 485", pp. 203-208.

Kassim, A., Workneh, T., and Bezuidenhout, C. (2013). A review on postharvest handling of avocado fruit. *African Journal of Agricultural Research* **8**, 2385-2402.

Khorram, F., Ramezanian, A., and Hosseini, S. M. H. (2017). Effect of different edible coatings on postharvest quality of ‘Kinnow’mandarin. *Journal of Food Measurement and Characterization* **11**, 1827-1833.

Kubheka, S. F., Tesfay, S., Mditshwa, A., and Magwaza, L. (2019). Efficacy of carboxymethyl cellulose and gum arabic edible coatings in combination with moringa leaf extract in improving postharvest quality of 'Maluma' avocado fruit. *In* "II International Symposium on Moringa 1306", pp. 293-300.

Kumar, N., Ojha, A., Upadhyay, A., Singh, R., and Kumar, S. (2021). Effect of active chitosan-pullulan composite edible coating enrich with pomegranate peel extract on the storage quality of green bell pepper. *LWT- Food Science and Technology* **138**, 110435.

Landahl, S., Meyer, M. D., and Terry, L. A. (2009). Spatial and temporal analysis of textural and biochemical changes of imported avocado cv. Hass during fruit ripening. *Journal of Agricultural and Food Chemistry* **57**, 7039-7047.

Langa, S. (2018). Effects of edible coatings and moringa extracts on postharvest quality of papaya fruits (Doctoral Dissertation).

Le, K. H., Nguyen, M. D.-B., Dai Tran, L., Thi, H. P. N., Van Tran, C., Van Tran, K., Thi, H. P. N., Thi, N. D., Yoon, Y. S., and Nguyen, D. D. (2021). A novel antimicrobial ZnO nanoparticles-added polysaccharide edible coating for the preservation of postharvest avocado under ambient conditions. *Progress in Organic Coatings* **158**, 106339.

Liu, W., Zhang, M., and Bhandari, B. (2020). Nanotechnology–A shelf life extension strategy for fruits and vegetables. *Critical Reviews in Food Science and Nutrition* **60**, 1706-1721.

Liu, X., Sievert, J., Arpaia, M. L., and Madore, M. A. (2002). Postulated physiological roles of the seven-carbon sugars, mannoheptulose, and perseitol in avocado. *Journal of the American Society for Horticultural Science* **127**, 108-114.

Maftoonazad, N., and Ramaswamy, H. (2005). Postharvest shelf-life extension of avocados using methyl cellulose-based coating. *LWT-Food science and technology* **38**, 617-624.

Magwaza, L. S., and Tesfay, S. Z. (2015). A review of destructive and non-destructive methods for determining avocado fruit maturity. *Food and Bioprocess Technology* **8**, 1995-2011.

Malmiri, J. H., Osman, A., Tan, C., and Rahman, A. R. (2011). Evaluation of effectiveness of three cellulose derivative-based edible coatings on changes of physico-chemical characteristics of'Berangan'banana (*Musa sapientum* cv. Berangan) during storage at ambient conditions. *International Food Research Journal* **18**, 1381.

Maringgal, B., Hashim, N., Tawakkal, I. S. M. A., and Mohamed, M. T. M. (2020). Recent advance in edible coating and its effect on fresh/fresh-cut fruits quality. *Trends in Food Science and Technology* **96**, 253-267.

Milbury, P. E., Chen, C.-Y., Dolnikowski, G. G., and Blumberg, J. B. (2006). Determination of flavonoids and phenolics and their distribution in almonds. *Journal of Agricultural and Food Chemistry* **54**, 5027-5033.

Mwelase, S., Mditshwa, A., Magwaza, L. S., and Tesfay, S. Z. (2022). Maturity Indexing and Postharvest Performance of Newly Developed 'Lamb Hass' Avocado Fruit. *International Journal of Fruit Science* **22**, 453-470.

Nair, M. S., Saxena, A., and Kaur, C. (2018). Effect of chitosan and alginate based coatings enriched with pomegranate peel extract to extend the postharvest quality of guava (*Psidium guajava* L.). *Food Chemistry* **240**, 245-252.

Obeng, E., Kpodo, F., Tettey, C., Essuman, E., and Adzinyo, O. (2020). Antioxidant, total phenols and proximate constituents of four tropical leafy vegetables. *Scientific African* **7**, e00227.

Panahirad, S., Dadpour, M., Peighambardoust, S. H., Soltanzadeh, M., Gullón, B., Alirezalu, K., and Lorenzo, J. M. (2021). Applications of carboxymethyl cellulose-and pectin-based active edible coatings in preservation of fruits and vegetables: A review. *Trends in Food Science and Technology* **110**, 663-673.

Panahirad, S., Naghshiband-Hassani, R., Ghanbarzadeh, B., Zaare-Nahandi, F., and Mahna, N. (2019). Shelf life quality of plum fruits (*Prunus domestica* L.) improves with carboxymethylcellulose-based edible coating. *HortScience* **54**, 505-510.

Paniagua, A., East, A., Hindmarsh, J., and Heyes, J. (2013). Moisture loss is the major cause of firmness change during postharvest storage of blueberry. *Postharvest Biology and Technology* **79**, 13-19.

Payasi, A., Mishra, N. N., Chaves, A. L. S., and Singh, R. (2009). Biochemistry of fruit softening: an overview. *Physiology and Molecular Biology of Plants* **15**, 103-113.

Peretto, G., Du, W.-X., Avena-Bustillos, R. J., De J. Berrios, J., Sambo, P., and McHugh, T. H. (2017). Electrostatic and conventional spraying of alginate-based edible coating with natural antimicrobials for preserving fresh strawberry quality. *Food and Bioprocess Technology* **10**, 165-174.

Riaz, A., Aadil, R. M., Amoussa, A. M. O., Bashari, M., Abid, M., and Hashim, M. M. (2021). Application of chitosan-based apple peel polyphenols edible coating on the preservation of strawberry (Fragaria ananassa cv Hongyan) fruit. *Journal of Food Processing and Preservation* **45**, e15018.

Rivera, S. A., Ferreyra, R., Robledo, P., Selles, G., Arpaia, M. L., Saavedra, J., and Defilippi, B. G. (2017). Identification of preharvest factors determining postharvest ripening behaviors in 'Hass' avocado under long term storage. *Scientia horticulturae* **216**, 29-37.

Romanazzi, G., Feliziani, E., and Sivakumar, D. (2018). Chitosan, a biopolymer with triple action on postharvest decay of fruit and vegetables: Eliciting, antimicrobial and film-forming properties. *Frontiers in Microbiology* **9**, 2745.

Sharma, L., Saini, C. S., Sharma, H. K., and Sandhu, K. S. (2019). Biocomposite edible coatings based on cross linked-sesame protein and mango puree for the shelf life stability of fresh-cut mango fruit. *Journal of Food Process Engineering* **42**, e12938.

Shezi, S., Magwaza, L. S., Tesfay, S. Z., and Mditshwa, A. (2020). Simple and multiple linear regression models for predicting maturity of 'Mendez# 1'and 'Hass' avocado fruit harvested from inside and outside tree canopy positions. *International Journal of Fruit Science* **20**, S1969-S1983.

Singh, P., Magalhães, S., Alves, L., Antunes, F., Miguel, M., Lindman, B., and Medronho, B. (2019). Cellulose-based edible films for probiotic entrapment. *Food Hydrocolloids* **88**, 68-74.

Sivakumar, D., and Bautista-Baños, S. (2014). A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. *Crop Protection* **64**, 27-37.

Sivakumar, D., Tuna Gunes, N., and Romanazzi, G. (2021). A comprehensive review on the impact of edible coatings, essential oils, and their nano formulations on postharvest decay anthracnose of avocados, mangoes, and papayas. *Frontiers in Microbiology* **12**, 711092.

Tesfay, S. Z., and Magwaza, L. S. (2017). Evaluating the efficacy of moringa leaf extract, chitosan and carboxymethyl cellulose as edible coatings for enhancing quality and extending postharvest life of avocado (*Persea americana* Mill.) fruit. *Food Packaging and Shelf Life* **11**, 40-48.

Tesfay, S. Z., Magwaza, L. S., Mbili, N., and Mditshwa, A. (2017). Carboxyl methylcellulose (CMC) containing moringa plant extracts as new postharvest organic edible coating for Avocado (*Persea americana* Mill.) fruit. *Scientia Horticulturae* **226**, 201-207.

Thakur, R., Pristijono, P., Golding, J., Stathopoulos, C. E., Scarlett, C., Bowyer, M., Singh, S., and Vuong, Q. (2018). Development and application of rice starch based edible coating to improve the postharvest storage potential and quality of plum fruit (*Prunus salicina*). *Scientia Horticulturae* **237**, 59-66.

Wang, S. Y., and Gao, H. (2013). Effect of chitosan-based edible coating on antioxidants, antioxidant enzyme system, and postharvest fruit quality of strawberries *(Fragaria x aranassa* Duch.). *LWT-Food Science and Technology* **52**, 71-79.

Wolstenholme, B. (2012). Developments in the world avocado industry, and their relevance to the South African and African industries. *In* "II All Africa Horticulture Congress 1007", pp. 865-871.

Wróblewska-Krepsztul, J., Rydzkowski, T., Borowski, G., Szczypiński, M., Klepka, T., and Thakur, V. K. (2018). Recent progress in biodegradable polymers and nanocomposite-

based packaging materials for sustainable environment. *International Journal of Polymer Analysis and Characterization* **23**, 383-395.

