Assessing gaseous ozone and edible coatings as postharvest treatments for mango (*Mangifera indica* L.) fruit

By

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Declaration

I, Nonjabulo L. Bambalele, student no. 214582697, declare that the research reported in this thesis is my own work. The data sourced from other scholars, is clearly referenced. The data from this study has not been submitted to any institution for obtaining a degree.

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Summary

This research examines the potential of gaseous ozone and edible coatings in preserving postharvest quality and extending the shelf-life of 'Keitt' mango fruit. A critical review of the literature focused on the recent postharvest technologies used to preserve the quality of mango fruit. The prospects of using non-chemical postharvest treatments such as gaseous ozone and edible coatings were also reviewed. A screening study was conducted to determine the optimum ozone (O_3) application time for effectively maintaining fruit quality and extending shelf-life. Mango fruit were intermittently exposed to gaseous ozone for twelve, twenty-four, thirty-six, or forty-eight hours, and the control fruit were untreated. Fruit were stored at 10°C for twenty-one days and seven days shelf-life at ambient temperature. The findings showed that the O_3 treatment should be applied at the pre-climacteric stage to achieve optimum results. Ozone treatment for 24 or 36 hours effectively maintained firmness and carotenoids content, delayed color changes, decay incidence, and mass loss. Therefore, an ozone exposure time of 24 and 36 hours was adopted for the study. These exposure times were incorporated into edible coatings (moringa leaf extract and carboxymethyl cellulose) for further investigation.

The study on the effect of gaseous O_3 incorporated with edible coatings on sensory attributes and physicochemical parameters showed that EC and EC + O_3 (36 h) were more effective in delaying the ripening process and maintaining the postharvest quality. Overall, consumers preferred the fruit coated with EC due to its attractive color, smell, and sweetness. The study on the postharvest effect of gaseous O_3 and EC on antioxidants and the biochemical properties of mango fruit is discussed in Chapter Five. The findings of this study demonstrated that the treatment combination of EC and gaseous O_3 (36 h) effectively maintained antioxidants, membrane integrity and enhanced the quality of mango fruit during storage. The effect of gaseous O_3 and EC on postharvest diseases of mango fruit, specifically *Colletotrichum gloeosporioides* (anthracnose) and *Lasiodiplodia theobromae* (stem-end

rot), was also investigated. This study revealed that EC + O_3 (24 h) effectively controlled stem-end rot and anthracnose in mango fruit. The treatment combination of EC and O_3 (36 h) reduced the mycelial growth and disease incidence of *Lasiodiplodia theobromae* and *Colletotrichum gloeosporioides.*

The study of EC and O_3 in the volatile compounds of mango fruit is discussed in Chapter Seven. The fruit treated with EC had a high content of volatile compounds compared to other treatments. The treatment combination of EC + O_3 (24 h) was not effective in maintaining the volatile compounds of mango fruit during storage. The findings of the current study suggest that EC + O_3 (36 h) can be used as postharvest treatment of mango fruit. Additional research is required to gain more insights in understanding the EC+ O_3 mode of action in maintaining volatile compounds and controlling mango postharvest diseases.

List of publications and conference presentations

The chapters of this thesis were written using journal manuscript format because they are intended forpublication. Three research chapters have already been published.

Published articles

1. Bambalele, N.L., Mditshwa, A., Magwaza, L.S., Tesfay, S.Z. 2021. Recent advances on postharvest technologies of mango fruit: A review, International Journal of Fruit Science, 21:1, 565-586, doi:10.1080/15538362.2021.1918605.

2. Bambalele, N., Mditshwa, A., Tesfay, S.Z., Magwaza, L.S. 2019. Moringa leaf extract infused into carboxymethyl cellulose edible coating combined with gaseous ozone as postharvest treatments maintain quality and extend shelf life of mango (*Mangifera indica* L.) fruit. Acta Hortic, 1306, 225-232. doi: 10.17660/ActaHortic.2021.1306.28

3. Bambalele, N.L., Mditshwa, A., Magwaza, L.S., Tesfay, S.Z., 2021. The effect of gaseous ozone and moringa leaf–carboxymethyl cellulose edible coating on antioxidant activity and biochemical properties of 'Keitt' mango fruit. Coatings 11, 1406. https://doi.org/10.3390/coatings11111406.

Conference presentation

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maintain quality and extend shelf life of mango (*Mangifera indica* L.) fruit. International Symposium on Moringa. Pretoria, 11 November 2019. Oral presentation.

Preface

This thesis is an anthology of various manuscripts presented disjointedly. Each chapter represents an independent article, and reiteration between chapters was unavoidable.

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1   Introduction

The major postharvest problems faced by the South African mango industry are fruit softening and diseases, including anthracnose and stem-end rot (Donkin and Oosthuyse, 1996; Fivaz, 2006; Sivakumar et al., 2011). Fruit softening and postharvest diseases have a devastating effect on mango fruit quality, resulting in significant economic losses (DAFF, 2015). Fungicide treatment and low temperature storage are often utilized to reduce postharvest diseases and quality deterioration (Jabbar et al., 2011; Barman and Asrey, 2014). While fungicide application in fruit is essential, its negative impact on the environment and human health is alarming. Fungicides such as prochloraz are used to enhance quality and suppress microbial infections in mango fruit (Sivakumar et al., 2011). However, fungicide residues may end up in the final commodity and pose serious health hazards to the consumers (Swart and Broekhuizen, 2004; Mutengwe et al., 2016). As a result, recent research has focused on biodegradable, non-residual, and non-chemical postharvest treatments (Wei et al., 2007). The non-residual treatments include edible coatings, ozone, hot water, and UV-C.

Edible coatings are an environmentally friendly thin material layer that forms a modified atmosphere, thus decreasing interior oxygen and moisture loss (Ncama et al., 2018). These coatings are made from either proteins, polysaccharides, lipids, or resins (Khaliq et al., 2016b; Ncama et al., 2018). The efficacy of edible coatings such as chitosan, carboxymethyl cellulose (CMC), gum arabic, and *Aloe vera* has been studied as a postharvest treatment in fresh produce. The edible coatings have been evaluated in apple, tomato, avocado, mango, citrus, plum, and strawberry (Pandit et al., 2009; Shao et al., 2012; Ali et al., 2013; Bill et al., 2014; Chowdappa et al., 2014; Youssef et al., 2015; Tahir et al., 2018). The findings indicate that edible coatings preserved antioxidant activity, prolonged shelf-life,

decreased weight loss, respiration rate, while maintaining firmness and color in fruits. Furthermore, the edible coatings were effective as an antimicrobial agent in inhibiting the development of *Colletotrichum gloeosporioides* associated with anthracnose in avocado and mango as well as *Penicillium expansum* and *Botrytis cinerea* causing decay in apples (Shao et al., 2012; Bill et al., 2014; Chowdappa et al., 2014).

Moringa leaf extract (MLE) used as an edible coating can extend the shelf-life of fruit without leaving residues. For instance, Tesfay and Magwaza (2017) reported high firmness retention and reduced the respiration rate in avocado fruit coated with moringa ethanolic leaf extract before storage. The researchers concluded that fruit quality was enhanced through high C7 sugar content, low mesocarp electrical conductivity, polyphenol oxidase activity that maintained membrane integrity, decreasing browning, and extended shelf-life. Further studies by Kubheka et al. (2020) revealed that gum Arabic (15%) + moringa combined with CMC (1%) inhibited the mycelial growth of *Colletotrichum gloeosporioides*.

Over the past decade, research interests on ozone (O_3) as a postharvest treatment for horticultural crops have notably increased. The postharvest application of O_3 in fresh produce has been evaluated (Wei et al., 2007; Cayuela et al., 2010; Huyskens-Keil et al., 2012). Researchers concluded that O_3 could extend shelf-life and maintain postharvest quality by decreasing weight loss, delaying ripening, and inhibiting fungal diseases. Ozone blocks ethylene biosynthesis by reducing the 1-aminocyclopropane-1-carboxylic acid (ACC) levels in the fruit cell wall (Minas et al., 2014a). This is achieved by inhibiting the gene expression of 1-aminocyclopropane-1-carboxylase synthase (ACS) (Minas et al., 2014a). Ozone is an unstable compound that decomposes to form hydroxyl, superoxide, and hydroperoxyl, leaving no chemical residues (Brodowska et al., 2018b; Simpson and Mitch, 2020).

Ozone has oxidizing potential and antimicrobial properties against a broad spectrum of bacteria, viruses, and fungi (Contigiani et al., 2018; De Santis et al., 2021).

Mangoes continue to experience high postharvest losses and lack an eco-friendly postharvest treatment. The potential of O_3 as a postharvest treatment presents an excellent opportunity for the mango industry to adopt a non-chemical and an environmentally friendly technology. There is no literature discussing the effect of using edible coating like moringa under ozonized storage conditions. Moreover, there is no scientific data about the treatment combination of moringa leaf extract with commercial edible coatings and O_3 during cold storage of mangoes in South Africa. Thus, there is a need to evaluate these treatment combinations as this could provide a potential solution to reduce mango postharvest and economic losses.

2   Research Rationale

Mango (*Mangifera indica* L.) is one of the most nutritional fruit containing intrinsic compounds such as carbohydrates, amino acids, potassium, proteins, calcium, carotenoids, and vitamins (Aziz et al., 2012). Mango has a strong aroma and flavor, which makes it attractive to consumers. Aroma and flavor are essential characteristics that influence the fruit's quality, price, and consumer acceptability. The interaction between volatile compounds, sugars, and acids creates mango flavor (Malundo et al., 1996). The significant volatile compounds of mango include terpenes, ketones, and aldehydes (Laohaprasit et al., 2011). The compounds limonene, (E)-β-ionone, (E)-β-damascenone, ethyl butanotae, δ-3-carene, and α-copaene are responsible for aroma and flavor in mango (Pino, 2012; Bonneau et al., 2016). The main cultivars grown in South Africa include Keitt, Heidi, Tommy Atkins, Sensation, Kent, and Zill. The mango industry produces approximately 84 000 tons annually (NAMC, 2020).

This contributes 6.5 % to the subtropical fruit's gross production value in South Africa (DAFF, 2015). While mango production has significantly increased over the years, the limited shelf-life and high postharvest losses restrict the potential to contribute to economic growth. Mango is a climacteric fruit, and it is characterized by postharvest biochemical and physiological modifications resulting in a short shelf- life compared to other fruits. Such biochemical changes include a burst in ethylene production accompanied by increased respiration rate and modification of enzyme activities, leading to fruit softening, change in chemical composition, and antioxidants (Zaharah et al., 2012; Hossain et al., 2014). Fruit quality is vital as consumers purchase fruits based on appearance and freshness (Yun et al., 2012). The fruit is of high quality when it is disease-free, can withstand long shipment distances, and has a good appearance. Therefore, fruit quality must be maintained throughout the shelf life.

3   Aim

This research examines the potential of gaseous ozone and edible coatings in preserving postharvest quality and extending the shelf-life of 'Keitt' mango fruit. The research will also investigate the possible mechanisms of action employed by these treatments to retain fruit quality.

4   Objectives

The specific objectives of this study were to:

1. Evaluate the influence of gaseous ozone on physicochemical quality attributes of mango fruit (cv. Keitt) during cold storage.

2. Assess the efficacy of CMC+MLE incorporated with ozone to maintain mango fruit quality, delay ripening, and extend shelf-life.

3. Investigate the effect of gaseous O_3 and moringa leaf extract–CMC based edible coating on the

antioxidant activities and biochemical properties of 'Keitt' mango fruit.

4. Investigate the antifungal activity of edible coating (moringa leaf extract and carboxymethyl cellulose) and O_3 on the growth of *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae* in mango fruit.

5. Analyse changes in mango volatile compounds as affected by edible coatings (MLE and CMC) and ozone treatment during cold storage and fruit ripening.

5 References

Ali, A., Maqbool, M., Alderson, P.G., Zahid, N. 2013. Effect of gum arabic as an edible coating on antioxidant capacity of tomato (*Solanum lycopersicum* L.) fruit during storage. Postharvest Biol. Technol. 76, 119-124.

Barman, K., Asrey, R. 2014. Salicylic acid pre-treatment alleviates chilling injury, preserves bioactive compounds and enhances shelf life of mango fruit during cold storage. J. Sci. Ind. Res. 73, 713-718

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

Bonneau, A., Boulanger, R., Lebrun, M., Maraval, I., Gunata, Z. 2016. Aroma compounds in fresh and dried mango fruit (*Mangifera indica* L. cv. Kent): impact of drying on volatile composition. Int.l J. Food Sci. Technol.51, 789-800.

Brodowska, A.J., Nowak, A., Śmigielski, K. 2018. Ozone in the food industry: Principles of ozone treatment, mechanisms of action, and applications: An overview. Crit. Rev. Food Sci. Nutr. 58, 2176-2201.

Cayuela, J.A., Vázquez, A., Pérez, A.G., García, J.M. 2010. Control of table grapes postharvest decay by ozone treatment and resveratrol induction. Food Sci. Technol. Int. 15, 495-502.

Chowdappa, P., Gowda, S., Chethana, C., Madhura, S. 2014. Antifungal activity of chitosan-silver nanoparticle composite against *Colletotrichum gloeosporioides* associated with mango anthracnose. Afr. J. Microbiol. Res. 8, 1803-1812.

Contigiani, E.V., Jaramillo-Sánchez, G., Castro, M.A., Gómez, P.L., Alzamora, S.M. 2018. Postharvest quality of strawberry fruit (*Fragaria x Ananassa* Duch cv. Albion) as affected by ozone washing: fungal spoilage, mechanical properties, and structure. Food Bioproc. Tech. 11, 1639-1650.

De Santis, D., Garzoli, S., Vettraino, A.M. 2021. Effect of gaseous ozone treatment on the aroma and clove rot by *Fusarium proliferatum* during garlic postharvest storage. Heliyon 7, e06634.

Donkin, D., Oosthuyse, S. 1996. Quality evaluations of sea-exported South African mangoes in Europe during the 1995/96 season. South African Mango Growers' Association Yearbook (South Africa).

Fivaz, J. 2006. Mango production in South Africa as compared to the rest of the world, VIII International Mango Symposium 820, 29-46.

Hossain, M., Rana, M., Kimura, Y., Roslan, H.A. 2014. Changes in biochemical characteristics and activities of ripening associated enzymes in mango fruit during the storage at different temperatures. BioMed Res. Int. Article ID 232969. http://dx.doi.org/10.1155/2014/232969

Huyskens-Keil, S., Hassenberg, K., Herppich, W. 2012. Impact of postharvest UV-C and ozone treatment on textural properties of white asparagus (*Asparagus officinalis* L.). J. Appl. Bot. Food Qual. 84, 229.

Jabbar, A., Malik, A., Islam-UD-Din, Anwar, R., Ayub, M., Rajwana, I., Amin, M., Khan, A., Saeed, M. 2011. Effect of combined application of fungicides and hot water quarantine treatment on postharvest diseases and quality of mango fruit. Pak. J. Bot. 43, 65-73.

Khaliq, G., Muda Mohamed, M.T., Ghazali, H.M., Ding, P., Ali, A. 2016. Influence of gum arabic coating enriched with calcium chloride on physiological, biochemical and quality responses of mango (*Mangifera indica* L.) fruit stored under low temperature stress. Postharvest Biol. Technol. 111, 362-369.

Kubheka, S.F., Tesfay, S.Z., Mditshwa, A., 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. HortSci. 55, 410-415.

Laohaprasit, N., Ambadipudi, D., Srzednicki, G. 2011. Optimisation of extraction conditions of volatile compounds in'Nam Dok Mai'mangoes. Int. Food Res. J.18 (3): 1043-1049.

Malundo, T., Baldwill, E., Ware, G., Shewfelt, R. 1996. Volatile composition and interaction influence flavor properties of mango (*Mangifera indica* L.). Proceedings-Florida State Horticultural Society, 109, 264-268.

Minas, I.S., Vicente, A.R., Dhanapal, A.P., Manganaris, G.A., Goulas, V., Vasilakakis, M., Crisosto, C.H., Molassiotis, A. 2014. Ozone-induced kiwifruit ripening delay is mediated by ethylene biosynthesis inhibition and cell wall dismantling regulation. Plant Sci. 229, 76-85.

Mutengwe, M., Chidamba, L., Korsten, L. 2016. Pesticide residue monitoring on South African fresh produce exported over a 6-year period. J. Food Prot. 79, 1759-1766.

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

Pandit, S.S., Kulkarni, R.S., Chidley, H.G., Giri, A.P., Pujari, K.H., Köllner, T.G., Degenhardt, J., Gershenzon, J., Gupta, V.S. 2009. Changes in volatile composition during fruit development and ripening of 'Alphonso'mango. J. Sci. Food Agric. 89, 2071-2081.

Pino, J.A. 2012. Odour-active compounds in mango (*Mangifera indica* L. cv. Corazón). International J. Food Sci. Technol. 47, 1944-1950.

Shao, X., Tu, K., Tu, S., Tu, J. 2012. A combination of heat treatment and chitosan coating delays ripening and reduces decay in "Gala" apple fruit. J. Food Qual. 35, 83-92.

Simpson, A.M.-A., Mitch, W.A. 2020. Chlorine and ozone disinfection and disinfection by products inpostharvest food processing facilities: A review. Crit. Rev. Environ. Sci. Technol. 1-43.

Sivakumar, D., Jiang, Y., Yahia, E.M. 2011. Maintaining mango (*Mangifera indica* L.) fruit quality during the export chain. Food Res. Int. 44, 1254-1263.

Swart, S., Broekhuizen, W.v. 2004. Evaluation of post-harvest disease control with regards to the new maximum residue level of five ppm for prochloraz on mangoes in the European Union. South African Mango Growers' Association 24, 73-77.

Tahir, H.E., Xiaobo, Z., Jiyong, S., Mahunu, G.K., Zhai, X., Mariod, A.A. 2018. Quality and postharvest shelf life of cold-stored strawberry fruit as affected by gum arabic (*Acacia senegal*) edible coating. J. Food Biochem. 42, e12527.

Tesfay, S.Z., 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 Packag. Shelf Life 11, 40-48.

Wei, K., Zhou, H., Zhou, T., Gong, J. 2007. Comparison of aqueous ozone and chlorine as sanitizers in the food processing industry: impact on fresh agricultural produce quality. Ozone Sci. Eng. 29, 113-120.

Youssef, A.R., Ali, E.A., Emam, H.E. 2015. Influence of postharvest applications of some edible coatings on storage life and quality attributes of Navel orange fruit during cold storage. Int. J. Chem. Res. 8, 2189-2200.

Yun, Z., Jin, S., Ding, Y., Wang, Z., Gao, H., Pan, Z., Xu, J., Cheng, Y., Deng, X. 2012. Comparative transcriptomics and proteomics analysis of citrus fruit, to improve understanding of the effect of low temperature on maintaining fruit quality during lengthy post-harvest storage. J. Experimental Bot. 63, 2873-2893.

Zaharah, S.S., Singh, Z., Symons, G.M., Reid, J.B. 2012. Role of brassinosteroids, ethylene, abscisic acid, and indole-3-acetic acid in mango fruit ripening. J. Plant Growth Regul. 31, 363-372.

Chapter 2

Recent advances on postharvest technologies of mango fruit: A review

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Abstract

Mango is the third most important fruit in the tropics due to its nutritional properties and delicious flavor. The fruit is exceptionally perishable due to its climacteric nature, which decreases the quality and shelf-life. Preserving fruit quality and preventing losses during postharvest is one of the critical solutions in sustaining human dietary demands. Postharvest treatments such as 1-methylcyclopropene, edible coatings, and hot water treatment have shown to be effective in preserving fruit quality. However, developing environmental-friendly postharvest technologies that ensure the safety of consumers remains a challenge. Gaseous ozone, controlled atmosphere (CA), and pulsed electric field (PEF) are some of the emerging technologies with great potential for the mango fruit industry. The use of such technologies has been demonstrated to be effective in maintaining the sensory, nutritional, and physicochemical quality of the mango fruit. However, the mode of action of the emerging technologies is not yet understood. This review provides an overview of various postharvest techniques used to

preserve mango fruit quality. The potential of the emerging postharvest technologies to maintain mango fruit quality during storage and shelf-life is also discussed.

Keywords: Chemical treatments, ozone, carboxymethyl cellulose, shelf-life, fruit quality

1 Introduction

Mango (*Mangifera indica* L.) is one of the most nutritional and commonly consumed fruit in tropical and subtropical agroclimatic regions (Aziz et al., 2012). As of 2017, global mango production was 47 133 thousand tonnes (Altendorf, 2017), with Asia leading the world mango production, followed by India and Africa (Altendorf, 2017). Mango fruit is a good source of polyphenols, ascorbic acid, carotenoids, vitamins, and carbohydrates (Singh et al., 2013). The nutritional properties of mango, especially antioxidants, are essential for human health as they are known to boost the immune system and also prevent cardiovascular diseases, cataracts, and various types of cancer (Sivakumar et al., 2011; Muhammad et al., 2014). The fruit is susceptible to various postharvest diseases such as anthracnose and physiological disorders, including chilling injury, spongy tissue, and lenticel spot. Unfortunately, these individual problems or their combination may result in postharvest losses as well as the loss of revenue for the producers and everyone involved in the postharvest value chain. A significant proportion of losses of mango occur during storage and transportation as a result of poor handling and improper facilities (Sivakumar et al., 2011).

Postharvest technologies such as chemical and non-chemical treatments are used to maintain fruit quality during storage. For instance, 1-methylcyclopropene (1-MCP) and nitric oxide (NO) have been demonstrated to be effective chemical treatments for preserving mango fruit quality (Faasema et al., 2012; Tran et al., 2015). Postharvest 1-MCP treatments inhibit ethylene biosynthesis, which retards the respiration rate, retain firmness and delay fruit ripening (Wang et al., 2009; Hong et al., 2014;

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Razzaq et al., 2015). Nitric oxide, as a postharvest treatment, is known for prolonging the shelf-life by reducing the incidence of postharvest pathogens and chilling injury (Barman et al., 2014). The use of these treatments has shown to be a promising strategy to enhance fruit quality during storage and postharvest handling chain. However, there are growing concerns regarding the postharvest application of chemical treatments mainly because they do not only harm the environment but also pose various risks to human health. As a result, the focus of postharvest research for mangoes has recently shifted towards environmental-friendly and non-chemical treatments.

Edible coatings are biodegradable postharvest treatments applied to fruit and vegetables. They form a thin layer of material over the fruit surface, creating a protective barrier to oxygen, solute movement of food, and moisture (Baldwin et al., 1995; Bourtoom, 2008). The advantage of edible coatings is that they are natural, contain antioxidants and sometimes vitamins which are beneficial to the consumers. They also possess anti-browning and anti-microbial properties, which maintain fruit quality (Ducamp-Collin et al., 2009; Gurjar et al., 2018). Natural edible coatings such as chitosan, Gum Arabic, and carboxymethyl cellulose (CMC) can control postharvest disorders and diseases such as anthracnose, stem-end rot, and black spot in mango (Zhu et al., 2008; Gava et al., 2018).

Heat treatment is another non-chemical technique that has proved to be effective in decreasing postharvest diseases, fruit softening, and maintaining mango fruit color (Le et al., 2010, Luria et al., 2014; Wang et al., 2016; Dautt-Castro et al., 2018). Cell wall degrading enzymes, such as β-galactosidase and polygalacturonase (PG) are reportedly inhibited by heat treatments (Dautt-Castro et al., 2018). However, the response of mango fruit to heat treatment significantly depends on various factors including cultivar, temperature, and exposure time. For certain cultivars such as 'Kent' and 'cat Hoa loc', high temperature and increased exposure time can damage the fruit peel, leading to fruit softening and susceptibility to diseases.

Table 2.1 presents a list of published review articles on postharvest treatments of mango fruit. Notably, these reviews have largely focused on the causes of quality loss and commercially adopted postharvest treatments of mango fruit. Non-chemical postharvest treatments, as well as innovative and environmental-friendly technologies, have received little attention from researchers. Therefore, the current review provides an extensive overview of different postharvest techniques currently used on mango fruit, focusing on non-chemical treatments. Anthracnose and chilling injury are the most commercially important postharvest challenges affecting the mango industry. This review further discusses the potential of emerging postharvest technologies to preserve fruit quality and control postharvest diseases. Research gaps, as well as prospects for future research of the postharvest research in mangoes, are also highlighted.

Table 2.1: Published literature reviews on postharvest technologies of mango fruit

Focus area	Reference
Quality properties of harvested mango fruit and regulating technologies	Tian et al. (2010)
Maintaining mango fruit quality during the export chain	Sivakumar et al. (2011)
Postharvest biology and biotechnology of mangoes	Singh et al. (2013)
A review of postharvest treatments to maintain mango (*Mangifera Indica* L.) quality	Asio and Cuaresma (2016)
Modified atmosphere packaging and postharvest treatments on mango preservation	Liu et al. (2018)

2 Postharvest chemical treatments

The use of chemical treatments is quite an old postharvest management practice, especially for perishable horticultural crops. Chemical treatments such as nitric oxide, salicylic acid, 1-MCP and oxalic acid are commercially used by the mango industry (Ding et al., 2007; Hong et al., 2014; Razzaq

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et al., 2015, Junmatong et al., 2015). Their use has shown to be effective in maintaining fruit quality and extend shelf-life.

2.1 1-Methylcyclopropene

1-Methylcyclopropene, commercially known as SmartFresh®, is a well-known ethylene antagonistthat is used in various fresh horticultural fruits and vegetables. Its efficacy as a mango postharvest treatment has been well researched and documented. For instance, recent studies have shown that 1-MCP (1 µL L^{-1} for 12 hours) reduced ethylene production and respiration rate in 'Kensington Pride' mango fruit after 16 days of storage at ambient temperature (Razzaq et al., 2015). Wang et al. (2009) reported a low 1-aminocyclopropane-1-carboxylic acid (ACC) and 1-aminocyclopropane-1-carboxylate oxidase (ACO) concentrations in 1-MCP (1 µL L^{-1} for 24 hours) treated mango fruit (cv. 'Tainong') compared to the untreated control after 16 days of storage at 20 °C. 1-MCP inhibits the initial step in ethylene biosynthesis, leading to delayed ethylene production and fruit ripening.

Ripening in mango fruit is characterized by an increase in total soluble solids (TSS) and a decrease in titratable acidity. Previous research revealed that 1-MCP (652 µg L^{-1} for 5 minutes) reduced the accumulation of TSS in 'Kent' mangoes stored at 12°C (Osuna-Garcia et al., 2015); however, contrasting results have been observed in other varieties (Table 2.2). Further studies of 1-MCP (1µL L^{-1}) treatment of 'Carabao' mangoes stored at 5°C showed an increase in TSS content (Castillo-Israel et al., 2015). An increase in TSS designates the inability of 1-MCP to retard biochemical reactions associated with fruit ripening. Accumulation of TSS indicates an increase in fruit sweetness, as starch is hydrolyzed to the predominant soluble sugars (sucrose, fructose, and glucose) during ripening (Singh et al., 2013). The 1-MCP (1 µL L^{-1}) treatment delayed the accumulation of sucrose and total sugars of 'Kensington Pride' mango (Razzaq et al., 2015).

Fruit texture is one of the critical fruit quality parameters. Textural properties include firmness, adhesiveness, springiness, cohesiveness, and gumminess (Valente et al., 2011). The application of 1-MCPhas been reported to have an enormous effect on mango fruit firmness. For example, Razzaq et al. (2015) reported that 1-MCP treated fruit had high rheological properties such as springiness and stiffness. Fruit quality and rheological properties are affected by moisture loss. Previous studies revealed that 1-MCP (5 µL L^{-1} for 12 hours) treatment decreased electrical conductivity, thereby maintaining the membrane integrity of 'Irwin' mango fruit stored at 10 °C for twenty-five days (Wongmetha and Ke, 2013). Polysaccharides, hemicellulose, and pectin are depolymerized during mango ripening leading to fruit softening (Yashoda et al., 2006). The process of textural changes is due to enzyme activities, and the modification of cell wall polymers.

The 1-MCP treatment affects cell wall degrading enzymes in mango fruit (Figure 2.1). For instance, Razzaq et al. (2015) observed a reduced enzyme activity of endo- polygalacturonase *(endo-*PG), pectinesterase (PE), and endo-1,4-β-D-glucanase (EGase) in 1-MCP treated fruit. EGase gene *MiCel1* was suppressed in 100 µL L^{-1} 1-MCP treated mango fruit (Chourasia et al., 2008). Endoglucanase is partially responsible for the depolymerization of cellulose and hemicellulose (Chourasia et al., 2008).
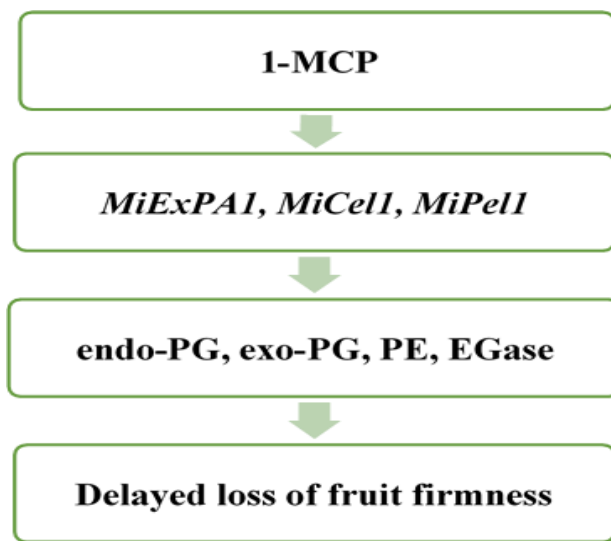
Figure 2.1: The mechanism of 1-MCP in maintaining fruit firmness

The 1-MCP (100 µL L^{-1} for 12 hours) treatment also delayed the accumulation of the *MiPel1* gene in mango (Chourasia et al., 2006). Pectate lyases gene *MiPel1* is related to ripening in 'Dashehari' mango and correlated to pectin solubilization (Chourasia et al., 2006). Accordingly, a delayed accumulation of *MiPel1* gene causes a decrease in enzyme activities of pectate lyases. Total pectin decreases during fruit ripening resulting in cell wall degradation (Chourasia et al., 2006). Sane et al. (2005) reported that 1-MCP (100 µL L^{-1} for 12 hours) treatment reduced the levels of *MiExPA1*. The *MiExPA1* expansion gene is ripening-and-ethylene related, and it is also strongly linked to the late stages of mango fruit softening (Sane et al., 2005). Clearly, there is enough empirical evidence to conclude that the postharvest application of 1-MCP inhibits the accumulation of genes and enzyme activities involved in cell wall modification, thus maintaining the firmness and delaying ripening. Therefore, the manipulation of these genes could play a vital role in developing mango cultivars that can retain fruit firmness during long-term storage and shipping to distant overseas markets.

Table 2.2: The effect of postharvest application of 1-MCP on physiochemical and biochemical attributes of mango fruit

Concentration	Exposure time	Mango cultivar	Key findings	References
2000 ppb	18 & 24 h	'Kesar'	Maintained ascorbic acid (AA) content & delayed TSS accumulation	Sakhale et al. (2018)
			Decreased the respiration rate	
1 µL L^{-1}	12 h	'Kensington' 'Pride'	Retarded enzyme activities of PE, EGase, *endo* & *exo*-PG	Razzaq et al. (2015)
			Decreased respiration rate & ethylene production	
1 µL L^{-1}	24 h	'Tainong'	Reduced concentration of superoxide radicals and hydrogen peroxide	Wang et al. (2009)
			Inhibited superoxide dismutase, catalase & ascorbate peroxidase enzyme activities	
300 nL L^{-1}	20 h	'Kent'	Delayed fruit firmness and ripening	Osuna-García et al. (2009)
0.5 or 1 µL/L	24 h	'Keitt'	Maintained the green colour, delayed fruit softening & ripening	Ngamchuachit et al. (2014)
5 µL L^{-1}	12 h	'Irwin'	Decreased electrolyte leakage & maintained firmness	Wongmetha et al. (2013)
1 or 2 ppm	24 h	'Peter', 'Julie', 'Brokin'	Not effective in reducing TSS accumulation & acidity loss	Faasema et al. (2012)
			Extended shelf-life up to 25 days	

2.2 Nitric oxide

Nitric oxide is a free radical gas that is highly reactive. It is a signaling molecule with a crucial role in various physiological and biochemical processes, especially during fruit ripening (Romero-Puertas et al., 2004; Freschi, 2013). The NO treatment is strongly linked with inhibiting ethylene biosynthesis during postharvest handling (Tran et al., 2015). A study by Hong et al. (2014) showed that treating 'Zill' mango fruit with NO (100 µM) for 30 minutes significantly reduced ethylene production and delayed climacteric peak. Similarly, a reduced respiration rate has been reported in 'Kensington Pride' mangoes fumigated with NO (20 µL L^{-1} for 2 hours) after seven days of storage at 13°C (Zaharah and Singh, 2011a). The NO mechanism of action is linked to its ability to bind ACO thereby forming an ACO-NO binary complex (Zaharah and Singh, 2011a). Interestingly, biochemical studies have demonstrated that the ACC-ACO-NO trinomial complex, a product of ACO-NO chelation by ACC, reduces ethylene production (Freschi, 2013; Hong et al., 2014). There is a growing body of knowledge suggesting that ethylene biosynthesis genes are affected by NO treatment. For instance, Hong et al. (2014) reported a lower expression of *MiACO* mRNA gene after NO treatment in mango fruit. These researchers also noted that *MiETR1* mRNA, a well-known ethylene receptor gene, was upregulated while *MiERS1* mRNA was suppressed in mango peel during storage at 25°C. Ethylene receptors act as negative regulators, suppressing the ethylene signaling pathway (Wang et al., 2002). It can be hypothesized that ethylene production is inhibited by the suppression of ethylene-related genes and enzymes.

The use of NO treatment has also been reported to affect the physicochemical attributes of mango fruit. For example, postharvest application of NO (1 mM) aqueous solution minimised weight loss and maintained firmness in 'Nam Dok Mai Si Thong' mango fruit after seven days of storage at 22 °C (Tran et al., 2015). Change in fruit texture is due to water loss, rupture, and cell wall weakening

(Zerbini et al. 2015; Vázquez-Celestino et al., 2016). Modification of fruit firmness is associated with ripening, softening, and senescence. Notably, Zaharah and Singh (2011b) and Zaharah and Singh (2013) reported that mango mesocarp tissue fumigated with 20 µL L^{-1} or 40 µL L^{-1} NO had high adhesiveness, firmness, chewiness, stiffness, and springiness. The high firmness retention is partly due to the fact that fruit exposure to NO reduces cell wall linked enzyme activities such as polygalacturonase (exo-PG) and endo-1,4-β-D-glucanase (EGase) (Zaharah and Singh, 2011a). A high PE enzyme activity in NO fumigated mango fruit has also been reported. Pectinesterase has various roles during postharvest biochemical processes, including the depolymerization of pectin molecules into soluble pectate and methanol. Thus, the released pectate interacts with calcium, leading to higher PE and improved cell wall integrity (Zaharah and Singh, 2011a). Therefore, it can be deduced that the decrease in these enzyme activities reduces cell wall breakdown, thereby maintaining fruit firmness.

Nitric oxide is effective in controlling postharvest diseases such as anthracnose in mango fruit. For instance, Hu et al. (2014) reported that NO (0.1 mM sodium nitroprusside (SNP) treatment) reduced the natural disease incidence and lesion diameter in 'Guifei' mango stored at ambient temperature for ten days. NO increased the defense-related enzyme activities such as phenylalanine ammonia-lyase (PAL), cinnamate-hydroxylase (C$_4$H), peroxide (POD), chitinase (CHI) and β-1,3-glucanase (GLU) (Hu et al., 2014). Recently, Zheng et al. (2017) reported that NO (0.2 mM SNP aqueous solution, 10 min at 25 °C) upregulated gene expression of POD, CHI and PAL in kiwifruit during storage at ambient temperature for thirteen days. The increased enzyme activity initiates the biosynthesis of anti-fungal metabolites such as flavonoids, phenolics, phytoalexins, and tannins (Hu et al., 2014; Zheng et al., 2017). These secondary metabolites form a protective barrier against pathogen infection, thus inhibiting infection and pathogen growth on the fruit (Zheng et al., 2017). The mode of action of NO in inducing defense against postharvest pathogens is through the activation of pathogenesis-related proteins and phenylpropanoid metabolism (Hu et al., 2014). The resistance of mango fruit to

anthracnose is through the increased enzyme activities and accumulation of secondary metabolites, causing hypersensitive response to cell death, and strengthening fruit immunity (Scheler, et al., 2013; Hu, etal., 2014; Zheng et al., 2017).

2.3 Salicylic acid

Salicylic acid (SA) is a plant hormone that regulates various physiological processes in plants (He et al., 2016). Such physiological processes include fruit ripening, tolerance to chilling injury (CI), and resistance to postharvest diseases (Zainuri et al., 2001; Ding et al., 2007). Chilling injury occurs in mango fruit when exposed to temperatures below 13°C, depending on canopy position and cultivars (Barman and Asrey, 2014; Sudheeran et al., 2018). Sudheeran et al. (2018) reported that mango fruit (cv. 'Shelly') from the outside canopy had less CI than the inside canopy stored at 5 °C for twenty-one days. Phakawatmongkol et al. (2004) reported that cultivars such as 'Nam Dok Mai' and 'Okrong' were more and least susceptible to CI, respectively. The CI symptoms include sunken lesions, shriveling, pitting, discoloration of the peel, susceptibility to decay, and uneven ripening (Li et al., 2015). Severe CI symptoms have been reported in fruit at ambient temperature during shelf-life after cold storage (Ntsoane et al., 2019b). Mango fruit induce CI resistance through the accumulation of anthocyanin and flavonoids (Sivankalyani et al., 2016; Ntsoane et al., 2019b).