Yaman, Ö., and Bayoındırlı, L. (2002). Effects of an edible coating and cold storage on shelf-life and quality of cherries. *LWT-Food Science and Technology* **35**, 146-150.

Zahedi, S. M., Hosseini, M. S., Karimi, M., and Ebrahimzadeh, A. (2019). Effects of postharvest polyamine application and edible coating on maintaining quality of mango (*Mangifera indica* L.) cv. Langra during cold storage. *Food Science and Nutrition* **7**, 433-441.

Zhang, X., Zhang, X., Liu, X., Du, M., and Tian, Y. (2019). Effect of polysaccharide derived from *Osmunda japonica* Thunb-incorporated carboxymethyl cellulose coatings on preservation of tomatoes. *Journal of Food Processing and Preservation* **43**, e14239.

Chapter 4

Green Synthesis of Zinc Oxide Nanoparticles Using *Moringa Oleifera* Leaf Extract and its Antifungal Effect Against *Colletotrichum gloeosporioides* in Avocado

4.1 Abstract

Avocado, one of the most commercially important fruits, is susceptible to several postharvest diseases, including anthracnose caused by *Colletotrichum gloeosporioides*, which renders the fruit unpalatable. Prochloraz, a synthetic postharvest chemical treatment, is currently used to control anthracnose in avocados. However, several studies have reported adverse effects of prochloraz on human health due to its metabolite. Thus, there is an imposed ban on this fungicide on fruits destined for the European Union, which is the primary target and lucrative market for the South African avocado industry. The present study evaluated the efficacy of *Moringa oleifera* leaf extract (MLE) on the green synthesis of zinc oxide (ZnO) nanoparticles (NPs) (ZnO NPs) and its antifungal effect against *C. gloeosporioides* on 'Hass' avocado fruit. Green synthesis of ZnO NPs was done using zinc acetate dihydrate (0.2 M) as a source of zinc ions and moringa leaf extract (5 %) as a reducing and stabilizing agent. The antifungal activity of the synthesized ZnO NPs was tested against *C. gloeosporiodes* in the potato dextrose agar potato dextrose agar (PDA) amended with ZnO NPs (0.25, 0.5, 1, and 2%) and observing the mycelial growth on a two-day basis for nine days. The antioxidant radical scavenging activity of the formed ZnO NPs were found to be dependent on the concentrations, hence increased with increasing concentrations. Moreover, compared to the control, all the tested concentrations (0.25, 0.5, 1, and 2%) of moringa-based ZnO NPs significantly inhibited the radial growth of *C. gloeosporioides* isolates in the *in vitro* study. The highest inhibition percentage (72%) of mycelial growth was observed in isolates treated with 1% ZnO-NPs. The findings from this study showed a strong effect of ZnO NPs against *C. gloeosporioides;* therefore, it could be recommended as an effective antifungal agent to control anthracnose in avocados.

Keywords:

Prochloraz; anthracnose; nanotechnology; mycelial growth; antioxidants; *in vitro*

4.2 Introduction

A significant amount of avocado fruit is lost postharvest due to diseases and the perishability nature of this fruit. This impedes the economy since avocado-producing industries in South Africa are export-orientated; thus, the quality of this fruit is crucial. Maintaining avocado fruit quality throughout the export process is still challenging. *Colletotrichum gloeosporioides*, an anthracnose causal agent, is one of the most economically devastating postharvest pathogens that is responsible for massive spoilage of fresh produce (Freeman et al., 1996). Although it starts developing pre-harvest while the fruit is still attached to the tree, the symptoms are commonly observed in storage during fruit ripening. This pathogen compromises the quality of fruit, which results in the decline of the market value, and, in the case of severe infection, the fruit becomes inedible. The control of anthracnose has depended almost entirely upon prochloraz, especially for South African avocado fruit. However, there is an imposed ban on prochloraz by some of the important international markets (Sivakumar et al., 2021). This follows from the US Environmental Protection Agency (EPA) associating some of these fungicide metabolites with elevated chronic diseases, such as cancer, and marking them as a priority pollutant (Shimshoni et al., 2020). In fact, the adverse effects of prochloraz on human health and the environment are documented as well (Xu et al., 2018; Obianom et al., 2019; Sivakumar et al., 2021; Lin et al., 2023). Moreover, various pathogens have developed resistance to most chemical treatments. This, therefore, necessitates continued research aiming to develop or improve environmentally safe organic postharvest treatments to replace synthetic fungicides.

Nanotechnology has gained popularity for its wide applications in different fields, including renewable energy, environmental remediation, surface disinfection, medicine, and agriculture (Sharma et al., 2020; Jobe et al., 2022). This is due to unique properties, such as crystallinity, size, shape, and morphology resembled by nanoparticles (NPs) (Shinde, 2015). Various metals and metal oxides, such as copper (Cu), copper oxide (CuO), zinc oxide (ZnO), Titanium oxide (TiO_2), silver (Ag), Palladium (Pd), and Iron (Fe) and its oxides have been used as source of NPs and featured in horticultural commodities postharvest preservative studies as well (Suryavanshi et al., 2017; Iliger et al., 2021; Sharifan et al., 2021; Wang et al., 2023).

Commonly, the resulting NPs maintain the quality and increase the shelf life of most horticultural produce (de Oliveira Filho et al., 2021; de Oliveira Filho et al., 2022). However, most authors have demonstrated a great potential for ZnO NPs, mainly due to their high piezoelectric properties, excellent biocompatibility, large binding energy, non-toxicity, and low cost (Jiang et al., 2018; Jain et al., 2020). Furthermore, ZnO NPs have unique chemical and physical properties, giving these compounds a strong antibacterial and antifungal activity at low concentrations, owing to their high surface area to volume ratio (Espitia et al., 2013).

Different techniques, such as chemical, physical, and biological methods, have been used to synthesize NPs (Rane et al., 2018). However, some methods, such as chemical and physical, are not sustainable for the environment and consumers. For example, the chemical method involves the use of toxic chemicals, which makes this approach unfriendly, whereas, on the other hand, there is more energy, area, and time required by the physical approach (Thema et al., 2015). There is a consistently increasing interest in the biological synthesis of nanoparticles, and more focus has been put on optimizing green chemistry technology to synthesize such materials. Biological synthesis involves using plant extracts and other microorganisms with biomedical applications. Various authors have presented the potential of different plant parts, such as stems, roots, leaves, and the actual fruit, in the synthesis of nanoparticles (Elia et al., 2014; Ghaffari-Moghaddam et al., 2014; Niraimathee et al., 2016; Kumar et al., 2017; Santhoshkumar et al., 2017; Behravan et al., 2019). These plant parts present the phytochemicals that act as stabilizing and reducing agents during synthesis (Ramesh et al., 2015; Thema et al., 2015). Thus, synthesizing nanoparticles by such an approach raises no concerns as it is an environmentally friendly, safe, cost-effective, and biocompatible green approach.

Moringa oleifera is one of the most widely cultivated crops for its nutritional and medicinal properties (Matthew, 2016). There has been extensive research on the antimicrobial and antioxidant activity of different parts of this crop (Tesfay et al., 2011; Kumar et al., 2012; Ndhlala et al., 2014; Mohammed et al., 2019; Tshabalala et al., 2020). Moreover, extracts from different parts of this crop have been featured in different pre- and postharvest fruit treatment research and responded positively (Adetunji et al., 2013; Kubheka et al., 2020; Mahmoud et al., 2020; Nasir et al., 2020). However, little research has been conducted to optimize the synthesis of nanoparticles using this crop. This paper, therefore, focused on the synthesis of ZnO-NPs using *Moringa oleifera* leaf extract following a greener approach and evaluating its

morphological, structural, and antifungal properties against *C. gloeosporioides* on 'Hass' avocado fruit.

4.3 Materials and Methods

4.3.1 Materials

The fresh moringa leaf powder used in this study was purchased from the Agricultural Research Council (ARC), located in Pretoria, South Africa. All the chemicals and reagents were bought from Merck Life Science (Pty) Ltd, Johannesburg, South Africa.

4.3.2 Plant extract preparation and synthesis of zinc oxide nanoparticles

The plant extract was prepared following a slightly modified method previously described by Bhuyan et al. (2015) and Hassan et al. (2021). Briefly, the extract was prepared by mixing 10 g of dried moringa leaf powder with 100 mL of distilled water in a 250 mL Erlenmeyer flask. The mixture was after that heated at 75 ℃ for 15 minutes. This was followed by allowing the mixture to cool at room temperature and filtering through Whatman No.1 filter paper. The filtrate was collected and stored in a refrigerator at 4 ℃ until further use.

Zinc acetate dihydrate (0.2 M) was prepared in 100 mL deionized water under constant stirring on a hot plate magnetic stirrer at 85 ℃ for 60 minutes. After 20 minutes, 25 mL of 2 M sodium hydroxide was slowly added until the pH of the solution was 12. After 1 hour, 25 mL of moringa leaf extract was slowly added under continuous stirring for 2 hours using a magnetic stirrer. The resulting deep yellow coloured paste was collected, oven-dried overnight at 60 ℃, transferred to a ceramic crucible cup, and heated in a furnace at 460 ℃ for 2 hours. The resulted powder material was collected, put in an air-tight bottle, and stored at room temperature until further use.