Storing mango fruit at low temperature induces free radicals such as hydrogen peroxide (H_2O_2) and superoxide radicals (O_2^-), causing oxidative stress and CI (Junmatong et al., 2015). Increased ROS levels can cause lipid peroxidation leading to reduced membrane integrity and fruit firmness. Junmatong et al. (2015) reported that SA (1mM) inhibited the accumulation of H_2O_2 and O_2^- in 'Nam Dok Mai No. 4' mangoes stored at 5 °C for forty-two days. These authors further found that the SA treatment increased the activities of CAT, ascorbate peroxidase (APX), and SOD. SA is a signaling molecule that activates the gene expression of *CAT*, *SOD,* and *APX* at cold temperatures (Figure 2.2).

The SOD dismutate O_2^- into H_2O_2, which is detoxified by APX and CAT (Ding et al., 2007). The enzyme CAT, APX, and SOD can scavenge ROS, leading to fruit adapting to cold temperatures and reducing CI.
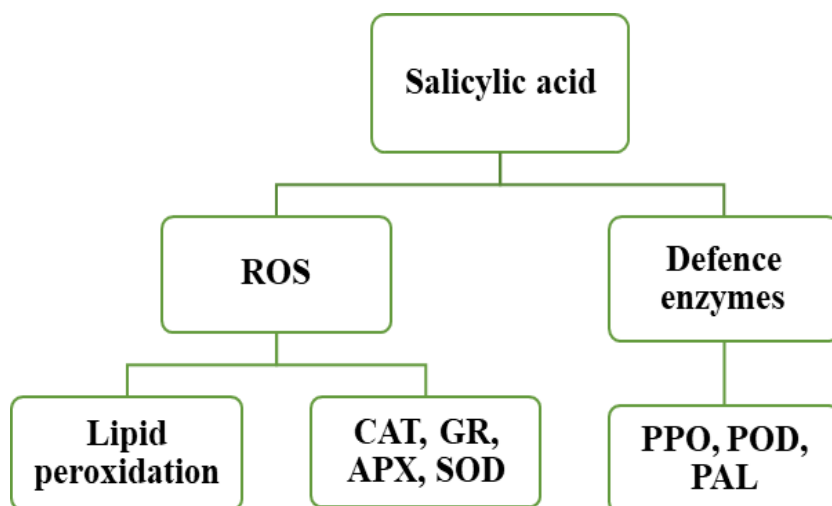
Figure 2.2: Salicylic acid induces resistance to CI in the fruit (Modified from Asghari and Aghdam, 2010). Application of SA in fruit prior to chilling temperatures induce ROS scavenging and avoidance genes such as APX, SOD and CAT. The increased antioxidant capacity of the cells leads to fruit adapting to cold temperatures, thereby reducing the incidence of postharvest disorders such as chilling injury

SA is known to control anthracnose caused by *Colletotrichum gloeosporioides* in mango fruit. Zeng at al. (2006) reported that treating 'Matisu' mango fruit with SA (1 mmolL^{-1}) reduced the disease incidence and lesion diameter of *Colletotrichum gloeosporioides*. In their *in vitro* study, He et al. (2006) revealed that mycelial growth was significantly reduced by SA (2 and 5 mM) treatment in 'Tainong' mangoes. These researchers also reported that SA (2 mM) increased the enzyme activities of CHI and GLU. These enzymes are involved in inducing resistance against diseases. The GLU is reported to cause disease resistance at the early stages of fruit ripening (Zeng et al., 2006). Mango fruit treated with SA has been shown to accumulate more polyphenoloxidase (PPO), POD and record low phenolic content (Zeng et al., 2006). The enzyme PPO plays a vital role in the defense against diseases

as it catalyzes phenolics into quinines. Thus, it can be concluded that SA induces resistance against anthracnose by stimulating CHI, GLU and PPO activities during postharvest handling.

3 Edible coatings

Edible coatings are semipermeable membranes on the fruit skin, creating a modified internal atmosphere, decreasing moisture loss, and respiration rate (Bourtoom, 2008). They are composed of proteins, polysaccharides, lipids, and resins (Baldwin et al., 1995). The efficacy of edible coatings as a postharvest treatment in horticultural crops has extensively been evaluated (Table 2.3). Among many benefits, edible coatings preserve antioxidant activity, prolong shelf-life, reduce mass loss, reduce respirationrate, while maintaining firmness and colour in treated fruits.

3.1 Chitosan

Chitosan is derived from the deacetylation of beta 1, 4-D-glucosamine, a natural polymer (Gurjar et al., 2018). The coating is biodegradable, non-toxic, and characterized by anti-microbial properties. Chitosan is effective in inhibiting postharvest diseases such as *Colletotrichum gloeosporioides*, *Alternaria alternate,* and *Dothoriella* spp. Recent research by Gurjar et al. (2018) revealed that chitosan coating is effective in suppressing microbial growth in processed 'Mallika' mangoes. Similarly, chitosan coating at 2% suppressed the incidence and reduced the lesion diameter of *Colletotrichum gloeosporioides* associated with anthracnose in 'Tainong' mango fruit (Zhu et al., 2008). Lower incidences of stem-end rot incidence caused by *Dothoriella* spp. (Wang et al., 2007) as well as reduced germination and mycelia growth of *Alternaria alternata* associated with black spot (López-Mora et al., 2013) have been reported in chitosan-coated mangoes. Chitosan is positively charged and thus interacts with the negatively charged cell membrane, therefore affecting the permeability of the cell (Cissé et al., 2015). The mechanism of chitosan antibacterial activity is

associated with low pH and attributes of the cell surface. It is speculated that chitosan interacts with the outer membrane of the pathogen cell surface, causing leakage of the intracellular substances leading to cell death.

Except for reducing postharvest diseases, chitosan also regulates various physiological and biochemical processes that have an enormous effect on mango fruit quality. For example, Cosme Silva et al. (2017) observed a delayed climacteric peak and decreased respiration rate in 'Palmer' mango treated with 3% chitosan. Coating 'Tainong' mango with a 2% chitosan reduced the respiration rate (Wang et al., 2007). Chitosan coating is reported to be selective to CO_2 permeability than O_2. For instance, Cissé et al. (2015) observed decreased O_2 consumption and increased CO_2 production in 'Kent' mango fruit coated with 1 or 1.5 % chitosan after eight days of storage at ambient temperature. The increased CO_2 could enhance the succinic acid and inhibit succinic dehydrogenase activity, resulting in decreased respiration rate (Mathooko, 1996; Deng et al., 2006). Respiration plays a crucial role in fruit metabolic activity and affects shelf-life. Chitosan forms a permeable barrier against carbon dioxide, moisture, and oxygen, resulting in reduced water loss, respiration, and oxidation reaction rate. However, the protective barrier formed by chitosan in fruit can lead to off-flavours. For example, Wang et al. (2007) reported that chitosan (2%) resulted in poor taste in 'Tainong' mango after thirty-five days of storage at 15°C. The off flavours could be attributed to the anaerobic respiration caused by the coating (Sothornvit and Rodsamran, 2008). Alcohol and acetaldehyde are produced during anaerobic respiration resulting in bad odour and off flavours (Sothornvit and Rodsamran, 2008).

When detached from the tree, fruits continue to respire, consuming all the oxygen inside the fruit. Continuous respiration and loss of water through transpiration lead to the weight loss of the fruit. Fortunately, edible coatings such as chitosan are effective in reducing both water loss as well as fruit weight loss. For instance, 3% chitosan reduced weight loss in processed mango fruit (*cv*. 'Mallika') during storage at 8 °C for eight days (Gurjar et al., 2018). Fruit weight loss leads to shriveling and

decreased aesthetic quality. Consumers buy fruit based on visual appearance, and weight loss can affect the acceptability of the fruit because severe mass loss results in shriveling. Furthermore, like other fruits, mango is sold on a weight basis, therefore losing more weight could result in the loss of profit.

Chitosan coating is effective in maintaining firmness in mango during storage. Cissé et al. (2015) reported that chitosan coating retained firmness in 'Kent' mango fruit. The loss of membrane integrity is an indicator of fruit ripening and senescence. Chitosan coating retarded the increase of malondialdehyde (MDA) content and maintained membrane integrity (Khaliq et al., 2017). The MDA is a secondary end-product resulting from free radicals damaging lipid peroxidation. Lipid degradation alters cellular membrane structure and function. The protective barrier formed by the coating reduces lipid peroxidation leading to decreased electrolyte leakage, thus retaining fruit firmness and delaying senescence.

3.2 Carboxymethyl cellulose

Carboxymethyl cellulose (CMC) is derived from cellulose and composed of linear chains of β (1-4) glucosidic units with carboxyl substituent, hydroxypropyl, and methyl (Salinas-Roca et al., 2018).

Table 2.3: The effect of edible coatings on postharvest quality of mango fruit

Edible coating material	Concentration	Cultivar	Key findings	Reference
Aloe vera	50 %	'Alphonse'	Increased total sugars, extended shelf-life, retained firmness	Abd El-Gawad et al. (2019)
Gum Arabic	10 %	'Choke Anan'	Inhibited ethylene production, retained ascorbic acid and firmness	Khaliq et al. (2015)
Gum Arabic	10 %	'Apple'	Extended shelf-life, decreased weight loss, delayed TSS accumulation	Daisy et al. (2020)
Chitosan Gallate	25-75 ml L^{-1}	'Hindi-Besennara'	Maintained membrane integrity, delayed ripening	Awad et al. (2017)
Chitosan	8 %	'Fa-lun'	Inhibited microbial growth, decreased weight loss	Nongtaodum & Jangchud (2009)
Chitosan	2 %	'Tainong'	Decreased weight loss, maintained firmness, suppressed anthracnose incidence	Zhu et al. (2008)
Chitosan	1 %	'Nam Dok Mai'	Inhibited disease severity, mycelia growth & *Colletotrichum gloeosporioides* spore germination	Jitareerat et al. (2007)
CMC	10 g kg^{-1}	'Tommy Atkins', 'Kent'	Maintained firmness, maintained high scores during sensory evaluation	Plotto et al. (2010)
CMC	0.1 %	'Tommy Atkins'	Suppressed disease incidence & lesion diameter of *Colletotrichum dianesei*	Gava et al. (2018)

CMC coating can form a semipermeable barrier, thereby modifying the internal fruit atmosphere, limiting the exchange of gases into and out of fruit, and hence reducing respiration rate and delaying senescence. There is a considerable amount of published research illustrating the potential of CMC as the postharvest treatment of mango fruit. For instance, Phaiphan and Rattanapanone (2008) reported that mango fruit coated with 1% CMC had a lower respiration rate compared to their uncoated counterparts. The efficacy of CMC is partly linked to its ability to modify the biochemical processes causing partial anaerobic respiration. A decline in respiration rate and shift of climacteric peak leads to delayed fruit senescence. An increase in respiration rate is associated with fruit colour changes from green to yellow, indicating fruit ripening. Coating mango fruit with 2% CMC is effective in maintaining fruit colour during storage (Abbasi et al., 2011). Plotto et al. (2004) reported delayed colour changes in CMC-treated mango fruit.

The use of CMC as a standalone treatment has been reported to be ineffective for some cultivars and postharvest handling conditions. For instance, Salinas-Rosa et al. (2018) reported that 2% CMC failed to reduce mold, yeast, and psychrophilic bacteria in fresh-cut 'Tommy Atkins' mango fruit stored at 4 °C for ten days. Interestingly, the researchers found that incorporating CMC with gum rabic coating proved to be effective in reducing microbial growth in 'Button' mushrooms stored at 4 °C for twelve days (Srivastava and Bala, 2016). The anti-microbial effect of CMC is complex and not well understood. The microbial count was reduced in 'Tommy Atkins' mango fruit coated with CMC (5 g kg^{-1}) after twenty-one days of storage at 5°C (Plotto et al., 2010).

Recent attempts to improve the efficacy of edible coatings have focused on combining two or more coating agents to improve the anti-fungal and physical properties. The use of plant extracts such as moringa, *Aloe vera* gel with CMC, or chitosan has been shown to be

effective in various horticultural fresh vegetables and fruits (Tesfay and Magwaza, 2017). Ali et al. (2012) reported that treatment combination of Gum Arabic (GA) 10% + chitosan 1% effectively decreased the disease index and mycelia growth of *Colletotrichum gloeosporioides* in 'Eksotika II' papaya stored at 12 °C for twenty-eight days. The combination of GA that contains no antifungal properties with chitosan, which has antimicrobial properties, augmented the coating, thus decreasing anthracnose in the fruit (Ali et al., 2012; Siddiqui and Ali, 2014). Edible coatings that contain natural antimicrobial and antioxidants have shown high resistance against microorganisms (Ali et al., 2017). However, so far, very little research has focused on the combinational use of plant extracts with edible coatings as postharvest treatment of mango fruit. Thus, postharvest research assessing the effect of plant extracts and the commercially used edible coatings is warranted.

3.3 Gum Arabic

Gum arabic (GA) is a natural polysaccharide obtained from the branches and stem of *Acacia* species (Ali et al., 2010). Although it is commonly used as a stabilizer and thickener by the food industry, recent studies have demonstrated its potential as an edible coating (Khaliq et al., 2016b). Gum arabic does not only affect the physical attributes of the fruit, the biochemical and nutritional qualities are also influenced by the treatment. Vitamin C is one of the prominent nutritional attributes in mango fruit, it is water-soluble and highly beneficial for human health(Muhammad et al., 2014; Mditshwa et al., 2017). A recent study by Daisy et al. (2020) demonstrated that 15% GA coating effectively maintained the AA in 'Apple' mango stored at23°C for fifteen days. The AA is a powerful antioxidant that reduces oxidative stress caused by ROS (Khaliq et al., 2015). Khaliq et al. (2016a) reported that GA (10%) coating reduced H_2O_2 and O_2^- in 'Choke Anan' mango fruit during storage at 6°C for twenty-eight days. The GA coating improves the antioxidant pool (Khaliq et al., 2016a), which scavenges

excess ROS, damage to the fruit. Additionally, the high content of vitamin C in GA treated mangoes improves nutritional fruit quality. The consumption of vitamin C-rich foods is known for boosting the immune system and reducing the risk of cardiovascular diseases and various types of cancer (Muhammad et al., 2014).

Ethylene production is a well-known indicator of the metabolic activity and has tremendous influence on shelf-life and quality of mango fruit. During fruit ripening, there is an upsurge in ethylene production, modulating biochemical changes such as aroma, texture, and colour changes (Khaliq et al., 2015, Daisy et al., 2020). Khaliq et al. (2015) reported that GA (10%) delayed the ethylene climacteric peak for twenty-one days in 'Choke Anan' mango stored at 6°C. Lawson et al. (2019) reported that ethylene is negatively correlated to firmness during fruit ripening. The decreased ethylene production slows down the rate of fruit ripening and softening. Khaliq et al. (2016b) reported that GA (10%) coating maintained firmness in 'Choke Anan' mango during storage at 13°C for twenty-eight days. The loss of fruit firmness in mango is due to the changes in cell wall composition and structure (Khaliq et al., 2015). The barrier formed by the GA coating could decrease the cell wall degrading enzyme activity, thus delaying loss of fruit firmness.

4   Non-chemical treatments

Non-chemical treatments such as ultraviolet irradiation and heat treatment have shown to be effective in maintaining the postharvest quality of mango (Table 2.4). These treatments have been used successfully for controlling postharvest diseases and extending the shelf-life. Among these treatments, heat treatment is used commercially by the mango industry, as it is cost-effective and easily adapted by mango producers (Sivakumar et al., 2011).

4.1 Heat treatment

4.1.1   Hot water treatment

Postharvest heat technology has been used in horticultural crops to sanitize and extend shelf-life. Hot air (HAT) and hot water treatment (HWT) are some of the cheap and commonly used heat treatments. The use of heat as a postharvest treatment of mango fruit has been well-researched and documented. For instance, HWT at 55 °C for ten-minutes suppressed respiration in 'Tainong' mango fruit during storage at 20°C for six days (Zhang et al., 2012). Similarly, studies on 'Ivory' mango revealed that hot water treatment at 60°C for one-minute inhibited ethylene production and respiration rate (Wang et al., 2016). The efficacy of HWT is strongly linked to its potential to regulate key enzymatic activities affecting quality attributes of fresh horticultural produce. The activities of ACC oxidase have been demonstrated to inhibit in hot water treated (46 °C, 90-minutes) 'Keitt' mango (Bender et al., 2003). ACC oxidase regulates ethylene biosynthesis; therefore, inhibition of this enzyme delays production of ethylene. Ethylene production activates the physical and biochemical processes involved in fruit softening (Khaliq et al., 2015).

Increased firmness retention in fruit subjected to HWT before long-term cold storage has been reported (Ding and Mijin, 2013). Notably, heat treatment may cause stress resistance by stimulating the antioxidant activities and protective enzymes of the treated fruit. Enzymes such as PG, β-galactosidase, α-mannosidase, and β-hexosaminidase are involved in cell wall modification and softening in mango fruit (Abu-Sarra and Abu-Goukku, 1992; Hossain et al., 2014). HWT (60 °C for 1 minute) inhibits the cell wall degrading enzyme PG after ten days of storage at 25°C (Wang et al., 2016). Previous studies by Ketsa et al. (1998) revealed that HWT

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at 33 °C for three days increased the activity of β-galactosidase caused in 'Nam Dokmai' mango after eight days of storage at 25°C. This suggests that β-galactosidase might play a prominent role in mango fruit softening than PG. Sripong et al. (2015) reported a rapid fruit softening in 'Chok-Anan' mango dipped in 55 °C for five minutes. Dautt-Castro et al. (2018) indicated that HWT (47°C for 5 minutes) upregulates cell wall genes of β-galactosidase (*MiBGAL c23904*), pectate lysase (*MiPL c20761*), polygalacturonases (*MiPG c21885*), ram-nogalaturonase (*MiRGL c23797*) and small heat shock proteins (*MiHSP20 c12121*). Ram-nogalaturonase *MiRGL c23797* gene is involved in rhamnogalacturonan degradation and has a physiological role in abiotic stress (Dautt-Castro et al., 2018). The upregulation of these genes causes an increase in their related enzymes, leading to rapid fruit softening and ripening. The variation in temperature and HWT time could trigger different reactions with regard to gene expression and enzyme activities.

HWT has an enormous effect on the organoleptic and physicochemical quality attributes of mangoes. A recent study by Dautt-Castro et al. (2018) demonstrated that HWT (47 °C for 5 minutes) increased TSS accumulation in 'Ataulfo' mango during storage at 20°C for eight days. Hot water is known to upregulate beta-amylase gene *MiBAM c23077* involved in starch hydrolyzes (Dautt-Castro et al., 2018). Sucrose synthase gene *MiSS, c10928,* is upregulated by HWT (Dautt-Castro et al., 2018). The upregulation of these genes could lead to rapid starch degradation, an increase in TSS accumulation, thus enhanced fruit ripening. It should, however, be noted that the effect of heat treatments on some quality attributes is cultivar dependent. Le et al. (2010) reported vapor heat treatment at 46.5 °C for 40 minutes did not affect TSS accumulation in 'Tuu Shien' mango stored at 12 °C for three weeks. Thus, it is critical to design an appropriate post-harvest protocol for each mango cultivar.

Research has also shown that HWT maintains colour and appearance of the fruit peel. For instance, 'Tuu Shien' mango fruit treated with hot water at 50 ℃ for ten minutes retained the green colour during storage (Le et al., 2010). Dautt-Castro et al. (2018) reported that hot water down-regulates *chloroplastic-like* (*LHCIIb*) (*EC4.99.1.1*) genes involved in chlorophyll biosynthesis. These researchers found that HWT increased gene expression of *anthocyanin 5-aromatic* (*anthocyanin5a*) (*EC:2.3.1.144*) and *UD-Pglycosyltransferase 85a2-like* (*85A2*) (*EC:2.4.1.115*) which are involved in anthocyanin accumulation. An increased chlorophyll degradation and increased production of anthocyanin resulted in homogenous colour development of mango fruit.

Hot water treatment is effective in suppressing the severity of CI in mango fruit. Zang et al. (2012) reported that HWT (55 ℃ for 10 minutes) reduced the CI in 'Tainong 1' mango fruit during storage (21 days, 5 ℃ and 5 days, 20 ℃). The HWT activates lipid-related metabolism in mango fruit during low-temperature storage. Vega-Alvarez et al. (2020) observed high levels of linolenic acid in 'Keitt' mango treated with hot water (46.1 ℃ for 90 minutes) and stored at 5 ℃ for twenty-one days followed by seven days at 21 ℃. Similarly, Yimyong et al. (2011) observed high levels of lipoxygenase (LOX) protein in 'Okrong' mango treated with hot water at 50 ℃ for ten minutes and stored at 8 ℃ for fifteen days. The increased levels of LOX and fatty acids could induce the CI tolerance in mango fruit.

Table 2.4: Mango fruit quality as influenced by heat treatment and UV-C irradiation

Treatment	Cultivar	Key findings	Reference
HWT (50 °C, 20 min)	'Carabao'	Reduced the incidence of anthracnose & stem-end rot	Alvindia and Acda (2015)
HWT (46.1 °C, 75 min)	'Keitt'	Increased ascorbic acid; decreased CI, total phenolics & flavonoids	López-López et al. (2018)
HWT (60 °C, 1 min)	'Ivory'	Increased flavonoids and total phenolics. Reduced POD & PPO activities	Wang et al. (2016)
HWT (50 °C, 10 min)	'Okrong'	Inhibited APX activity & ethylene production	Yimyong et al. (2011)
HWT (55 °C, 10 min)	'Tainong 1'	Decreased chilling injury & β-galactosidase activity; increased PME & PG activity	Zhang et al. (2012)
HAT (47 °C, 180 min)	'cat Hoa loc'	A high percentage of burned fruit; increased weight loss	Hoa et al. (2010)
Heat vapor (46.5 °C, 40 min)	'Tuu Shien'	Maintained peel colour & fruit firmness; delayed TSS accumulation	Le et al. (2010)
UV-C (254 nm, 60 min)	'Chokanan'	Delayed TSS accumulation, increased ascorbic acid content; maintained total polyphenol & antioxidant content; decreased sensory attributes	George et al. (2015)
UV-C (250-280 nm, 10 min)	'Tommy Atkins'	Decreased fruit decay as well as simple sugars & organic acids	González-Aguilar et al. (2001)

4.1.2   Hot air treatment

Hot air treatments (HAT) influence postharvest pathological disorders and diseases. For example, studies have shown that a combined treatment of heat vapor at 46.5 °C for 40 minutes and hot water at 55 °C for three minutes decreased the incidence of anthracnose caused by *Colletotrichum gloeosporioides* and *Alternaria alternate* associated with black spot in 'Tuu Shien' mango (Le et al., 2010). Further studies on heat treatment indicated that hot water vapour at 55 °C for 15-20 seconds decreased the incidence of black spot caused by *Alternaria alternate* in 'Shelly' mango stored at 12 °C for twenty-one days (Luria et al., 2014). Defense-related genes associated with salicylic acid and jasmonic acid such as *Syntaxin-121-like* (*Syn121*), *glutaredoxin (EC 1.20.4.1)*, and *Allene oxide synthase* (*AOS)* were upregulated by heat treatment in mango fruit (Luria et al., 2014). Syntaxin is a plant defense protein that causes resistance against disease and pathogen penetration (Shukla et al., 2010). This suggests that heat treatment regulates cellular defense genes inducing resistance against pathogens, thus reducing decay of mango fruit during storage.

It is crucial to note that there are various factors affecting the efficacy of heat treatments. These factors include the maturity stage, cultivars, exposure time, and temperature. Fruit size is another critical factor that has to be considered when applying heat treatments. It is well known that small fruit are easily damaged compared to larger fruit (Sivakumar and Fallik, 2013). Moreover, immature fruit are less heat tolerant than mature fruit; due to internal breakdown that can occur when they are exposed to heat (Sivakumar et al., 2011; Sivakumar and Fallik, 2013). Mechanical damage and poor quality has been reported following heat treatment. For instance, Osuna-Garcia et al. (2015) observed lenticel damage and dark browning spots in

'Kent' mango treated with 46.1°C for 90 minutes. Increasing the temperature or exposure time can cause heat-induced injury, firmness, and weight loss leading to rapid fruit decay. Thus, it is important to consider all these factors when heat is used as the treatment for fresh mango fruit.

4.2 Ultraviolet-C (UV-C) radiation

Short-wave ultraviolet is a non-thermal technology with a wavelength of 190-280 nm (Mohamed et al., 2017). UV-C irradiation is used as a postharvest treatment to enhance fruit quality and extend the shelf-life of fresh fruits and vegetables. The use of UV-C as a postharvest treatment has been reported in mangoes. For example, George et al. (2015) reported that UV-C treatment at 254 nm preserved quality and increased the shelf-life of fresh-cut mango (cv. 'Chokanan') up to fifteen days. UV-C treatment (250 nm for 15 minutes) preserved sensory attributes of 'Chokanan' mangoes stored at 4 °C for fifteen days. The taste and aroma are closely correlated and influence how the consumer perceives the fruit. Loss of either one of these attributes may result in the fruit being rejected by the consumers.

Antioxidants are an important quality attribute in mangoes as they have a prominent role in human health. There is growing literature evidence demonstrating that UV-C treatment does affect the accumulation of phytochemicals, such as antioxidants. For instance, UV-C irradiation (250-280 nm for 10 minutes) of 'Tommy Atkin' mango fruit increased the total phenolic and flavonoid content after fifteen days of storage at 5 °C (González-Aguilar et al., 2007). The high antioxidant activity in mangoes provides a much desired health benefit to the consumers. Various physiological and postharvest quality-linked enzymatic activities are also influenced by irradiation. For example, Safitri et al. (2015) demonstrated that UV-C irradiation at 4.93

kJ m^{-2} reduced respiration rate in 'Nam Dok Mai Si Thong' mango fruit stored at 14 °C for twenty days. The expression of 1-aminocyclopropane carboxylate synthase (ACS) and ACO was significantly inhibited in UV-C treated 'Chikanan' mangoes (George et al., 2016). As mentioned above, ACS and ACO are strongly involved in ethylene biosynthesis; therefore, their reduction may delay ripening, minimize fruit decay and prolong shelf-life.

Experimental studies have also demonstrated that irradiation has the potential of inhibiting various postharvest diseases and disorders. A recent *in vitro* study by Terao et al. (2015) showed that UV-C treatment at 20 kJ m^{2} reduced the mycelia growth of *Colletotrichum gloeosporioides* and *Botryosphaeria dothidea*. Romero et al. (2017) reported that UV-C treatment (2.064 kJ m^{-2} for 5 minutes) decreased *Escherichia coli* and *Listeria innocua* growthin 'Tommy Atkins' sliced mango stored at 4°C for fifteen days. Biochemically, UV-C irradiation induces defense-related enzyme activities of GLU, POD, PAL and CHI (Sripong et al., 2015). The increased expression of GLU and CHI in UV-C treated fruit could be an indication of their involvement in degrading the cell wall of the postharvest pathogens, while PAL creates an unconducive environment for pathogen growth and development.

5   Storage technologies

Preservation of mango fruit quality using techniques such as controlled atmosphere (CA) and modified atmosphere packaging (MAP) has been used over the past decade (Table 2.5). In thesestorage conditions, the oxygen (O_2) concentration is reduced while carbon dioxide (CO_2) is increased to extend fruit shelf-life. The benefits of decreasing O_2 and increasing CO_2 levels include the reduction of respiration rate, which slows down the metabolic processes, resulting in reduced fruit senescence (Costa et al., 2018; Ntsoane et al., 2019a).

5.1 Controlled atmosphere

Controlled atmosphere (CA) incorporated with optimum low temperatures has been used to maintain the quality of mango fruit during storage (Sumual et al., 2017). The adequate level of CO_2 and O_2 are important factors affecting fruit quality. Ullah et al. (2010) reported that CA treatment of 3% O_2 and 6% CO_2 retained sweetness and flavour in 'Alphonso' mangoes stored at 10 °C for twenty-one days. However, CA storage has been reported to compromise fruit aroma during storage. A study by Rattanapanone et al. (2001) reported that aroma diminished in 'Tommy Atkins' mango fruit stored at 4 kPa O_2 and 10 kPa CO_2 for eight days at 10°C. The loss of aroma in mango fruit could be linked to drastic changes in the accumulation of volatile compounds under low oxygen and high carbon dioxide storage conditions. A reduction of aroma volatile compounds such as esters, ketones and aldehydes has been reported in 'Kensington Pride' mangoes stored at CA of 2% O_2 and 9% CO_2 at 13°C for thirty-five days (Lalel and Singh, 2003). High CO_2 concentrations in the storage chambers can easily trigger anaerobic respiration. Thus, an appropriate gas composition is critical for ensuring the superior nutritional and physicochemical quality of mangoes stored in CA.

5.2 Modified atmosphere packaging

Modified atmosphere packaging (MAP) is used to increase shelf-life and maintain the postharvest quality of horticultural crops. MAP has been shown to be effective in combination with other treatments such as HWT and coatings. Ramayya et al. (2012) reported that unperforated oriented polypropylene bags decreased weight loss in 'Alphanso' mangoes stored at 10°C for twenty-one days. The storage temperature may determine the success of MAP storage. Increased weight loss has been reported in 'Tommy Atkins' mangoes wrapped in

flexible Xtend® and stored at 25 °C compared to those at 12 °C for twenty-one days (Costa et al. 2018). These studies highlight the importance of cold storage in decreasing weight loss and fruit spoilage in MAP. Preserving fruit quality is dependent on optimum treatment combination of MAP and cold storage. The combination of MAP with low temperatures is crucial for reducing respiration rate and other metabolic processes that may compromise fruit quality during postharvest storage and shelf-life.

5.3 Low oxygen storage

Low oxygen storage (LOS) is an emerging technology that allows fresh horticultural produce to be stored under extremely low O_2 levels (Wright et al., 2015). Firmness retention and reduced respiration rate are some of the benefits associated with LOS storage. A recent study by Ntsoane et al. (2019a) reported that combing CA storage with 1% O_2 reduced the respiration rate in 'Shelly' mango during storage at 13°C for twenty-one days. An earlier experiment by de Almeida Teixeira and Durigan (2011) also revealed that storing 'Palmer' mangoes in CA + 1% or 5% O_2 notably retarded respiration rate for twenty-eight days. The reduced respiration rate could be attributed to low levels of O_2 concentration, which also inhibits ethylene biosynthesis.

Table 2.5: Effect of MAP, CA and LOS on mango fruit quality

Treatment	Atmospheric condition	Film type	Cultivar	Key findings	Reference
CA	5.0 kPa O_2 + 20 kPa CO_2	-	'Palmer'	Increased weight and firmness loss	de Almeida Teixeira et al. (2018)
CA	3% O_2 + 6% CO_2	-	'Alphonso'	Retained firmness, decreased weight loss & increased TSS accumulation	Ullah et al. (2010)
CA	3% O_2 + 5% CO_2	-	'Kensington Pride'	Increased carotenoids, retained ascorbic acid, decreased organic acids	Sumual et al. (2017)
MAP	Not reported	Unperforated oriented polypropylene	'Alphonso'	Maintained colour, firmness, ascorbic acid & eating quality	Ramayya et al. (2012)
MAP	Not reported	Xtend®	'Sufaid Chaunsa'	Retained firmness & green colour	Hafeez et al. (2016)
MAP		Polyethylene	'Alphonso'	Maintained firmness, decreased weight loss. Increased TSS accumulation	Ullah et al. (2012)
MAP	9.52% O_2 + 0.23% CO_2	Cellophane	'Namdok Mai'	Poor sensory quality, presence of off-flavors, increased browning incidence	Sothornvit and Rodsamran (2010)
LOS	2% O2	-	'Kensington Pride'	Increased fatty acids & aroma volatile compounds	Lallel and Singh (2004)

| LOS | 1 or 5% O2 | - | 'Palmer' | Delayed fruit ripening & firmness loss; delayed accumulation of reducing and total sugars | de Almeida Teixeira and Durigan (2011) |

Although LOS offers many benefits to mango producers, it should be noted that it may cause internal browning, off-flavours as well as peel discolouration (Wright et al., 2015). In fact, Ntsoane et al. (2019a) reported that consumers rejected 'Shelly' mangoes stored at 1% O_2 for twenty-one days at 13 °C due to off-flavour and bad odour. Interestingly, these researchers also found that elevating O_2 concentration to 10% eliminated off-flavours. This suggests that storing mangoes below their oxygen limit can result in anaerobic conditions as well as accumulation of undesired volatile compounds, resulting in poor sensory quality and marketability. Thus, proper LOS protocols must be developed for each mango cultivar as varieties might have a different response. Moreover, the interaction between LOS and maturity must be investigated as the mango fruit's response to low oxygen levels might be influenced by the physiological and biochemical status at harvest.

6   Emerging postharvest technologies

Postharvest technologies such as ozone and pulsed electric field are gaining the attention of researchers. More research is done to explore the potential of these technologies to preserve fruit quality. However, limited data is available on the use of such technologies as postharvest treatments of mango fruit.

6.1 Ozone

Ozone (O_3) is a triatomic oxygen molecule with a high oxidative reduction potential (Sandhu et al., 2011). It is highly unstable and when it decomposes, it forms radicals such as carbon dioxide, hydrogen, carbon monoxide as well as water (Anglada et al., 1999). The use of O_3 to maintain the quality and extend the

shelf-life of horticultural crops has been documented (Shezi et al., 2020a). Emerging evidence indicates that O_3 is effective in preserving firmness, decreasing weight loss and shelf-life in fruits (Tran et al., 2013; Minas et al., 2014; Shezi et al., 2020b).

The use of ozone as a postharvest treatment has been evaluated on mangoes, even though the research in this area remains very insignificant. Tran et al. (2013) observed a reduced respiration rate in mango fumigated with 10 µL L^{-1} O_3 for 10 minutes during storage at 25 °C for six days. Although the mechanism of action is not yet fully understood, ozone is reported to inhibit ethylene biosynthesis by reducing the ACC levels in fruit cell walls (Minas et al., 2014). Additionally, ACO protein and ACO1, an enzyme responsible for ethylene production, are suppressed and down-regulated, respectively, by postharvest ozone treatments (Tzortzakis et al., 2013).

Due to its anti-microbial activity, ozone seems to be effective against various postharvest pathogens. Barbosa-Martínez et al. (2002) reported that ozone inhibited the spore germination of various pathogens, including *Fusarium oxysporum* and *Colletotrichum gloeosporioides*. Recent studies by da Silva Neto et al. (2019) has shown that O_3 at 3.3 ppm reduced the disease incidence and severity of anthracnose in papaya fruit stored at room temperature for twelve days. In an in vitro study, Ong and Ali (2015) reported that O_3 (3.5 µL/L or 5 µL/L for twenty-four hours) treatment generates ROS, which degrades mitochondria of *Colletotrichum gloeosporioides* spores. ROS causes oxidative stress in fungi, which modifies the endoplasmic reticula and mitochondria structure (Ong and Ali, 2015). Ozone inhibits the defense-related genes *Chi3a*, *Chi9b, Gluac*, *Glubs* and plant defense gene *Pdf1.2* (Tzortzakis et al., 2011). The possible mechanism of O_3 in inducing disease resistance in fruit is through the accumulation of phenolic compounds and down regulation of defense-related genes (Minas et al., 2010; Tzortzakis et

al., 2011).

The effectiveness of O₃ against pathogens is influenced by storage conditions, particularly relative humidity and temperature (Miller et al., 2013; Batakliev et al., 2014; Egorova et al., 2015). While the potential of ozone has extensively been evaluated for other fresh horticultural products, it has received little attention from the mango industry. For instance, the best ozone concentration and exposure time for mangoes are currently not known. Moreover, the relationship between harvest maturity and ozone concentration has never been determined. Thus, concerted research efforts must be made to ascertain the possible role of ozone as a postharvest treatment of mangoes.

6.2 Pulsed electric field

Pulsed electric field (PEF) is a non-thermal technology used for microbial inactivation of food items. The use of PEF is linked with reduced respiration rate as well as retention of nutritional quality attributes such as ascorbic acid and antioxidants (González-Casado et al., 2018). Although the effect of PEF on fresh mangoes has not yet been reported, treating 'Mallika' mango nectar with PEF has been shown to inactivate microflora and maintain sensory quality attributes (Kumar et al., 2015). Ascorbic acid and alpha-tocopherol are some of the key antioxidants that prevent off-flavour development in mango fruit (Kaur et al., 2020). Similarly, a recent report by Kumar et al. (2019) demonstrated that PEF (70-120 Hz pulse frequency, 15-24 µs pulse width) treatment significantly prolonged the shelf-life of mango nectar stored at 5°C for ninety days. The authors linked the prolonged shelf-life with the ability of PEF to kill pathogens without compromising food quality. The mechanism of PEF on the inactivation of microorganisms is not well understood. Moreover, there is limited data available on PEF as a postharvest treatment of mango

fruit. Considering that PEF is non-thermal and could be cost-effective compared to the currently used postharvest treatments, research assessing its potential for the mango fruit industry is warranted.

7   Conclusion and research prospects

The literature provides exciting findings on emerging technologies such as PEF and O_3. While the use of such postharvest treatments has yielded impressive results, they are yet to be commercially adopted. Obtaining a balance between fruit quality and consumer safety is required for the product to be accepted by consumers and industry. Further research is necessary to gain understanding and explore the potential use of these technologies on the quality of fresh produce. The ozone decomposition mechanism is intricate, and research should aim at enhancing the efficacy of ozone and explore different exposure times suitable for each harvest maturity and mango cultivar. The potential of O_3 in combination with other treatments such as edible coatings to decrease postharvest diseases and enhance fruit quality requires further investigation. There is a gap of knowledge on postharvest use of O_3 incorporated with edible coatings such as CMC and moringa in mango fruit. Moreover, further research is needed to develop useful cellulose-based coatings to prolong shelf-life. Future research should focus on understanding the mode of action of O_3 incorporated with other postharvest treatments and commercializing these postharvest technologies.

8   References

Abbasi, K.S., Anjum, N., Sammi, S,. Masud, T., Ali, S. 2011. Effect of coatings and packaging material on the keeping quality of mangoes (*Mangifera indica* L.) stored at low temperature. Pakistan J. Nutr. 10(2): 129-138.

Abd El-Gawad, M.G., Zaki, Z.A., Ekbal. Z.A. 2019. Effect of some postharvest treatments on quality of "Alphonse" mango fruits during cold storage. Middle East J. 8 (4): 1067-1079.

Abu-Sarra, A.F., Abu-Goukhi, A.A. 1992. Changes in pectinesterase, polygalacturonse and cellulase activity during mango fruit ripening. J. Hortic. Sci. 67(4): 561-568. https://doi.org/10.1080/00221589.1992.11516284.

Ali, A., M. Maqbool, P.G. Alderson Zahid, N. 2012. Efficacy of biodegradable novel edible coatings to control postharvest anthracnose and maintain quality of fresh horticultural produce. Acta Horticulturae 945: 39-44. doi:10.17660/ActaHortic.2012.945.3

Ali, A., Maqbool, M., Ramachandran, S., Alderson. P.G., 2010. Gum arabic as a novel edible coating for enhancing shelf-life and improving postharvest quality of tomato (*Solanum lycopersicum* L.) fruit. Postharvest Biol. Tech. 58, 42-47. doi: 10.1016/j.postharvbio.2010.05.005.

Ali, A., Yeoh, W. K., Forney, C., Siddiqui, M. W. 2017. Advances in postharvest technologies to extend the storage life of minimally processed fruits and vegetables. Crit. Rev. Food Sci. Nutr. https://doi.org/10.1080/10408398.2017.1339180.

Altendorf, S. 2017. Global prospects for major tropical fruits. Short-term outlook, challenges and opportunities in a vibrant global marketplace. Food outlook. http://www.fao.org/fileadmin/templates/est/COMM_MARKETS_MONITORING/Tropical_Fruits/Documents/Tropical_Fruits_Special_Feature.pdf.

Alvindia, D.G., Acda, M.A. 2015. Revisiting the efficacy of hot water treatment in managing anthracnose and stem-end rot diseases of mango cv. 'Carabao'. Crop Prot. 67, 96-101. http://dx.doi.org/10.1016/j.cropro.2014.09.016.

Anglada, J.M., Crehuet, R., Bofill, J.M. 1999. The ozonolysis of ethylene: a theoretical study of the gas-phase reaction mechanism. Chem. Eur. J. 5(6): 1809-1822. https://doi.org/10.1002/(SICI)1521-3765(19990604)5:6<1809::AIDCHEM1809>3.0.CO;2-N.

Asghari, M., Aghdam, M.S. 2010. Impact of salicylic acid on post-harvest physiology of horticultural crops. Trends in Food Sci. Technol. 21, 502-509. doi: 10.1016/j.tifs.2010.07.009.

Asio, L.G., Cuaresma. F.D. 2016. A review of postharvest treatments to maintain mango (*Mangifera Indica* L.) quality. Annals of Trop. Res. 38(1): 81-93. doi: 10.32945/atr3817.2016.

Awad, M.A., Al-Qurashi, A.D., Mohamed, S.A., 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. J. Food Sci. Technol. 54(13): 4139-4148. https://doi.org/10.1007/s13197-017-2762-x.

Aziz, N.A.A., Wong, L.M., Bhat, R., Cheng, L.H. 2012. Evaluation of processed green and ripe mango peel and pulp flours (*Mangifera indica* var. Chokanan) in terms of chemical composition, antioxidant compounds and functional properties. J. Sci. Food Agric. 92, 557-563. https://doi.org/10.1002/jsfa.4606.

Baldwin, E.A., Nisperos-Carriedo, M.O., Baker, R.A. 1995. Edible coatings for lightly processed fruits and vegetables. Hort. Sci. 30(1): 35-38.

Barbosa-Martínez, C., Ponce de León-García, L., Sepúlveda-Sánchez, J. 2002. Effects of ozone, iodine and chlorine on spore germination of fungi isolated from mango fruits. Revista Mexicana de

Fitopatología 20(1): 60-65

Barman, K., Asrey, R. 2014. Salicylic acid pre-treatment alleviates chilling injury, preserve bioactive compounds and enhances shelf life of mango fruit during cold storage. J. Sci. Ind. Resh. 73, 713-718.

Barman, K., Asrey, R. Pal, R.K. Jha, S.K. Bhatia, K. 2014. Post-harvest nitric oxide treatment reduces chilling injury and enhances the shelf-life of mango (*Mangifera indica* L.) fruit during low-temperature storage. J. Hort. Sci. Biotech. 89(3): 253-260. http://dx.doi.org/10.1080/14620316.2014.11513076.

Batakliev, T., V. Georgiev, M., Anachkov, S., Rakovsky, G.E., Zaikov. 2014. Ozone decomposition. Interdisc. Toxicol. **7**(2): 47-59. doi: 10.2478/intox-2014-0008.

Bender, R.J., Seibert, E., Brecht, J.K. 2003. Heat treatment effects on ACC oxidase activity of 'Keitt' mangoes. Braz. J. Plant Physiol. 15(3): 145-148. https://doi.org/10.1590/S1677-04202003000300003.

Bourtoom, T. 2008. Edible films and coatings: characteristics and properties. Int. Food Res. J. 15(3): 237-248.

Castillo-Israel, K.A.T., Gandia, J.B.L., Velez, A.C.G., Absulio, W.L., Bainto, L.C. 2015. Storage quality of fresh-cut Philippine 'Carabao' mango (*Mangifera indica* L. cv. 'Carabao') fruits with 1-methylcyclopropene (1-MCP) post-cutting treatment. Int. Food Res. J. 22(6): 2196-2202.

Chourasia, A., Sane, V.A., Nath, P. 2006. Differential expression of pectate lyase during ethylene-induced postharvest softening of mango (*Mangifera indica* var. Dashehari). Physiologia Plantarum 128, 546-555. https://doi.org/10.1111/j.1399-3054.2006.00752.x.

Chourasia, A., Sane, V.A., Singh, R.K., Nath, P. 2008. Isolation and characterization of the MiCel1 gene from mango: ripening related expression and enhanced endoglucanase activity during softening. Plant Growth Regul. 56, 117-127. https://doi.org/10.1007/s10725-008-9292-5.

Cissé, M., Polidori, J., Montet, D., Loiseau, G., Ducamp-Collin, M.N. 2015. Preservation of mango quality by using functional chitosan lactoperoxidase systems coatings. Postharvest Biol. Tec. 101, 10-14. https://doi.org/10.1016/j.postharvbio.2014.11.003.

Cosme Silva, G.M., Silva, W.B., Medeiros, D.B., Salvador, A.R., Cordeiro, M.H.M., da Silva, N.M., Santana, D.B., Mizobutsi. G.P. 2017. The chitosan affects severely the carbon metabolism in mango (*Mangifera indica* L. cv. Palmer) fruit during storage. Food Chem. 237, 372-378. https://doi.org/10.1016/j.foodchem.2017.05.123.

Costa, J.D.D.S., Figueiredo Neto, A., Almeida, F.D.A.C., Costa. M.D.S. 2018. Conservation of 'Tommy Atkins' mangoes stored under passive modified atmosphere. Rev. Caatinga. 31(1): 117-125. http://dx.doi.org/10.1590/1983-21252018v31n114rc.

da Silva Neto, O.P., da Silva Pinto, E.V., Ootani, M.A., da Silva Junior, J.L., da Silva Bentes Lima, J. L., de Sousa. A.E.D. 2019. Ozone slows down anthracnose and increases shelf life of papaya fruits. Rev. Bras. Frutic., Jaboticabal. 41(5): e-439. doi: http://dx.doi.org /10.1590/0100-29452019439

Daisy, L.L., Nduko, J.M., Joseph, W. M., Richard, S.M. 2020. Effect of edible gum Arabic coating on the shelf life and quality of mangoes (*Mangifera indica*) during storage. J. Food Sci. Tech. 57(1): 79-85. https://doi.org/10.1007/s13197-019-04032-w.

Dautt-Castro, M., A. Ochoa-Leyva, C.A. Contreras-Vergara, A. Muhlia-Almazán, M. Rivera-Domínguez, S. Casas-Flores, Martinez-Tellez, M.A., Sañudo-Barajas, A., Osuna-Enciso, T., Baez-Sañudo, M.A., Quiroz-Figueroa, F.R., Kuhn, D.N., Islas-Osuna, M.A. 2018. Mesocarp RNA-Seq analysis of mango (*Mangifera indica* L.) identify quarantine postharvest treatment effects on gene expression. Sci. Hortic. 227, 146-153. https://doi.org/10.1016/j.scienta.2017.09.031.

de Almeida Teixeira, G.H., Durigan, J.F. 2011. Storage of 'Palmer' mangoes in low-oxygen atmospheres. Fruits 66(4): 279-289. doi: 10.1051/fruits/2011037.

de Almeida Teixeira, G.H., Santos, L.O., Júnior, L.C.C., Durigan, J.F. 2018. Effect of carbon dioxide (CO_2) and oxygen (O_2) levels on quality of 'Palmer' mangoes under controlled atmosphere storage. J. Food Sci. Tech. 55(1): 145-156. doi: 10.1007/s13197-017-2873-4.

Denga, Y., Wu, Y. Li. Y. 2006. Physiological responses and quality attributes of 'Kyoho' grapes to controlled atmosphere storage. LWT 39, 584-590. doi:10.1016/j.lwt.2005.05.001.

Diaz-Sobac, R., Pérez-Flores, L., Vernon-Carter, E.J. 2000. Emulsion coating to extend postharvest life of mango (*Mangifera indica* cv. Manila). J. Hortic. Sci. Biotech. 75(1): 126-128. https://doi.org/10.1111/j.1745-4549.1996.tb00742.x.

Ding, P., Mijin. S. 2013. Physico-chemical characteristics of Chok Anan mango fruit after hot water treatment. Pertanika J. Trop. Agric. Sci. 36(4): 359-372

Ding, Z.S., Tian, S.P., Zheng, X.L., Zhou, Z.W., Xu, Y. 2007. Responses of reactive oxygen metabolism and quality in mango fruit to exogenous oxalic acid or salicylic acid under chilling temperature stress. Physiol. Plant. 130, 112-121. doi: 10.1111/j.1399-3054.2007.00893.x.

Ducamp-Collin, M.N., Reynes, M., Lebrun, M., Freire Jr. M. 2009. Fresh cut mango fruits: Evaluation of edible coatings. Proc. VIIIth Int. Mango Symposium, Acta Hort. 820, ISHS.

Egorova, G.V., Voblikova, V.A., Sabitova, L.V., Tkachenko, I. S.,Tkachenko, S. N., Lunin, V.V. 2015. Ozone solubility in water. Vestn. Mosk. Univ. Khimiya. 5, 261-265. doi: 10.3103/S0027131415050053.

Faasema, J., Alakali, J.S. Abu, J.O. 2012. Effects of storage temperature on 1-methylcyclopropene-treated mango (*Mangnifera indica*) fruit varieties. J. Food Process. Pres. 38, 289-295. https://doi.org/10.1111/j.1745-4549.2012.00775.x.

Freschi, L. 2013. Nitric oxide and phytohormone interactions: Current status and perspectives. Front. Plant Sci. doi: 10.3389/fpls.2013.00398.

Gava, C.A.T., de Castro, A.P.C., Pereira, C.A., Fernandes-Júnior, P.I. 2018. Isolation of fruit colonizer yeasts and screening against mango decay caused by multiple pathogens. Biol. Control. 117, 137-146. https://doi.org/10.1016/j.biocontrol.2017.11.005.

George, D.S., Razali, Z., Santhirasegaram, V., Somasundram, C. 2015. Effects of ultraviolet light (UV-C) and heat treatment on the quality of fresh-cut Chokanan mango and Josephine pineapple. Journal of Food Sci. 80(2): 426-434. doi: 10.1111/1750-3841.12762.

George, D.S., Razali, Z., Santhirasegaram, V., Somasundram, C. 2016. Effect of postharvest ultraviolet-C treatment on the proteome changes in fresh cut mango (*Mangifera indica* L. cv. Chokanan). J. Sci. Food Agric. 96, 2851-2860. https://doi.org/10.1002/jsfa.7454.

González-Aguilar, G.A., Wang, C.Y., Buta, J.G., Krizek, D.T. 2001. Use of UV-C irradiation to prevent decay and maintain postharvest quality of ripe `Tommy Atkins' mangoes. Int. J. Food Sci. Tech. 36, 767-773. https://doi.org/10.1111/j.1365-2621.2001.00522.x.

González-Aguilar, G.A., M.A. Villegas-ochoa, M.A. Martínez-Téllez, A.A. Gardea, Ayala-Zavala, J.F. 2007. Improving antioxidant capacity of fresh-cut mangoes treated with UV-C. J. Food Sci.: Sens. Nutr. Qual. Food 72(3): 197-202. https://doi.org/10.1111/j.1750-3841.2007.00295.x.

González-Casado, S., Martín-Belloso, O., Elez-Martínez, P., Soliva-Fortuny, R. 2018. Enhancing the carotenoid content of tomato fruit with pulsed electric field treatments: Effects on respiratory activity and quality attributes. Postharvest Biol. Tech. 137, 113-118. https://doi.org/10.1016/j.postharvbio.2017.11.017.

Gurjar, P.S., Garg, N., Yadav, K.K., Lenka, J., Shukla, D.K. 2018. Effect of chitosan on biochemical and microbial quality of minimally processed mango (*Mangifera indica* L.) cubes during storage. Appl. Biol. Res. 20(1): 98-103. doi: 10.5958/0974-0112.2018.00022.1.

Hafeez, O., Malik, U., AKhalid, M.S., Amin, M., Khalid, S., Umar, M. 2016. Effect of modified atmosphere packaging on postharvest quality of mango cv. Sindhri and Sufaid Chaunsa during storage. Turk. J. Agric.-Food Sci. Tech. 4(12): 1104-1111.

He, J., Ren, Y., Chen, C., Liu, J., Liu, H., Pei. Y. 2016. Defense responses of salicylic acid in mango fruit against postharvest anthracnose, caused by *Colletotrichum gloeosporioides* and its possible mechanism. J. Food Safety 37. doi: 10.1111/jfs.12294.

Hoa, T.T., Self, G., Ducamp, M.N. 2010. Effects of hot air treatment on postharvest quality of 'cat Hoa loc' mangoes. Fruits 65(4): 237-244. doi: 10.1051/fruits/2010019.

Hong, K., Gong, D., Xu, H.S., Wang, S., Jia, Z., Chen, J.L., Zhang, L. 2014. Effects of salicylic acid and nitric oxide pretreatments on the expression of genes involved in the ethylene signaling pathway and the quality of postharvest mango fruit. N. Z. J. Crop Hortic. Sci. 42 (3): 205-216. http://dx.doi.org/10.1080/01140671.2014.892012.

Hossain, M.A., Rana, M.M., Kimura, Y. Roslan,H.A. 2014. Changes in biochemical characteristics and activities of ripening associated enzymes in mango fruit during the storage at different temperatures. Hindawi Publishing Corporation BioMed Res. Int. ID 232969. http://dx.doi.org/10.1155/2014/232969 .

Hu, M., Yanga, D., Huber, D.J., Jiang, Y., Lia, M., Gao, Z., Zhang, Z. 2014. Reduction of postharvest anthracnose and enhancement of disease resistance in ripening mango fruit by nitric oxide treatment. Postharvest Biol. Technol. 97, 115-122. http://dx.doi.org/10.1016/j.postharvbio.2014.06.013.

Jitareerat, P., Paumchai, S., Kanlayanarat, S., Sangchote, S. 2007. Effect of chitosan on ripening, enzymatic activity, and disease development in mango (*Mangifera indica*) fruit. N. Z. J. Crop Hortic. Sci. 35, 211-218. https://doi.org/10.1080/01140670709510187.

Junmatong, C., Faiyue, B., Rotarayanont, S., Uthaibutra, J., Boonyakiat, D., Saengnil, K. 2015. Cold storage in salicylic acid increases enzymatic and non-enzymatic antioxidants of Nam Dok Mai No. 4 mango fruit. Sci. Asia 41, 12-21. doi: 10.2306/scienceasia1513-1874.2015.41.012.

Kaur, K., Kaur, G., Brar, J.S. 2020. Pre-harvest application of hexanal formulations for improving post-harvest life and quality of mango (*Mangifera indica* L.) cv. Dashehari. J Food Sci. Technol. 57,4257-4264. https://doi.org/10.1007/s13197-020-04464-9.

Ketsa, S., Chidtragool, S., Klein, J.D., Lurie, S. 1998. Effect of heat treatment on changes in softening, pectic substances and activities of polygalacturonase, pectinesterase and β-galactosidase of ripening mango. J. Plant Physiol. 153, 457-461.

Khaliq, G., Mohamed, M.T.M. Ali, A., Ding, P., Ghazali, H.M. 2015. Effect of gum arabic coating combined with calcium chloride on physico-chemical and qualitative properties of mango (*Mangifera indica* L.) fruit during low temperature storage. Sci. Hortic. 190, 187-194. http://dx.doi.org/10.1016/j.scienta.2015.04.020.

Khaliq, G., Mohamed, M.T.M., Ghazali, H.M., Ding, P., Ali, A. 2016a. Influence of gum arabic coating enriched with calcium chloride on physiological, biochemical and quality responses of mango (*Mangifera indica* L.) fruit stored under low temperature stress. Postharvest Biol. Tech. 111, 362-369. http://dx.doi.org/10.1016/j.postharvbio.2015.09.029.

Khaliq, G., Mohamed, M.T.M., Ding, P., Ghazali, H.M., Ali, A. 2016b. Storage behaviour and quality responses of mango (*Mangifera indica* L.) fruit treated with chitosan and gum arabic coatings during cold storage conditions. Int. Food Res. J. 23(Suppl): S141-S148.

Khaliq, K., Mehar, U.N. Muhammad, R. Naimatullah, K. 2017. Textural properties and enzyme activity of mango (*Mangifera indica* L.) fruit coated with chitosan during storage. J. Agric. Stud. 5(2). https://doi.org/10.5296/jas.v5i2.10946.

Kumar, R., Bawa, A.S., Kathiravan, T., Nadanasabapathi, S. 2015. Optimization of pulsed electric field parameters for mango nectar processing using response surface methodology. Int. Food Res. J. 22(4):1353- 1360.

Kumar, R., Vijayalakshmi, S., Rajeshwara, R., Sunny, K., Nadanasabapathi, S. 2019. Effect of storage on thermal, pulsed electric field and combination processed mango nectar. J. Food Meas. Charact. 13(1): 131-143. https://doi.org/10.1007/s11694-018-9926-x.

Lalel, H.J.D., Singh, Z. 2004. Biosynthesis of aroma volatile compounds and fatty acids in 'Kensington Pride' mangoes after storage in a controlled atmosphere at different oxygen and carbon dioxide concentrations. J. Hortic. Sci. Biotech. 79(3): 343-353. http://dx.doi.org/10.1080/14620316.2004.11511771.

Lalel, H.J.D., Singh, Z., Tan, S.C. 2003.Elevated levels of CO_2 in controlled atmosphere storage affects shelf life, fruit quality and aroma volatiles of mango. In: XXVI International Horticultural Congress:Issues and Advances in Postharvest Horticulture 628, 407-413.

Lawson, T., Lycett, G.W., Ali, A., Chin, C. F. 2019. Characterization of Southeast Asia mangoes (*Mangifera indica* L.) according to their physicochemical attributes. Sci. Hortic. 243, 189-196. https://doi.org/10.1016/j.scienta.2018.08.014.

Le, T., Shiesh, C., Lin, H. 2010. Effect of vapor heat and hot water treatments on disease incidence and quality of Taiwan native strain mango fruits. Int. J. Agric. Biol. 12, 673-678.

Li, P., Zheng, X. 2015. Prestorage application of oxalic acid to alleviate chilling injury in mango fruit. HortScience 50(12):1795-1800.

Liu, X., Fu, Y., Guo, P., Xu, W. 2018. Modified atmosphere packaging and postharvest treatments on mango preservation: a review. In: Applied Sciences in Graphic Communication and Packaging. 511-516. Springer, Singapore.

López-López, M.E., López-Valenzuela, J.A., Delgado-Vargas, F., López-Angulo, G., Carrillo-López, A., Ayón-Reyna, L.E., Vega-Garcí. M.O. 2018. A treatment combining hot water with calcium lactate improves the chilling injury tolerance of mango fruit. HortScience 53 (2): 217-223. https://doi.org/10.21273/HORTSCI12575-17.

López-Mora, L.I., Gutiérrez-Martínez, P., Bautista-Baños, S., Jiménez-García, L.F., Zavaleta-Mancera, H.A. 2013. Evaluation of antifungal activity of chitosan in *Alternaria alternata* and in the quality of 'Tommy Atkins' mango during storage. Rev. Chapingo Ser. Hortic. 19(3): 315-331. doi: 10.5154/r.rchsh.2012.07.038.

Luria, N., N. Sela, Yaari, M., Feygenberg, O., Kobiler, I, Lers, A., Prusky, D. 2014. De-novo assembly of mango fruit peel transcriptome reveals mechanisms of mango response to hot water treatment. BMC Genomics. 15, 957. http://www.biomedcentral.com/1471-2164/15/957.

Mathooko, F.M. 1996. Regulation of respiratory metabolism in fruits and vegetables by carbon dioxide. Postharvest Biol. Technol. 9, 247-264.

Mditshwa, A., Magwaza, L.S., Tesfay, S.Z. Opara, U.L. 2017. Postharvest factors affecting vitamin C content of citrus fruits: A review. Sci. Hortic. 218, 95-104. http://dx.doi.org/10.1016/j.scienta.2017.02.024.

Miller, F.A., Silva, C.L.M., Brandão, T.R.S. 2013. A review on ozone-based treatments for fruit and vegetables preservation. Food Eng. Rev. 5, 77-106. doi: 10.1007/s12393-013-9064-5.

Minas, I.S., Vicente, A.R. Dhanapal, A.P. Manganaris, G.A. Goulas, V. Vasilakakis, M. Crisosto, C.H. Molassiotis, A. 2014. Ozone-induced kiwifruit ripening delay is mediated by ethylene biosynthesis inhibition and cell wall dismantling regulation. Plant Sci. 229, 76-85. http://dx.doi.org/10.1016/j.plantsci.2014.08.016

Minas, I.S., Karaoglanidis, G. S. Manganaris, G.A. Vasilakakis, M. 2010. Effect of ozone application during cold storage of kiwifruit on the development of stem-end rot caused by *Botrytis cinerea*. Postharvest Biol. Technol. 58, 203-210. doi:10.1016/j.postharvbio.2010.07.002.

Mohamed, N.T.S., Ding, P., Kadir, J., Ghazali, H.M. 2017. Potential of UVC germicidal irradiation in suppressing crown rot disease, retaining postharvest quality and antioxidant capacity of *Musa* AAA "Berangan" during fruit ripening. Food Sci. Nutr. 5, 967-980. doi: 10.1002/fsn3.482.

Muhammad, I., Ashiru, S., Kanoma, A.I., Sani, I. Garba, S. 2014. Effect of ripening stage on vitamin C content in selected fruits. Int. J. Agric. Fores. Fish. 2(3): 60.

Ngamchuachit, P., Barrett, D.M., Mitcham, E.J. 2014. Effects of 1-methylcyclopropene and hot water quarantine treatment on quality of "Keitt" mangos. J. Food Sci. 79 (4). doi: 10.1111/1750-3841.12380.

Nongtaodum, S., Jangchud, A. 2009. Effects of edible chitosan coating on quality of fresh-cut mangoes (Fa-lun) during storage. Kasetsart J. (Nat. Sci.) 43, 282 -289. https://www.researchgate.net/publication/266466570.

Ntsoane, M.L., Luca, A., Zude-Sasse, M., Sivakumar, D., Mahajan, P.V. 2019a. Impact of low oxygen storage on quality attributes including pigments and volatile compounds in 'Shelly' mango. Sci. Hortic. 250, 174-183. https://doi.org/10.1016/j.scienta.2019.02.041.

Ntsoane, M.L., Zude-Sasse, M., Mahajan, P., Sivakumar, D. 2019b. Quality assesment and postharvest technology of mango: A review of its current status and future perspectives. Sci. Hortic. 249, 77-85. https://doi.org/10.1016/j.scienta.2019.01.033.

Ong, M.K., Alia, A. 2015. Antifungal action of ozone against *Colletotrichum gloeosporioides* and control of papaya anthracnose. Postharvest Biol. Technol. 100, 113-119. http://dx.doi.org/10.1016/j.postharvbio.2014.09.023.

Osuna-Garcia, J.A., Brecht, J.K., Huber, D.J., Nolasco-Gonzalez, Y. 2015. Aqueous 1-methylcyclopropene to delay ripening of 'Kent' mango with or without quarantine hot water treatment. HortTechnology 25(3): 349-357. https://www.researchgate.net/publication/282265613.

Osuna-García, J.A., Pérez-Barraza. M.H., Beltran, J.A. Vázquez-Valdivia, V. 2009. Methylcyclopropene (1-MCP), a new approach for exporting 'Kent' mangos to Europe and Japan. Proc.VIIIth Int. Mango Symposium. Acta Hort. 820, 721-724.

Pandit, S.S., Kulkarni, R.S., Giri, A.P., Köllner, T.G., Degenhardt, J., Gershenzon, J. Gupta, V.S. 2010. Expression profiling of various genes during the fruit development and ripening of mango. Plant Physiol. Biochem. 48, 426-433. doi: 10.1016/j.plaphy.2010.02.012.

Phaiphan, A., Rattanapanone, N. 2008. Effect of edible coatings on quality of mango fruit(*Mangifera indica*) 'Chok-Anan' during storage. Acta Hortic. 773, 227-232.

Phakawatmongkol, W., Ketsa, S., van Doorn, W.G. 2004. Variation in fruit chilling injury among mango cultivars. Postharvest Biol. Tec. 32, 115-118. doi: 10.1016/j.postharvbio.2003.11.011.

Plotto, A., Narciso, J.A., Rattanapanone, N., Baldwin, E.A. 2010. Surface treatments and coatings to maintain fresh-cut mango quality in storage. J. Sci. Food Agric. 90, 2333-2341. doi: 10.1002/jsfa.4095.

Plotto, A., Goodner, Baldwin, K.L., E.A., Bai, J. Rattanapanone, N. 2004. Effect of polysaccharide coating on quality of fresh cut mangoes (*Mangifera indica*). Proc. Fla. State Hort. Soc. 117, 382-388.

Promyou, S., Supapvanich, S. 2016. Physicochemical changes in 'Kaew Kamin' mango fruit illuminated with ultraviolet-c (UV-C) during storage. J. Agr. Sci. Tech. 18, 145-154.

Ramayya, N., Niranjan, K., Duncan, E. 2012. Effects of modified atmosphere packaging on quality of 'Alphonso' mangoes. J. Food Sci. Tech. 49(6): 721-728. doi: 10.1007/s13197-010-0215-x.

Rattanapanone, N., Lee, Y., Wu, T., Watada, A.E. 2001. Quality and microbial changes of fresh-cut mango cubes held in controlled atmosphere. HortScience 36(6): 1091-1095.

Razzaq, K., Singh, Z., Khan, A.S., Khan, S.A.K.U., Ullah, S. 2015. Role of 1-MCP in regulating 'Kensington Pride' mango fruit softening and ripening. Plant Growth Regul. doi: 10.1007/s10725-015-0101-7.

Romero, L., Colivet, J., Aron, N.M., Ramosvillarroel, A. 2017. Impact of ultraviolet light on quality attributes of stored fresh-cut mango. The Annals of the University of Dunarea de Jos of Galati. Fascicle VI. Food Technology 41(1): 62-80.

Romero-Puertas, M.C., Perazzolli, M., Zago, E.D., Delledonne, M. 2004. Nitric oxide signalling functions in plant-pathogen interactions. Cell. Microbiol. 6(9): 795-803. doi: 10.1111/j.1462-5822.2004.00428.x.

Safitri, A., Theppakorn, T., Naradisorn, M., Setha, S. 2015. Effects of UV-C irradiation on ripening quality and antioxidant capacity of mango fruit cv. Nam Dok Mai Si Thong. J. Food Sci. Agric. Tech. 1(1): 164-170. http://jfat.mfu.ac.th.

Sakhale, B.K., Gaikwad, S.S., Chavan. R.F. 2018. Application of 1-methylcyclopropene on mango fruit (cv. Kesar): potential for shelf life enhancement and retention of quality. J. Food Sci. Technol. 55(2): 776-781. https://doi.org/10.1007/s13197-017-2990-0.

Salinas-Roca, B., Guerreiro, A., Welti-Chanes, J., Antunes, M.D.C., Martín-Belloso, O. 2018. Improving quality of fresh-cut mango using polysaccharide-based edible coatings. Int. J. Food Sci. Tech. 53, 938-945. doi: 10.1111/ijfs.13666.

Sandhu, H.P.S., Manthey, F.A., Simsek, S. 2011. Quality of bread made from ozonated wheat (*Triticum aestivum* L.) flour. J. Sci Food Agric. 1(91): 1576-1584. doi: 10.1002/jsfa.4350.

Sane, V.A., Chourasia, A., Nath, P. 2005. Softening in mango (*Mangifera indica* cv. Dashehari) is correlated with the expression of an early ethylene responsive, ripening related expansin gene, *MiExpA1*. Postharvest Biol. Tech. 38, 223-230.

Scheler, C., Durner, J., Astier, J. 2013. Nitric oxide and reactive oxygen species in plant biotic interactions. Curr. Opin. Plant Biol. 16, 534-539. http://dx.doi.org/10.1016/j.pbi.2013.06.020.

Shezi, S., Magwaza, L.S., Mditshwa, A., Tesfay, S.Z. 2020a. Changes in biochemistry of fresh produce in response to ozone postharvest treatment. Sci. Hortic. 269, 109397. https://doi.org/10.1016/j.scienta.2020.109397.

Shezi, S., Tesfay, S.Z., Magwaza, L.S., Mditshwa, A., Motsa, N.M. 2020b. Gaseous ozone and plant extract-based edible coatings as postharvest treatments for 'Hass' avocado (*Persea americana* Mill.) fruit. Acta Hortic. 1275, 53-60. doi: 10.17660/ActaHortic.2020.1275.8.

Shukla, S., Srivastava, P. Choubey, S.K. Gomase, V.S. 2010. Comparative analysis and structure elucidation of Syntaxin- A novel component tends to defense mechanism in plant proteomics. J. Plant Genomics 1(1): 01-08.

Siddiqui, Y., Asgar, A. 2014. *Colletotrichum gloeosporioides* (Anthracnose). Postharvest Decay. Academic Press. 337-371. https://doi.org/10.1016/B978-0-12-411552-1.00011-9.

Singh, Z., Singh, R.K., Sane, V.A., Nath, P. 2013. Mango - postharvest biology and biotechnology. Crit. Rev. Plant Sci. 32, 217-236. doi: 10.1080/07352689.2012.743399.

Sivakumar, D., Fallik, E. 2013. Influence of heat treatments on quality retention of fresh and fresh-cut produce. Food Rev. Int. 29, 294-320. doi: 10.1080/87559129.2013.790048.

Sivakumar, D., Jiang, Y., Yahia, E.M. 2011. Maintaining mango (*Mangifera indica* L.) fruit quality during the export chain. Food Res. Int. 44, 1254-1263. doi: 10.1016/j.foodres.2010.11.022.

Sivankalyani, V., Feygenberg, O., Diskin, S., Wright, B., Alkan, N. 2016. Increased anthocyanin and flavonoids in mango fruit peel are associated with cold and pathogen resistance. Postharvest Biol. Tech. 111, 132-139. http://dx.doi.org/10.1016/j.postharvbio.2015.08.001.

Sophia, O., Robert, G.M., Ngwela, W.J. 2015. Effect of *Aloe vera* gel coating on postharvest quality and shelf life of mango (*Mangifera indica* L.) fruits var. Ngowe. J. Hortic. For. 7(1): 1-7.

Sothornvit, R., Rodsamran, P. 2008. Effect of a mango film on quality of whole and minimally processed mangoes. Postharvest Biol. Tech. 47(3): 407-415. doi: 10.1016/j.postharvbio.2007.08.005.

Sothornvit, R., Rodsamran, P. 2010. Mango film coated for fresh-cut mango in modified atmosphere packaging. Int. J. Food Sci. Tech. 45(8): 1689-1695.

Sripong, K., Jitareerat, P., Tsuyumu, S., Uthairatanakij, A., Srilaong, V., Wongs-Aree, C., Ma, G., Zhang, L., Kato, M. 2015. Combined treatment with hot water and UV-C elicits disease resistance against anthracnose and improves the quality of harvested mangoes. Crop Prot. 77, 1-8. http://dx.doi.org/10.1016/j.cropro.2015.07.004.

Srivastava, S., Bala, K.L. 2016. Effect of arabic gum-carboxymethylcellulose edible coatings on shelf life of button mushroom (*Agaricus bisporus*). Int. J. Res. Eng. Technol. 5(6): 484-494. http://ijret.esatjournals.org.

Sudheeran, P.K., Feygenberg, O. Maurer, D. Alkan, N. 2018. Improved cold tolerance of mango fruit with enhanced anthocyanin and flavonoid contents. Molecules 23, 1832. doi: 10.3390/molecules23071832.

Sumual, M.F., Singh, Z., Singh, S.P., Tan, S.C. 2017. Fruit ripening and quality of 'Kensington Pride' mangoes following the controlled atmosphere storage. Jurnal Teknologi Pertanian (Agricultural Technology Journal) 8(1).

Terao, D., de Carvalho Campos, J.S., Benato, E.A., Hashimoto, J.M. 2015. Alternative strategy on control of postharvest diseases of mango (*Mangifera indica* L.) by use of low dose of ultraviolet-c irradiation. Food Eng. Rev. 7, 171-175. doi: 10.1007/s12393-014-9089-4.

Tesfay, S.Z., 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 Packag. Shelf Life 11, 40-48. http://dx.doi.org/10.1016/j.fpsl.2016.12.001.

Tian, S.P., Liu, J. Zhang, C.F. Meng, X.H. 2010. Quality properties of harvested mango fruits and regulating technologies. New trends in postharvest management of fresh produce II. Fresh Produce 4, 49-54.

Tran, T.T.L., Aimla-or, S., Srilaong, V., Jitareerat, P., Wongs-Aree, C., Uthairatanakij, A. 2013. Fumigation with ozone to extend the storage life of mango fruit cv. Nam Dok Mai No. 4. Agric. Sci. J. 44(2): 663-672.

Tran, T.T.L., Aiamla-or, S., Srilaong, V., Jitareerat, P., Wongs-Aree, C., Uthairatanakij, A. 2015. Application of nitric oxide to extend the shelf life of mango fruit. Acta Hortic.: II Southeast Asia Symposium on Quality Management in Postharvest Systems. doi: 10.17660/ActaHortic.2015.1088.11.

Tzortzakis, N., Taybi, T. Antony, E. Singleton, I., Borland, A., Barnes, J. 2013. Profiling shifts in protein complement in tomato fruit induced by atmospheric ozone-enrichment and/or wound-inoculation with *Botrytis cinerea*. Postharvest Biol. Tech. 78, 67-75. http://dx.doi.org/10.1016/j.postharvbio.2012.12.005.

Tzortzakis, N., Taybi, T., Roberts, R., Singleton, I., Borland, A., Barnes, J. 2011. Low-level atmospheric ozone exposure induces protection against *Botrytis cinerea* with down-regulation of ethylene-, jasmonate- and pathogenesis-related genes in tomato fruit. Postharvest Biol. Technol. 61, 152-159. doi:10.1016/j.postharvbio.2011.02.013.

Ullah, H., Ahmad, S., Thompson, A.K., Ahmad, W., Nawaz, M.A. 2010. Storage of ripe mango (*Mangifera indica* L.) cv. Alphonso in controlled atmosphere with elevated CO_2. Pak. J. Bot. 42(3): 2077-2084.

Ullah, H., Ahmad, S., Amjad, M., Khan, M.A. 2012. Response of mango cultivars to modified atmosphere storage at an ambient temperature cv. Alphanso and Chounsa. Pak. J. Agri. Sci. 49(3): 323-329. http://www.pakjas.com.pk.

Valente, M., Ribeyre, F., Self, G., Berthiot, L., Assemat, S. 2011. Instrumental and sensory characterization of mango fruit texture. J. Food Qual. 34, 413-424. doi: 10.1111/j.1745-4557.2011.00412.x.