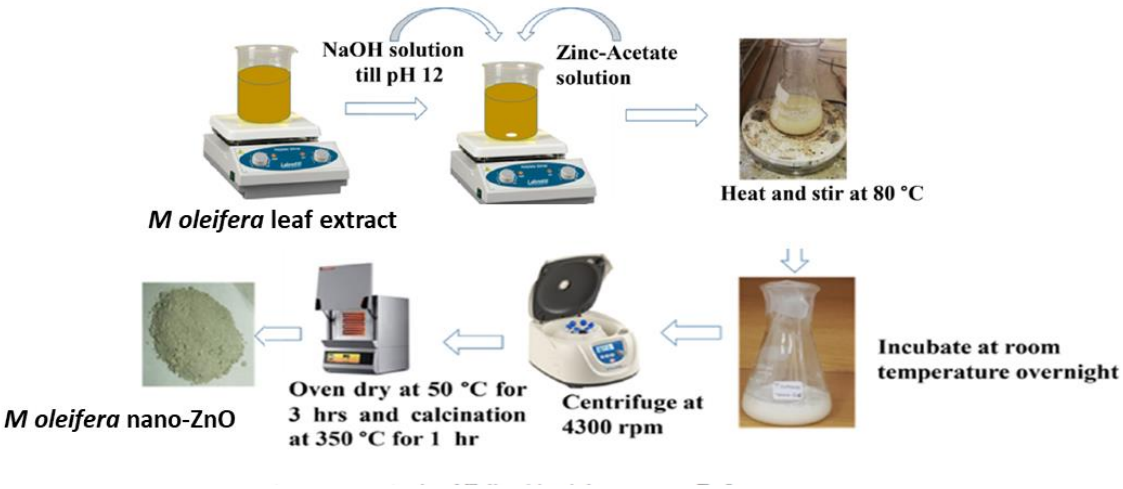

Figure 0.1: The schematic diagram of the stages involved in the biosynthesis of zinc oxide nanoparticles using moringa extract.

4.3.3 Characterization of zinc oxide nanoparticles

The synthesized ZnO NPs were confirmed by evaluating the size, structure, and shape of the crystal using Zeiss EVO scanning electron microscopy (Zeiss, Oberkochen, Germany) (SEM) and JEOL JEM 1400 transmission electron microscopy (JEOL, Beijing, Shanghai, China) (TEM). For TEM analysis, the ZnO NPs were suspended in absolute ethanol and sonicated for 15 minutes for clear dispersion of particles. A drop of the resulting sonicated solution was cast onto a carbon-coated copper grid, dried in a mercury lamp for 10 minutes, and examined under TEM. For SEM analysis, the ZnO NPs were mounted onto stubs and coated three times with gold deposited by a quorum sputter (Q150R ES) under a vacuum using argon gas. This was followed by viewing the NPs under the Zeiss EVO SEM in high vacuum mode.

4.3.4 Antioxidant activity of zinc oxide nanoparticles

The scavenging activity of ZnO NPs was evaluated using 2,2-diphenyl-1-picrylhydrazyl assay following a method previously described by Safawo et al. (2018), with slight amendments. Briefly, 1 mL of different concentrations of ZnO NPs (0.25, 0.5, 1, 2 % v/w) dissolved in ethanol were separately mixed with 1 mL of methanolic DPPH (0.1 mM) and vortexed for 1 minute to mix the solution thoroughly. Consequently, the mixture was incubated in the dark

for 30 minutes at room temperature. The absorbances for the prepared solutions were read at 520 nm wavelength using the UV-1800 Spectrophotometer (Shimadzu Scientific Instruments INC., Columbia, USA), and the experiment was repeated three times. The control contained only methanol and DPPH solution. The scavenging activity was estimated using Eq. 4.1 below.

$$\% \text{ inhibition} = \frac{Control\ absorbance - Sample\ absorbance}{Control\ absorbance} x\ 100 \quad (4.1)$$

4.3.5 Isolation of pathogen and media preparation

The pathogen, *C. gloeosporioides* was isolated from avocado fruit by aseptically cutting internally infected tissues using an alcohol-sterilized scalpel. The tissues were surface sterilized for 30 seconds in 70% ethanol, followed by rinsing twice in distilled water, placed on the potato dextrose agar (PDA) plates, and incubated for seven days at 28 ℃ to allow the fungal growth. To prepare the PDA media, 15.5 g of potato dextrose was mixed with 400 mL of distilled water and autoclaved at 121 ℃ for 15 minutes. The mixture was allowed to cool to 50 ℃ in water and poured into 90 mm Petri dishes. The pure culture was obtained by subculturing the isolated colonies into fresh PDA plates.

4.3.6 Confirmation of pathogen isolates

The isolates were confirmed by evaluating their morphological structures under the light microscope at 40x and 100x magnifications based on their hyphae orientation and the shape of their spores. Based on visual observation, the pathogen had fast-growing white to off-white colonies with dense whitish mycelia. On the reverse side of the Petrie dishes, the colour was yellowish towards the centre. The light microscopy confirmed the formation of cylindric and nonseptate conidia with obtuse ends (Figure 0.2). All these observed characteristics match the ones reported for *Colletotrichum gloeosporioides* in a study by Hassan et al. (2018).

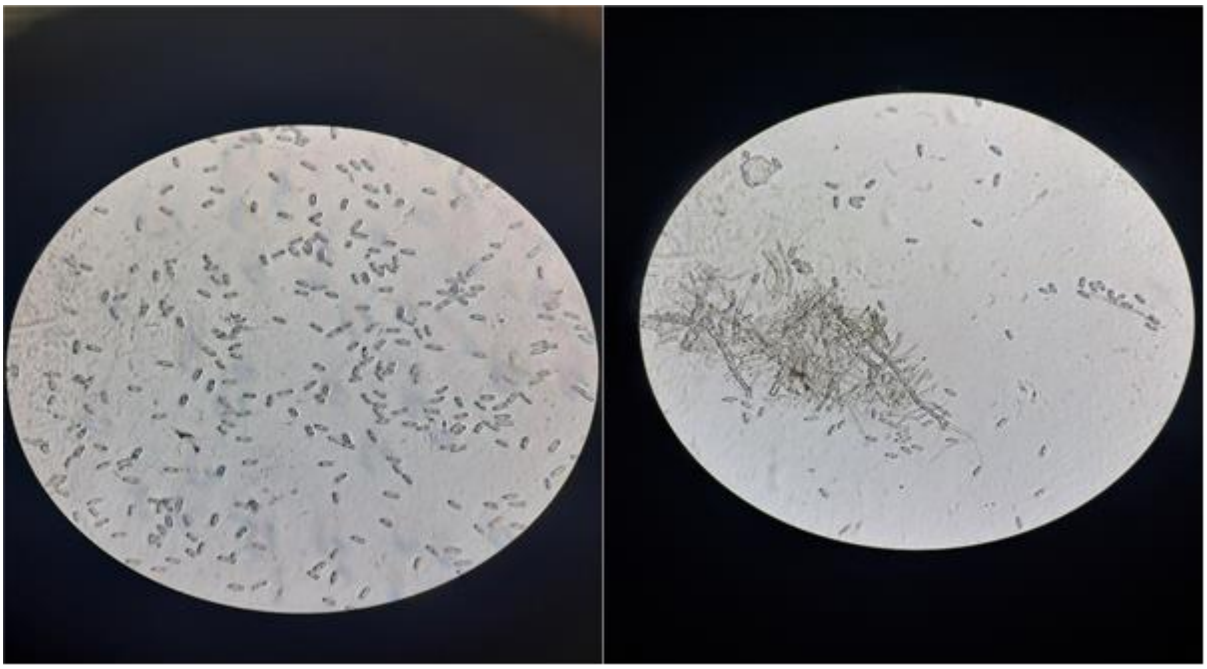

Figure 0.2: Morphology of *C. gloeosporioides* confirmed under light microscope

4.3.7 Preparation of ZnO-based treatments and in-vitro assay

The antifungal activity of the synthesized ZnO NPs was tested at different concentrations (0, 0.25, 0.5,1, and 2% v/w) against *C. gloeosporioides* isolates in Petri dishes. The used concentrations were amended from those previously tested by Le et al. (2021) on avocado. Different treatments were obtained by separately amending the prepared PDA agar with ZnO NPs (0.25, 0.5, 1, and 2 %), and the pure (unamended) PDA served as a control. After cooling, about 20 mL of the amended PDA was poured into 90 mm sterile Petri dishes, and a disc of mycelium (3 mm diameter) excised from the pure culture was inoculated. This was followed by incubating the petri dishes for nine days at 25 ℃. At this time, the mycelial growth was evaluated by measuring the diameter of the colony on a two-day basis during the incubation period. The treatments were completely randomized, and each treatment had three replicate cultures with two measurements recorded per replicate and per measuring day. The inhibition percentage was determined using the following Eq. 4.2 (González-Merino et al., 2021).

% Inhibition = $\frac{MGC\ (mm) - MGT\ (mm)}{MGC\ (mm)} x\ 100\ \%$; where MGC = mycelial growth in the control; MGT= mycelial growth in the ZnO NPs treatment. (2.2)

4.4 Statistical analysis

A completely randomized design was used for this experiment with three replicates. The GenStat statistical software (GenStat Twentieth Edition, VSN International Ltd, UK) was used for the analysis of variance (ANOVA) of the data analysis. The mean separation was performed using Duncan's Multiple Range Test (DMRT) at a 5 % significance level.