Vázquez-Celestino, D., Ramos-Sotelo, H., Rivera-Pastrana, D.M., Vázquez-Barrios, M.E., Mercado-Silva, E.M. 2016. Effects of waxing, microperforated polyethylene bag, 1-methylcyclopropene and nitric oxide on firmness and shrivel and weight loss of 'Manila' mango fruit during ripening. Postharvest Biol. Tech. 111, 398-405. http://dx.doi.org/10.1016/j.postharvbio.2015.09.030.

Vega-Alvarez, M., Salazar-Salas, N.Y., López-Angulo, G., Pineda-Hidalgo, K.V., López-López, M.E., Vega-García, M.O., Delgado-Vargas, F., López-Valenzuela, J.A. 2020. Metabolomic changes in mango fruit peel associated with chilling injury tolerance induced by quarantine hot water treatment. Postharvest Biol. Technol. 169, 111299. https://doi.org/10.1016/j.postharvbio.2020.111299.

Wang, B., Wang, J., Feng, X., Lin, L., Zhao, Y., Jiang, W. 2009. Effects of 1-MCP and exogenous ethylene on fruit ripening and antioxidants in stored mango. Plant Growth Regul. 57, 185-192. doi: 10.1007/s10725-008-9335-y.

Wang, H., Yang, Z., Song, F., Chen, F.W., Zhao, S. 2016. Effects of heat treatment on changes of respiration rate and enzyme activity of Ivory mangoes during storage. J. Food Process. Preserv. 41. doi: 10.1111/jfpp.12737.

Wang, J., Wang, B., Jiang, W., Zhao, Y. 2007. Quality and shelf life of mango (*Mangifera indica* L. cv. 'Tainong') coated by using chitosan and polyphenols. Food Sci. Tech. Int. 13(4):317-322. doi: 10.1177/1082013207082503.

Wang, K.L.C., Li, H., and Ecker, J.R. 2002. Ethylene biosynthesis and signaling networks. Plant Cell S131-S151. www.plantcell.org/cgi/doi/10.1105/tpc.001768.

Wongmetha, O., Ke, L. 2013. Changes in quality attributes and storage life of mango during storage. Adv. Mater. Res. 610, 3518-3521. doi: 10.4028/www.scientific.net/AMR.610-613.3518.

Wongmetha, O., Ke, L., Liang, Y. 2013. The changes in physico-chemical compositions of 'Irwin' mango fruit after postharvest treatments under low temperature storage. Thai J. Agric. Sci. 46(1): 11-19.

Wright, A.H., Delong, J.M., Arul, J., Prange, R.K. 2015. The trend toward lower oxygen levels during apple (*Malus × domestica* Borkh) storage. J. Hortic. Sci. Biotech. 90(1): 1-13. http://dx.doi.org/10.1080/14620316.2015.11513146.

Yashoda, H.M., Prabha, T.N., Tharanathan, R.N. 2006. Mango ripening: changes in cell wall constituents in relation to textural softening. J. Sci. Food Agric. 86, 713-721. doi: 10.1002/jsfa.2404.

Yimyong, S., Datsenka, T.U., Handa, A.K., Seraypheap, K. 2011. Hot water treatment delays ripening-associated metabolic shift in 'Okrong' mango fruit during storage. J. Amer. Soc. Hort. Sci. 136(6): 441-451.

Zaharah, S.S., Singh, Z. 2011a. Mode of action of nitric oxide in inhibiting ethylene biosynthesis and fruit softening during ripening and cool storage of 'Kensington Pride' mango. Postharvest Biol. Technol. 62(3): 258-266. doi: 10.1016/j.postharvbio.2011.06.007.

Zaharah, S.S., Singh, Z. 2011b. Post-harvest fumigation with nitric oxide at the pre-climacteric and climacteric-rise stages influences ripening and quality in mango fruit. J. Hortic. Sci. Biotech. 86(6): 645-653. doi: 10.1080/14620316.2011.11512817.

Zaharah, S.S., Singh, Z. 2013. Nitric oxide fumigation delays mango fruit ripening. Acta Hortic. doi: 10.17660/ActaHortic.2013.992.67.

Zaharah, S.S., Singh, Z., Symons, G.M., Reid, J.B. 2012. Role of brassinosteroids, ethylene, abscisic acid, and indole-3-acetic acid in mango fruit ripening. J. Plant Growth Regul. 31, 363-372. doi: 10.1007/s00344-011-9245-5.

Zainuri, D.C. Joyce, Wearing, A.H., Coates, L., Terry, L. 2001. Effects of phosphonate and salicylic acid treatments on anthracnose disease development and ripening of 'Kensington Pride' mango fruit. Aust. J. Exp. Agric. 41, 805-813.

Zeng, K., Cao, J., Jiang, W. 2006. Enhancing disease resistance in harvested mango (*Mangifera indica* L. cv. 'Matisu') fruit by salicylic acid. J. Sci. Food Agric. 86, 694-698. doi: 10.1002/jsfa.2397.

Zerbini, P.E., Vanoli, M., Rizzolo, A., Grassi, M., de Azevedo Pimentel, R.M., Spinelli, L., Torricelli, A. 2015. Optical properties, ethylene production and softening in mango fruit. Postharvest Biol. Technol. 101, 58-65. http://dx.doi.org/10.1016/j.postharvbio.2014.11.008.

Zhang, Z., Gao, Z., Li, M., Hu, M., Gao, H., Yang, D., Yang, B. 2012. Hot water treatment maintains normal ripening and cell wall metabolism in mango (*Mangifera indica* L.) fruit. HortScience 47(10):1466-1471.

Zheng, X., Hu, B., Song, L., Pan, J., Liu, M. 2017. Changes in quality and defense resistance of kiwifruit in response to nitric oxide treatment during storage at room temperature. Sci. Hortic. 222, 187-192. http://dx.doi.org/10.1016/j.scienta.2017.05.010.

Zhu, X., Wang, Q., Cao, J., Jiang, W. 2008. Effects of chitosan coating on postharvest quality of mango

(*Mangifera Indica* L. cv. Tainong) fruits. J. Food Process. Preserv. 32, 770

Chapter 3

Determination of gaseous ozone exposure time for storage of 'Keitt' mango fruit

Abstract

Ozone is a postharvest technology with no chemical residue and poses no health threats to the environment and human health. A screening study was conducted to optimize fruit exposure intervals to gaseous ozone (O_3) as a postharvest treatment of mangoes. Mango fruit *cv* 'Kiett' were exposed to O_3 (0.25 mg L^{-1}) intermittently for twelve, twenty-four, thirty-six, or forty-eight hours (i.e. Day 0, Day 7 or Day 14); and the control fruit were untreated. Fruit were stored at 10°C for twenty-one days, simulating shipment to European export markets and ripened at ambient temperature for seven days. Fruit quality parameters measured were titratable acidity (TA), mass loss (%), total soluble solids (TSS), pulp color, and total carotenoids content. Data was collected at seven-day intervals. Mass loss of untreated fruit (30.91%) was significantly higher compared to O_3 (D0 & 21), O_3 (D0), O_3 (D14), O_3 (D0 & 7), O_3 (D0, 7 & 14) and O_3 (D0, 7, 14 & 21) which were 29.35%, 28.48%, 27.00%, 25.90%, 20.54% and 20.49%, respectively. Fruit treated with O_3 (D0, 7 & 14) significantly maintained firmness, delayed TSS accumulation, and decreasedTA loss. The untreated fruit had a high decay percentage compared to other treatments at the end of storage. Treating mango fruit with O_3 (D0, 7, 14 & 21) enhanced the concentration of total carotenoids during storage. The concentration of total carotenoids was 490.19 µg g^{-1} for D0 and increased to 1613.92 µg g^{-1} in D21. The shelf-life of mango fruit was also improved by postharvest O_3 treatments with D0, 7 14, & 21 extending shelf-life by two days compared to the control fruit. The current study indicates that O_3 could be used effectively as an environmentally friendly gaseous or non-chemical postharvest treatment to maintain the quality and shelf-life of mango fruit.

Keywords: Ozone, mango, postharvest quality, shelf-life, carotenoids, decay index

1 Introduction

Mango (*Mangifera indica* L.) is one of the economically-important fruits due to its high nutritional properties (Hmmam et al., 2021). The fruit contains essential dietary compounds such as proteins, vitamins, flavonoids, phenolics, and carotenoids (Maldonado-Celis et al., 2019). However, the fruit is highly perishable with a limited shelf-life and is susceptible to fungal spoilage and postharvest decay (Nakakoga et al., 2021). Various postharvest technologies are used to enhance fruit quality, and these include ozone (O_3), hot water, edible coatings, and chemical treatment. Ozone is produced by electric corona discharge or ultraviolet radiation (Pandiselvam et al., 2019). It has high oxidizing power and antimicrobial properties that are effective against an extensive spectrum of microorganisms (Brodowska et al., 2018a; Contigiani et al., 2018). The benefit of O_3 is that it doesn't have chemical residues that pose threats to the environment and human health. Ozone is an unstable gas that decomposes rapidly to form highly reactive oxidative radicals such as superoxide anion and hydroxyl radical (Perry and Yousef, 2011). It is speculated that hydroxyl radicals are responsible for the antimicrobial efficacy of O_3 (Perry and Yousef, 2011). The United States of America Food and Drug Administration (FDA, 2001) declared O_3 as Generally Recognized as Safe (GRAS). The FDA permitted O_3 as an additive in food and vegetables. This has positively influenced O_3 as a postharvest treatment in fresh fruit and vegetables.

Ozone is reported to extend fruit shelf-life by delaying ripening and senescence. For instance, Terao et al. (2019) reported that O_3 (3 mg mL^{-1}) reduced the respiration rate in 'THB' papaya during storage at 10°C for twelve days. Ozone oxidizes ethylene in fresh produce during storage (Aslam et al., 2020). The

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respiration rate influences biochemical changes that initiate cell wall degradation processes, resulting in loss of firmness and ripening. Previous research has shown that O_3 maintains firmness in various fresh horticultural products (Terao et al., 2019; Mustapha et al., 2020). For instance, Mustapha et al. (2020) reported that aqueous ozone (0.85 mg/L, 15 minutes) maintained firmness in cherry tomato during storage at 4°C for twenty-one days. Fruit softening is characterized by modification of cell wall components, including hemicellulose, pectin, and polysaccharides leading to loss of fruit firmness (Toti et al., 2018). Enzyme activities play an essential role in cell wall degradation. For instance, Cardenas-Perez et al. (2018) reported an increase in enzyme activities of polygalacturonase (PG) and pectin methylesterase (PME) during mango ripening. The cell wall pectin methyl esters are hydrolyzed by the PME enzyme (Toti et al., 2018). The PG enzyme degrades pectic polysaccharides into water-soluble galacturonides resulting in fruit softening (Cardenas-Perez et al., 2018).

Although ozone has been assessed as a postharvest treatment for various fruits and vegetables, there is currently very little available data on the use of O_3 in mangoes. Ozone could provide a potential solution in maintaining fruit quality and extending the shelf-life of mango fruit. The objective of this study was to determine the optimum O_3 exposure interval that effectively enhances fruit quality and prolongs shelf-life during cold storage.

2 Materials and methods

2.1 Fruit material

Mango fruit (*cv.* 'Keitt') were harvested from Goedgelegen Farm of Westfalia (Pty) Ltd, a commercial farm located in Tzaneen, South Africa. Fruit were transported overnight under cold storage to the University of KwaZulu-Natal (Pietermaritzburg Campus), Postharvest Research Laboratory. On arrival at the laboratory, fruits were graded for uniformity according to color and size and stored in ventilated carton boxes. Mango fruit were subjected to gaseous ozone for 12 hours at seven-day intervals during cold storage. Figure 3.1 illustrates the ozone treatment and exposure intervals used in the experiment, while the control fruit were untreated. Fruit were stored at 10°C and 90% relative humidity (RH) for twenty-one days, simulating shipment to the European markets (Sivakumar et al., 2012). After cold storage, fruit were ripened at ambient temperature for seven days. Data was collected after every seven days.

2.2 Ozone treatment

Corona discharge ozone generator (Ozone Purification Technology, Johannesburg, South Africa) was used to generate gaseous ozone. As mentioned above, mango fruit were treated with O_3 (0.25 mg L^{-1}) at various levels during cold storage. The O_3 levels resulted in sixteen treatments, and the results of eight treatments were reported. These treatments were selected based on their performance and classified according to best, average, and poor performance. The following treatments are reported:

Control

T1: O_3 day 0

T2: O_3 day14

T3: O_3 day 0 & 7

T4: O_3 day 0 & 21

T5: O_3 day 14 &21

T6: O_3 day 0, 7 & 14

T7: O_3 day 0, 7, 14 & 21
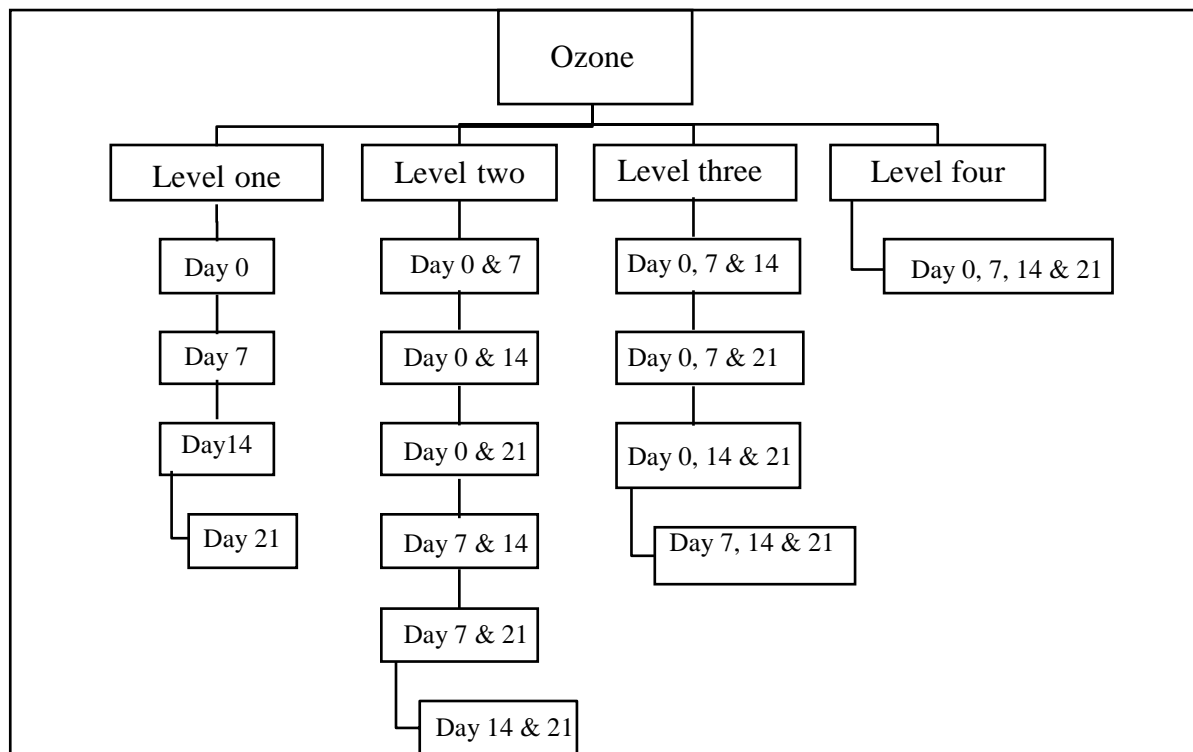
Figure 3.1: Gaseous ozone application intervals

1.1 Percentage weight loss

Fruit were weighed on day zero (i.e. on arrival at the lab) and at the end of each storage interval, using a separate sample of three replicates for each treatment. The mass loss was calculated using Equation One by Akhtar et al. (2010a):

$$\text{Mass loss (\%)} = \frac{A-B}{A} x 100 \qquad (1)$$

Where A indicates the fruit mass at harvest and B is the fruit mass after storage.

1.2 Fruit firmness

Mango firmness was measured as described by González-Aguilar et al. (2001), using the handheld firmness tester (Bareiss, Germany) equipped with an 8 mm plunger tip. Firmness was measured on opposite cheeks of the fruit, and the results were expressed as newton (N).

1.3 Fruit color

Color changes in mango pulp color were determined as described by González-Aguilar et al. (2001), using a chromameter (Chroma Meter, Konica Minolta Sensing, INC., Japan). The color parameters considered were Luminosity (*L*), *a*, *b*, °Hue angle (H), and Chromaticity (c). Where L = 100 to L = 0 indicates the lightness from white to black. The +*a*, −*a* indicate the degree of red and green, and +b, −*b* show yellow toblue. In triplicates, readings were done on the surface of half-cut mango pulp. Prior to scanning fruit, thechromameter was calibrated by scanning a white reference brick, y = 0.3215, Y = 87.0, X = 0.3.

1.4 Titratable acidity (TA) and total soluble solids (TSS)

Fruit were peeled, cut into cubes, and homogenized using a blender. The puree was filtered three times using a muslin cloth. The juice was used for TSS and TA analysis. The TSS was analyzed using a digital

refractometer (B+S RFM340+ refractometer, Bellingham and Stanley Ltd, Tunbridge Wells, Kent, United Kingdom) and reported as a percentage. The TA was tested using the automated titration (Rondolino G 20's Compact Titrator, Mettler Toledo, Schwerzenbach, Switzerland). Before titration, the sensor was calibrated using buffer standards with pH 4 and 7, with the slope and zero point (offset): Slope: - 59.16 mV/pH @ 25 °C and Zero point: +/- 0 mV or pH 7.00. Distilled water (40 mL) was mixed with 8 mL of juice and titrated to the endpoint (pH 8.1) with 0.1 M of sodium hydroxide (NaOH) and expressed as % malic acid using Equation Two:

$$\%\text{TA} = \frac{0.067 \ x \ Titre \ x \ (NaOH) \ 100}{Juice \ used} \tag{2}$$

1.5 Decay index (DI)

For each treatment, nine fruits were evaluated for the DI on the peel. Fruits were classified into four groups according to their severity (Score 0-4). Score 0 (none) means no visible decay, 1 means that there is few (1%) scattered decay, 2 means that 2-20% of fruit surface is covered by decay, 3 means that there is 21-50% decay which covers the fruit and 4 means that there is > 50% fruit decay. The DI index was calculated as defined by Khaliq et al. (2016a):

$$\text{DI} = \Sigma \ \frac{(Disease \ scale \ x \ number \ of \ fruit \ in \ each \ class) \ x100}{Number \ of \ total \ fruit \ x \ highest \ disease \ scale} \tag{3}$$

1.6 Total carotenoids

The concentration of total carotenoids was measured as described by More and Rao (2019) with

modification. In triplicates, a sample (1 g) was added to 14 mL of hexane: acetone (3:2 *v/v*) and incubated for 1.5 hours in the dark. Thereafter, samples were centrifuged (Avanti J-265 XP, Beckaman Coulter, Indianapolis, USA) at 10 000 rpm for ten minutes at 4°C. The extract was transferred to a volumetric flask and adjusted to 25 mL using hexane: acetone. The absorbance was measured at 450 nm using a spectrophotometer (Shimadzu Scientific Instruments INC., Columbia USA) against hexane: acetone as ablank. Results were expressed as μg g^{-1} DM.

1.7 Statistical analysis

The collected data was subjected to the analysis of variance (ANOVA) using GenStat statistical software (GenStat®, 18 edition, VSN International, UK). Mean separation was performed using Fischer's least significant difference (LSD) at a 5% level of significance. Pearson correlation coefficient was used to determine relationships between each physicochemical parameter.

1 Results

1.1 Fruit mass loss

Fruit mass loss (%) increased sharply during storage in treated and untreated mango fruit (Figure 3.2). Ozone significantly ($p < 0.05$) reduced the mass loss percentage in mango fruit. At day fourteen, mass loss of O_3(D0 &7), O_3 (D0 & 21), O_3 (0, 7 & 14) and O_3 (0, 7, 14 & 21) was 7.85%, 8.03%, 7.33% and 6.98%, respectively. At the end of storage, untreated fruit had the highest mass loss compared to O_3 (D0 & 21), O_3 (D0), O_3 (D14), O_3 (D0 & 7), O_3 (D0, 7 & 14). The treatment means of O_3 (D0), O_3 (D14), O_3 (D0, 7 & 21) were not significantly different at the end of storage.
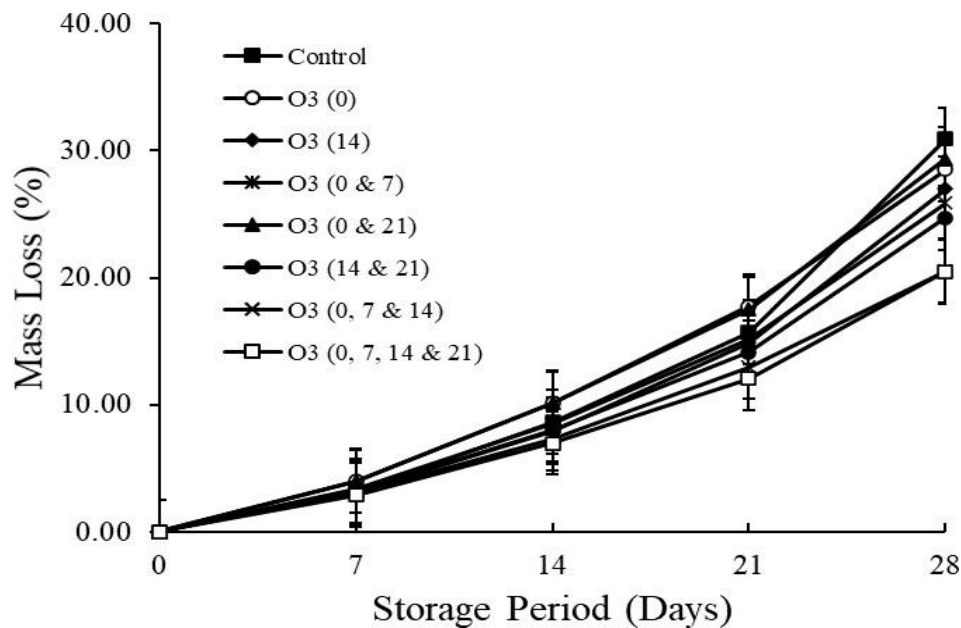
Figure 3.2: Effect of gaseous ozone on fruit mass loss stored at 10°C for twenty-one daysand seven days shelf-life (±SE, *n* = 9)

1.2 Fruit firmness

Fruit firmness decreased significantly ($p < 0.01$) in all treatments during storage (Table 3.1). The low fruit firmness was observed in fruit treated with O_3 (D14) compared to other treatments at the end of storage. However, no significant differences were observed between treatment means of O_3 (D0) and the untreated fruit. The firmness of fruit treated with O_3 (D0 & 21) was higher compared to O_3 (D0, 7 &14), O_3 (D0, 7,14 & 21), O3 (D14), and the untreated fruit. In the current study, treated fruit O_3 (D0 &7), O_3 (D0, 7 & 14), and O_3 (0, 7, 14 & 21) had high fruit firmness compared to other treatments from day fourteen till the end of storage.

Table 3.1: Effect of gaseous ozone on mango fruit texture (N) stored at 10°C for twenty-one days and seven days shelf-life at ambient temperatures

Treatments	Storage time (Days)				
	0	**7**	**14**	**21**	**28**
Control	62.21^{opr}	59.46^{lmno}	52.84^{ghi}	47.96^{cdef}	40.51^{b}
O₃ (D0)	62.46^{opqr}	59.60^{lmnop}	54.9^{hij}	50.02^{efg}	40.51^{b}
O₃ (D14)	62.98^{opqr}	60.63^{mnopq}	54.71^{hij}	49.04^{def}	36.60^{a}
O₃ (D0 & 7)	63.38^{pqr}	59.71^{lmnop}	56.71^{jkl}	52.27^{g}	46.74^{cde}
O₃ (D0 & 21)	62.21^{pqr}	58.54^{kln}	55.12^{hij}	50.44^{fg}	47.95^{df}
O₃ (D14 & 21)	65.05^{pqr}	64.61^{r}	56.79^{kjl}	52.37^{gh}	45.99^{cd}
O₃ (D0, 7 & 14)	63.05^{pqr}	58.88^{klmn}	56.12^{jk}	53.12^{ghi}	45.61^{c}
O₃ (D0, 7, 14 & 21)	63.80^{pqr}	60.46^{mnopq}	56.96^{jkl}	54.12^{hij}	44.86^{c}

Means sharing the same letter between rows and within the columns are not significantly different (LSD = 2.95; *n* = 9)

1.3 Fruit color

Ozone treatments significantly affected the color of mango pulp during storage. There were no

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significant differences between treatment means regarding the °hue and b* value of mango fruit. The lightness significantly ($p < 0.05$) decreased in all treatments during storage (Table 3.2). On day fourteen, fruit treated with O_3 (D0) had a high L* value compared to other treatments. There were no significant differences between the treatment means of O_3 (D14), O_3 (D0 &7), and O_3 (D14 &21) at day fourteen. At the end of storage, fruit treated with O_3 (D 14 & 21) had a higher L* value compared to O_3 (D0, 7, 14 & 21), O_3 (D0), O_3 (D0 & 21) and O_3 (D0 & 7).

Table 3.2: Changes in the L* value of mango fruit treated with gaseous ozone and stored at 10°C fortwenty-one days and seven days shelf-life at ambient temperature

Treatment	Storage time (Days)				
	0	**7**	**14**	**21**	**28**
Control	61.52^{ghij}	57.73^{efghi}	50.64^{bcde}	49.63^{cdef}	47.26^{abc}
O$_3$ (0)	64.58^{ijk}	58.53^{fghi}	61.84^{ghij}	47.68^{abc}	49.99^{cdef}
O$_3$ (14)	64.22^{ijk}	52.06^{cdef}	58.20^{ghi}	48.25^{abc}	42.73^{a}
O$_3$ (0 & 7)	67.61^{jk}	56.24^{defgh}	58.80^{fghi}	48.56^{bcd}	45.94^{ab}
O$_3$ (0 & 21)	68.50^{jk}	50.79^{bcdef}	54.30^{def}	50.00^{def}	47.72^{abc}
O$_3$ (14 & 21)	62.49^{hijk}	54.84^{defgh}	56.24^{efgh}	47.10^{abc}	52.12^{def}
O$_3$ (0, 7 & 14)	70.65^{k}	54.97^{defgh}	52.90^{cdefg}	49.80^{cdef}	47.09^{abc}
O$_3$ (0, 7, 14 & 21)	69.21^{jk}	53.89^{defgh}	49.09^{bcde}	49.16^{bcde}	51.57^{def}

Means sharing the same letter between rows and within the columns are not significantly different (LSD = 2.65; n = 9)

The chroma values varied among treatments during storage (Table 3.3). Chroma significantly ($p < 0.05$) decreased in all treatments from day fourteen till the end of storage. There were no significant differences between treatment means of O$_3$ (D0 & 7), O3 (D0, 7 & 14), and O$_3$ (D0, 7, 14 & 21) at day twenty-one. The fruit treated with O$_3$ (D0, 7, 14 & 21) had the highest chroma values compared to other treatments. There were no significant differences between the treatment means of O$_3$ (D0 &7), O$_3$ (D0 & 21), and O$_3$ (D14 & 21) at the end of the storage period.

Table 3.3: Changes in the chroma of mango fruit treated with gaseous ozone and stored at 10°C for twenty-one days and seven days shelf-life at ambient temperature

Treatments	Storage time (Days)				
	0	**7**	**14**	**21**	**28**
Control	44.73^{hijkl}	37.90^{defghi}	34.14^{bcdef}	32.40^{bcdef}	31.89^{abcd}
O$_3$ (0)	45.15^{ijkl}	40.53^{ghijk}	44.82^{hijkl}	30.42^{abc}	35.76^{bcdefg}
O$_3$ (14)	47.56^{jkl}	32.40^{cdef}	41.10^{ghijk}	30.27^{abc}	27.55^{a}
O$_3$ (0 & 7)	47.01^{jkl}	40.67^{ghijk}	38.73^{efghij}	30.91^{abc}	31.59^{abcd}
O$_3$ (0 & 21)	51.34^{l}	33.63^{cdef}	37.86^{efghi}	33.76^{abcdef}	33.88^{bcdef}
O$_3$ (14 & 21)	43.05^{ghijk}	37.23^{defghi}	38.15^{efghi}	30.22^{abc}	35.25^{bcdefg}
O$_3$ (0, 7 & 14)	52.41^{l}	36.04^{cdefg}	35.31^{cdefg}	35.28^{bcdefg}	28.84^{ab}
O$_3$ (0, 7, 14 & 21)	46.73^{kl}	30.13^{abc}	36.31^{cdefgh}	32.02^{bcde}	40.04^{ghijk}

Means sharing the same letter between rows and within the columns are not significantly different (LSD = 7.08; *n* = 9)

The a* value of mango fruit significantly ($p < 0.05$) increased in all treatments during the storage period (Table 3.4). There were no significant differences between treatment means of all treatments at day zero andseven. Fruit treated with O_3 (D14) had a higher a* value compared to other treatments. At the end of storage, untreated fruit had a higher a* value compared to O_3 (D0 & 21), O_3 (D14), O_3 (D14 &21), and O_3 (D0).

Table 3.4: Changes in the a* value of mango fruit treated with gaseous ozone and stored at 10°C fortwenty-one days and seven days shelf-life at ambient temperature

Treatment	Storage time (Days)				
	0	**7**	**14**	**21**	**28**
Control	-4.56[a]	1.81[b]	6.12[defg]	12.29[jklm]	18.57[n]
O_3 (0)	-3.87[a]	1.19[b]	7.95[efgh]	10.20[ijk]	14.79[klm]
O_3 (14)	-3.33[a]	1.73[b]	9.93[ghij]	10.06[ghi]	15.80[m]
O_3 (0 & 7)	-4.22[a]	0.92[b]	4.56[cde]	8.08[efgh]	11.76[jkl]
O_3 (0 & 21)	-4.10[a]	1.32[b]	5.73[cde]	11.0[6ijkl]	16.07[m]
O_3 (14 & 21)	-4.04[a]	1.78[b]	8.51[fghi]	10.51[ghij]	15.29[lm]
O_3 (0, 7 & 14)	-3.78[a]	1.46[b]	3.57[bc]	6.40[def]	9.14[fhij]
O_3 (0, 7, 14 & 21)	-5.22[a]	0.96[b]	2.64[bc]	5.12[def]	10.70[ijkl]

Means sharing the same letter between rows and within the columns are not significantly different (LSD = 1.82; $n = 9$)

1.4 TSS and TA

The TSS and TA are some of the well-known maturity indices for mango fruit. The TSS increased gradually during storage in both control and O_3 treated fruit (Table 3.5). The TSS of ozone-treated fruit was significantly ($p < 0.05$) different from that of the control. At day 14, the TSS of untreated fruit was highercompared to O_3 (D14), O3 (D14 & 21), O3 (D0, 7 & 14) and O_3 (D0, 7, 14 & 21). At the end of storage, the highest TSS was observed in untreated fruit and O_3 (D0), which were 17.38 and 17.45, respectively.

Table 3.5: Effect of gaseous ozone on mango fruit TSS (%) during storage at 10°C for twenty-one days and seven days shelf-life at ambient temperature

Treatment	Storage time (Days)				
	0	**7**	**14**	**21**	**28**
Control	9.85[a]	13.29[efgh]	15.40[klmno]	16.77[mnop]	17.38[q]
O$_3$ (0)	10.46[a]	12.72[def]	14.11[ghijk]	14.92[jkl]	17.45[q]
O$_3$ (14)	11.79[bcd]	12.61[def]	13.42[efgh]	14.98[kl]	16.02[mnop]
O$_3$ (0 & 7)	9.58[a]	12.86[defg]	14.38[ijkl]	14.61[ijkm]	15.33[mnop]
O$_3$ (0 & 21)	10.91[abc]	13.43[efgh]	14.22[hijk]	15.25[jk]	16.65[pq]
O$_3$ (14 & 21)	11.77[bcd]	12.30[cde]	13.05[defg]	15.23[jkl]	16.01[mnop]
O$_3$ (0, 7 & 14)	10.14[a]	12.17[cde]	13.30[efghi]	14.57[ijk]	16.47[opq]
O$_3$ (0, 7, 14 & 21)	9.72[a]	11.81[bcd]	13.81[fghi]	14.50[ijkl]	16.22[op]

Means sharing the same letter between rows and within the columns are not significantly different (LSD =1.25; $n = 9$)

The TA of treated and untreated mango fruit significantly decreased during the storage period (Table 3.6). Fruit treated with O$_3$ (0, 7 & 14) had high TA compared to other treatments from day fourteen until the end of the storage period. At day fourteen, highest TA was observed on O$_3$ (D0, 7, 14 & 21), compared to O$_3$ (D0, 7 & 14), O$_3$ (D0 &7), O$_3$ (D0 & 21) and O$_3$ (D14 & 21). At the end of cold storage, O$_3$ (D0) (0.63) had high TA compared to O$_3$ (D0, 7& 14), O$_3$ (D0 & 7) and O$_3$ (D0, 7, 14 & 21) which were 0.60, 0.57 and 0.56, respectively. There were no significant differences between the treatment

means of O_3 (D14), O_3 (D0 & 7), O_3 (D0 & 21), and O_3 (D14 & 21) at the end of the storage period.

Table 3.1: Effect of gaseous ozone on TA (%) of mango fruit, stored at 10 °C for twenty-one days and seven days shelf-life at ambient temperature

Treatments	Storage time (Days)				
	0	**7**	**14**	**21**	**28**
Control	1.19^q	0.98^{klmno}	0.77^{defgh}	0.71^{bcdef}	0.55^a
O_3 (0)	1.06^{nopq}	0.92^{ijklm}	0.73^{bcdefg}	0.65^{bcd}	0.63^{bc}
O3 (14)	1.13^{pq}	1.10^{opq}	0.77^{defgh}	0.78^{defgh}	0.55^a
O_3 (0 & 7)	1.09^{nopq}	1.00^{lmnop}	0.80^{efghi}	0.67^{bcde}	0.57^a
O_3 (0 & 21)	1.00^{lmnop}	1.01^{mnop}	0.82^{fghij}	0.75^{cdefgh}	0.56^a
O_3 (14 & 21)	1.07^{nopq}	1.01^{lmnop}	0.80^{efghi}	0.61^d	0.55^a
O_3 (0, 7 & 14)	1.07^{nopq}	0.96^{klmno}	0.86^{ghijk}	0.75^{cdefgh}	0.60^b
O_3 (0, 7, 14 & 21)	1.02^{mnop}	0.95^{jklmn}	0.87^{hijk}	0.66^{bcd}	0.56^a

Means sharing the same letter between rows and within the columns are not significantly different (LSD = 0.05; *n*=9)

1.5 Decay index

The decay index of mango fruit varied between treatments during the storage period (Table 3.7). Ozone treatment significantly ($p < 0.05$) decreased the decay in mango fruit. The fruit started showing signs of decay from day 7 for the control treatment, O_3 (D0) and O_3 (D14). Untreated fruit had high decay from day fourteen until the end of storage. At the end of storage, untreated fruit (42.83%) had high decay compared to O_3 (0), O_3 (D0 & 21), O_3 (D14 & 21), O_3 (D0, 7 & 14), O_3 (D0, 7, 14 & 21) which had decay indices of 36.26%, 31.07%, 31.09%, 20.52% and 19.29%, respectively.

Table 3.2: Effect of gaseous ozone on decay index (%) of mango fruit stored at 10 °C for twenty-one days and seven days shelf-life at ambient temperature

Treatments	Storage time (Days)				
	0	**7**	**14**	**21**	**28**
Control	0.00^a	1.48^a	7.41^{bc}	14.07^{bc}	42.83^g
O$_3$ (0)	0.00a	1.92^a	5.02^{ab}	8.28^{bc}	36.26^d
O3 (14)	0.00^a	1.48^a	4.57^{ab}	11.11^{bc}	30.63^{ef}
O$_3$ (0 & 7)	0.00^a	0.00^a	3.51^{ab}	6.67abc	28.22^e
O$_3$ (0 & 21)	0.00^a	0.74^a	4.44^{ab}	11.56^{bc}	31.07^{def}
O$_3$ (14 & 21)	0.00^a	0.00^a	4.69^{ab}	8.89^{bc}	31.09^{def}
O$_3$ (0, 7 & 14)	0.00^a	0.00^a	2.53^a	4.43^a	20.52^h
O$_3$ (0, 7, 14 & 21)	0.00^a	0.00^a	1.91^a	4.27^a	19.29^h

Means sharing the same letter between rows and within the columns are not significantly different (LSD = 4.31; $n = 9$)

1.6 Total carotenoids

The content of total carotenoids of mango fruit increased during the storage period (Table 3.8). Ozone treatment significantly ($p < 0.05$) enhanced the concentration of total carotenoids of mango fruit. The highest concentration of carotenoids was observed in O_3 (0, 7, 14 & 21) from day fourteen till the end of storage. On day fourteen, there were no significant differences between the treatment means of control, O_3 (D0), O_3 (D14), O_3 (D0 & 21), and O_3 (D14 & 21). Fruit treated with O_3 (D0 ,7 14& 21) (2341.68 µg g^{-1}) had significantly higher carotenoids concentration compared to O_3 (D0, 7 & 14), O_3 (D14), O_3 (D0), and O_3 (D0, & 7) which had 1227.37 µg g^{-1}, 16.02.88 µg g^{-1}, 1773.30 µg g^{-1}, and 2173.74 µg g^{-1}, respectively at the end of the storage period.

Table 3.3: Effect of gaseous ozone on total carotenoids ($\mu g\ g^{-1}$) content of mango fruit stored at 10 °C for twenty-one days and seven days shelf-life at ambient temperature

Treatments	Storage time (Days)				
	0	**7**	**14**	**21**	**28**
Control	528.31[ab]	538.75[abc]	725.91[abcd]	1130.08[fgh]	1684.29[kln]
O$_3$ (0)	475.29[ab]	470.91[ab]	843.90[cdef]	1343.56[hij]	1773.30[klmn]
O3 (14)	693.91[abcd]	549.50[abc]	906.76[cdef]	1365.39[hij]	1602.88[hijkl]
O$_3$ (0 & 7)	407.39[a]	533.82[abc]	1090.09[fgh]	1541.33[ijk]	2173.74[mo]
O$_3$ (0 & 21)	799.88[bcdef]	467.93[ab]	764.39[bcde]	1355.90[hij]	1944.63[lmno]
O$_3$ (14 & 21)	809.16[bcdef]	468.57[ab]	971.92[defg]	1254.78g[hij]	1890.00[lmn]
O$_3$ (0, 7 & 14)	492.56[ab]	615.00[abc]	1127.92[fgh]	1344.63[hij]	1227.37[fghij]
O$_3$ (0, 7, 14 & 21)	490.19[ab]	568.91[abc]	1613.94[ghi]	1613.92[jklm]	2341.68[o]

Means sharing the same letter between rows and within the columns are not significantly different (LSD = 442.5; *n* = 9)

1.7 Fruit shelf-life

Ozone treatment significantly ($p < 0.05$) extended fruit shelf-life. The shelf-life of mango fruit ranged between four and six days. Untreated fruit ripened faster compared to other treatments (Figure3.3). However, no significant differences were observed between the ripening time of O$_3$ at D14 and D0 & 21.The fruit exposed to O$_3$ (D0 & 7), O$_3$ (D0, 7 &14), and O$_3$ (D0, 7, 14 & 21) had a longer shelf-life

compared to other treatments. Ozone exposure time was effective in extending the shelf-life of mango fruit. Results from the current study suggest that O_3 effectively delayed the climacteric peak, thus delaying ripening.
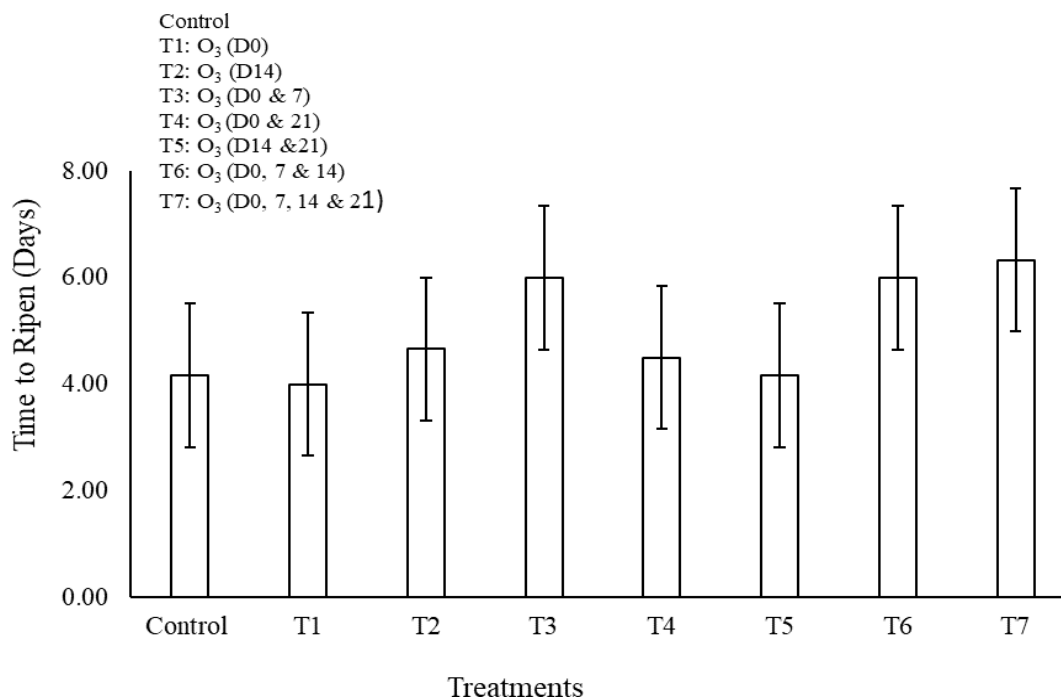
Figure 3.3: Effect of gaseous ozone on the shelf-life of mango fruit stored at ambienttemperature for seven days ($\pm$SE, $n = 9$)

1.8 Relationship between DI and mango fruit physicochemical parameters

Fruit firmness was negatively correlated to TSS ($R^2 = -0.65$), ML % ($R^2 = -0.84$), carotenoids ($R^2 = -0.53$) and DI ($R^2 = -0.59$). The TSS was positively correlated to ML % ($R^2 = 0.72$ and DI ($R^2 = 0.60$) Table 3.9). A positive correlation was observed between ML %, carotenoids ($R^2 = 0.65$) and DI ($R^2 = 0.65$). The TA was negatively correlated to ML % ($R^2 = -0.71$) and carotenoids ($R^2 = -0.50$). Moreover, luminosity was positively correlated to the chroma ($R^2 = 0.85$).

Table 3.4: Pearson correlations coefficient of firmness, TSS, TA, carotenoids, DI, L, and C in mango fruit stored at 10 °C for twenty-one days and seven days shelf-life.

	Firmness	*TSS*	*ML %*	*TA*	*Carotenoids*	*DI*	*L**	*a**	*c*
Firmness									
TSS	**-0.65**								
ML %	**-0.84**	**0.72**							
TA	**0.58**	**-0.59**	**-0.71**						
Carotenoids	**-0.53**	0.49	**0.65**	**-0.50**					
DI	**-0.59**	**0.60**	**0.65**	-0.40	0.38				
L	0.45	-0.47	**-0.50**	0.37	-0.32	-0.28			
a	-0.42	0.40	0.43	-0.38	0.30	0.24	-0.44		
c	0.36	-0.44	-0.44	0.34	-0.28	-0.25	**0.85**	-0.33	

2 Discussion

Fruit mass is an essential parameter as fresh horticultural produce is often sold on a mass basis. The effect of O_3 on mango fruit mass has previously been studied. da Silva et al. (2020) reported that aqueous O_3 (3 ppm) decreased the mass loss in 'Palmer' mango during storage at 20°C for twenty days. Physiological moisture loss occurs in fresh produce due to increased transpiration and respiration rates. The decreased fruit mass loss in O_3 treated fruit could be due to retarded respiration rate (Zhang et al., 2011). In mango

fruit, shriveling occurs when mass loss increases above 10% and the marketability of the fruit decreases (Barman et al., 2014; Vázquez-Celestino et al., 2016).

Loss of firmness occurs in mango fruit postharvest due to biochemical modifications resulting in softening (Yashoda et al., 2006). Zhang et al. (2021) reported that ozone (1 ppm, for 5 hours) maintained the firmness of cantaloupe melon during storage at 4°C for thirty days. Minas et al. (2014b) reported that O_3 (0.3 µL L^{-1}) inhibited the enzyme activities of polygalacturonase and endo-1,4-β-glucanase/1,4-β-glucosidase in kiwi fruit during storage. Zhang et al. (2021) reported that O_3 (1 ppm, for 5 hours) down-regulated pectinesterase and polygalacturonase gene expression in cantaloupe melon. In the current study, fruit treated with O_3 (D0 & 7) maintained the firmness of mango fruit during storage. The enhanced firmness could be attributed to O_3 inhibiting cell wall modifying enzymes, thus decreasing fruit softening and extending shelf-life. Current results indicate that O_3 oxidizes ethylene, thus regulating fruit softeningvia ethylene-dependent pathways.

Ozone treatment was effective in maintaining the interior color of mango fruit. These findings corroborate those of Jia et al. (2020), who reported that O_3 (200 mg m^{-3}) retained the L* value in 'Jin Qiu hong' peach. Similarly, Panou et al. (2021) observed a high L* value in 'Carbarosa' strawberries treated with O_3 (1 ppm). The changes from -a* to +a* value indicate the color change from green to red. The color changes from green to red were observed in all treatments. Panou et al. (2021) reported a low a* value in 'Carbarosa' strawberries treated with O_3 (1 ppm). Bolel et al. (2019) observed decreased chroma values in 'Hicaznar' pomegranates treated with O_3 (4 ppm). Current results indicate that O_3 (D0, 7, 14 & 21) delayed color changes in mango fruit. Chroma suggests the intensity of the color that is affected by pigment saturation (Sousa et al., 2021). The changes in color from green to yellow could be attributed to chlorophyll degradation and accumulation of pigments. The colour change from green to yellow indicates fruit ripening.

In the current study, O_3 treatment delayed TSS accumulation. Similar results were reported by Paico et al. (2018) in mango fruit treated with O_3. Similarly, Mustapha et al. (2020) reported that aqueous O_3 (0.2 mg L) delayed TSS accumulation in cherry tomato during storage. However, Tran et al. (2015) reported that O_3 (10 µL L^{-1}) had no significant effect on TSS of 'Nam Dok Mai No.4' mango fruit. Enzymatic starch degradation magnifies fruit sweetness. Proteins responsible for sugar metabolism, particularly sucrose synthase, are abundant in fruit during ripening (Muccilli et al., 2009). Enzymes such as β-galactosidase and α-galactosidase release secondary metabolites involved in sugar metabolism (Minas et al., 2012). Jia et al. (2020) reported that O_3 delayed the loss of TA in 'Jin Qiu hong' peach during storage. Barboni et al. (2010) reported that O_3 delayed the decrease of malic, citric, and quinic acids in 'Hayward' kiwifruit. The TSS and organic acids affect fruit flavor and ripening.

The decay reduces fruit quality and consumer acceptance. Jia et al. (2020) reported that O_3 (200 mg m^{-3}) decreased the decay percentage in 'Jin Qiu hong' peach during storage. In the current study, O_3 (D0, 7, 14 & 21) treatment delayed fruit decay. The reduced decay in treated fruit could be attributed to the oxidizing potential of O_3. Ozone is reported to be effective against fungal diseases such as anthracnose, stem-end rot, and grey mold (Savi and Scussel, 2014; Terao et al., 2019).

Carotenoids are known to increase during mango fruit ripening. Minas et al. (2010) reported that O_3 (0.3 μL L^{-1}) increased the carotenoids content in 'Hayward' kiwifruit. Similarly, Toti et al. (2018) reported that O_3 (0.3 ppm) was ineffective in maintaining cantaloupe melons carotenoid content during storage. The increased carotenoids content could be attributed to the enhanced chlorophyll degradation. Carotenoids biosynthesis occurs through the methylerythritol phosphate pathway (Ma et al., 2018). The gene expression of *PDS*, *PSY*, *ZDS*, *ZEP*, and *BCH* are upregulated during mango fruit ripening and correlates with β-carotene (Ma et al., 2018). Campayo et al. (2021) reported that aqueous O_3 (150 mL, 1000 mV) down-regulated *VviZISO1*, *VvLBCY2*, *VviCISO1,* and *VviZDS1* genes in microvine *ML1*. Ozone penetrates the fruit via lenticels or stomata into the cell membrane, where it breaks down to form reactive oxygen species (Campayo et al., 2021). The enhanced carotenoids content could be attributed to O_3 inducing the synthesis of secondary metabolites as a defense mechanism under oxidative stress.

3 Conclusion

This study provides an understanding of the effect of O_3 on mango fruit quality at various application times. The postharvest application time of O_3 is essential in preserving the quality and extending mango fruit shelf life. The current results indicate the O_3 treatment should be applied at the pre-climacteric stage to yield superior results. Superior results were observed in treatments that received O_3 before the climacteric stage, such as O_3 (0 & 7), O_3 (D0, 7 & 14), and O_3 (D0, 7, 14 & 21). Increasing ozone application from three to four levels yielded similar results in most physicochemical parameters. Therefore, the three-levels of O_3 application should be considered to minimize the costs. Current results provide the basis for developing postharvest O_3 protocols but warrant further investigation.

4 References

Abd El-Razek, E., Abd El-Motty, E.Z., Orabi, S.A., Abou-Elfotouh, A. 2019. Improving fruit quality of mango fruits cv. Zebda by coating with moringa and green tea leaves extracts under cold storage. Middle East J. Agric. Res 8, 1325-1343.

Adetunji, C., Fawole, O., Arowora, K., Nwaubani, S., Ajayi, E., Oloke, J., Majolagbe, O., Ogundele, B., Aina, J., Adetunji, J. 2012. Quality and safety of *Citrus sinensis* coated with hydroxypropylmethylcellulose edible coatings containing *Moringa oleifera* extract stored at ambient temperature. Global J. Sci. Frontier Res. 12, 6.

Akhtar, A., Abbasi, N.A., Hussain, A. 2010a. Effect of calcium chloride treatments on quality characteristics of loquat fruit during storage. Pak. J. Bot. 42, 181-188.

Akhtar, A., Abbasi, N.A., Hussain, A. 2010b. Effect of calcium chloride treatments on quality characteristics of loquat fruit during storage. Pak. J. Bot. 42, 181-188.

Ali, A., Maqbool, M., Alderson, P.G., Zahid, N. 2013. Effect of gum arabic as an edible coating on antioxidant capacity of tomato (*Solanum lycopersicum* L.) fruit during storage. Postharvest Biol. Technol.76, 119-124.

Aslam, R., Alam, M.S., Saeed, P.A. 2020. Sanitization potential of ozone and its role in postharvest quality management of fruits and vegetables. Food Eng. Rev. 12, 48-67.

Barboni, T., Cannac, M., Chiaramonti, N. 2010. Effect of cold storage and ozone treatment on physicochemical parameters, soluble sugars and organic acids in *Actinidia deliciosa*. Food Chem. 121, 946-951.doi:10.1016/j.foodchem.2010.01.024.

Barman, K., Asrey, R. 2014. Salicylic acid pre-treatment alleviates chilling injury, preserves bioactive compounds and enhances shelf life of mango fruit during cold storage. J. Sci. Ind. Res. 73, 713-718.

Barman, K., Asrey, R., Pal, R.K., Jha, S.K., Bhatia, K. 2014. Post-harvest nitric oxide treatment reduces chilling injury and enhances the shelf-life of mango (*Mangifera indica* L.) fruit during low-temperature storage. J. Hortic. Sci. Biotechnol. 89, 253-260.

Berkovich, L., Earon, G., Ron, I., Rimmon, A., Vexler, A., Lev-Ari, S. 2013. *Moringa oleifera* aqueous leaf extract down-regulates nuclear factor-kappaB and increases cytotoxic effect of chemotherapy in pancreatic cancer cells. BMC Complement. Altern. Med. 13, 1-7.

Bill, M., Sivakumar, D., Korsten, L., Thompson, A.K. 2014. 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 Prot. 64, 159-167.

Bolel, H., Koyuncu, M.A., ErbaŞ, D. 2019. The combined effect of controlled atmosphere with ozone and prochloraz treatment on storage life and quality of pomegranate cv. Hicaznar. Akademik Ziraat Dergisi 10.29278/azd.555195.doi:10.29278/azd.555195.

Bonneau, A., Boulanger, R., Lebrun, M., Maraval, I., Gunata, Z. 2016. Aroma compounds in fresh and dried mango fruit (*Mangifera indica* L. cv. Kent): impact of drying on volatile composition. Int. J. of Food Sci. Technol. 51, 789-800.

Brodowska, A.J., Nowak, A., Smigielski, K. 2018a. Ozone in the food industry: Principles of ozone treatment, mechanisms of action, and applications: An overview. Crit. Rev. Food Sci. Nutr. 58, 2176-2201.doi:10.1080/10408398.2017.1308313.

Brodowska, A.J., Nowak, A., Śmigielski, K. 2018b. Ozone in the food industry: Principles of ozone treatment, mechanisms of action, and applications: An overview. Crit. Rev. Food Sci. Nutr. 58, 2176-2201.

Campayo, A., Savoi, S., Romieu, C., López-Jiménez, A.J., de la Hoz, K.S., Salinas, M.R., Torregrosa, L., Alonso, G.L. 2021. The application of ozonated water rearranges the *Vitis vinifera* L. leaf and berry transcriptomes eliciting defence and antioxidant responses. Sci. Rep. 11, 1-14.

Cardenas-Perez, S., Chanona-Perez, J.J., Guemes-Vera, N., Cybulska, J., Szymanska-Chargot, M., Chylinska, M., Koziol, A., Gawkowska, D., Pieczywek, P.M., Zdunek, A. 2018. Structural, mechanical and enzymatic study of pectin and cellulose during mango ripening. Carbohydr. Polym. 196, 313-321.doi:10.1016/j.carbpol.2018.05.044.

Cayuela, J.A., Vázquez, A., Pérez, A.G., García, J.M. 2010. Control of table grapes postharvest decay by ozone treatment and resveratrol induction. Food Sci. Technol. Int.15, 495-502.doi:10.1177/1082013209350539.

Chowdappa, P., Gowda, S., Chethana, C., Madhura, S. 2014. Antifungal activity of chitosan-silver nanoparticle composite against *Colletotrichum gloeosporioides* associated with mango anthracnose. Afr. J. Microbiol. Res. 8, 1803-1812.

Contigiani, E.V., Jaramillo-Sánchez, G., Castro, M.A., Gómez, P.L., Alzamora, S.M. 2018. Postharvest quality of strawberry fruit (*Fragaria x ananassa* Duch cv. Albion) as affected by ozone washing: fungal spoilage, mechanical properties, and structure. Food Biopr. Technol. 11, 1639-1650.doi:10.1007/s11947-018-2127-0.

da Silva, E., Neto, A.F., Silva, J., Donzeli, V., Costa, M., Costa, J., Figueiredo, R. 2020. Effects of sanitizers on the control of *Alternaria* sp. fungus for 'Palmer' mango conservation. J. Agric. Sci. 12 (9): 216-226

De Santis, D., Garzoli, S., Vettraino, A.M. 2021. Effect of gaseous ozone treatment on the aroma and clove rot by *Fusarium proliferatum* during garlic postharvest storage. Heliyon 7, e06634.

de Souza, L.P., Faroni, L.R.D.A., Heleno, F.F., Cecon, P.R., Gonçalves, T.D.C., da Silva, G.J., Prates, L.H.F. 2018. Effects of ozone treatment on postharvest carrot quality. Lwt 90, 53-60.

Donkin, D., Oosthuyse, S. 1996. Quality evaluations of sea-exported South African mangoes in Europe during the 1995/96 season. South African Mango Growers' Association Yearbook (South Africa).

Es-Safi, N.-E., Cheynier, V., Moutounet, M. 2003. Implication of phenolic reactions in food organoleptic properties. J. Food Compos. Anal. 16, 535-553.

Fivaz, J. 2006. Mango production in South Africa as compared to the rest of the world. VIII International Mango Symposium 820, pp. 29-46.

Gonçalves, A.A. 2009. Ozone: an emerging technology for the seafood industry. Braz. Arch. Biol. Technol. 52, 1527-1539.

González-Aguilar, G.A., Buta, J.G., Wang, C.Y. 2001. Methyl jasmonate reduces chilling injury symptoms and enhances colour development of 'Kent' mangoes. J. Sci. Food Agric. 81, 1244-1249. doi:10.1002/jsfa.933.

González-Aguilar, G.A., Buta, J.G., Wang, C.Y. 2001. Methyl jasmonate reduces chilling injury symptoms and enhances colour development of 'Kent' mangoes. J. Sci. Food Agric. 81, 1244-1249.

Gurjar, P., Garg, N., Yadav, K., Lenka, J., Shukla, D. 2018. Effect of chitosan on biochemical and microbial quality of minimally processed mango (*Mangifera indica* L.) cubes during storage. Indian J. Hortic. 75, 130-134.

Hmmam, I., Zaid, N.m., Mamdouh, B., Abdallatif, A., Abd-Elfattah, M., Ali, M. 2021. Storage behavior of "Seddik" mango fruit coated with CMC and guar gum-based silver nanoparticles. Horticulturae 7, 44. doi:10.3390/horticulturae7030044.

Hossain, M., Rana, M., Kimura, Y., Roslan, H.A. 2014. Changes in biochemical characteristics and activities of ripening associated enzymes in mango fruit during the storage at different temperatures. BioMed Res. Int. 2014. Article ID 232969. http://dx.doi.org/10.1155/2014/232969.

Huyskens-Keil, S., Hassenberg, K., Herppich, W. 2012. Impact of postharvest UV-C and ozone treatment on textural properties of white asparagus (*Asparagus officinalis* L.). J. Appl. Bot. Food Qual. 84, 229-243.

Jabbar, A., Malik, A., Islam-UD-Din, Anwar, R., Ayub, M., Rajwana, I., Amin, M., Khan, A., Saeed, M. 2011. Effect of combined application of fungicides and hot water quarantine treatment on postharvest diseases and quality of mango fruit. Pak. J. Bot. 43, 65-73.

Jia, X., Li, J., Du, M., Zhao, Z., Song, J., Yang, W., Zheng, Y., Chen, L., Li, X. 2020. Combination of low fluctuation of temperature with TiO_2 photocatalytic/ozone for the quality maintenance of postharvest peach. Foods 9, 234-249.

Khaliq, G., Mohamed, M.T.M., Ding, P., Ghazali, H., Ali, A. 2016a. Storage behaviour and quality responses of mango (*Mangifera indica* L.) fruit treated with chitosan and gum arabic coatings during cold storage conditions. Int. Food Res. J. 23, S141-S148.

Khaliq, G., Muda Mohamed, M.T., Ghazali, H.M., Ding, P., Ali, A. 2016b. Influence of gum arabic coating enriched with calcium chloride on physiological, biochemical and quality responses of mango (*Mangifera indica* L.) fruit stored under low temperature stress. Postharvest Biol. Technol. 111, 362-369. doi:10.1016/j.postharvbio.2015.09.029.

Kubheka, S.F., Tesfay, S.Z., Mditshwa, A., 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. Hort. Sci. 55, 410-415.

Laohaprasit, N., Ambadipudi, D., Srzednicki, G. 2011. Optimisation of extraction conditions of volatile compounds in 'Nam Dok Mai' mangoes. Int. Food Res. J. 18, 1043-1049.

Lawless, H.T., Heymann, H., 2010. Sensory evaluation of food: principles and practices. Springer.

Ma, X., Zheng, B., Ma, Y., Xu, W., Wu, H., Wang, S. 2018. Carotenoid accumulation and expression of carotenoid biosynthesis genes in mango flesh during fruit development and ripening. Scientia Horticulturae 237, 201-206.

Maldonado-Celis, M.E., Yahia, E.M., Bedoya, R., Landazuri, P., Loango, N., Aguillon, J., Restrepo, B., Guerrero Ospina, J.C. 2019. Chemical composition of mango (*Mangifera indica* L.) fruit: nutritional and phytochemical compounds. Front. Plant Sci. 10, 1073-1094. doi:10.3389/fpls.2019.01073.

Malundo, T., Baldwill, E., Ware, G., Shewfelt, R. 1996. Volatile composition and interaction influence flavor properties of mango (*Mangifera indica* L.), Proceedings-Florida State Horticultural Society. Florida State Horticultural Society109, 264-268.

Minas, I.S., Karaoglanidis, G.S., Manganaris, G.A., Vasilakakis, M. 2010. Effect of ozone application during cold storage of kiwifruit on the development of stem-end rot caused by *Botrytis cinerea*. Postharvest Biol. Technol. 58, 203-210.

Minas, I.S., Tanou, G., Belghazi, M., Job, D., Manganaris, G.A., Molassiotis, A., Vasilakakis, M. 2012. Physiological and proteomic approaches to address the active role of ozone in kiwifruit post-harvest ripening. J. Exp. Bot. 63, 2449-2464. doi:10.1093/jxb/err418.

Minas, I.S., Vicente, A.R., Dhanapal, A.P., Manganaris, G.A., Goulas, V., Vasilakakis, M., Crisosto, C.H., Molassiotis, A. 2014a. Ozone-induced kiwifruit ripening delay is mediated by ethylene biosynthesis inhibition and cell wall dismantling regulation. Plant Sci. 229, 76-85.

Minas, I.S., Vicente, A.R., Dhanapal, A.P., Manganaris, G.A., Goulas, V., Vasilakakis, M., Crisosto, C.H., Molassiotis, A. 2014b. Ozone-induced kiwifruit ripening delay is mediated by ethylene biosynthesis inhibition and cell wall dismantling regulation. Plant Sci. 229, 76- 85. doi:10.1016/j.plantsci.2014.08.016.

More, S., Rao, T.R. 2019. Elicitor-mediated sanitization in combination with edible coatings improve postharvest shelf life and antioxidant potential of mango fruit. Environ. Exp. Biol. 17, 107-114.

Muccilli, V., Licciardello, C., Fontanini, D., Russo, M.P., Cunsolo, V., Saletti, R., Recupero, G.R., Foti, S. 2009. Proteome analysis of *Citrus sinensis* L. (Osbeck) flesh at ripening time. J. Proteom. 73, 134-152.

Mustapha, A.T., Zhou, C., Wahia, H., Amanor-Atiemoh, R., Otu, P., Qudus, A., Fakayode, O.A., Ma, H. 2020. Sonozonation: Enhancing the antimicrobial efficiency of aqueous ozone washing techniques on cherry tomato. Ultrason. Sonochem. 64, 105059.

Mutengwe, M., Chidamba, L., Korsten, L. 2016. Pesticide residue monitoring on South African fresh produce exported over a 6-year period. J. Food Protect. 79, 1759-1766.

Nakakoga, Y., Tada, Y., Sakoda, T., Tazawa, K., Ichikawa, H. 2021. Sterilization of stem-end rot of mango using dielectric barrier discharge and investigation of sterilization factors. Electron. Commun. Jpn. 104, e12325.

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

Paico, M.J.P., Jittanit, W., Jarupan, L., Chonhenchob, V. 2018. Effect of ozone on prolonging the shelf life of mango and broccoli during cold storage and distribution, The 21st IAPRI World Conference on Packaging.

Pandiselvam, R., Subhashini, S., Banuu Priya, E., Kothakota, A., Ramesh, S., Shahir, S. 2019. Ozone based food preservation: a promising green technology for enhanced food safety. Ozone Sci. Eng. 41, 17-34.

Pandit, S.S., Kulkarni, R.S., Chidley, H.G., Giri, A.P., Pujari, K.H., Köllner, T.G., Degenhardt, J., Gershenzon, J., Gupta, V.S. 2009. Changes in volatile composition during fruit development and ripening of 'Alphonso' mango. J. Sci. Food Agric. 89, 2071-2081.

Panou, A.A., Akrida-Demertzi, K., Demertzis, P., Riganakos, K.A. 2021. Effect of gaseous ozone and heat treatment on quality and shelf life of fresh strawberries during cold storage. Int. J. Fruit Sci. 21, 218-231.

Perry, J.J., Yousef, A.E. 2011. Decontamination of raw foods using ozone-based sanitization techniques. Ann. Rev. Food Sci. Technol. 2, 281-298.

Pino, J.A. 2012. Odour-active compounds in mango (*Mangifera indica* L. cv. Corazón). Int. J. Food Sci. Technol. 47, 1944-1950. doi:10.1111/j.1365-2621.2012.03054.x.

Salinas-Roca, B., Guerreiro, A., Welti-Chanes, J., Antunes, M.D., Martín-Belloso, O. 2018. Improving quality of fresh-cut mango using polysaccharide-based edible coatings. Int. J. Food Sci. Technol. 53, 938-945.

Savi, G.D., Scussel, V.M. 2014. Effects of ozone gas exposure on toxigenic fungi species from *Fusarium, Aspergillu*s, and *Penicillium* genera. Ozone Sci. Eng. 36, 144-152.

Shao, X., Tu, K., Tu, S., Tu, J. 2012. A combination of heat treatment and chitosan coating delays ripening and reduces decay in "Gala" apple fruit. J. Food Qual. 35, 83-92.

Simpson, A.M.-A., Mitch, W.A. 2020. Chlorine and ozone disinfection and disinfection byproducts in postharvest food processing facilities: A review. Crit. Rev. Environ. Sci. Technol. 1-43.

Singh, Z., Singh, R.K., Sane, V.A., Nath, P. 2013. Mango - postharvest biology and biotechnology. Crit. Rev. Plant Sci. 32, 217-236. doi:10.1080/07352689.2012.743399.

Sivakumar, D., Jiang, Y., Yahia, E.M. 2011. Maintaining mango (*Mangifera indica* L.) fruit quality during the export chain. Food Res. Int. 44, 1254-1263.

Sivakumar, D., Van Deventer, F., Terry, L.A., Polenta, G.A., Korsten, L. 2012. Combination of 1-methylcyclopropene treatment and controlled atmosphere storage retains overall fruit quality and bioactive compounds in mango. J. Sci. Food Agric. 92, 821-830. doi:10.1002/jsfa.4653.

Sousa, F.F., Pinsetta Junior, J.S., Oliveira, K., Rodrigues, E.C.N., Andrade, J.P., Mattiuz, B.H. 2021. Conservation of 'Palmer' mango with an edible coating of hydroxypropyl methylcellulose and beeswax. Food Chem. 346, 128925. doi:10.1016/j.foodchem.2020.128925.

Swart, S., Broekhuizen, W.V. 2004. Evaluation of post-harvest disease control with regards to the new maximum residue level of five ppm for prochloraz on mangoes in the European Union. Research Journal-South African Mango Growers' Association 24, 73-77.

Tahir, H.E., Xiaobo, Z., Jiyong, S., Mahunu, G.K., Zhai, X., Mariod, A.A. 2018. Quality and postharvest-shelf life of cold-stored strawberry fruit as affected by gum arabic (*Acacia senegal*) edible coating. J. Food Biochem. 42, e12527.

Terao, D., de Lima Nechet, K., Frighetto, R.T.S., Sasaki, F.F.C. 2019. Ozonated water combined with heat treatment to control the stem-end rot of papaya. Scientia Horticulturae 257, 108722.

Tesfay, S.Z., 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 Packag. Shelf Life 11, 40-48.

Toti, M., Carboni, C., Botondi, R. 2018. Postharvest gaseous ozone treatment enhances quality parameters and delays softening in cantaloupe melon during storage at 6 degrees C. J. Sci. Food Agric. 98, 487-494. doi:10.1002/jsfa.8485.

Tran, T.T.L., Aiamla-or, S., Srilaong, V., Jitareerat, P., Wongs-Aree, C., Uthairatanakij, A. 2015. Ozone fumigation to delay ripening of mango 'Nam Dok Mai No 4' Proc. IInd Southeast Asia Symp. on Quality Management in Postharvest Systems.

Vázquez-Celestino, D., Ramos-Sotelo, H., Rivera-Pastrana, D.M., Vázquez-Barrios, M.E., Mercado-Silva, E.M. 2016. Effects of waxing, microperforated polyethylene bag, 1-methylcyclopropene and

nitric oxide on firmness and shrivel and weight loss of 'Manila' mango fruit during ripening. Postharvest Biol. Technol. 111, 398-405. doi:10.1016/j.postharvbio.2015.09.030.

Wei, K., Zhou, H., Zhou, T., Gong, J. 2007. Comparison of aqueous ozone and chlorine as sanitizers in the food processing industry: impact on fresh agricultural produce quality. Ozone Sci. Eng. 29, 113-120.

Yashoda, H.M., Prabha, T.N., Tharanathan, R.N. 2006. Mango ripening: Changes in cell wall constituents in relation to textural softening. J. Sci. Food Agric. 86, 713-721.

Youssef, A.R., Ali, E.A., Emam, H.E. 2015. Influence of postharvest applications of some edible coating on storage life and quality attributes of Navel orange fruit during cold storage. Int. J. Chemt. Res. 8, 2189-2200.

Yun, Z., Jin, S., Ding, Y., Wang, Z., Gao, H., Pan, Z., Xu, J., Cheng, Y., Deng, X. 2012. Comparative transcriptomics and proteomics analysis of citrus fruit, to improve understanding of the effect of low temperature on maintaining fruit quality during lengthy post-harvest storage. J. Exp. Bot. 63, 2873-2893.

Zaharah, S.S., Singh, Z., Symons, G.M., Reid, J.B. 2012. Role of brassinosteroids, ethylene, abscisic acid, and indole-3-acetic acid in mango fruit ripening. J. Plant Grow. Regul. 31, 363-372.

Zhang, X., Tang, N., Zhang, H., Chen, C., Li, L., Dong, C., Cheng, Y. 2021. Comparative transcriptomic analysis of cantaloupe melon under cold storage with ozone treatment. Food Res. Int. 140, 109993.

Zhang, X., Zhang, Z., Wang, L., Zhang, Z., Li, J., Zhao, C. 2011. Impact of ozone on quality of strawberry during cold storage. Front. Agric. China 5, 356-360.

<center>**Chapter 4**</center>

<center>**Moringa leaf extract infused into carboxymethyl cellulose edible coating combined with gaseous ozone as postharvest treatments maintain quality and extend shelf life of mango (*Mangifera indica L.*) fruit**</center>

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Abstract

This study assessed the efficacy of moringa leaf extract infused into carboxymethyl cellulose (EC) as postharvest treatments to maintain quality and extend shelf life of mango fruit stored under gaseous ozone (O_3). Mango fruit (*cv.* Keitt) were treated with ozone for 24 or 36 hours intermittently; EC; EC + O_3 (24 h) and EC + ozone (36 h), stored at 10°C and 95% RH for 21 days. The untreated fruit were used as the control treatment. The fruit were ripened at ambient temperature after cold storage. To assess the effect of treatments on physicochemical quality, fruit firmness, mass loss and total soluble solids (TSS) were measured. Sensory evaluation (smell, taste and texture) at the end of shelf-life was conducted using panel members to determine the consumer acceptance of the fruit. Fruit treated with EC + O_3 significantly retained firmness, delayed colour development of the pulp and TSS accumulation

compared to EC, ozone and untreated fruit. At the end of storage, untreated fruit had the highest mass loss (23.48 %) compared to O_3, EC and EC + O_3 (24 h) which had 20.24%, 17.68% and 18.06%, respectively. The coating significantly enhanced the sensory quality of mango compared to untreated, EC + O_3 (24 h) and O_3 treated fruit. The EC treatment had superior scores in taste and overall preferenceby consumers. The shelf-life of mango fruit was prolonged by EC and O_3 treatments. The current study indicated that coating mango fruit with EC and treating with O_3 delays ripening and enhances fruit quality. Further investigation is required to understand the mechanism of the combined effect ofcoating and O_3 and the potential for industrial use of this treatment.

Keywords: *Mangifera indica* L., sensory attributes, firmness, fruit quality, ripening, cold storage

1 Introduction

Mango is the third most important fruit in tropical and subtropical agriculture due to its nutritional properties (Singh et al., 2013). The fruit contains essential compounds such as amino acids, calcium, carotenoids, proteins and vitamins (Singh et al., 2013). However, the fruit is highly perishable due to its climacteric nature, which is characterized by high postharvest physiological and biochemical changes, leading to relatively short shelf-life. A burst in ethylene production initiates changes in enzyme activities resulting in colour change from green to yellow, alteration in chemical composition, aroma and fruit softening (Yashoda et al., 2006; Singh et al., 2013). Fruit softening accounts for major losses in the mango industry. Preserving fruit quality and preventing postharvest losses is one of the key solutions in ensuring and sustaining global food and nutritional security. Various innovative postharvest technologies have been

developed and used to preserve quality of horticultural crops. These include edible coatings, chemical and non-chemical treatments such as ultraviolet-C and heat treatments.

Edible coatings form a thin layer of a protective barrier that alters gaseous exchange, reduces moisture loss and enhances fruit quality. Edible coatings are biodegradable and non-toxic posing no serious health threats to humans when consumed. Notably, edible coatings contain antioxidants, antimicrobial and phytochemical compounds such as kaempferol, rhamnetin, isoquercitrin and kaempferitrin (Berkovich et al., 2013). *Moringa oleifera* leaf extract used as an edible coating has the potential to extend shelf-life of various horticultural crops. A combination of carboxymethyl cellulose (CMC) and moringa leaf extract (MLE) as an edible coating was previously reported to be effective in decreasing mass loss and maintaining firmness in avocado fruit (*cv.* 'Hass' and 'Fuerte') (Tesfay and Magwaza, 2017) and citrus fruit (Adetunji et al., 2012). While the use of edible coatings has mostly been effective in maintaining postharvest fruit quality, alternative sanitising technologies such as ozone (O_3) have also been researched.

Ozone is an allotrope of oxygen, composed of three oxygen atoms bonded together (Gonçalves, 2009). It is a powerful oxidizing and antimicrobial agent and effective against various microorganisms (Brodowska et al., 2018b). The advantage of O3 is that it leaves no chemical residues that may threaten environmentaland human health. The beneficial effects on O3 on shelf-life and postharvest quality have been reported. For instance, Zhang et al. (2011) reported that O3 significantly reduced respiration rate in strawberry during cold storage. Respiration rate is well-known for influencing biochemical processes that are linkedto cell wall degradation thereby resulting to firmness loss. Currently, to the best of our knowledge, there is no data available about the potential of combined use of O3, MLE and CMC in

mango fruit as a postharvest treatment. Moringa is cultivated in various provinces of South Africa and provides a cheap alternative as an edible coating. The combined treatment could provide an environmentally safe and cost-effective solution for reducing mango postharvest and economic losses. Therefore, the objective of the current study was to assess the efficacy of MLE + CMC incorporated with O_3 to maintain mango fruit quality, delay ripening and extend shelf-life.