4.5 Results and Discussion

4.5.1 Confirmation of ZnO nanoparticles through SEM and TEM

The formation of ZnO NPs was first confirmed by the yellow colour development during biosynthesis, which is regarded as the most reliable colour indicating the formation of NPs (Barzinjy and Azeez, 2020). Further evaluations using the SEM revealed amorphous ZnO NPs, while the TEM showed weakly agglomerated nanoparticles with a uniform spherical shape (Figure 0.3, Figure 0.4). The agglomeration occurred due to the narrow space between particles caused by the aqueous MLE and the high surface energy and electrostatic attraction of ZnO NPs (Ramesh et al., 2015; Darvishi et al., 2019). However, the weak agglomeration indicated the high potential of MLE to act as a stabilizing agent, thereby preventing the aggregation of the NPs (Holmes et al., 2003). This study reports the formation of ZnO NPs with a diameter estimated to range between 35 and 78 nm, as indicated in Figure 0.4. The overlapping of NPs contributed to the increased reported size. Various factors, such as the selected plant species, plant extract concentration, precursor concentration, reaction time, pH value, and calcination time, have been reported to influence the morphology of the synthesized NPs (Xu et al., 2021). Although this study did not compare the effect of different pH and calcination temperatures on the resulting NPs, increasing the pH to 12 and the high calcinating temperature (350 ℃) might have influenced the shape, size, and aggregation of the resulted NPs. Ochieng et al. (2015) previously reported a formation of weakly agglomerated homogeneous NPs at alkaline pH compared to acidic using *Spathodea campanulate* P. Beauv leaves extract. Another study by Karam and Abdulrahman (2022) reported that NPs are formed as non-orientation nanorods at low calcination temperatures and only join together and form various shapes at temperatures between 250 and 450 ℃ using thyme plant leaf extract (Karam and Abdulrahman, 2022).

Similarly, Zhu et al. (2021) observed a directly proportional trend between pH and ZnO NPs synthesized using *Cinnamomum camphora* (L.) Presl leaf extracts; the smallest average size was obtained at pH 7 (13.92 nm) while the highest (21.13 nm) was obtained at pH 9. Furthermore, this could be the reason for the ZnO NPs sizes reported in this study, given that the pH was raised to 12; however, it is important to mention that they were still within the nanoscale (>100 nm). There are, however, still some arguments about the effect of pH on the particle size of nanoparticles. Some authors claimed that the particle sizes decrease with an increased pH (Alias et al., 2010), while others claimed the opposite (Jay Chithra et al., 2015). This study has no conclusive results about the effect of pH on the green synthesis of ZnO NPs using moringa. Further studies still need to be conducted to determine various factors affecting the synthesis of these particles, following a similar approach. However, in general, the size of the ZnO NPs is increased with increased reaction period and calcination temperature and decreases with increased precursor and plant extract concentrations (Xu et al., 2021).

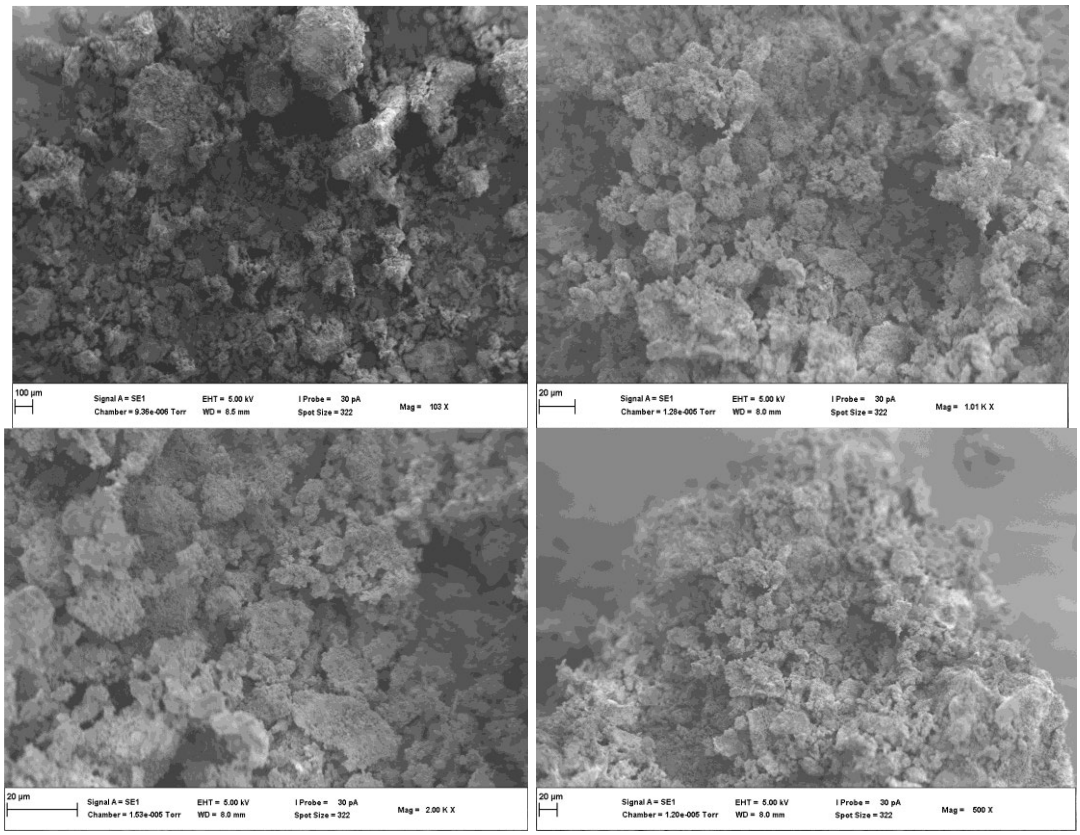

Figure 0.3: Scanning electron microscopy images of moringa-based ZnO nanoparticles at different magnifications.

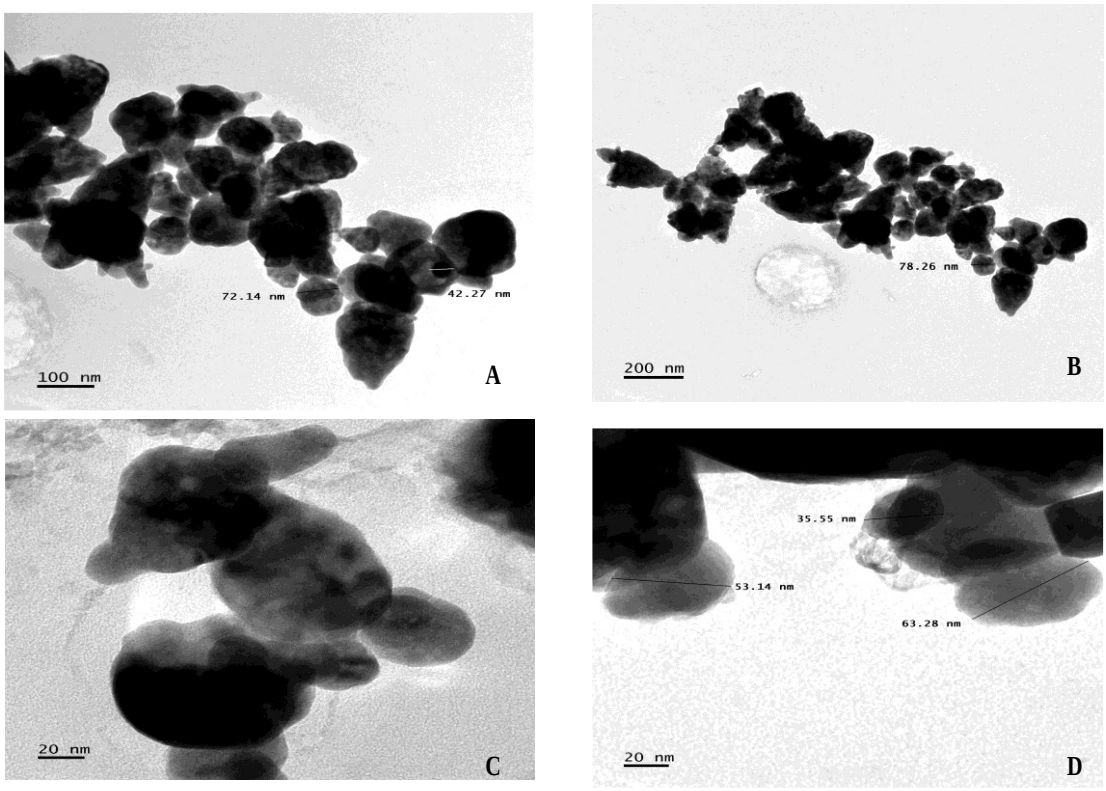

Figure 0.4: ZnO nanoparticles synthesized using moringa leaf extract confirmed under transmission electron miscroscopy at different magnifications.

4.5.2 Antioxidant activity of zinc oxide nanoparticles

The potential antioxidant activity of moringa leaf extract-based nanoparticles was evaluated using the DPPH method. Various authors have considered the DPPH method the most reliable and cheap method for determining the antioxidants' radical scavenging activity (Hossain et al., 2015; Ningsih et al., 2016). Figure 0.5 shows the results of the tested ZnO NPs at different concentrations against DPPH. The inhibition percentage increased with the increase in ZnO concentrations. The antioxidants present in these NPs were due to the MLE used in the biosynthesis. This could also indicate that the method used to synthesize moringa-based ZnO NPs was very effective and did not negatively affect the antioxidants of moringa and the resulting NPs. This observed moringa-based ZnO NPs antioxidants trend is similar to that generally displayed by ascorbic acid at different concentrations when used as a standard to determine antioxidants. The results in this study corroborate those by Zhu et al. (2021), who reported an increase in antioxidant activity with increased ZnO NPs synthesized with

Cinnamomum camphora (L.) Presl leaf extracts. Jobe et al. (2022) also reported that the lowest concentration of ZnO and Ag/ZnO NPs showed the lowest radical scavenging activity. Similarly, Mthana et al. (2022) demonstrated increased inhibition percentage with increasing concentrations of ZnO NPs synthesized following a green or conventional method. Generally, the antioxidant activity of greenly synthesized nanoparticles depends on the chemical composition of the extract. Therefore, the resulting nanoparticles will have a higher scavenging activity if the extract exhibits higher phenolics and flavonoids. Thus, this depicts that moringa leaves are a great source of antioxidants.