2 Materials and methods

2.1 Fruit material

Mango fruit (*cv.* 'Keitt') were harvested from a commercial orchard at Goedgelegen Farm of Westfalia (Pty) Ltd, in Tzaneen, South Africa. Harvested fruit were immediately transported in an overnight courier service to the Postharvest Laboratory of the University of KwaZulu-Natal. Upon arrival in the laboratory, fruit were graded for uniformity (in size and colour) and assigned to different postharvest treatments. The treatments used in the study were: control: untreated, T1: O_3 (24 hours); T2: O_3 (36 hours); T3: MLE 1% + CMC 1% (EC); T4: EC + O_3 (24 h); T5: EC+ O_3 (36 h). Moringa leaves were sourced from Ukulinga Research Farm of the University of KwaZulu-Natal and extracted as described by Tesfay et al. (2017). Briefly, moringa plant tissues (100 g) were extracted with 70% ethanol (1 L) for 12 h, with constant agitation.

2.2 Ozone treatment

Ozone (0.25 mg L^{-1}) was applied for 12 hours intermittently at different sampling days. Ozone treatment

for 24 hours, was applied at days 0 and 7 while for 36 hours fruit were treated at days 0, 7 and 14 during

storage. Fruit were coated prior to ozone exposure. The untreated fruit were used as the control treatment.

Fruit were stored at 10°C (±0.5) and 90% relative humidity for 21 days, simulating shipment to export

market (Sivakumar et al., 2011). Data was collected at seven-day intervals during storage. After 21 days,

fruit were ripened at ambient temperature for 7 days, simulating shelf life conditions at retail.

2.3 Mass loss (%)

Mango fruit were weighed upon arrival (day 0) and at every sampling day during storage. Mass loss

percentage was computed using Equation 1, as described by Akhtar et al. (2010b):

$$\text{Weight loss (\%)} = \frac{A - B}{A} \times 100 \tag{1}$$

Where *A* indicates the fruit weight at the time of harvest and *B* indicates the fruit weight after storage

intervals.

2.3 Firmness

Fruit firmness was measured as described by González-Aguilar et al. (2001), using a hand-held firmness

tester (Bareiss, Germany). Firmness was measured on opposite cheeks and results expressed as Newton (N).

2.4 Titratable acidity (TA) and total soluble solids (TSS)

Fruit were peeled, flesh cut into cubes and homogenized using a blender (Bennett Read, Tornado Tech Cyclonic Action, Tevo). The puree was filtered three times using a muslin cloth. The juice was used for TSS and TA analysis. The TA was measured using an automated titrator (Rondolino G 20's Compact Titrator, Mettler Toledo, Schwerzenbach, Switzerland). Prior to titration, the sensor was calibrated using buffer standards with pHs 4 and 7, with the slope and zero point (offset): Slope: - 59,16 mV/pH @ 25°C and Zero point: +/- 0 mV or pH 7.00. Distilled water (40 mL) was mixed with 8 mL of juice and titrated to the end point (pH 8.1) with 0.1 M of sodium hydroxide (NaOH) and TA expressed as % malic acid using Equation 2.

$$\%TA = \frac{0.067 \; x \; Titre \; x \; (NaOH) \; x \; 100}{Juice \; used} \tag{2}$$

TSS was analyzed using a digital desktop refractometer (B+S RFM340+ refractometer, Bellingham and Stanley Ltd, Tunbridge Wells, Kent, United Kingdom), and reported as a percentage.

2.5 Sensory evaluation

Sensory evaluation was conducted as previously described by Lawless and Heymann (2010) using a 9-point hedonic scale (1: dislike extremely, 5: neither like nor dislike and 9: like extremely). A panel of 41 untrained judges (ages 18-55 years) was used for the analysis. The consumers evaluated the fruit samples based on quality parameters (colour, smell, taste, texture and overall liking). The samples were

prepared on the day of tasting.

2.6 Statistical analysis

The collected data were subjected to the analysis of variance (ANOVA), using GenStat statistical software (GenStat®, 17.1 edition, VSN International, UK). Mean separation was performed using Fischer's least significant difference (LSD) at 5% level of significance.

3 Results and discussion

3.1 Total soluble solids and titratable acidity

For both TA and TSS, a significant ($p < 0.05$) interaction between postharvest treatments and time effect was observed, an indication that the effect of postharvest treatments dependent on storage time (Figure 4.1).The TSS gradually increased while TA decreased during shelf-life (Figure 4.2).
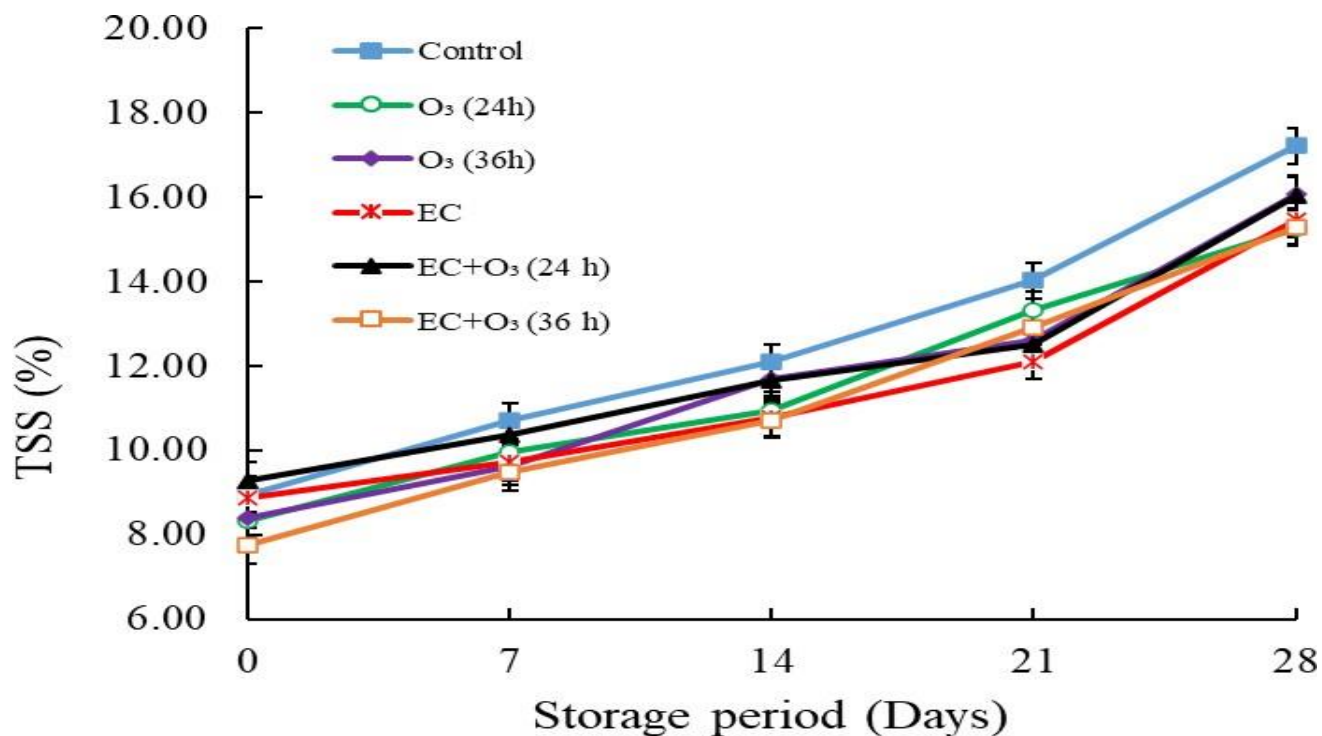
Figure 4.1: Effect of edible coatings and gaseous ozone on the TSS (%) of mango fruit, stored at 10°C for twenty-one days and seven days shelf-life at ambient temperature. Data represent means (±SE) of 3 fruit per replicate

The combination of EC + O_3 significantly ($p < 0.05$) delayed the accumulation of TSS and the decrease of TA. At the end of shelf-life, highest TSS (17.22) was measured in the untreated fruit compared to O_3 (36 hr) (16.09); EC + O_3 (24 hr) (16.05); EC (15.47); EC + O_3 (36 hr) (15.30) and O_3 (24 hr) (15.27). These results are comparable to those previously reported by de Souza et al. (2018) and Gurjar et al. (2018) who observed a delayed accumulation of TSS in carrot and mango treated with O_3 and chitosan, respectively.
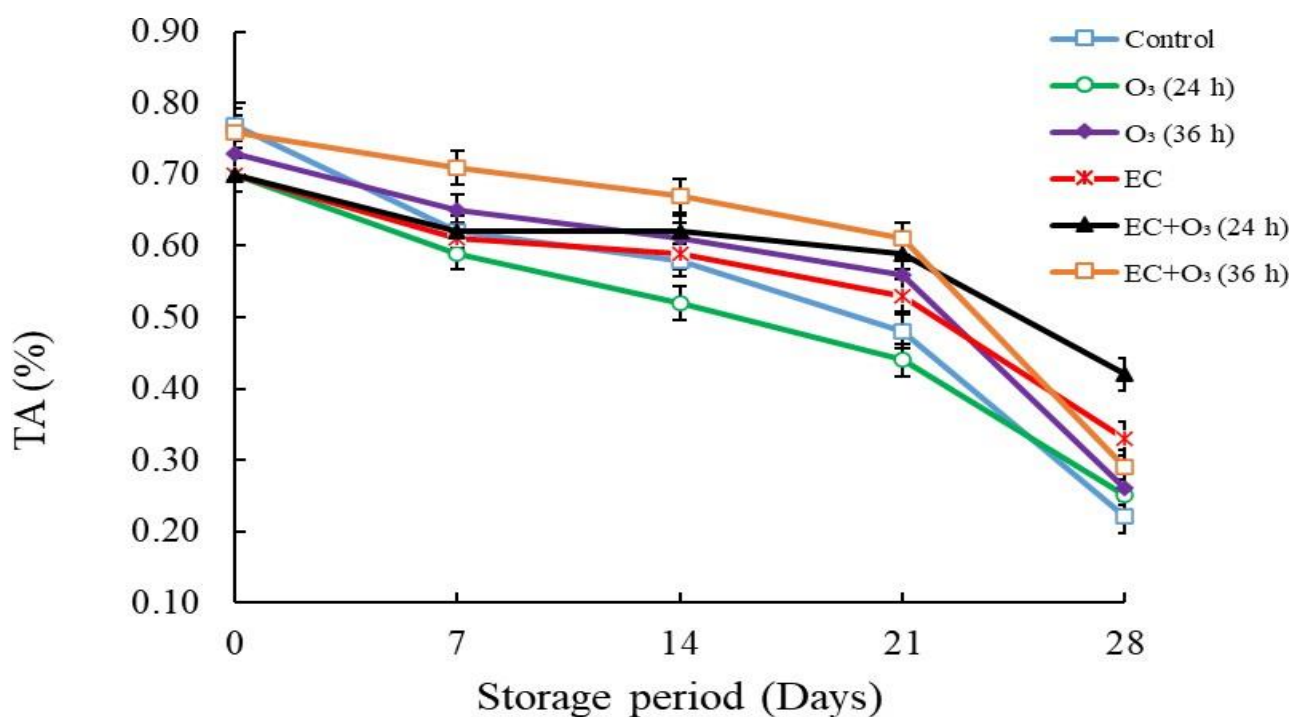
Figure 4.2: Effect of edible coatings and gaseous ozone on the TA (%) of mango fruit, stored at 10°C for twenty-one days and seven days shelf-life at ambient temperature. Data represent means (±SE) of 3 fruitper replicate

The increase in TSS observed in O_3 could be attributed to the inability of O_3 to inhibit biochemical changes such as starch hydrolyses to soluble sugars during ripening. Starch is the predominant polysaccharide in mango fruit that is converted to soluble sugars during ripening (Singh et al., 2013). The increase in TSS and decrease in TA observed in this study indicate that fruit ripening continued during storage, although the rate was slower on treated fruit compared to their untreated counterparts.

3.2 Fruit mass loss

Fruit mass loss (%) increased continuously during storage (Figure 4.3). The EC coating significantly (*p*

< 0.05) decreased mass loss percentage of mango fruit. Mass loss of untreated fruit was high throughout the storage period compared to other treatments. At the end of shelf-life, untreated fruit had the highest mass loss (23.36 %) compared to EC + O_3 (24h); O_3 (36 hr); O_3 (24 h); EC + O_3 (36 h) and EC which had mass loss of 21.25 %, 20.24 %, 19.48 %, 18.06 % and 17.68 %, respectively. Current results are similar to those reported by Tesfay and Magwaza (2017) where MLE + CMC coating retarded mass loss in avocado fruit. It is well known that fruit continue to transpire and respire postharvest and undergo biochemical processes leading to mass loss.
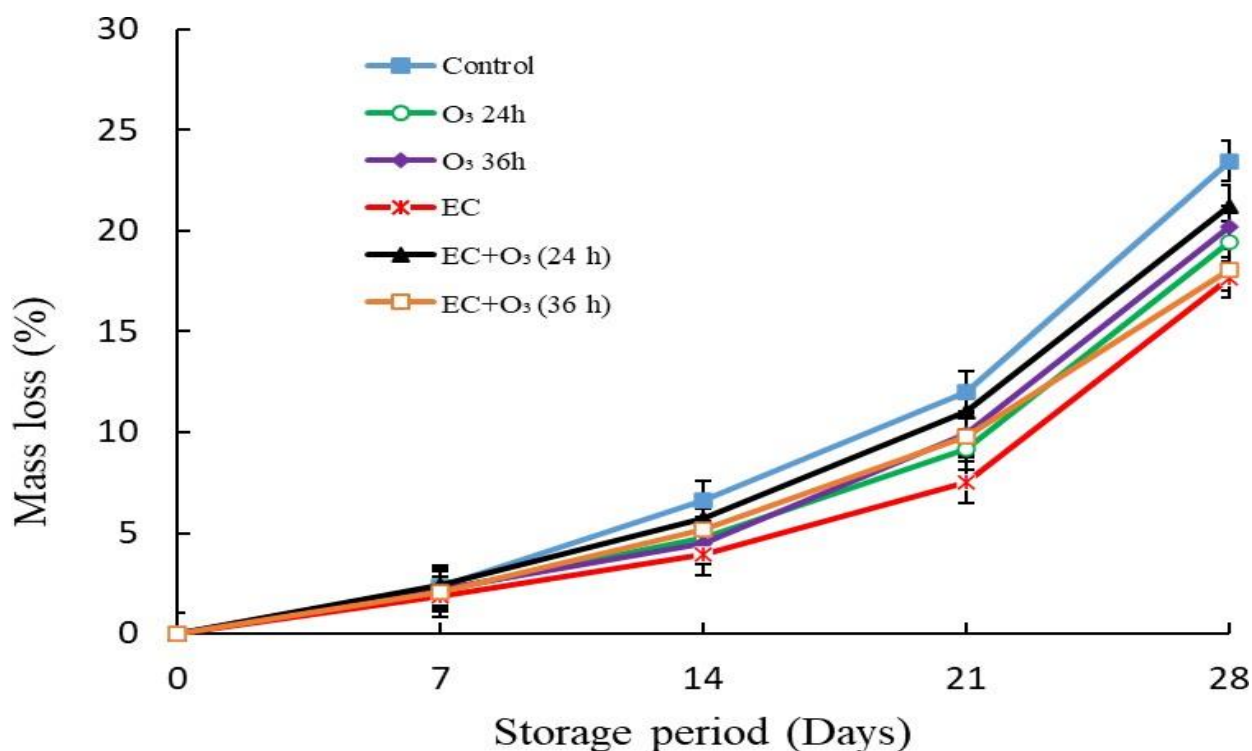
Figure 4.3: Effect of various treatments on mango mass loss (%), stored at 10°C for twenty-one days and seven days shelf-life at ambient temperature. Data represent means (±SE) of 3 fruit per replicate

3.3 Fruit firmness

The results of fruit firmness are shown in Table 4.1. Fruit treated with EC were significantly ($p < 0.01$) firmer throughout the storage period compared to other treatments and untreated fruit. At the end of the storage period, untreated fruit were softer compared to all treatments. The treatment combination of EC + O_3 (36 h) and EC had higher firmness retention compared to other treatments.

Table 4.1: Changes in fruit texture as influenced by various treatments during storage at 10 °C for twenty-one days and seven days shelf-life at ambient temperature.

Treatment	Storage time (Days)				
	0	**7**	**14**	**21**	**28**
Control	61.55^{no}	58.29^{m}	48.72^{ij}	38.74^{e}	24.66^{a}
O_3 (24 h)	61.38^{no}	58.54^{m}	50.33^{jk}	43.25^{f}	27.33^{b}
O_3 (36 h)	61.05^{no}	58.29^{m}	51.45^{k}	45.82^{gh}	30.00^{cd}
EC	62.30^{n}	59.54^{mn}	53.70^{l}	$47.67hi$	29.18^{bcd}
EC + O_3 (24 h)	61.3^{no}	58.04^{m}	52.18^{kl}	44.94^{fg}	28.25^{bc}
EC + O_3 (36 h)	61.05^{no}	57.96^{m}	54.15^{l}	46.17^{gh}	31.15^{d}

Means with different letters within a column and between columns indicate a significant difference between treatments. Data represent means (LSD = 2.00) of 3 fruit per replicate.

This suggests that edible coating and O_3 treatments were effective in delaying fruit softening. During fruit ripening, mango fruit is characterised by changes in fruit texture, enzyme activity and biochemical

changes. The changes in fruit texture could be attributed to the modification of cell wall pectin and hemicellulose polymers occuring during ripening resulting in fruit softening (Singh et al., 2013). Degradation of large insoluble polymers to short alcohol soluble polymers occurs during fruit ripening (Yashoda et al., 2006). In this study, high firmness may be due to the ability of EC and O_3 treatment to inhibit the accumulation of cell wall degrading enzymes (Minas et al., 2014a).

3.4 Shelf-life

The shelf-life of the mango fruit significantly ($p < 0.05$) varied among treatments. Mango fruit from EC + O3 (36 h) and EC took longer to ripen compared to other treatments (Figure 4.4). On the other hand, the shortest time to ripen was observed in the untreated fruit which was only four days. The extended shelf-life in the treated fruit could be attributed to the reduced respiration rate (Khaliq et al., 2016b). A decreased respiration rate retards ethylene production and metabolic activities, resulting in extended shelf-life.
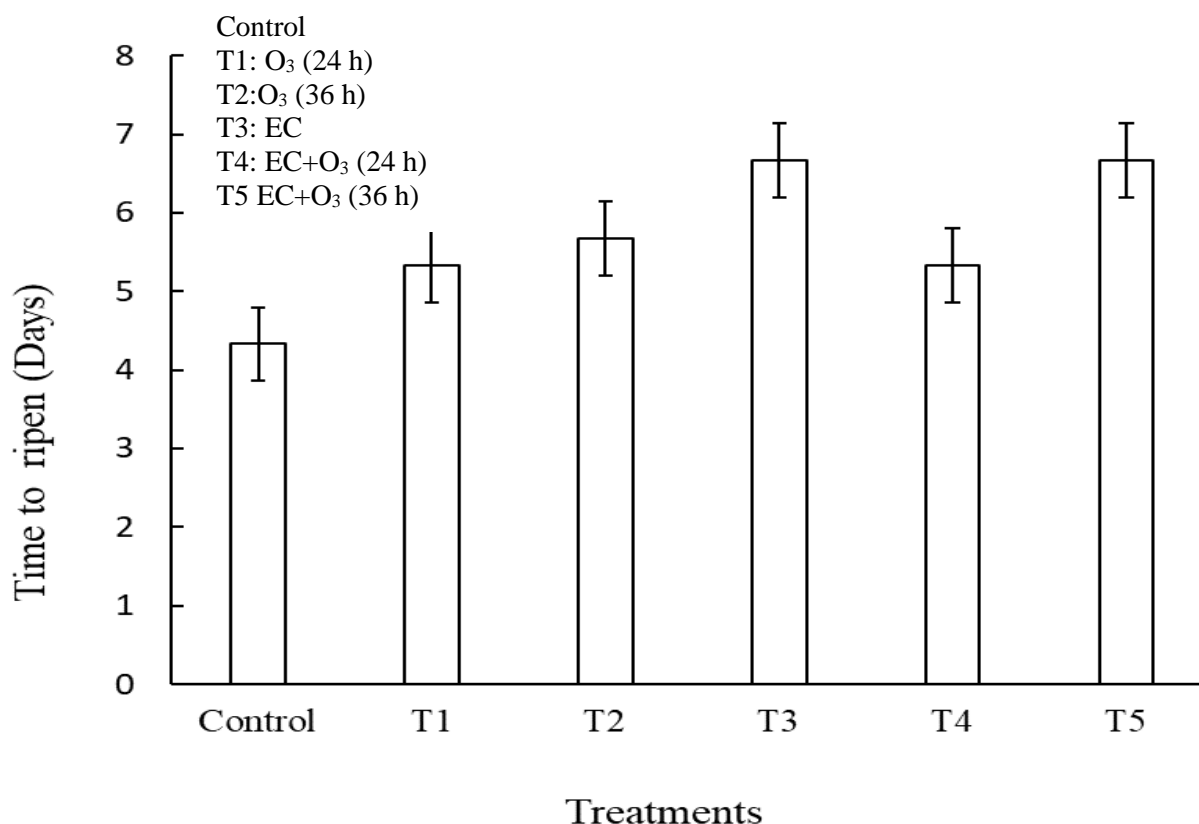
Figure 4.4: Effect of edible coatings and gaseous ozone on mango shelf-life during storage atambient temperature for seven days. Data represent means (±SE) of 3 fruit per replicate

3.5 Sensory quality

The results of the sensory evaluation are presented in Figure 4.5. The edible coating affected mango fruit sweetness. The fruit treated with EC (7.03) were rated high in sweetnesscompared to the control, O_3 (24 h); O_3 (36 h); EC + O_3 (24 h) and EC+ O_3 (36 h) which had 6.89, 6.59, 6.38, 5.36 and 5.00, respectively. The firmness showed a different trend to that of fruit sweetness. The untreated fruit were perceived as being soft compared to EC + O_3 (24 h); O_3 (36 h); EC + O_3 (24 h) and EC + O_3 (36 h). The untreated fruit (6.61) were rated as having a darker yellow colour compared to EC +O_3 (24 h) (5.76), EC (5.66) and EC + O_3 (36 h) (5.02).

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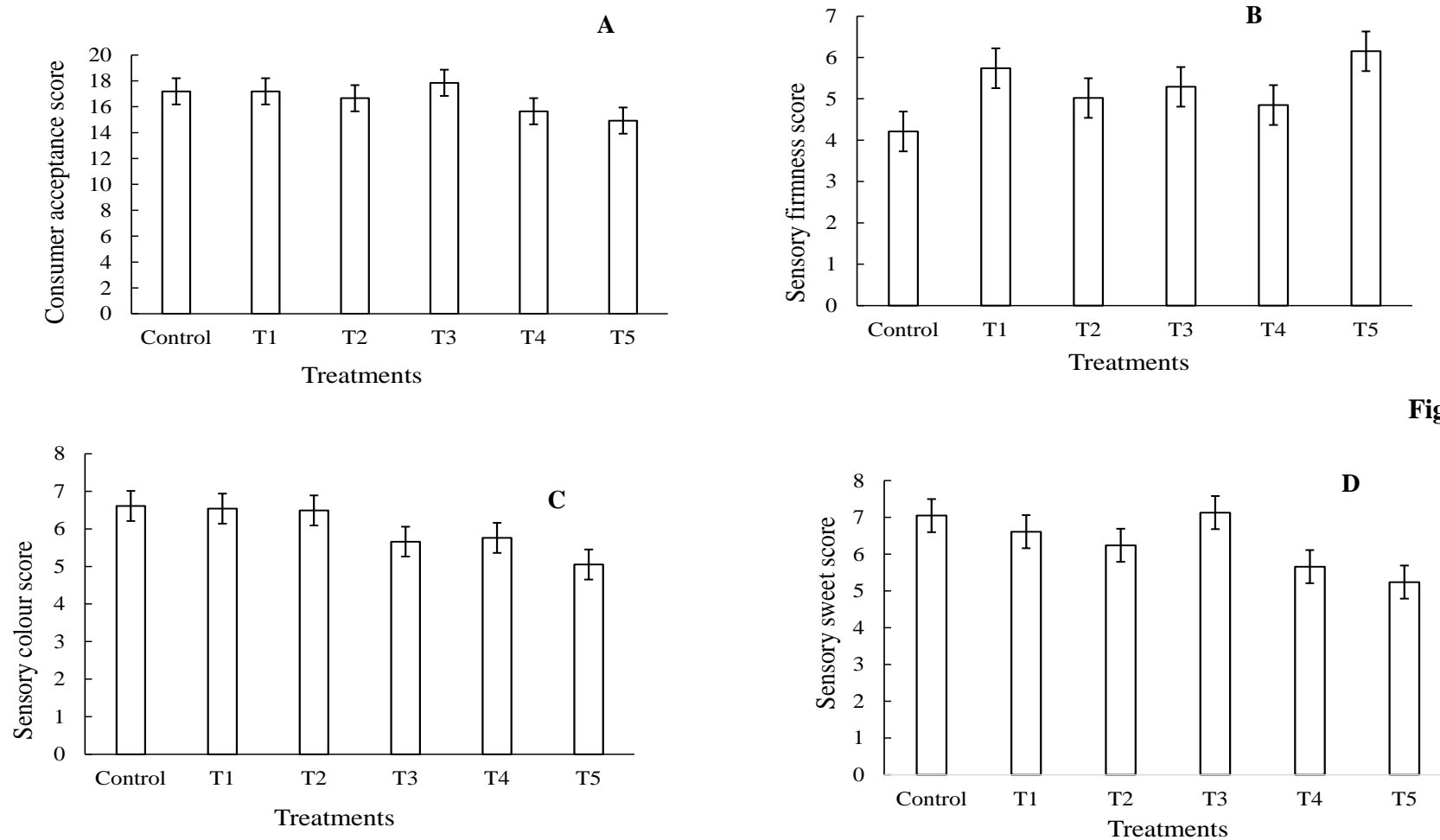
4.5: Effect of edible coatings and gaseous ozone on consumer acceptance (A), firmness (B), color (C) and sweetness (D) of mango fruit store at 10 °C for twenty-one days and seven days shelf-life at ambient temperature. Data represent means (±SE) of 3 fruit per replicate.

These results indicated that the fruit from the control, O3 (24 h) and O3 (36 h) treatments were riper compared to other treatments, hence a darker colour, decreased firmness and sweetness. The MLE + CMC coated fruit were overall preferred by the consumers. Current results are supported by those of Salinas-Roca et al. (2018) in mango fruit coated with chitosan. Preservation of fruit quality in coated fruit could be attributed to the antioxidant compounds present in moringa leaves (Abd El-Razek et al., 2019). Phenolic compounds contribute to the fruit sensory quality such as taste, colour and aroma (Es-Safi et al., 2003). The overall results of the current study indicate thatEC and EC + O$_3$ (24 h) were effective in enhancing the sensory quality of the fruit.

4   Conclusion

The results showed that EC and EC + O$_3$ (36 h) were more effective compared to O$_3$ in delaying the ripening process and maintaining the postharvest quality of mango fruit. Current results provide an understanding of the combination of EC and O$_3$ as a postharvest treatment in mango fruit. Preservation ofexternal and sensory quality in coated fruit has previously been attributed to the antioxidant (Abd El-Razeket al., 2019) and phenolic compounds (Es-Safi et al., 2003) found in moringa leaves. Further investigation is required to understand the mechanism of the combined effect of coating and O$_3$ and the potential for industrial use of this treatment.

5 References

Abd El-Razek, E., Abd El-Motty, E.Z., Orabi, S.A., Abou-Elfotouh, A. 2019. Improving fruit qualityof mango fruits cv. Zebda by coating with moringa and green tea leaves extracts under cold storage. Middle East J. Agric. Res. 8, 1325-1343.

Adetunji, C., Fawole, O., Arowora, K., Nwaubani, S., Ajayi, E., Oloke, J., Majolagbe, O., Ogundele, B., Aina, J., Adetunji, J. 2012. Quality and safety of *Citrus sinensis* coated with hydroxypropylmethylcellulose edible coatings containing *Moringa oleifera* extract stored at ambient temperature. Global J. Sci. Frontier Res. 12, 29-33.

Akhtar, A., Abbasi, N.A., Hussain, A. 2010a. Effect of calcium chloride treatments on quality characteristics of loquat fruit during storage. Pak. J. Bot. 42, 181-188.

Akhtar, A., Abbasi, N.A., Hussain, A. 2010b. Effect of calcium chloride treatments on quality characteristics of loquat fruit during storage. Pak. J. Bot. 42, 181-188.

Ali, A., Maqbool, M., Alderson, P.G., Zahid, N. 2013. Effect of gum arabic as an edible coating on antioxidant capacity of tomato (*Solanum lycopersicum* L.) fruit during storage. Postharvest Biol. Technol. 76, 119-124.

Aslam, R., Alam, M.S., Saeed, P.A. 2020. Sanitization potential of ozone and its role in postharvest quality management of fruits and vegetables. Food Eng. Rev. 12, 48-67.

Barboni, T., Cannac, M., Chiaramonti, N. 2010. Effect of cold storage and ozone treatment on physicochemical parameters, soluble sugars and organic acids in *Actinidia delicio*sa. Food Chem. 121, 946-951. doi:10.1016/j.foodchem.2010.01.024.

Barman, K., Asrey, R. 2014. Salicylic acid pre-treatment alleviates chilling injury, preserves bioactive compounds and enhances shelf life of mango fruit during cold storage. J. Sci. Ind. Res. 73, 713-718.

Barman, K., Asrey, R., Pal, R.K., Jha, S.K., Bhatia, K. 2014. Post-harvest nitric oxide treatment reduces chilling injury and enhances the shelf-life of mango (*Mangifera indica* L.) fruit during low-temperature storage. J. Hortic. Sci. Biotechnol. 89, 253-260.

Berkovich, L., Earon, G., Ron, I., Rimmon, A., Vexler, A., Lev-Ari, S. 2013. *Moringa oleifera* aqueous leaf extract down-regulates nuclear factor-kappaB and increases cytotoxic effect ofchemotherapy in pancreatic cancer cells. BMC Complement. Alt. Med. 13, 1-7.

Bill, M., Sivakumar, D., Korsten, L., Thompson, A.K. 2014. 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 Prot. 64, 159-167.

Bolel, H., Koyuncu, M.A., ErbaŞ, D. 2019. The combined effect of controlled atmosphere with ozone and prochloraz treatment on storage life and quality of pomegranate cv. Hicaznar. Akademik Ziraat Dergisi 10.29278/azd.555195. doi:10.29278/azd.555195.

Bonneau, A., Boulanger, R., Lebrun, M., Maraval, I., Gunata, Z. 2016. Aroma compounds in fresh and dried mango fruit (*Mangifera indica* L. cv. Kent): impact of drying on volatile composition. Int. J. Food Sci. Technol. 51, 789-800.

Brodowska, A.J., Nowak, A., Smigielski, K. 2018a. Ozone in the food industry: Principles of ozone treatment, mechanisms of action, and applications: An overview. Crit. Rev. Food Sci. Nutr. 58, 2176-2201. doi:10.1080/10408398.2017.1308313.

Brodowska, A.J., Nowak, A., Śmigielski, K. 2018b. Ozone in the food industry: Principles of ozone treatment, mechanisms of action, and applications: An overview. Crit. Rev. Food Sci. Nutr. 58, 176-220.

Campayo, A., Savoi, S., Romieu, C., López-Jiménez, A.J., de la Hoz, K.S., Salinas, M.R., Torregrosa, L., Alonso, G.L. 2021. The application of ozonated water rearranges the *Vitis vinifera* L. leaf and berry transcriptomes eliciting defence and antioxidant responses. Sci. Rep. 11, 1-14.

Cardenas-Perez, S., Chanona-Perez, J.J., Guemes-Vera, N., Cybulska, J., Szymanska-Chargot, M., Chylinska, M., Koziol, A., Gawkowska, D., Pieczywek, P.M., Zdunek, A. 2018. Structural, mechanical and enzymatic study of pectin and cellulose during mango ripening. Carbohydr. Polym. 196, 313-321. doi:10.1016/j.carbpol.2018.05.044.

Cayuela, J.A., Vázquez, A., Pérez, A.G., García, J.M. 2010. Control of table grapes postharvest decayby ozone treatment and resveratrol induction. Food Sci. Technol. Int. 15, 495- 502. doi:10.1177/1082013209350539.

Chowdappa, P., Gowda, S., Chethana, C., Madhura, S. 2014. Antifungal activity of chitosan-silver nanoparticle composite against *Colletotrichum gloeosporioides* associated with mango anthracnose. Afr. J. Microbiol. Res. 8, 1803-1812.

Contigiani, E.V., Jaramillo-Sánchez, G., Castro, M.A., Gómez, P.L., Alzamora, S.M. 2018. Postharvest quality of strawberry fruit (*Fragaria x ananassa* Duch cv. Albion) as affected by ozone washing: fungal spoilage, mechanical properties, and structure. Food Biopr. Technol. 11, 1639-1650. doi:10.1007/s11947-018-2127-0.

da Silva, E., Neto, A.F., Silva, J., Donzeli, V., Costa, M., Costa, J., Figueiredo, R. 2020. Effects of sanitizers on the control of *Alternaria* sp. fungus for 'Palmer' mango conservation. J. Agric. Scie. 12 (9): 216-226

De Santis, D., Garzoli, S., Vettraino, A.M. 2021. Effect of gaseous ozone treatment on the aroma and clove rot by *Fusarium proliferatum* during garlic postharvest storage. Heliyon 7, e06634.

de Souza, L.P., Faroni, L.R.D.A., Heleno, F.F., Cecon, P.R., Gonçalves, T.D.C., da Silva, G.J., Prates, L.H.F. 2018. Effects of ozone treatment on postharvest carrot quality. Lwt 90, 53-60.

Donkin, D., Oosthuyse, S. 1996. Quality evaluations of sea-exported South African mangoes in Europe during the 1995/96 season. South African Mango Growers' Association Yearbook (South Africa).

Es-Safi, N.-E., Cheynier, V., Moutounet, M. 2003. Implication of phenolic reactions in food

organoleptic properties. J. Food Comp. Anal. 16, 535-553.

Fivaz, J. 2006. Mango production in South Africa as compared to the rest of the world, VIII International Mango Symposium 820, 29-46.

Gonçalves, A.A. 2009. Ozone: an emerging technology for the seafood industry. Braz. Arch. Biol. Technol. 52, 1527-1539.

González-Aguilar, G.A., Buta, J.G., Wang, C.Y. 2001. Methyl jasmonate reduces chilling injury symptoms and enhances colour development of 'Kent' mangoes. J. Sci. Food Agric. 81, 1244-1249. doi:10.1002/jsfa.933.

Gurjar, P., Garg, N., Yadav, K., Lenka, J., Shukla, D. 2018. Effect of chitosan on biochemical and microbial quality of minimally processed mango (*Mangifera indica* L.) cubes during storage. Ind. J. Hortic. 75, 130-134.

Hmmam, I., Zaid, N.m., Mamdouh, B., Abdallatif, A., Abd-Elfattah, M., Ali, M. 2021. Storage behavior of "Seddik" mango fruit coated with CMC and guar gum-based silver nanoparticles. Horticulturae 7, 44. doi:10.3390/horticulturae7030044.

Hossain, M., Rana, M., Kimura, Y., Roslan, H.A. 2014. Changes in biochemical characteristics and activities of ripening associated enzymes in mango fruit during the storage at different temperatures. BioMed Res. Int. Article ID 232969. http://dx.doi.org/10.1155/2014/232969.

Huyskens-Keil, S., Hassenberg, K., Herppich, W. 2012. Impact of postharvest UV-C and ozone treatment on textural properties of white asparagus (*Asparagus officinalis* L.). J. Appl. Bot. Food Qual. 84 (2): 229-234.

Jabbar, A., Malik, A., Islam-UD-Din, Anwar, R., Ayub, M., Rajwana, I., Amin, M., Khan, A., Saeed, M. 2011. Effect of combined application of fungicides and hot water quarantine treatment on postharvest diseases and quality of mango fruit. Pak. J. Bot. 43, 65-73.

Jia, X., Li, J., Du, M., Zhao, Z., Song, J., Yang, W., Zheng, Y., Chen, L., Li, X. 2020. Combination of

low fluctuation of temperature with TiO_2 photocatalytic/ozone for the quality maintenance of postharvest peach. Foods 9, 234-249.

Khaliq, G., Mohamed, M.T.M., Ding, P., Ghazali, H., Ali, A. 2016a. Storage behaviour and quality responses of mango (*Mangifera indica* L.) fruit treated with chitosan and gum arabic coatings during cold storage conditions. Int. Food Res. J. 23, S141-148

Khaliq, G., Muda Mohamed, M.T., Ghazali, H.M., Ding, P., Ali, A. 2016b. Influence of gum arabic coating enriched with calcium chloride on physiological, biochemical and quality responses of mango (*Mangifera indica* L.) fruit stored under low temperature stress. Postharvest Biol. Technol. 111, 362-369. doi:10.1016/j.postharvbio.2015.09.029.