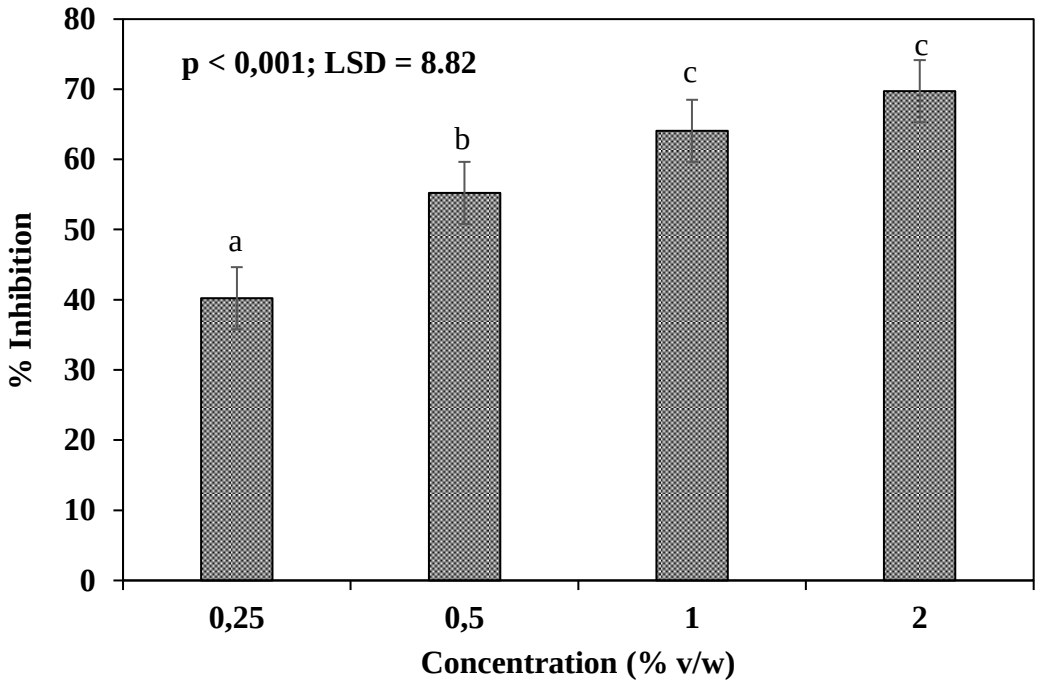

Figure 0.5: Scavenging activity of ZnO NPs at different concentrations against DPPH. *The vertical bars represent standard error (SE) at n = 3; means sharing the same letter are not statistically significant according to Duncan's Multiple Range Test (DMRT) (P = 0.05).

4.5.3 Effect of moringa-based ZnO NPS on the mycelia growth of *Colletotrichum gloeosporioides*

Figure 0.7 shows that the ZnO NPs significantly ($p < 0.001$) affected the mycelia growth of *C. gloeosporioides.* The growth of mycelia reached 24, 24, 17, 29, and 67 mm for *C.*

gloeosporioides cultures in 0.25, 0.5, 1, 2, and 0 % (control) concentrations of ZnO NPs, respectively. This marked a decrease of 64, 64, 71, and 56% in the mycelial growth of the *C. gloeosporioides* cultures exposed to 0.25, 0.5, 1, and 2%, respectively, compared to the control. Various authors have reported the antibacterial effect of green synthesized ZnO NPs (El-Kady et al., 2023; Ihsan et al., 2023; Mushtaq et al., 2023; Yilma et al., 2023), while few have examined their antifungal effect. In this study, ZnO NPs were synthesized using moringa leaf extract and evaluated for their potent antifungal effect against avocado *C. gloeosporioides*. The enhanced ZnO NPs efficacy presented in this study could be attributed to their unique characteristics, including their large surface area. Similar results were reported by Pariona et al. (2020), where all the concentrations used for ZnO NPs significantly inhibited the mycelial growth of *Fusarium solani*, *Fusarium oxysporum f.* sp. *lycopersici*, and *Colletotrichum gloeosporioides* with variable effectivity. However, the effectiveness of NPs may also be affected by the variation in shape, size, and fungi species. It was found that increasing the concentration of ZnO NPs to 2% resulted in the highest mycelial growth on day nine compared to the other concentrations used, except the control. Still, based on a visual perspective, it is important to mention that this treatment inhibited the pathogen from sporulating. Similarly, Arciniegas-Grijalba et al. (2017) reported a high antifungal effect of ZnO NPs against *Erythricium salmonocolor* (Berk. & Broome) on day 10, which was, thereafter, reduced overtime on day 22. Various authors have reported different levels of cellular damage caused by different concentrations of NPs to other pathogens, including *Colletotrichum gloeosporioides* (la Rosa-García et al., 2018). Although there were no cellular evaluations in the present study, the results obtained could indicate the ability of ZnO NPs to inhibit fungal growth by distorting and damaging its conidia (He et al., 2011). Furthermore, ZnO NPs are associated with the development of reactive oxygen species (ROS), which damage the cell (Lipovsky et al., 2011). A study by Espitia et al. (2012) further demonstrated that the resulting ROS on the cell wall of the fungi, due to the presence of ZnO NPs, causes protein denaturation and consequently destroys the DNA and the cell wall.

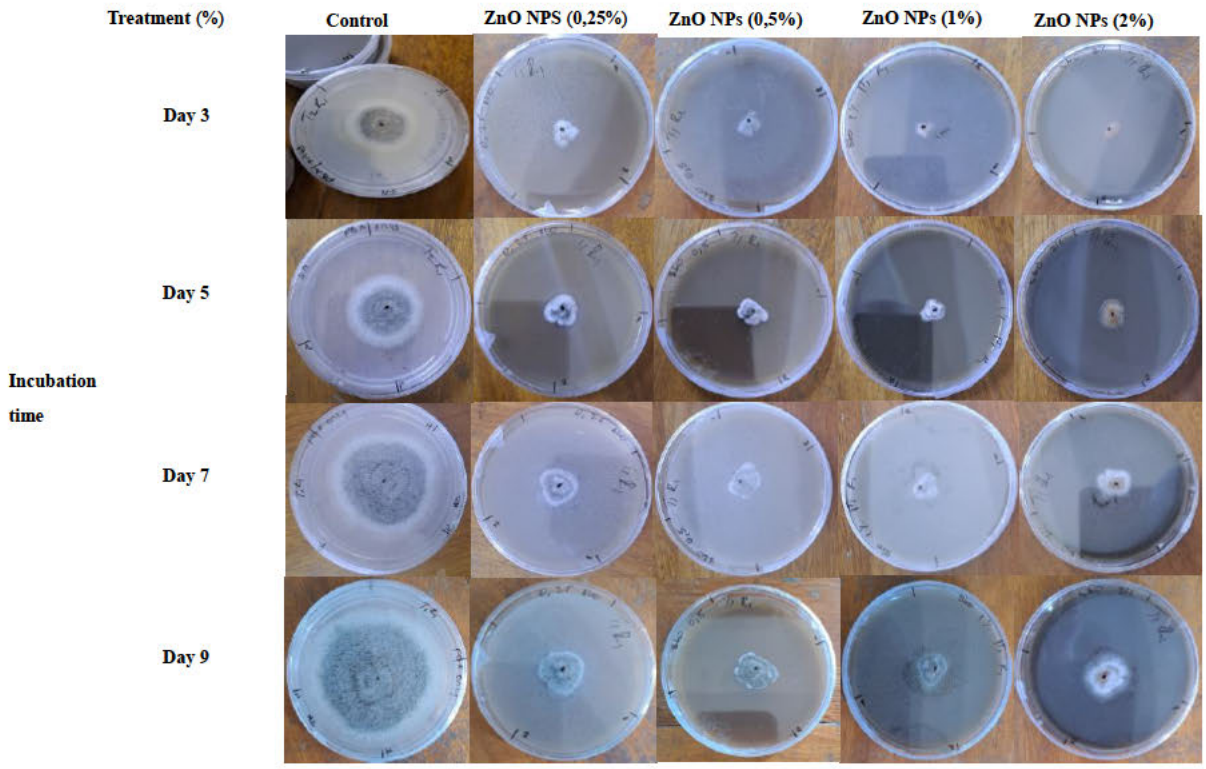

Figure 0.6: The mycelial growth of *Colletotrichum gloeosporioides* isolate during storage as influenced by the application of ZnO NPs at 0, 0.25, 0.5, 1, and 2% concentrations.