Konsue, W., Dethoup, T., Limtong, S. 2020. Biological control of fruit rot and anthracnose of postharvest mango by antagonistic yeasts from economic crops leaves. Microorganisms 8, 317-333.

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

Laohaprasit, N., Ambadipudi, D., Srzednicki, G. 2011. Optimisation of extraction conditions of volatile compounds in 'Nam Dok Mai' mangoes. Int. Food Res. J. 18 (3): 1043-1049

Lawless, H.T., Heymann, H. 2010. Sensory evaluation of food: principles and practices. Springer.

Ma, X., Zheng, B., Ma, Y., Xu, W., Wu, H., Wang, S. 2018. Carotenoid accumulation and expressionof carotenoid biosynthesis genes in mango flesh during fruit development and ripening. Sci. Hortic. 237, 201-206.

Maldonado-Celis, M.E., Yahia, E.M., Bedoya, R., Landazuri, P., Loango, N., Aguillon, J., Restrepo, B., Guerrero Ospina, J.C., 2019. Chemical composition of mango (*Mangifera indica* L.) fruit: nutritional and phytochemical compounds. Front. Plant Sci. 10,1073. doi:10.3389/fpls.2019.01073.

Malundo, T., Baldwill, E., Ware, G., Shewfelt, R. 1996. Volatile composition and interaction influence flavor properties of mango (*Mangifera indica* L.). Proceedings-Florida State Horticultural Society. Florida State Horticultural Society 109, 264-268.

Minas, I.S., Karaoglanidis, G.S., Manganaris, G.A., Vasilakakis, M. 2010. Effect of ozone application during cold storage of kiwifruit on the development of stem-end rot caused by *Botrytis cinerea.* Postharvest Biol. Technol. 58, 203-210.

Minas, I.S., Tanou, G., Belghazi, M., Job, D., Manganaris, G.A., Molassiotis, A., Vasilakakis, M. 2012. Physiological and proteomic approaches to address the active role of ozone in kiwifruit post-harvest ripening. J. Exp. Bot. 63, 2449-2464. doi:10.1093/jxb/err418.

Minas, I.S., Vicente, A.R., Dhanapal, A.P., Manganaris, G.A., Goulas, V., Vasilakakis, M., Crisosto, C.H., Molassiotis, A. 2014a. Ozone-induced kiwifruit ripening delay is mediated by ethylene biosynthesis inhibition and cell wall dismantling regulation. Plant Sci. 229, 76-85.

More, S., Rao, T.R. 2019. Elicitor-mediated sanitization in combination with edible coatings improve postharvest shelf life and antioxidant potential of mango fruit. Environ. Experim. Biol. 17, 107-114.

Muccilli, V., Licciardello, C., Fontanini, D., Russo, M.P., Cunsolo, V., Saletti, R., Recupero, G.R., Foti, S. 2009. Proteome analysis of *Citrus sinensis* L. (Osbeck) flesh at ripening time. J. Proteom. 73, 134-152.

Mustapha, A.T., Zhou, C., Wahia, H., Amanor-Atiemoh, R., Otu, P., Qudus, A., Fakayode, O.A., Ma, H. 2020. Sonozonation: Enhancing the antimicrobial efficiency of aqueous ozone washing techniques on cherry tomato. Ultrason. Sonochem. 64, 105059.

Mutengwe, M., Chidamba, L., Korsten, L. 2016. Pesticide residue monitoring on South African fresh produce exported over a 6-year period. J. Food Protect.79, 1759-1766.

Nakakoga, Y., Tada, Y., Sakoda, T., Tazawa, K., Ichikawa, H. 2021. Sterilization of stem-end rot of mango using dielectric barrier discharge and investigation of sterilization factors. Electron. Commun. Jpn. 104, e12325.

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

Paico, M.J.P., Jittanit, W., Jarupan, L., Chonhenchob, V. 2018. Effect of ozone on prolonging the shelf life of mango and broccoli during cold storage and distribution. The 21st IAPRI World Conference on Packaging.

Pandiselvam, R., Subhashini, S., Banuu Priya, E., Kothakota, A., Ramesh, S., Shahir, S. 2019. Ozone based food preservation: a promising green technology for enhanced food safety. Ozone Sci. Eng. 41, 17-34.

Pandit, S.S., Kulkarni, R.S., Chidley, H.G., Giri, A.P., Pujari, K.H., Köllner, T.G., Degenhardt, J., Gershenzon, J., Gupta, V.S. 2009. Changes in volatile composition during fruit development and ripening of 'Alphonso' mango. J. Sci. Food Agric. 89, 2071-2081.

Panou, A.A., Akrida-Demertzi, K., Demertzis, P., Riganakos, K.A. 2021. Effect of gaseous ozone and heat treatment on quality and shelf life of fresh strawberries during cold storage. Int. J. Fruit Sci. 21, 218-231.

Perry, J.J., Yousef, A.E. 2011. Decontamination of raw foods using ozone-based sanitization techniques. Ann. Rev. Food Sci. Technol. 2, 281-298.

Pino, J.A. 2012. Odour-active compounds in mango (*Mangifera indica* L. cv. Corazón). InternationalJ. Food Sci. Technol. 47, 1944-1950. doi:10.1111/j.1365-2621.2012.03054.x.

Salinas-Roca, B., Guerreiro, A., Welti-Chanes, J., Antunes, M.D., Martín-Belloso, O. 2018. Improving quality of fresh-cut mango using polysaccharide-based edible coatings. Int. J. Food Sci. Technol. 53, 938-945.

Savi, G.D., Scussel, V.M. 2014. Effects of ozone gas exposure on toxigenic fungi species from *Fusarium*, *Aspergillus*, and *Penicillium* genera. Ozone Sci. Engi. 36, 144-152.

Shao, X., Tu, K., Tu, S., Tu, J. 2012. A combination of heat treatment and chitosan coating delays ripening and reduces decay in "Gala" apple fruit. J. Food Qual. 35, 83-92.

Siddiqui, Y., Ali, A. 2014. *Colletotrichum gloeosporioides* (Anthracnose), Postharvest Decay. Elsevier, pp. 337-371.

Simpson, A.M.-A., Mitch, W.A. 2020. Chlorine and ozone disinfection and disinfection byproducts in postharvest food processing facilities: A review. Crit. Rev. Environ. Science Technol. 1-43.

Singh, Z., Singh, R.K., Sane, V.A., Nath, P. 2013. Mango - Postharvest Biology and Biotechnology. Crit. Rev. Plant Sci. 32, 217-236. doi:10.1080/07352689.2012.743399.

Sivakumar, D., Jiang, Y., Yahia, E.M. 2011. Maintaining mango (*Mangifera indica* L.) fruit quality during the export chain. Food Res. Int. 44, 1254-1263.

Sivakumar, D., Van Deventer, F., Terry, L.A., Polenta, G.A., Korsten, L. 2012. Combination of 1-methylcyclopropene treatment and controlled atmosphere storage retains overall fruit quality and bioactive compounds in mango. J. Sci. Food Agric. 92, 821-830. doi:10.1002/jsfa.4653.

Sousa, F.F., Pinsetta Junior, J.S., Oliveira, K., Rodrigues, E.C.N., Andrade, J.P., Mattiuz, B.H. 2021. Conservation of 'Palmer' mango with an edible coating of hydroxypropyl methylcellulose and beeswax. Food Chem. 346, 128925. doi:10.1016/j.foodchem.2020.128925.

Swart, S., Broekhuizen, W.V. 2004. Evaluation of post-harvest disease control with regards to the new maximum residue level of five ppm for prochloraz on mangoes in the European Union. South African Mango Growers' Association 24, 73-77.

Tahir, H.E., Xiaobo, Z., Jiyong, S., Mahunu, G.K., Zhai, X., Mariod, A.A. 2018. Quality and postharvest-shelf life of cold-stored strawberry fruit as affected by gum arabic (*Acacia senegal*) edible coating. J. Food Biochem. 42, e12527.

Terao, D., de Lima Nechet, K., Frighetto, R.T.S., Sasaki, F.F.C. 2019. Ozonated water combined with heat treatment to control the stem-end rot of papaya. Scientia Horticulturae 257, 108722.

Tesfay, S.Z., 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 Packag. Shelf Life 11, 40-48.

Toti, M., Carboni, C., Botondi, R. 2018. Postharvest gaseous ozone treatment enhances quality parameters and delays softening in cantaloupe melon during storage at 6 degrees C. J. Sci. Food Agric. 98, 487-494. doi:10.1002/jsfa.8485.

Tran, T.T.L., Aiamla-or, S., Srilaong, V., Jitareerat, P., Wongs-Aree, C., Uthairatanakij, A. 2015. Ozone fumigation to delay ripening of mango 'Nam Dok Mai No 4' Proc. IInd Southeast Asia Symp. on Quality Management in Postharvest Systems.

Vázquez-Celestino, D., Ramos-Sotelo, H., Rivera-Pastrana, D.M., Vázquez-Barrios, M.E., Mercado-Silva, E.M. 2016. Effects of waxing, microperforated polyethylene bag, 1-methylcyclopropene and nitric oxide on firmness and shrivel and weight loss of 'Manila' mango fruit during ripening. Postharvest Biol. Technol. 111, 398-405. doi:10.1016/j.postharvbio.2015.09.030.

Wei, K., Zhou, H., Zhou, T., Gong, J. 2007. Comparison of aqueous ozone and chlorine as sanitizers in the food processing industry: impact on fresh agricultural produce quality. Ozone Sci. Eng. 29, 113-120.

Yashoda, H.M., Prabha, T.N., Tharanathan, R.N. 2006. Mango ripening: changes in cell wall constituents in relation to textural softening. J. Sci. Food Agric. 86, 713-721.

Youssef, A.R., Ali, E.A., Emam, H.E. 2015. Influence of postharvest applications of some edible coating on storage life and quality attributes of Navel orange fruit during cold storage. Int. J. Chemt. Res. 8, 2189-2200.

Yun, Z., Jin, S., Ding, Y., Wang, Z., Gao, H., Pan, Z., Xu, J., Cheng, Y., Deng, X. 2012. Comparative transcriptomics and proteomics analysis of citrus fruit, to improve understanding of the effect of low temperature on maintaining fruit quality during lengthy post-harvest storage. J. Exper. Bot. 63, 2873-2893.

Zaharah, S.S., Singh, Z., Symons, G.M., Reid, J.B. 2012. Role of brassinosteroids, ethylene, abscisic acid, and indole-3-acetic acid in mango fruit ripening. J. Plant Grow. Regul. 31, 363-372.

Zhang, X., Tang, N., Zhang, H., Chen, C., Li, L., Dong, C., Cheng, Y. 2021. Comparative transcriptomic analysis of cantaloupe melon under cold storage with ozone treatment. FoodRes. Int. 140, 109993.

Zhang, X., Zhang, Z., Wang, L., Zhang, Z., Li, J., Zhao, C. 2011. Impact of ozone on quality of strawberry during cold storage. Front. Agric. China 5, 356-360.

Chapter 5

The effect of gaseous ozone and moringa leaf–carboxymethyl cellulose edible coating on antioxidant activity and biochemical properties of 'Keitt' mango fruit

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Abstract

This study evaluated the effect of edible coating and gaseous ozone on the antioxidant activities and biochemical properties of mango fruit. Mango fruit (*cv*. Keitt) were coated with moringa leaf extract and carboxymethyl cellulose (EC) before exposure to ozone (0.25 mg L^{-1}). Gaseous ozone (O_3) was administered intermittently for 24 or 36 h, and the control fruit were untreated. The fruit were stored at 10°C for twenty-one days, then ripened at ambient temperature for seven days. The parameters measured were ascorbic acid, lipid peroxidation, phenolic content, total sugars, and antioxidant capacity (FRAP and DPPH). At the end of storage, the EC + O_3 (36 h) had high phenolic content: 175.02 µg GEA/g DM compared to 151.87 µg GEA/g DM and 138.98 µg GEA/g DM for the O_3 (24 h)

and untreated fruit, respectively. Moreover, the combination of the EC and O_3 (36 h) had a higher effect ($p < 0.05$) on preserving the antioxidant capacity of the mangoes. The EC + O_3 (24 h) and EC significantly delayed fruit softening and maintained membrane integrity. Furthermore, the fruit treated with the EC reduced the accumulation of reducing (7.61 mg/mL) and total sugars (8.81 mg/mL) compared to the control treatment, which had a concentration of 12.74 mg/mL and 13.78 mg/mL, respectively. These findings demonstrate that EC combined with gaseous O_3 enhanced the antioxidants of mango fruit during storage.

Keywords: Mango fruit; sugars; phenolics; antioxidants; edible coating; ozone

1 Introduction

Mango (*Mangifera indica* L.) is an excellent source of vitamins, carotenoids, antioxidants, including phenolics, and ascorbic acid. Recent studies have highlighted the nutritional value of fruit and vegetables and their effect on human health (Abeysinghe et al., 2007). The high intake of fresh fruits that are rich in natural antioxidants is linked to a lower risk of diseases, such as cancer, Alzheimer's, cardiovascular diseases, and rheumatoid arthritis (Alothman et al., 2010; Skrovankova et al., 2012; Liu et al., 2018). Antioxidants are reactive against reactive oxygen species, such as hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) (Skrovankova et al., 2012; Shezi et al., 2020). Reactive oxygen species (ROS) can cause oxidative stress in the fruit, resulting in decreased sensory quality, leading to off flavors (Skrovankova et al., 2012; Gaikwad et al., 2020). In order to maintain the quality and high contents of antioxidants and bioactive compounds, postharvest chemical treatments are commonly used by the fresh produce industry (Gaikwad et al., 2020; Khaliq et al., 2020). Treatments such as nitric oxide, 1-methylcyclopropene and salicylic acid are some of the commonly used chemicals for preserving the antioxidants and overall quality of various horticultural produce (Barman and Asrey,

2014; Khaliq et al., 2016; Gaikwad et al., 2020; Khaliq et al., 2020). The increasing consumer demands for chemical-free food has necessitated searching for novel and environmentally friendly postharvest treatments. Edible coatings and gaseous ozone have gained interest among food scientists and postharvest researchers in recent years.

Edible coatings (EC) are produced from lipids, proteins, resins, and polysaccharides (Khaliq et al., 2016; Ncama et al., 2018). Polysaccharide-based coatings include chitosan, cellulose, starch, pectin, and gums (Bourtoom, 2008). These coatings form a pervious sheet on the fruit surface, causing a modified atmosphere, thus reducing water loss and internal oxygen and increasing internal carbon dioxide (Bourtoom, 2008; Baldwin et al., 1995). The edible coatings have effectively decreased postharvest disease, extended shelf-life, and preserved antioxidants, such as carotenoids, phenolic compounds, flavonoids, and ascorbic acid (Khaliq et al., 2016; Salinas-Roca et al., 2018). For instance, Daisy et al. (2020) reported that gum Arabic (15%) maintained the ascorbic acid and β-carotene in 'Apple' mango stored at ambient temperature for fifteen days. Likewise, a study by Ncama et al. (2021) revealed that moringa leaf extract (MLE) (2%) and carboxymethyl cellulose (CMC) (1%) preserved the ascorbic acid in 'Marsh' grapefruit during storage at 5°C for sixty-three days.

Ozone (O_3) is an unstable molecule with antimicrobial properties and high oxidation potential (Shezi et al., 2020). It decomposes to form radicals, such as superoxide, hydroxyl, and hydroperoxyl, leaving no chemical residues (Brodowska et al., 2018). Numerous studies have been conducted to evaluate the effect of O_3 on maintaining the quality of various horticultural crops (Alothman et al., 2010; Minas et al., 2012). For instance, ozone effectively preserved the ascorbic acid, carotenoids in 'Palmer' mango, and flavonoids in 'Qiushui' pear during storage (Zhao et al., 2013; de Almeida Monaco et al., 2016). Ozone treatment (0.3 µL L^{-1}) enhanced both the ferric reducing antioxidant power assay (FRAP) and

1.1-diphenyl-2-picrylhydrazy (DPPH) antioxidant capacity in 'Hayward' kiwifruit stored at 0°C for three months and ripened at 20-0 for twelve days (Minas et al., 2012). Ozone reduces the O_2 accumulation in the fruit surroundings, thus decreasing the rate of respiration and resulting in biochemical changes that lead to enhanced fruit quality. Firmness is an important quality indicator as consumers buy fresh fruit based on its appearance.

Edible coatings enhance the fruit quality by preventing water loss and delaying the accumulation of electrolyte leakage and lipid peroxidation (Tesfay et al., 2017; Escamilla-García et al., 2018). Kumaret al. (2021) observed the delayed softening in 'Sefada' mango coated with chitosan 2% + pullulan 2% stored at 4°C for fifteen days. Studies by Hmmam et al. (2021) revealed that CMC and guar gum-based silver nanoparticles (AgNPs) coating retained the firmness in 'Seddik' mangoes during storageat 13°C for thirty days. Edible coatings are linked to the modification of cell wall structures, which leads to fruit firmness retention (Escamilla-García et al., 2018).

Preserving antioxidants and quality during the postharvest treatment is vital for maintaining high nutritional value of the stored fruit. Although O_3 and edible coatings have been extensively studied in recent years, the combined effect of these treatments has not yet been investigated. Moreover, the infusion of moringa leaf extract into CMC has never been tested on mango fruit. Thus,the objective of this study was to investigate the effect of gaseous ozone and moringa leaf extract– CMC-based edible coating on the antioxidant activities and biochemical properties of 'Keitt' mango fruit.

2 Materials and Methods

2.1 Fruit material

Mango fruit (*cv*. 'Keitt') were harvested from Goedgelegen Farm of Westfalia (Pty) Ltd, a commercial farm located in Tzaneen, South Africa. Fruit were transported within 24 h to the Postharvest ResearchLaboratory of the University of KwaZulu-Natal (Pietermaritzburg Campus). The fruit used in the experiment were free from mechanical and physiological defects; they were also of the same size,color, and maturity. The experiment was replicated three times using three fruit per replicate. A total of 300 fruit were used for the experiment. The fruit were assigned to different treatments as follows:

Control: untreated

T1: O_3 (24 h)T2:

O_3 (36 h)

T3: EC (MLE 1% + CMC 1%)

T4: EC + O_3 (24 h)

T5: EC + O_3 (36 h)

2.2 Edible coating

Moringa leaf extract was prepared as described by Tesfay and Magwaza (2017), with some modification. Briefly, 100 g of moringa leaf powder was extracted with 1000 mL of 70% ethanol (v/v) for twelve hours at room temperature with constant agitation. The extract was evaporated at 37°C using a Genevac evaporator (Genevac® EZ 2.3; Ipswich, UK). The crude extract was suspended with 1000

mLof distilled water and incorporated to 10 g of CMC.

2.3 Ozone treatment

Gaseous ozone was generated using the corona discharge ozone generator (Ozone Purification Technology, Johannesburg, South Africa). Ozone treatment (0.25 mg L^{-1}) of mango fruit was done in cold storage for twelve hours at seven-day intervals. For the 24 h treatment, fruit were exposed to O$_3$ at day zero and seven, whereas, for 36 h, it was day zero, seven, and fourteen. The O$_3$ times used in the current study were selected based on the physicochemical results of a screening study conducted in 2018. Fruit were coated with moringa leaf extract–CMC before O$_3$ treatment. The control fruit were untreated, and all the fruit were stored at 10°C and 90% relative humidity for twenty-one days, mimicking shipment from South Africa to the European Union markets. After cold storage, fruit were transferred to shelf-life at ambient temperature for seven days.

2.4 Sample preparation

Mangoes were peeled, pulp removed from the kernel, and cut into small cubes. Afterward, the cubes were pureed using a blender (Bennett Read, Tornado Tech Cyclonic Action, Tevo, Durban, South Africa). The puree was filtered three times using a muslin cloth and centrifuged (Avanti J-265 XP, Beckaman Coulter, Indianapolis, IN, USA) at 1500 rpm for ten minutes to obtain a clear extract. The juice extract was stored in specimen bottles at -20°C until used for analysis. The peel was freeze-dried using a Vir Tis BenchTop Pro freeze drier (SP Scientific,Warminster, PA, USA). After that, samples were crushed into powder and stored at -20°C until used. The experiment was replicated three times, with three fruit per replicate. Fruit firmness was measured as described by González-Aguilar et al.

(2001) using a hand-held tester (Bareiss, Baiersbronn, Germany) and expressed as Newton (N).

2.5 Antioxidant activity

2.5.1   1.1-Diphenyl-2-picrylhydrazy (DPPH)

The 1.1-diphenyl-2-picrylhydrazy (DPPH) radical scavenging assay was determined as described by Alothman et al. (2010), with minor modifications. A 150 mg sample was added to 3 mL methanol 80% (*v/v*) and incubated for 1.5 h at 35°C. After that, samples were cooled down and filtered with a 0.45 µm syringe filter (Merck, Warmstadt, Germany). In triplicates, 3.9 mL of methanolic DPPH (2.5 mg/100 mL) was added to the extract (100 µL) and incubated for 60 min at room temperature. The absorbance was measured with a spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA) at 515 nm, using methanol as blank. The DPPH radical scavenging activity was calculated and plotted against Trolox standards and expressed as µM TE/g DM.

2.5.2   Ferric reducing antioxidant power assay (FRAP)

FRAP was determined as described by Alothman et al. (2010), with modification. Briefly, 3 mL of methanol, water and hydrochloric acid (70:29.5:0.5 *v/v*) were added to 150 mg sample and incubated at 35°C for 1.5 h. Samples were cooled down and centrifuged (Avanti J-265 XP, Beckaman Coulter, Indianapolis, IN, USA) for ten minutes at 13,000 rpm and filtered with a 0.45 µm syringe filter (Merck, Warmstadt, Germany). In triplicates, extract (50 µL) was added to 3.6 mL of FRAP solution and incubated at 37°C for ten minutes. The absorbance was measured with a spectrophotometer (Shimadzu

Scientific Instruments Inc., Columbia, MD, USA) at 590 nm, using deionized water as a blank. FRAP was expressed as µmol Fe (II)/g dry mass.

2.6 Total phenolic content

Total phenolics were determined as described by Lamien-Meda et al. (2008), with minor modification. Peel powder (1 g) was added to 10 mL acetone (80% *v/v*) at room temperature for 30 min with constant agitation. After that, samples were centrifuged (Avanti J-265XP, Beckaman Coulter, Indianapolis, IN, USA) for ten minutes at 10,000 rpm (5°C). Total phenolic compounds were determined using the Folin–Ciocalteau method. In triplicates, sample extract (0.1 mL) was added to 2.5 mL Folin–Ciocalteau reagent (2 N). After five minutes, 2 mL sodium carbonate (75 g/L) was added, and samples were incubated at 65°C for 2 h. Thereafter, samples were cooled down, and absorbance was measured with a spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA) at 750 nm using acetone as blank. Total phenolic compounds were expressed as µg (GAE)/g dry matter.

2.7 Ascorbic acid

Ascorbic acid was measured as described by More and Rao (2019), with slight modification. Sample powder (1 g) was added to 9 mL of metaphosphoric acid (1%) and sonicated in ice for 3 min. Thereafter, samples were centrifuged (Avanti J-265 XP, Beckaman Coulter, Indianapolis, IN, USA) at 10,000 rpm (4°C) for five minutes. In triplicates, the extract (1 mL) was added to 9 mL of 2,6-dichlorophenolindephenol dye (0.025%) and incubated in the dark at room temperature for ten minutes. The absorbance was measured with a spectrophotometer (Shimadzu Scientific Instruments Inc.,

Columbia, MD, USA) at 515 nm using 1% metaphosphoric acid as a blank. Ascorbic acid was expressed as mg/g dry mass.

2.8 Electrical conductivity

Electrical conductivity was determined as described by Jincy et al. (2017) with modification. Mango cubes (10 mm) were rinsed in deionized water three times, then incubated at 25°C in deionized water (10 mL) for four hours with constant shaking. Thereafter, samples were incubated for 60 min at 95°C and cooled down to 25°C. Electrical conductivity was measured with the Benchtop conductivity/TDS meter (Bante 510, Bante Instruments, Shanghai, China) before and after boiling. The relative electrolyte leakage (REL) was calculated using the following formula:

$$REL \% = \frac{Ei}{Ef} \; x \; 100 \qquad\qquad (1)$$

Where Ei and Ef are the initial and final readings, respectively.

2.9 Lipid peroxidation

Lipid peroxidation was measured as the amount of malondialdehyde (MDA) as described by Diperierro and De Leonardis (1997), with minor modifications. Mango peel (1 g) was homogenized in 10 mL of 1% trichloroacetic acid (*w/v*). Thereafter, the sample was centrifuged (Avanti J-265 XP, Beckaman Coulter, Indianapolis, IN, USA) at 10,000 rpm at 4°C for ten minutes. In triplicates, the sample extract (1 mL) was added to 20% trichloroacetic acid (4 mL) containing 0.5% thiobarbituric acid *(v/v)* and boiled for thirty minutes at 95°C. The samples were thereafter cooled down on the ice and centrifuged at 10,000 rpm for

ten minutes. The absorbance was measured with a spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA) at 532 nm and 600 nm. The MDA concentration was calculated using the Equation 2 and expressed as ngmol^{-1} g^{-1}.

Total MDA$= \frac{A532 - A600}{155} x\ 10^3$

Where A532 is the absorbance at 532 nm and A600 is the absorbance at 600 nm.

2.10   Total sugars

For the analysis of total sugars, juice extract was filtered with a 0.45 µm filter (Merck, Warmstadt, Germany). The extract (200 µL) was diluted with 800 µL of ultrapure water and vortexed for 20 s. Sugars were analyzed as described by Tesfay and Magwaza (2017) using an isocratic HPLC. Sample extracts were infused into the Rezex RCM monosaccharide Ca$^+$ (8%) of 300 mm X 7.8 mm column (Phenomenex, Torrance, CA, USA) with a Carbo-Ca^{2+} of 3 mm X 4 mm guard column (Phenomenex). The column temperature was 80°C, and the mobile phase was ultra-pure water. The concentration of sugars was determined by comparing the peak areas of the standard curve with those of samples.

2.11   Statistical analysis

Data were subjected to the analysis of variance (ANOVA) using GenStat statistical software (GenStat ®, 18 edition, VSN International, Hemel Hempstead, UK). Fischer's least significant difference (LSD)was used to separate means at 5% level of significance. Pearson correlation coefficient was used to determine relationships   between   lipid   peroxidation,   phenolic   content,   and   antioxidants.

3 Results

3.1 Firmness

The untreated fruit showed a rapid decrease in firmness compared to the other treatments (Figure 5.1). There was a significant ($p < 0.05$) difference between the treatment means of the untreated fruit, O_3 (36 h), EC, EC + O_3 (24 h), and EC + O_3 (36 h), from day fourteen till the end of storage. High firmness was observed at the end of storage in the fruit treated with EC+ O_3 (36 h), O_3 (36 h), and EC.
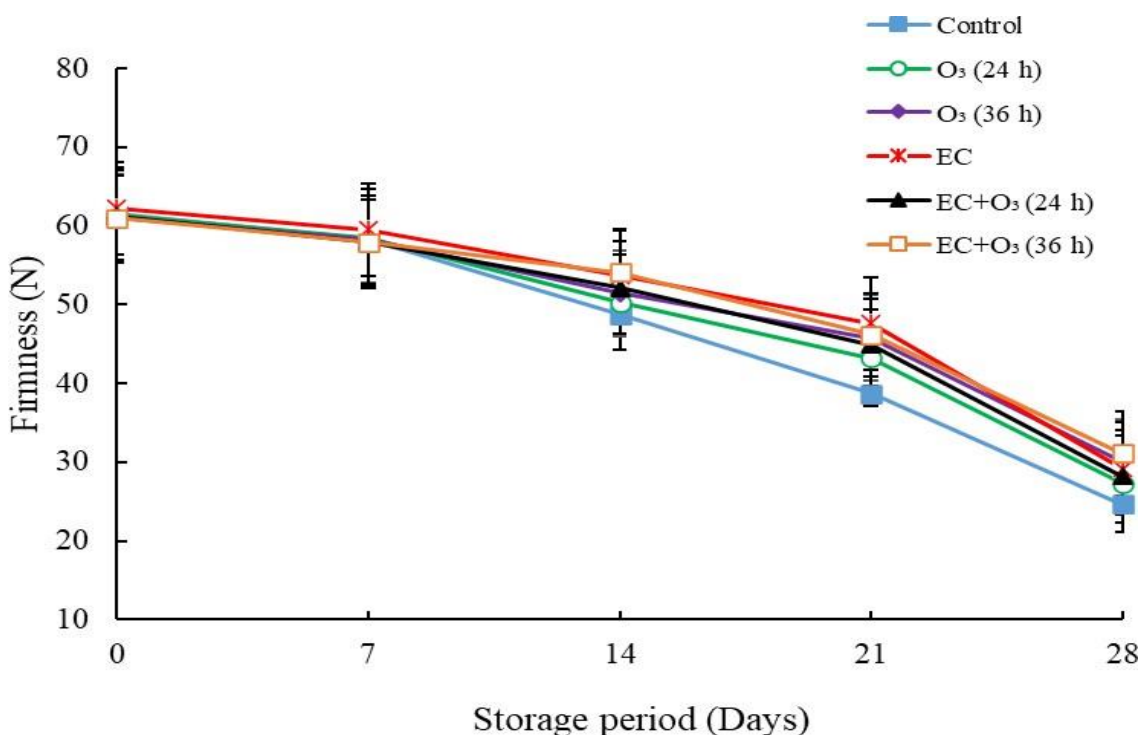
Figure 5.1: Effect of edible coating and gaseous ozone on mango fruit firmness stored at 10°C for twenty-one days and seven days shelf-life at ambient temperature (±SE, $n = 9$)

3.2 Antioxidant activity

The DPPH scavenging activity in all the treatments increased up to day fourteen, then gradually decreased until the end of storage. The fruit coated with EC had significantly ($p < 0.05$) higher scavenging activity from day fourteen up to the end of storage compared to the other treatments (Figure 5.2). The DPPH activity of the untreated fruit was low compared to the other treatments, except at the end of storage when it was comparable to O_3 (24 h). At the end of storage, the DPPH activities of the EC + O_3 (24 h) and O_3 (36 h) were not significantly different.
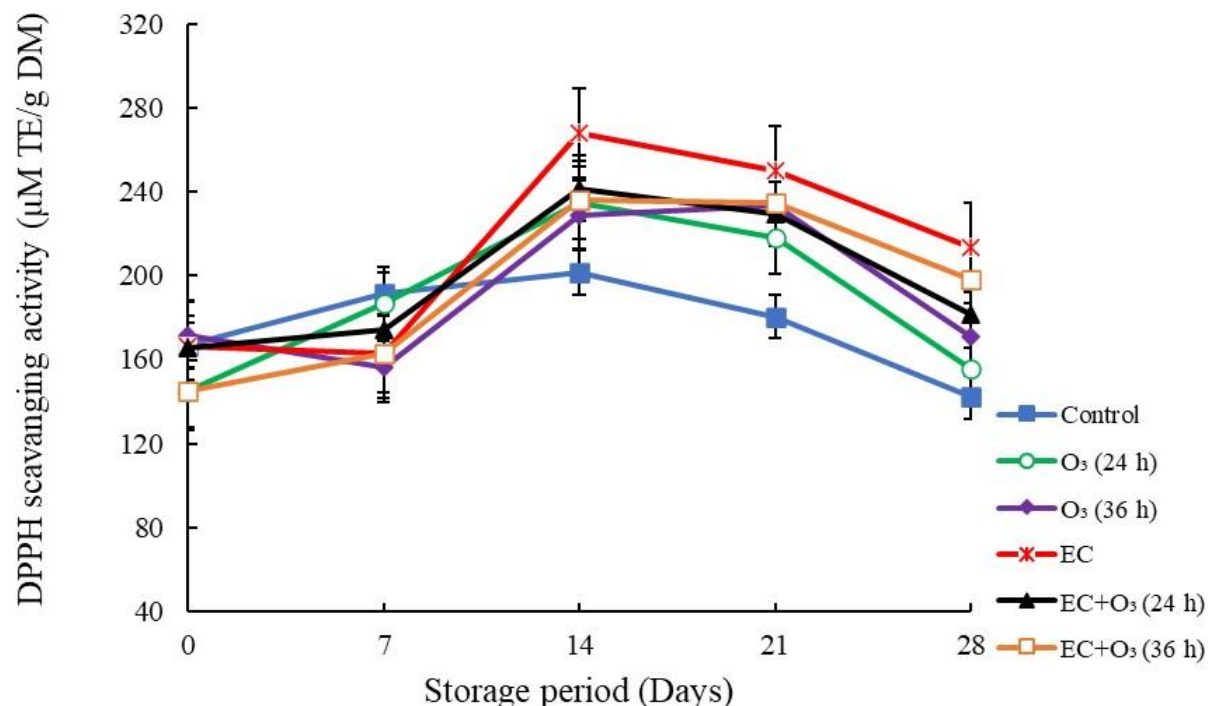
Figure 5.2: Effect of edible coating and gaseous ozone on DPPH scavenging activity of mango peel during storage at 10 °C for twenty-one days and shelf-life at ambient temperature for seven days ($\pm$SE, $n = 9$).

The antioxidant activity, measured by the FRAP, of the mango fruit decreased in all the treatments during storage (Figure 5.3). The FRAP activity of the O_3 (24 h) was low compared to the other treatments throughout the storage period. At the end of storage, the EC + O_3 (36 h) had a high FRAP antioxidant compared to the control, O_3 (24 h), and EC + O_3 (24 h). However, there were no significant differences between the treatment means of O_3 (24 h), EC, EC + O_3 (24 h), and the control at the end of storage.
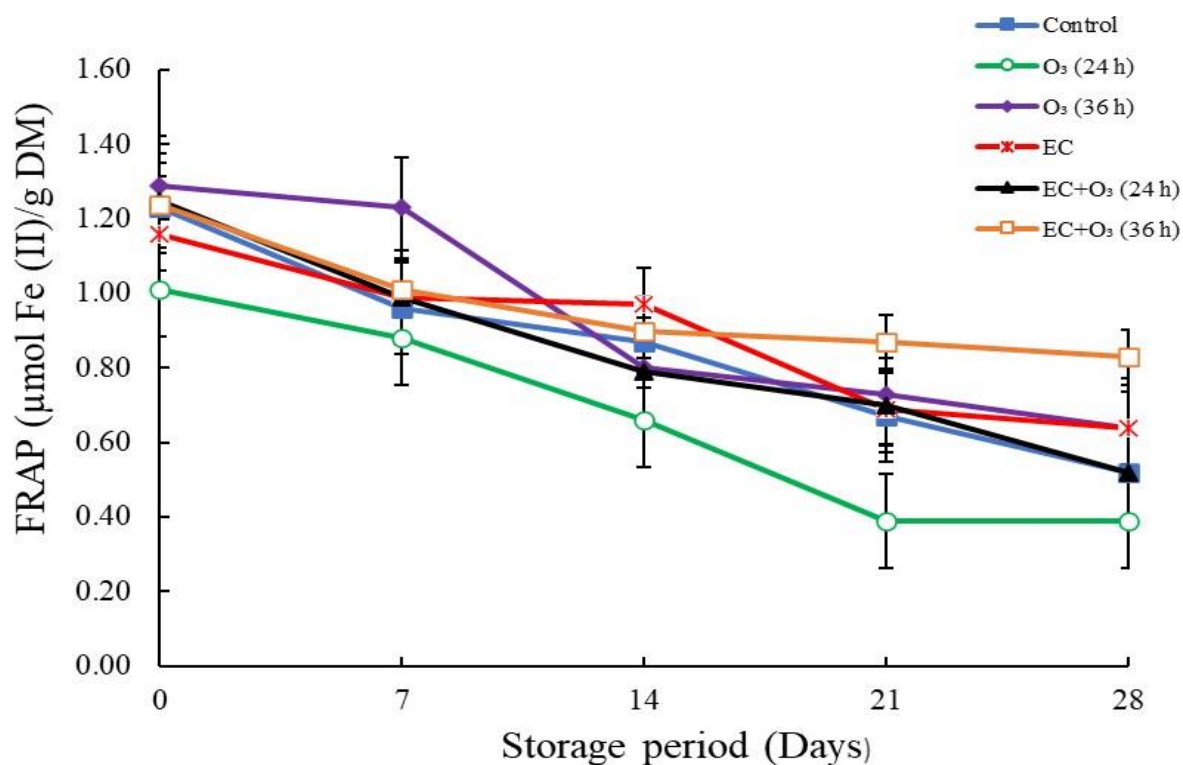
Figure 5.3: Effect of edible coating and gaseous ozone on FRAP activity in mango peel during storage at 10 °C for twenty-one days and shelf-life at ambient temperature for seven days (±SE, $n = 9$)

3.4 Total phenolics

The total phenolic compounds in the mango fruit significantly decreased during storage. However, the phenolic content of the treated fruit decreased gradually compared to the rapid reduction in the untreated fruit (Figure 5.4). The phenolic content of the mango fruit was significantly ($p < 0.05$) affected by the different treatments. The phenolic content in the EC and EC+ O_3 (36 h) was higher than the other treatments from day fourteen until the end of storage. At the end of storage, low phenolic content was observed in the O_3 (24 h), EC+ O_3 (24 h), and untreated fruit.
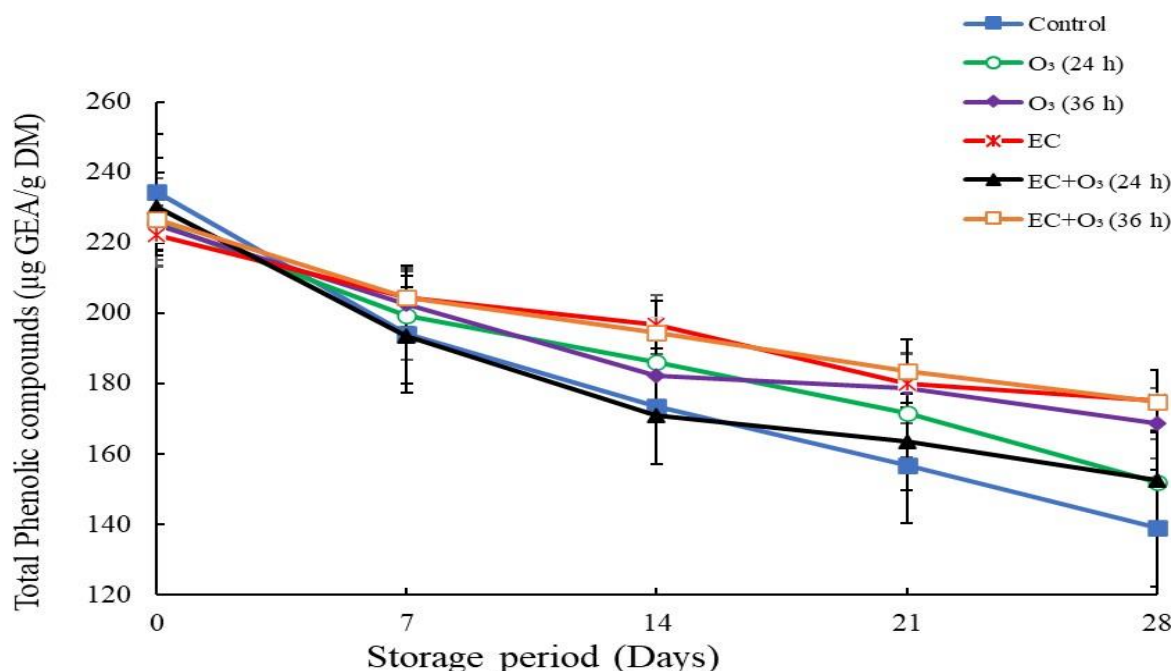
Figure 5.4: Effect of edible coating and gaseous ozone on total phenolics in mango peel during storage at 10 °C for twenty-one days and shelf-life at ambient temperature for seven days (±SE, $n = 9$)

3.5 Ascorbic acid

There were significant ($p < 0.05$) differences in ascorbic acid (AA) between the treatment means over storage time (Figure 5.5). The fruit coated with the EC had the highest AA compared to the other treatments from day fourteen till the end of storage. However, there was no significant difference between the treatment means of the EC and EC + O_3 (24 h) at the end of storage. The fruit treated with the O_3 (36 h) inhibited a decrease in AA compared to the O_3 (24 h) treatment during storage. The AA in the EC + O_3 (24 h) and EC + O_3 (36 h) was similar throughout the storage period. At the end of storage, the highest AA was observed in the EC (98.70 mg/g DM) treatment compared to the EC + O_3 (24 h), O_3 (24 h), and untreated fruit, which had 87.30 mg/g DM, 77.10 mg/g DM, 70.20 mg/g DM, 50.70 mg/g DM, and 37.41 mg/g DM, respectively.