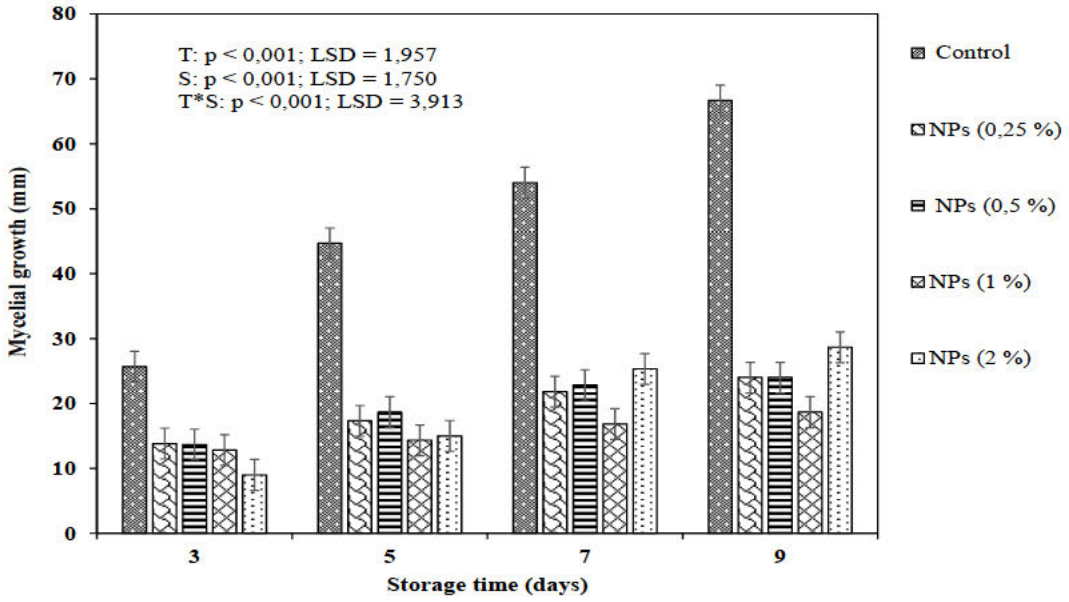

Figure 0.7: The mycelial growth of *Colletotrichum gloeosporiodes* isolate as influenced by ZnO NPs and storage period. *The vertical bars represent standard error (SE) at n = 3; T, treatment; S, storage period.

4.6 Conclusion

The biological approach for the synthesis of ZnO NPs using moringa leaf extract has been proven to be a simple, environmentally friendly, and effective approach. Using plant-based materials as reducing and stabilizing agents minimizes the usage of synthetic harmful and toxic materials. Furthermore, given the potential of these particles in controlling the postharvest *Colletotrichum gloeosporioides,* they can be recommended as an environmentally friendly substitute for the currently used fungicide at packhouses. However, further in vivo assessment is still required since this study was only in vitro. Nanotechnology has great potential, and more research needs to be done on the mechanism of the formation of nanoparticles to have complete control of the characteristics of the resulting product, such as size and shape. This is necessary due to the relationship between the morphology and the properties of the nanoparticles; therefore, it is much more important to synthesize the NPs with the morphology that best suits the aim intended.

4.7 References

Adetunji, C., Fawole, O., Arowora, K., Nwaubani, S., Oloke, J., Adetunji, J., and Ajani, A. (2013). Postharvest quality and safety maintenance of the physical properties of *Daucus carota* L. fruits by Neem oil and Moringa oil treatment: a new edible coatings. *Agrosearch* **13**, 131-142.

Alias, S., Ismail, A., and Mohamad, A. (2010). Effect of pH on ZnO nanoparticle properties synthesized by sol–gel centrifugation. *Journal of Alloys and Compounds* **499**, 231-237.

Arciniegas-Grijalba, P., Patiño-Portela, M., Mosquera-Sánchez, L., Guerrero-Vargas, J., and Rodríguez-Páez, J. (2017). ZnO nanoparticles (ZnO-NPs) and their antifungal activity against coffee fungus *Erythricium salmonicolor*. *Applied Nanoscience* **7**, 225-241.

Barzinjy, A. A., and Azeez, H. H. (2020). Green synthesis and characterization of zinc oxide nanoparticles using *Eucalyptus globulus* Labill. leaf extract and zinc nitrate hexahydrate salt. *SN Applied Sciences* **2**, 991.

Behravan, M., Panahi, A. H., Naghizadeh, A., Ziaee, M., Mahdavi, R., and Mirzapour, A. (2019). Facile green synthesis of silver nanoparticles using *Berberis vulgaris* leaf and root aqueous extract and its antibacterial activity. *International Journal of Biological Macromolecules* **124**, 148-154.

Bhuyan, T., Mishra, K., Khanuja, M., Prasad, R., and Varma, A. (2015). Biosynthesis of zinc oxide nanoparticles from *Azadirachta indica* for antibacterial and photocatalytic applications. *Materials Science in Semiconductor Processing* **32**, 55-61.

Darvishi, E., Kahrizi, D., and Arkan, E. (2019). Comparison of different properties of zinc oxide nanoparticles synthesized by the green (using *Juglans regia* L. leaf extract) and chemical methods. *Journal of Molecular Liquids* **286**, 110831.

de Oliveira Filho, J. G., Bertolo, M. R. V., da Costa Brito, S., Malafatti, J. O. D., Bertazzo, G. B., Colacique, M. N., Paris, E. C., Bogusz Junior, S., and Ferreira, M. D. (2022). Recent advances in the application of nanotechnology to reduce fruit and vegetable losses during post-harvest. *Brazilian Journal of Physics* **52**, 126.

de Oliveira Filho, J. G., Miranda, M., Ferreira, M. D., and Plotto, A. (2021). Nanoemulsions as edible coatings: A potential strategy for fresh fruits and vegetables preservation. *Foods* **10**, 2438.

El-Kady, A. M., S. Hassan, A., Mohamed, K., Alfaifi, M. S., Elshazly, H., Alamri, Z. Z., Wakid, M. H., Gattan, H. S., Altwaim, S. A., and Al-Megrin, W. A. I. (2023). Zinc oxide nanoparticles produced by *Zingiber officinale* ameliorates acute toxoplasmosis-induced pathological and biochemical alterations and reduced parasite burden in mice model. *PLOS Neglected Tropical Diseases* **17**, e0011447.

Elia, P., Zach, R., Hazan, S., Kolusheva, S., Porat, Z. e., and Zeiri, Y. (2014). Green synthesis of gold nanoparticles using plant extracts as reducing agents. *International Journal of Nanomedicine*, 4007-4021.

Espitia, P. J. P., Soares, N. d. F. F., Coimbra, J. S. d. R., de Andrade, N. J., Cruz, R. S., and Medeiros, E. A. A. (2012). Zinc oxide nanoparticles: synthesis, antimicrobial activity and food packaging applications. *Food and Bioprocess Technology* **5**, 1447-1464.

Espitia, P. J. P., Soares, N. d. F. F., Teófilo, R. F., Vitor, D. M., Coimbra, J. S. d. R., de Andrade, N. J., de Sousa, F. B., Sinisterra, R. D., and Medeiros, E. A. A. (2013). Optimized dispersion of ZnO nanoparticles and antimicrobial activity against foodborne pathogens and spoilage microorganisms. *Journal of Nanoparticle Research* **15**, 1-16.

Freeman, S., Katan, T., and Shabi, E. (1996). Characterization of Colletotrichum gloeosporioides isolates from avocado and almond fruits with molecular and pathogenicity tests. *Applied and environmental microbiology* **62**, 1014-1020.

Ghaffari-Moghaddam, M., Hadi-Dabanlou, R., Khajeh, M., Rakhshanipour, M., and Shameli, K. (2014). Green synthesis of silver nanoparticles using plant extracts. *Korean Journal of Chemical Engineering* **31**, 548-557.

González-Merino, A. M., Hernández-Juárez, A., Betancourt-Galindo, R., Ochoa-Fuentes, Y. M., Valdez-Aguilar, L. A., and Limón-Corona, M. L. (2021). Antifungal activity of zinc oxide nanoparticles in *Fusarium oxysporum-Solanum lycopersicum* pathosystem under controlled conditions. *Journal of Phytopathology* **169**, 533-544.

Hassan, O., Jeon, J. Y., Chang, T., Shin, J. S., Oh, N. K., and Lee, Y. S. (2018). Molecular and morphological characterization of *Colletotrichum* species in the *Colletotrichum gloeosporioides* complex associated with persimmon anthracnose in South Korea. *Plant Disease* **102**, 1015-1024.

Hassan, S. A., Mujahid, H., Ali, M. M., Irshad, S., Naseer, R., Saeed, S., Firyal, S., and Arooj, F. (2021). Synthesis, characterization and protective effect of green tea-mediated zinc oxide nanoparticles against ochratoxin A induced hepatotoxicity and nephrotoxicity in albino rats. *Applied Nanoscience* **11**, 2281-2289.

He, L., Liu, Y., Mustapha, A., and Lin, M. (2011). Antifungal activity of zinc oxide nanoparticles against *Botrytis cinerea* and *Penicillium expansum*. *Microbiological Research* **166**, 207-215.

Holmes, J. D., Lyons, D. M., and Ziegler, K. J. (2003). Supercritical fluid synthesis of metal and semiconductor nanomaterials. *Chemistry–A European Journal* **9**, 2144-2150.

Hossain, M. M., Mondal, M., Hossain, M. P., Uddin, M. S., Uddin, N., Hasan, M. M., Hossain, M. S., Mahmudul Islam, A., and Rana, M. S. (2015). *In vitro* antioxidant, brine shrimp lethality and antimicrobial activities of methanol and petroleum ether extracts of *Cassia renigera* fruit. *Journal of Food Measurement and Characterization* **9**, 68-75.

Ihsan, M., Din, I. U., Alam, K., Munir, I., Mohamed, H. I., and Khan, F. (2023). Green fabrication, characterization of zinc oxide nanoparticles using plant extract of *momordica charantia* and *Curcuma zedoaria* and their antibacterial and antioxidant activities. *Applied Biochemistry and Biotechnology* **195**, 3546-3565.

Iliger, K. S., Sofi, T. A., Bhat, N. A., Ahanger, F. A., Sekhar, J. C., Elhendi, A. Z., Al-Huqail, A. A., and Khan, F. (2021). Copper nanoparticles: Green synthesis and managing fruit rot disease of chilli caused by *Colletotrichum capsici*. *Saudi Journal of Biological Sciences* **28**, 1477-1486.

Jain, D., Shivani, Bhojiya, A. A., Singh, H., Daima, H. K., Singh, M., Mohanty, S. R., Stephen, B. J., and Singh, A. (2020). Microbial fabrication of zinc oxide nanoparticles and evaluation of their antimicrobial and photocatalytic properties. *Frontiers in chemistry* **8**, 778.

Jay Chithra, M., Sathya, M., and Pushpanathan, K. (2015). Effect of pH on crystal size and photoluminescence property of ZnO nanoparticles prepared by chemical precipitation method. *Acta Metallurgica Sinica (English Letters)* **28**, 394-404.

Jiang, J., Pi, J., and Cai, J. (2018). The advancing of zinc oxide nanoparticles for biomedical applications. *Bioinorganic Chemistry and Applications* **2018**.

Jobe, M. C., Mthiyane, D. M., Mwanza, M., and Onwudiwe, D. C. (2022). Biosynthesis of zinc oxide and silver/zinc oxide nanoparticles from Urginea epigea for antibacterial and antioxidant applications. *Heliyon* **8**.

Karam, S. T., and Abdulrahman, A. F. (2022). Green synthesis and characterization of ZnO nanoparticles by using thyme plant leaf extract. *In* "Photonics", Vol. 9, pp. 594. MDPI.

Kubheka, S. F., Tesfay, S. Z., Mditshwa, A., and Magwaza, L. S. (2020). Evaluating the efficacy of edible coatings incorporated with moringa leaf extract on postharvest of 'Maluma'avocado fruit quality and its biofungicidal effect. *HortScience* **55**, 410-415.

Kumar, B., Smita, K., Cumbal, L., and Debut, A. (2017). Green synthesis of silver nanoparticles using Andean blackberry fruit extract. *Saudi Journal of Biological Sciences* **24**, 45-50.

Kumar, V., Pandey, N., Mohan, N., and Singh, R. P. (2012). Antibacterial & antioxidant activity of different extract of *Moringa oleifera* Leaves–an *in vitro* study. *International Journal of Pharmaceutical Sciences Review and Research* **12**, 89-94.

la Rosa-García, D., Susana, C., Martínez-Torres, P., Gómez-Cornelio, S., Corral-Aguado, M. A., Quintana, P., and Gómez-Ortíz, N. M. (2018). Antifungal activity of ZnO and MgO nanomaterials and their mixtures against *Colletotrichum gloeosporioides* strains from tropical fruit. *Journal of Nanomaterials* **2018**, 1-9.

Le, K. H., Nguyen, M. D.-B., Dai Tran, L., Thi, H. P. N., Van Tran, C., Van Tran, K., Thi, H. P. N., Thi, N. D., Yoon, Y. S., and Nguyen, D. D. (2021). A novel antimicrobial ZnO nanoparticles-added polysaccharide edible coating for the preservation of postharvest avocado under ambient conditions. *Progress in Organic Coatings* **158**, 106339.

Lin, Q., Huang, Y., Li, G., Luo, Z., Wang, L., Li, D., Xiang, Y., Liu, L., Ban, Z., and Li, L. (2023). The journey of prochloraz pesticide in *Citrus sinensis*: Residual distribution, impact on transcriptomic profiling and reduction by plasma-activated water. *Journal of Hazardous Materials* **448**, 130931.

Lipovsky, A., Nitzan, Y., Gedanken, A., and Lubart, R. (2011). Antifungal activity of ZnO nanoparticles—the role of ROS mediated cell injury. *Nanotechnology* **22**, 105101.

Mahmoud, T. S. M., Shaaban, F. K. M., and El-Hadidy, G. A. E.-M. (2020). Enhancement of antioxidant and storability of Hollywood plum cultivar by preharvest treatments with moringa leaf extract and some nutrients. *Bulletin of the National Research Centre* **44**, 1-11.

Mohammed, F., Tesfay, S., Modi, A., Mabhaudhi, T., and Chimonyo, V. (2019). Distribution of antioxidants in different parts of Moringa oleifera seedlings. *In* "II International Symposium on Moringa 1306", pp. 157-162.

Mthana, M. S., Mthiyane, D. M., Onwudiwe, D. C., and Singh, M. (2022). Biosynthesis of ZnO nanoparticles using capsicum Chinense fruit extract and their *in vitro* cytotoxicity and antioxidant assay. *Applied Sciences* **12**, 4451.

Mushtaq, W., Ishtiaq, M., Maqbool, M., Mazhar, M. W., Casini, R., Abd-ElGawad, A. M., and Elansary, H. O. (2023). Green synthesis of zinc oxide nanoparticles using viscum album extracts: unveiling bioactive compounds, antibacterial potential, and antioxidant activities. *Plants* **12**, 2130.

Nasir, M., Khan, A., Basra, S., and Malik, A. (2020). Improvement in growth, productivity and quality of 'Kinnow'mandarin fruit after exogenous application of *Moringa olifera* leaf extract. *South African Journal of Botany* **129**, 263-271.

Ndhlala, A. R., Mulaudzi, R., Ncube, B., Abdelgadir, H. A., Du Plooy, C. P., and Van Staden, J. (2014). Antioxidant, antimicrobial and phytochemical variations in thirteen *Moringa oleifera* Lam. cultivars. *Molecules* **19**, 10480-10494.

Ningsih, I. Y., Zulaikhah, S., Hidayat, M. A., and Kuswandi, B. (2016). Antioxidant activity of various Kenitu (*Chrysophyllum cainito* L.) leaves extracts from Jember, Indonesia. *Agriculture and Agricultural Science Procedia* **9**, 378-385.

Niraimathee, V., Subha, V., Ravindran, R. E., and Renganathan, S. (2016). Green synthesis of iron oxide nanoparticles from Mimosa pudica root extract. *International Journal of Environment and Sustainable Development* **15**, 227-240.

Obianom, C., Romanazzi, G., and Sivakumar, D. (2019). Effects of chitosan treatment on avocado postharvest diseases and expression of phenylalanine ammonia-lyase, chitinase and lipoxygenase genes. *Postharvest Biology and Technology* **147**, 214-221.

Ochieng, P., Iwuoha, E., Michira, I., Masikini, M., Ondiek, J., Githira, P., and Kamau, G. (2015). Green route synthesis and characterization of ZnO nanoparticles using *Spathodea campanulata*. *International Journal of BioChemiPhysics* **23**, e61.

Pariona, N., Paraguay-Delgado, F., Basurto-Cereceda, S., Morales-Mendoza, J., Hermida-Montero, L., and Mtz-Enriquez, A. I. (2020). Shape-dependent antifungal activity of ZnO particles against phytopathogenic fungi. *Applied Nanoscience* **10**, 435-443.

Ramesh, M., Anbuvannan, M., and Viruthagiri, G. (2015). Green synthesis of ZnO nanoparticles using *Solanum nigrum* leaf extract and their antibacterial activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **136**, 864-870.

Rane, A. V., Kanny, K., Abitha, V., and Thomas, S. (2018). Methods for synthesis of nanoparticles and fabrication of nanocomposites. *In* "Synthesis of inorganic nanomaterials", pp. 121-139. Elsevier.

Safawo, T., Sandeep, B., Pola, S., and Tadesse, A. (2018). Synthesis and characterization of zinc oxide nanoparticles using tuber extract of anchote (*Coccinia abyssinica* (Lam.) Cong.) for antimicrobial and antioxidant activity assessment. *OpenNano* **3**, 56-63.

Santhoshkumar, J., Kumar, S. V., and Rajeshkumar, S. (2017). Synthesis of zinc oxide nanoparticles using plant leaf extract against urinary tract infection pathogen. *Resource-Efficient Technologies* **3**, 459-465.

Sharifan, H., Noori, A., Bagheri, M., and Moore, J. M. (2021). Postharvest spraying of zinc oxide nanoparticles enhances shelf life qualities and zinc concentration of tomato fruits. *Crop and Pasture Science* **73**, 1-2.

Sharma, S., Kumar, K., Thakur, N., Chauhan, S., and Chauhan, M. (2020). The effect of shape and size of ZnO nanoparticles on their antimicrobial and photocatalytic activities: a green approach. *Bulletin of Materials Science* **43**, 1-10.

Shimshoni, J. A., Bommuraj, V., Chen, Y., Sperling, R., Barel, S., Feygenberg, O., Maurer, D., and Alkan, N. (2020). Postharvest fungicide for avocado fruits: antifungal efficacy and peel to pulp distribution kinetics. *Foods* **9**, 124.

Shinde, S. S. (2015). Antimicrobial activity of ZnO nanoparticles against pathogenic bacteria and fungi. Journal of *Science of Medicine Nanotechnology and Nanomedicine* **3**, 1033. JSM Nanotechnology & Nanomedicine

Sivakumar, D., Tuna Gunes, N., and Romanazzi, G. (2021). A comprehensive review on the impact of edible coatings, essential oils, and their nano formulations on postharvest decay anthracnose of avocados, mangoes, and papayas. *Frontiers in Microbiology* **12**, 711092.

Suryavanshi, P., Pandit, R., Gade, A., Derita, M., Zachino, S., and Rai, M. (2017). *Colletotrichum* sp.-mediated synthesis of sulphur and aluminium oxide nanoparticles and its in vitro activity against selected food-borne pathogens. *LWT-Food Science and Technology* **81**, 188-194.

Tesfay, S., Bertling, I., Odindo, A., Workneh, T. S., and Mathaba, N. (2011). Levels of anti-oxidants in different parts of moringa (*Moringa oleifera*) seedling. *African Journal of Agricultural Research* **6**, 5123-5132.

Thema, F., Manikandan, E., Dhlamini, M., and Maaza, M. (2015). Green synthesis of ZnO nanoparticles via *Agathosma betulina* natural extract. *Materials Letters* **161**, 124-127.

Tshabalala, T., Ndhlala, A., Ncube, B., Abdelgadir, H., and Van Staden, J. (2020). Potential substitution of the root with the leaf in the use of *Moringa oleifera* for antimicrobial, antidiabetic and antioxidant properties. *South African Journal of Botany* **129**, 106-112.

Wang, Y., Chen, G., Wang, D., Zhang, J., You, C., Wang, X., and Liu, H. (2023). Post-harvest application of nanoparticles of titanium dioxide (NPs-TiO_2) and ethylene to improve the coloration of detached apple fruit. *Foods* **12**, 3137.

Xu, J., Huang, Y., Zhu, S., Abbes, N., Jing, X., and Zhang, L. (2021). A review of the green synthesis of ZnO nanoparticles using plant extracts and their prospects for application in antibacterial textiles. *Journal of Engineered Fibers and Fabrics* **16**, 15589250211046242.

Xu, X., Chen, J., Li, B., and Tang, L. (2018). Carbendazim residues in vegetables in China between 2014 and 2016 and a chronic carbendazim exposure risk assessment. *Food Control* **91**, 20-25.

Yilma, T., Kassaw, M., Murthy, H., and Dekebo, A. (2023). ZnO Nanoparticles synthesized using aerial extract of *Ranunculus multifidus* plant: antibacterial and antioxidant activity. *Journal of Nanomaterials* **2023**, 1-10.

Zhu, W., Hu, C., Ren, Y., Lu, Y., Song, Y., Ji, Y., Han, C., and He, J. (2021). Green synthesis of zinc oxide nanoparticles using *Cinnamomum camphora* (L.) Presl leaf extracts and its antifungal activity. *Journal of Environmental Chemical Engineering* **9**, 106659.

Chapter 5

GENERAL DISCUSSION

Postharvest technologies significantly minimize food postharvest losses by maintaining the quality and extending the shelf life of fresh produce. Avocado is one of the most economically important fruits that is highly perishable and susceptible to several postharvest diseases. This is due to the climacteric nature of this fruit, which is associated with increased ethylene production during storage, fast-forwarding the ripening and senescence processes. Postharvest technologies, therefore, seek to reduce the rate of metabolic processes associated with fruit ripening and protect the fruit from microbial and physiological disorders (Mahajan et al., 2014).

This research study first reviewed the literature on the currently used postharvest treatments and the potential of emerging technologies, particularly nanotechnology, on climacteric fruit postharvest quality. Besides the basic technology of temperature conditioning, various techniques were reviewed, which include the use of physical (edible coatings, heat, and irradiation), chemical (fungicides, ethylene inhibitors, antioxidants, and anti-browning agents), and gaseous treatments (low oxygen storages and controlled atmosphere) (Mahajan et al., 2014; Usall et al., 2016; Barman et al., 2018). In addition, far-ultraviolet radiation (UV-C) and nanotechnology seem to be promising technologies due to their unique properties.

The first experiment was designed to evaluate the effect of composite edible coating of carboxymethyl cellulose (CMC) and moringa leaf extract (MLE) on the postharvest quality of "Hass" avocado fruit harvested at different maturity stages. The second experiment was conducted to synthesize zinc oxide (ZnO) nanoparticles (NPs) (ZnO NPs) using MLE and evaluate its antifungal effect against *Colletotrichum gloeosporioides* isolated from "Hass" avocado fruit.

5.1 The Effect of Composite Edible Coating: Carboxymethyl Cellulose and Moringa Leaf Extract on the Postharvest Quality of 'Hass' Avocado Fruit treated at Different Harvest Maturity Stages.

In this study, two concentrations of moringa (8 and 16 %) were extracted using chilled (100%) ethanol and non-chilled ethanol (50 %). The prepared extracts were mixed with CMC and used to treat "Hass' avocado, harvested at different maturities based on their dry matter (DM). The treatments significantly affected the change in fruit firmness over the 28 days of cold storage at 5.5 ℃ and 90 % RH and seven days of shelf life at 23 ℃. The firmness loss was found to be delayed on treated fruit compared to untreated. The fruit mass loss was also found to be reduced by all the treatments; however, CMC and 16 % chilled MLE were the most effective. This indicated that the CMC and MLE treatments were able to inhibit the transfer of water between the fruit and the environment, thereby reducing both mass and firmness loss as they are correlated.

The ripening process is accompanied by the colour change from green to purple or black in avocado fruit, mainly the "Hass" cultivar. This is the first characteristic that consumers use to judge the quality of fruit and decide whether to purchase the produce. As expected, all the fruit showed colour change; however, the treated fruit tended to have a delayed overall colour change. This was associated with the potential of MLE and CMC composite coating to delay the change of chloroplast into chromoplasts, which is the one containing the yellow and red pigments (Sharma et al., 2019). Furthermore, the total antioxidants of fruit were found to be not affected by the treatment. This indicated that CMC and MLE are the best treatments to extend the shelf life of avocado without compromising their compounds of interest, such as phenolics and flavonoids.

The concentration of C7 sugars, mannoheptulose and perseitol, decreased throughout the storage period. However, the reduction was more pronounced in untreated fruit. As the avocado fruit ripens, there is an accumulation of carbohydrates, and C7 sugars have a significant contribution to the total concentration of carbohydrates. The C7 sugars were also reported to have a major role in the ripening process of avocado, in such a manner that its concentration must drop below a threshold (20 mg/g DW) for the fruit ethylene production and respiration to occur (Liu et al., 2012). This means that the MLE and CMC treatment was able to delay the avocado physiological processes that trigger the decline of C7 sugar and, therefore, delay the ripening of the treated fruit.

5.2 Green Synthesis of Zinc Oxide Nanoparticles Using *Moringa Oleifera* Leaf Extract and its Antifungal Effect Against *Colletotrichum gloeosporioides* in Avocado

This study successfully synthesized ZnO NPs with a uniform spherical shape using zinc acetate dihydrate as the source of zinc ions and MLE as the stabilizing and reducing agent. Further evaluations revealed that the overall antioxidants of the synthesized ZnO NPs were not compromised during the synthesis. The antioxidants, estimated following the DPPH method, were found to be concentration dependent. Thus, increasing the ZnO NPs resulted in an increase in antioxidants. This proves moringa to be rich in antioxidants since resulting NPs characteristics are influenced by the plant specie used. The characteristics of the resulting NPs also depend on the pH, calcination time, reaction concentration, and extract concentration.

The *in vitro* study showed the synthesized ZnO NPs to be very effective in suppressing the mycelial growth of *Colletotrichum gloeosporioides* isolated from avocado. Different ZnO concentrations (0.25, 0.5, 1, 2 % v/w) significantly affected the growth of this pathogen in Petrie dishes during the 9-day storage period at 25 ℃. However, 1 % of ZnO NPs was found to be the most effective one. This shows great potential for greenly synthesized NPs to be used in packhouses as a substitute for the hazardous chemicals that are currently used.

5.3 CONCLUSION AND FUTURE OUTLOOKS

Based on the results of this study, moringa-based treatments can be recommended for use to preserve the postharvest quality of avocado fruit. This study demonstrated the great potential of moringa-based edible coating and moringa-based ZnO NPs in preserving the avocado quality and suppressing the mycelial growth of *Colletotrichum gloeosporioides*, respectively. This study only tested the efficacy of MLE combined with CMC as an edible coating and disjointly evaluated moringa potential in optimizing the green synthesis of nanoparticles, especially ZnO NPs. Future studies must, therefore, evaluate the effect of combining the ZnO NPs with edible coating on the quality of avocado and other fruits. Different metals or metal oxides may also be synthesized in the future using moringa. In addition, only the *in vitro* study was conducted; further, *in vivo* studies are still required to verify the antifungal effectiveness

of ZnO NPs synthesized using moringa. However, due to the sizes of NPs, they can easily penetrate the fruit; therefore, the concentration of NPs present in the edible portion of the fruit must be determined. This will help to align the zinc concentration with that recommended for human consumption, thereby not compromising the consumers' health.

5.4 References

Barman, K., Sharma, S., and Siddiqui, M. W. (2018). "Emerging postharvest treatment of fruits and vegetables," CRC Press.

Liu, J., Sui, Y., Wisniewski, M., Droby, S., Tian, S., Norelli, J., and Hershkovitz, V. (2012). Effect of heat treatment on inhibition of *Monilinia fructicola* and induction of disease resistance in peach fruit. *Postharvest Biology and Technology* **65**, 61-68.

Mahajan, P. V., Caleb, O. J., Singh, Z., Watkins, C. B., and Geyer, M. (2014). Postharvest treatments of fresh produce. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* **372**, 20130309.

Sharma, L., Saini, C. S., Sharma, H. K., and Sandhu, K. S. (2019). Biocomposite edible coatings based on cross linked-sesame protein and mango puree for the shelf life stability of fresh-cut mango fruit. *Journal of Food Process Engineering* **42**, e12938.

Usall, J., Ippolito, A., Sisquella, M., and Neri, F. (2016). Physical treatments to control postharvest diseases of fresh fruits and vegetables. *Postharvest Biology and Technology* **122**, 30-40.