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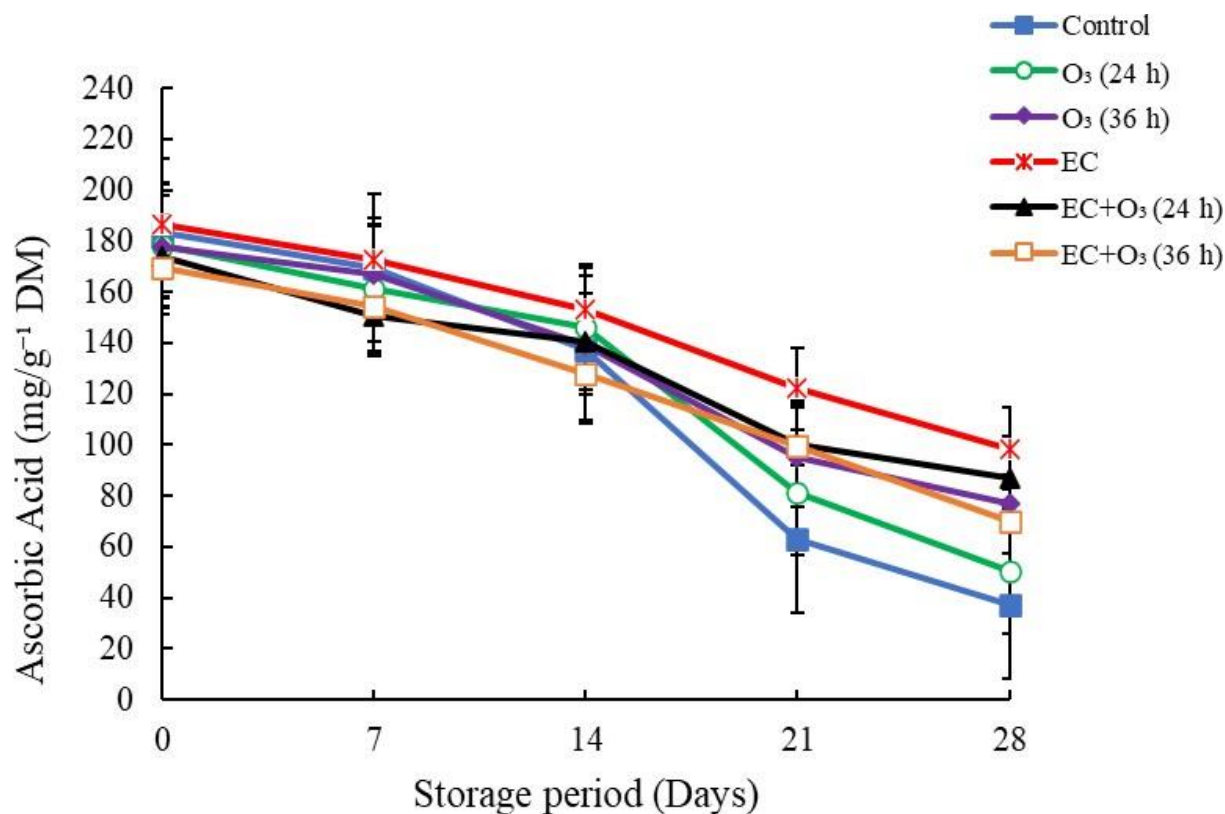
Figure 5.5: Effect of edible coating and gaseous ozone on AA in mango fruit during storage for twenty-one days at 10 °C and shelf-life for seven days at ambient temperature (±SE, $n = 9$)

3.6 Electrical conductivity

The results of the relative electrolyte leakage (REL) are shown in Table 5.1. The REL significantly ($p < 0.05$) increased in all the treatments throughout storage. However, a sharp increase was observed in the untreated fruit compared to the other treatments. The low percentage of REL was observed in the EC, EC + O_3 (24 h), and EC + O_3 (36 h) from day fourteen till the end of storage. The fruit treated with the EC + O_3 (24 h) (22.52%) maintained membrane integrity compared to the EC (24.40%), O_3 (36 h) (26. 46%), and the untreated fruit (39.77%) at the end of storage.

Table 5.1: Effect of edible coating and gaseous ozone on relative electrolyte leakage (REL) (%) of mango fruit during storage for twenty-one days at 10 °C and shelf-life for seven days at ambient temperature

Treatment	Storage time (Days)				
	0	**7**	**14**	**21**	**28**
Control	$4,13^a$	$9,57^{bc}$	$18,11^{hij}$	$27,79^l$	$39,77^m$
O₃ (24 h)	$4,10^a$	$9,47^{bc}$	$14,25^{efg}$	$20,11^j$	$27,94^l$
O₃ (36 h)	$4,47^a$	$9,45^{bc}$	$15,05^{fg}$	$19,29^{ij}$	$26,46^l$
EC	$3,99^a$	$9,94^{bc}$	$13,51^{def}$	$18,52^{ij}$	$24,40^k$
EC+O₃ (24 h)	$4,14^a$	$8,00^b$	$11,51^{cd}$	$16,19^{gh}$	$22,52^k$
EC+O₃ (36 h)	$3,92^a$	$7,81^b$	$12,19^{de}$	$17,37^{hi}$	$23,74^k$

Means with the same letters within a column and between columns indicate no significant difference between treatments. Data represent means (LSD = 2.00, $n = 9$)

3.7 Lipid peroxidation

The MDA content in the mango fruit significantly ($p < 0.05$) increased during storage. The fruit treated with the EC and O_3 (24 h) inhibited the accumulation of the MDA content during cold storage (Table 5.2). At the end of storage, high MDA content was observed in the untreated fruit compared to the EC+ O_3 (24 h), EC + O_3 (36 h), and O_3 (24 h). The MDA content of the EC + O_3 (24 h) and EC + O_3 (36 h) was not significantly different at the end of storage.

Table 5.2: Effect of edible coating and gaseous ozone on mango peel lipid peroxidation (MDA ngmol^{-1} g^{-1}) during storage for twenty-one days at 10 °C and shelf-life for seven days at ambient temperature

Treatment	Storage time (Days)				
	0	7	14	21	28
Control	0,47^{ab}	0,65^{abc}	1,68^{e}	2,65^{f}	5,12^{j}
O₃ (24 h)	0,42^{a}	0,66^{abc}	1,43^{de}	2,68^{f}	4,03^{gh}
O₃ (36 h)	0,42^{a}	0,65^{abc}	1,49^{de}	2,37^{f}	3,51^{g}
EC	0,49^{ab}	0,73^{abc}	1,04^{bcd}	2,54^{f}	3,87^{gh}
EC+O₃ (24 h)	0,43^{a}	0,73^{abc}	1,09^{cd}	2,26^{f}	4,64^{ij}
EC+O₃ (36 h)	0,44^{a}	0,70^{abc}	1,04^{bcd}	2,44^{f}	4,39^{hi}

Means with the same letters within a column and between columns indicate no significant difference between treatments. Data represent means (LSD = 0.50, $n = 9$)

3.8 Soluble sugars

The treatments significantly ($p < 0.05$) affected the sugar content of the mango fruit. The reducing sugars (RS) increased in all the treatments during storage (Figure 5.6). A rapid increase in the RS content was observed in the untreated fruit compared to the other treatments. At the end of storage, the low RS content was observed in the fruit treated with the EC and EC + O$_3$ (36 h), whereas it was notably high in the untreated fruit.
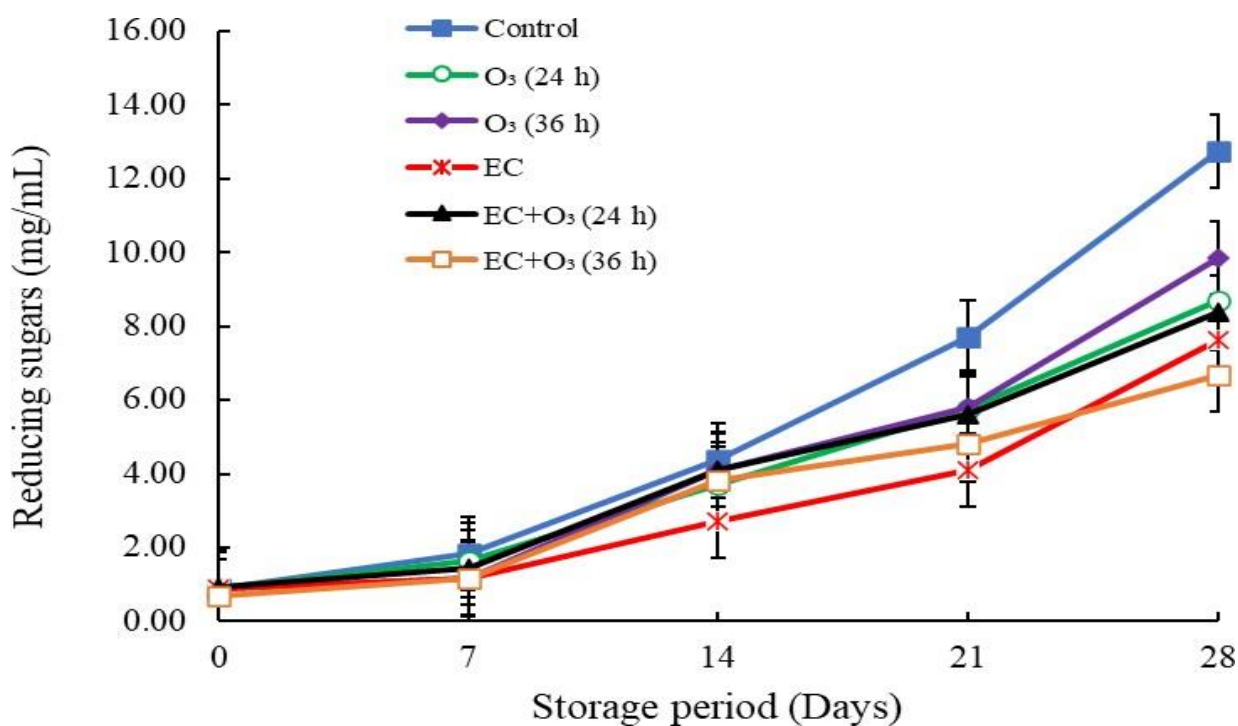
Figure 5.6: Effect of edible coating and gaseous ozone on reducing sugars of mango fruit during storage at 10 °C for twenty-one days and shelf-life at ambient temperature for seven days (±SE, *n* = 9)

The non-reducing sugars (NRS) increased in all the treatments from day zero till day fourteen, then slowly decreased (Figure 5.7). At the end of storage, there was no significant difference between the treatment means of the O_3 (24 h), O_3 (36 h), and EC + O_3 (24 h). The total sugars (TS) significantly increased in all the treatments during storage (Figure 5.8).
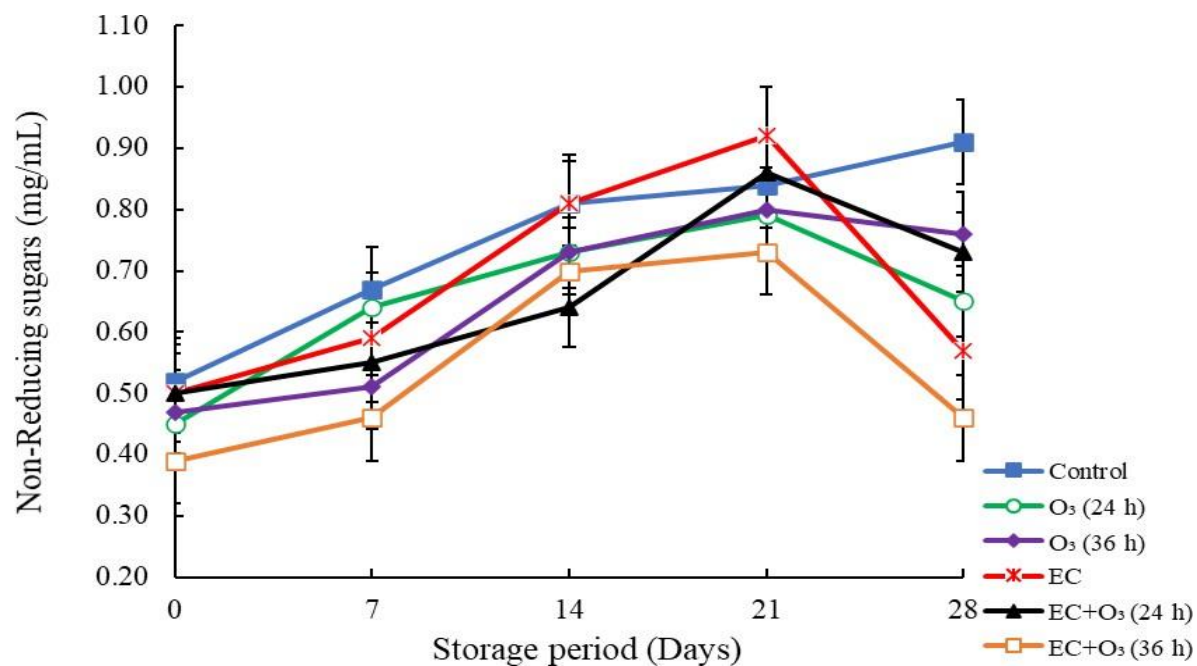
Figure 5.7: Effect of edible coating and gaseous ozone on non-reducing sugars of mango fruit during storage at 10 °C for twenty-one days and shelf-life at ambient temperature for seven days (±SE, $n = 9$)
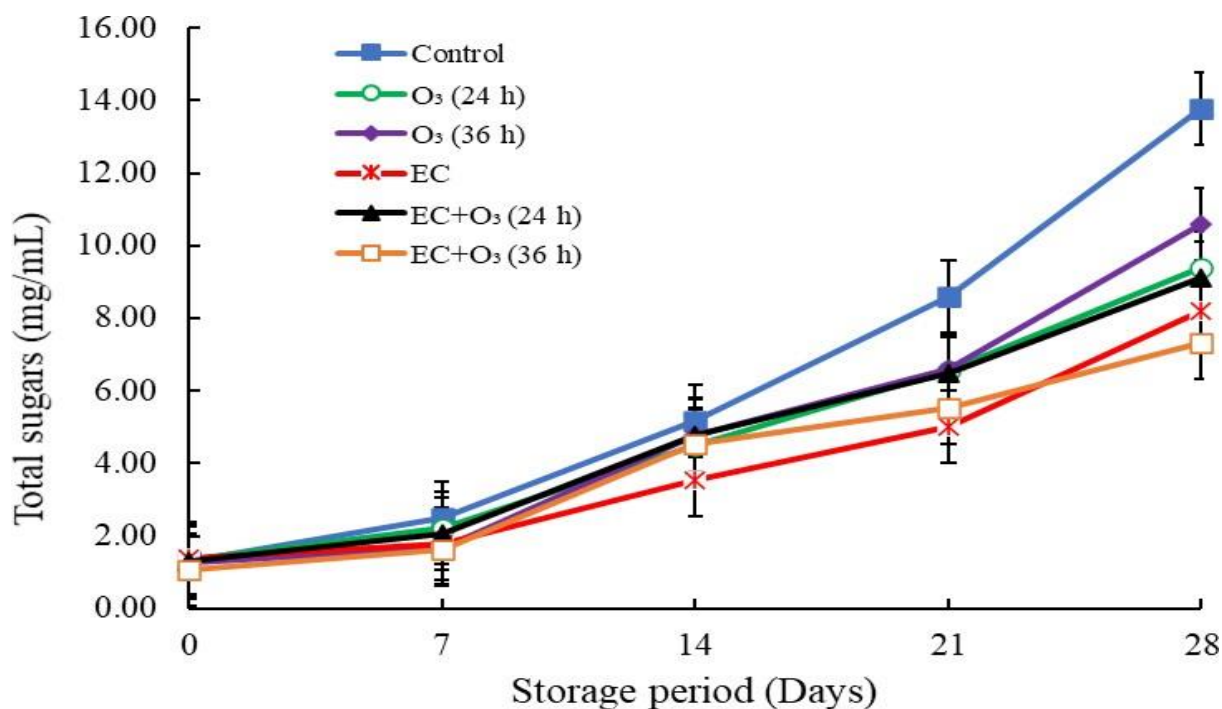
Figure 5.8: Effect of edible coating and gaseous ozone on total sugars of mango fruit during storage at 10°C for twenty-one days and shelf-life at ambient temperature for seven days (±SE, n=9)

3.9 Correlations of mango firmness and biochemical parameters

The fruit firmness was positively correlated to the FRAP ($R^2 = 0.51$) and had a strong negative correlation with the MDA ($R^2 = -0.77$), REL ($R^2 = -0.75$), and RS ($R^2 = -70$). A positive correlation ($R^2 = 0.61$) was observed between the FRAP and phenolic content (Table 5.3). The ascorbic acid was negatively correlated with all the parameters measured. The DPPH had a positive correlation to the MDA ($R^2 = 0.64$), RS ($R^2 = 0.59$), and REL ($R^2 = 0.59$). A medium to strong positive correlation was observed between the MDA, RS ($R^2 = 0.72$), NRS ($R^2 = 0.51$), TS ($R^2 = 0.73$), and REL ($R^2 = 0.89$).

Table 5.3: Pearson correlation coefficient of AA, FRAP, DPPH, phenolics, MDA, RS, NRS, TS, REL in mango fruit

	Firmness	AA	FRAP	DPPH	Phenolics	MDA	REL	RS	NRS
AA	0.25								
FRAP	**0.51**	0.17							
DPPH	**-0.60**	-0.20	-0.34						
Phenolics	0.48	0.15	**0.61**	-0.38					
MDA	**-0.77**	-0.26	-0.45	**0.64**	**-0.60**				
REL	**-0.75**	-0.18	-0.36	**0.59**	**-0.63**	**0.89**			
RS	**-0.70**	-0.16	-0.35	**0.59**	-0.47	**0.72**	**0.72**		
NRS	-0.43	0.05	-0.17	0.37	-0.32	**0.51**	**0.53**	**0.54**	
TS	**-0.70**	-0.15	-0.34	**0.59**	-0.47	**0.73**	**0.72**	**1.00**	**0.58**

4   Discussion

The antioxidant activities of the mango fruit in various treatments were measured by FRAP and DPPH. Sousa et al. (2021) observed an increased DPPH activity of 'Palmer' mango fruit treated with hydroxypropyl methylcellulose and beeswax (10%) during storage at 21°C for fifteen days. To the best of our knowledge, there are no data on the effect of O_3 on DPPH scavenging activity in mango fruit. This study is the first to seek an understanding of the ozone and antioxidant activity of mango fruit. Ozone is reported to enhance the antioxidant activity of mango fruit during storage (de Almeida Monaco et al., 2016). The current results are comparable to those reported by Minas et al. (2012) in 'Hayward' kiwifruit, where O3 increased the DPPH scavenging activity during storage. Alothman et al.

(2010) reported that O_3 (8 mL/s, 30 min) reduced the DPPH scavenging activity of fresh-cut guava fruit during storage. The increased DPPH scavenging activity in the treated fruit could be attributed to the O_3 acting as a signalling molecule in inducing an antioxidant stress response in mango fruit through oxidative stress during cold storage. The FRAP assay of the O_3 treated fruit increased with the exposure time. Similar results have been reported by Minas et al. (2010) in 'Hayward' kiwifruit treated with O_3 (0.3 μL L^{-1}). De Almeida et al. (2016) observed a decrease in the FRAP of 'Palmer' mango treated with ozonated water (1 mg L^{-1} s^{-1}) for 20 min.

Phenolic compounds are secondary plant metabolites that are produced through the shikimic acid pathway. The current findings confirm Awad et al. (2017), who reported that chitosan coating decreased the total phenolic content in 'Hindi-Besennara' mangoes during storage. Similarly, More and Rao (2019) reported that ozonated water (400 mg h^{-1}) decreased the phenolic content of 'Alphanso' mango stored at ambient temperature for fifteen days. The higher phenolic content observed in the EC could be attributed to the MLE antioxidants infusing into the mango fruit. The EC forms a semipermeable barrier on the fruit surface, allowing the infusion of antioxidants into the fruit (Mditshwa et al., 2017; Ncamas et al., 2018). Furthermore, a modified atmosphere caused by the EC in the fruit could decrease the respiration rate, leading to delayed biochemical processes associated with phenolic compound synthesis. The current results of the O_3 treatment could be attributed to the phenylalanine ammonialyase enzyme (PAL) synthesizing phenolic compounds (Shezi et al., 2020). Ozone could induce the synthesis of phenolic compounds by the PAL enzyme, thus alleviating oxidation stress (Alothman et al., 2010). Minas et al. (2012) reported that kiwifruit phenolic extracts treated with ozone (0.3 μL L^{-1}) suppressed the damage caused by peroxynitrite and hydroxyl radicals. The ozone could activate the antioxidant defense mechanism through the biosynthesis of the phenolic compounds linked to the shikimate pathway. Therefore, the high phenolic content in the O_3 treated fruit

could be responsible for suppressing the DNA damage caused by peroxynitrite and hydroxyl radicals during oxidative stress. Our results show that incorporating the EC with O_3 maintains the phenolic content in mango fruit during storage.

The ascorbic acid content of mangoes decreases during storage due to the oxidation process (González-Aguilar et al., 2001). In the current study, the EC preserved the AA content of mango fruit during storage. Sausa et al. (2021) reported that HPMC and 10% beeswax maintained the AA content in 'Palmer' mangoes during storage at 21℃ for fifteen days. The retention of AA content in coated fruit could be attributed to EC concentration and reduced oxygen permeability, which can prevent the oxidation of AA. Abd El-Razek et al. (2019) observed that moringa coating (10%) decreased the oxygen permeability and preserved the AA content of 'Zelda' mangoes during storage at 10℃ for forty-two days. The mangoes treated with ozone (36 h) had a high content of AA compared to the untreated fruit. The current results are similar to de Almeida (2016) in 'Palmer' mangoes soaked in ozonated water for twenty minutes. The ozone and EC maintained the AA, which could be scavenging reactive oxygen species, thus reducing the oxidative stress and delaying senescence.

Ozone decreases the respiration rate and ethylene production, delaying all the biochemical processes involved in fruit ripening (Minas et al., 2014). Studies by Aday and Caner (2014) revealed that O_3 (0.07 mg/L) treatment maintained the textural properties of strawberries stored at 4℃ for thirty-five days. The current results of the EC are comparable to those of Escamilla-García et al. (2018). The authors reported that chitosan-starch coating (1%) maintained the firmness of 'Maradol' papaya during storage at ambient temperature for fifteen days. Tesfay and Magwaza (2017) reported that moringa 2% + CMC 1% delayed the electrolyte leakage in 'Hass' avocado fruit during storage at 5.5℃ for twenty-one days. It could be hypothesized that EC creates a modified atmosphere within the fruit, leading to

biochemical changes of the cell membrane, thus decreasing electrolyte leakage and fruit softening. In the current study, the increasing REL during storage could be an indication of the modification of the cell wall structure and decreased fruit texture.

The MDA is the end product of unsaturated fatty acids peroxidation and a crucial measure of fruit quality (Abd El-Razek et al., 2019). The current results are comparable to Tesfay and Magwaza (2017), where moringa 2% and CMC 1% decreased the MDA content in 'Hass' avocados stored at 5.5°C for twenty-one days. The oxidative stress causes excessive oxygen reactive species production, which leads to cell membrane injury (Abd El-Razek et al., 2019). Similar results have been reported in 'Soreli' kiwifruit treated with O_3 (300 ppb) stored at 2°C for sixty days. Ozone reacts with fatty acids to form ozonides, which affects lipid fluidity (Carbon et al., 2015). The current results indicate that preserving the membrane integrity in mango fruit is associated with delaying the accumulation of MDA and REL and reduces the rate at which phenolics are used. Additionally, these results indicate that O3 modifies the lipid membrane, which inhibits oxidative stress and prevents membrane damage.

During mango fruit ripening, the NRS decreases and the RS increases due to starch hydrolysis. The current results of the NRS contradict those of Ncama et al. (2021), who reported that MLE + CMC (2%) had no significant effect in 'Marsh' grapefruit. Barboni et al. (2010) observed an increase in the RS in 'Hayward' kiwifruit treated with O_3 (4 mg/h) and stored at 0°C for twenty-three days. Studies by Shalluf et al. (2012) reported a decline in the sucrose and increased fructose and glucose content of 'Elegance' tomatoes treated with O_3 (0.5 mg O3/g) and stored at 15°C for five days. The increase in the soluble sugars could be attributed to the biosynthesis of sucrose by the sucrose phosphate synthase enzyme (SPS). Sucrose synthase (SS), SPS, acid invertase, and neutral invertases affect the fruit's glucose, sucrose, and fructose ratio (Wang et al., 2019). The SPS and SS are the key enzymes involved

in sucrose metabolism in mango fruit during fruit ripening (Wei et al., 2013). Tanou et al. (2015) reported a low level of sucrose phosphate synthase expression in 'Hayward' kiwifruit treated with SNP (100 µM) and ozone (0.3 µL L^{-1}) during storage (0°C for two months, 20 °C for eight days). In mango fruit, the increase in the soluble sugars is associated with fruit ripening and the accumulation of sweetness taste.

5   Conclusions

This is the first study to present findings on the combined effect of moringa leaf extract–CMC coating and gaseous O_3 on the postharvest treatment of mango fruit. The results indicated that the combination of the EC and gaseous O_3 (36 h) effectively maintained the firmness and delayed electrolyte leakage and MDA accumulation. These treatments also decreased the accumulation of sugars, delaying ripening in mango fruit. The enhanced phenolic content was associated with an increased FRAP. Moreover, the EC effectively maintained the AA content of the mango fruit. The current results suggest that increasing the O_3 exposure time does not negatively affect the total phenolic content and soluble sugars during the storage of mango fruit. The present results provide the mechanism of action and application time of ozone in maintaining the antioxidant activity and quality of mango fruit. These results indicate that the postharvest treatment combination of EC and O_3 can preserve the membrane integrity, antioxidants, and   enhance   the   fruit   quality.

6   References

Abd El-Razek, E., Abd El-Motty E.Z., Orabi S.A., Abou-Elfotouh A. 2019. Improving fruit quality of mango fruits cv. Zebda by coating with moringa and green tea leaves extracts under cold storage. Middle East J. Agric. Res. 8(4): 1325-1343.

Abeysinghe, D., Li X.; Sun C., Zhang W., Zhou C.; Chen K. 2007. Bioactive compounds and antioxidant capacities in different edible tissues of citrus fruit of four species. Food Chem. 104(4): 1338-1344.

Aday, M.S., Caner C. 2014. Individual and combined effects of ultrasound, ozone and chlorine dioxide on strawberry storage life. LWT-Food Sci. Technol. 57(1): 344-351.

Alothman, M., Kaur B., Fazilah A., Bhat R., Karim A.A. 2010. Ozone-induced changes of antioxidant capacity of fresh-cut tropical fruits. Innov. Food Sci. Emerg. Technol. 11(4): 666-671.

Awad, M.A., Al-Qurashi A.D., Mohamed S.A., 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. J. Food Sci. Technol. 54(13): 4139-4148.

Baldwin, E., Nisperos-Carriedo M., Baker R. 1995. Edible coatings for lightly processed fruits and vegetables. HortScience 30(1): 35-38.

Barboni, T., Cannac M., Chiaramonti N. 2010. Effect of cold storage and ozone treatment on physicochemical parameters, soluble sugars and organic acids in *Actinidia deliciosa*. Food Chem. 121(4): 946-951.

Barman, K., Asrey R. 2014. Salicylic acid pre-treatment alleviates chilling injury, preserves bioactive compounds and enhances shelf life of mango fruit during cold storage. J. Sci. Ind. Res. 73, 713-718.

Bourtoom, T. 2008. Edible films and coatings: Characteristics and properties. Int. Food Res. J. 15(3): 237-248.

Brodowska, A.J., Nowak A., Śmigielski K. 2018. Ozone in the food industry: Principles of ozone treatment, mechanisms of action, and applications: An overview. Crit. Rev. Food Sci. Nutr. 58(13): 2176-2201.

Carbone, K., Mencarelli F. 2015. Influence of short-term postharvest ozone treatments in nitrogen or air atmosphere on the metabolic response of white wine grapes. Food Biopr. Technol.8(8): 1739-1749.

Daisy, L.L., Nduko J.M., Joseph W.M., Richard S.M. 2020. Effect of edible gum Arabic coating on the shelf life and quality of mangoes (*Mangifera indica*) during storage. J. Food Sci.Technol. 57(1): 79-85.

de Almeida Monaco, K., Costa S.M., Minatel I.O., Correa C.R., Calero F.A., Vianello F., Lima G.P.P. 2016. Influence of ozonated water sanitation on postharvest quality of conventionally and organically cultivated mangoes after postharvest storage. Postharvest Biol. Technol. 120: 69-75.

Dipierro, S., De Leonardis S. 1997. The ascorbate system and lipid peroxidation in stored potato (*Solanum tuberosum* L.) tubers. J. Experim. Bota. 48(3): 779-783.

Escamilla-García, M., Rodríguez-Hernández M., Hernández-Hernández H., Delgado-Sánchez L., García-Almendárez B., Amaro-Reyes A., Regalado-González C. 2018. Effect of an edible coating based on chitosan and oxidized starch on shelf life of *Carica papaya* L., and its physicochemical and antimicrobial properties. Coatings 8(9): 318.

Gaikwad, S., Sakhale B., Chavan R. 2020. Effect of 1–MCP concentration, exposure time and storage temperature on post-harvest quality of mango fruit cv. Alphanso. Food Res. 4(3): 746-752.

González-Aguilar, G.A., Buta J.G., Wang C.Y. 2001. Methyl jasmonate reduces chilling injury symptoms and enhances colour development of 'Kent' mangoes. J. Sci. Food Agric. 81(13): 1244-1249.

Hmmam, I., Zaid N.M., Mamdouh B., Abdallatif A., Abd-Elfattah M., Ali M. 2021. Storage behavior of "Seddik" mango fruit coated with CMC and guar gum-based silver nanoparticles. Horticulturae 7(3): 44.

Jincy, M., Djanaguiraman M., Jeyakumar P., Subramanian K.S., Jayasankar S., Paliyath G. 2017. Inhibition of phospholipase D enzyme activity through hexanal leads to delayed mango (*Mangifera indica* L.) fruit ripening through changes in oxidants and antioxidant enzymes activity. Sci. Hortic. 218, 316-325.

Khaliq, G., Muda Mohamed M.T., Ghazali H.M., Ding P., Ali A. 2016. Influence of gum arabic coating enriched with calcium chloride on physiological, biochemical and quality responses of mango (*Mangifera indica* L.) fruit stored under low temperature stress. Postharvest Biol. Technol. 111, 362-369.

Khaliq, G., Ullah M., Memon S.A., Ali A.; Rashid M. 2020. Exogenous nitric oxide reduces postharvest anthracnose disease and maintains quality of custard apple (*Annona squamosa* L.) fruit during ripening. J. Food Measur. Character. 15(1): 707-716.

Kumar, N., Pratibha, Neeraj, Petkoska A.T., Al-Hilifi S.A., Fawole O.A. 2021. Effect of chitosan-pullulan composite edible coating functionalized with pomegranate peel extract on the shelf life of mango (*Mangifera indica*). Coatings 11(7): 764.

Lamien-Meda, A., Lamien C.E., Compaoré M.M., Meda R.N., Kiendrebeogo M., Zeba B., Millogo J.F., Nacoulma O.G. 2008. Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. Molecules 13(3): 581-594.

Liu, Z., Ren Z., Zhang J., Chuang C.C., Kandaswamy E., Zhou T., Zuo L. 2018. Role of ROS and nutritional antioxidants in human diseases. Front. Physiol. 9, 477.

Mditshwa, A., Magwaza L.S., Tesfay S.Z., Opara U.L. 2017. Postharvest factors affecting vitamin C content of citrus fruits: A review. Sci. Hortic. 218, 95-104.

Minas, I.S., Karaoglanidis G.S., Manganaris G.A., Vasilakakis M. 2010. Effect of ozone application during cold storage of kiwifruit on the development of stem-end rot caused by *Botrytis cinerea*. Postharvest Biol. Technol. 58(3): 203-210.

Minas, I.S., Tanou G., Belghazi M., Job D., Manganaris G.A., Molassiotis A., Vasilakakis M. 2012. Physiological and proteomic approaches to address the active role of ozone in kiwifruit post-harvest ripening. J. Experim. Bot. 63(7): 2449-2464.

Minas, I.S., Vicente A.R., Dhanapal A.P., Manganaris G.A., Goulas V., Vasilakakis M., Crisosto C.H., Molassiotis A. 2014. Ozone-induced kiwifruit ripening delay is mediated by ethylene biosynthesis inhibition and cell wall dismantling regulation. Plant Sci. 229, 76-85.

More, S., Rao T.R. 2019. Elicitor-mediated sanitization in combination with edible coatings improve postharvest shelf life and antioxidant potential of mango fruit. Environ. Exper.l Biol.17(3): 107-14.

Ncama, K., Magwaza L.S., Mditshwa A., Tesfay S.Z., Mbili N.C. 2021. The efficacy of moringa leaf extract as an active ingredient in organic coating of 'Marsh' grapefruit. Acta Hortic. (1306): 241-248.

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

Salinas-Roca, B., Guerreiro A., Welti-Chanes J., Antunes M.D., Martín-Belloso O. 2018. Improving quality of fresh-cut mango using polysaccharide-based edible coatings. Int. J. Food Sci. Techno. 53(4): 938-945.

Shalluf, M.A. 2012. Study of effect of different ozone doses on sugar content in tomatoes at different stages of ripening. Int. J. Nutr. Food Eng. 6(4): 182-184.

Shezi, S., Magwaza L.S., Mditshwa A., Tesfay S.Z. 2020. Changes in biochemistry of fresh produce in response to ozone postharvest treatment. Sci. Hortic. 269, 109397.

Skrovankova, S., Misurcova L., Machu L., 2012. Antioxidant activity and protecting health effects of common medicinal plants. Adv. Food Nutr. Res. 67, 75-139.

Sousa, F.F., Pinsetta Junior J.S., Oliveira K., Rodrigues E.C.N., Andrade J.P., Mattiuz B.H. 2021. Conservation of 'Palmer' mango with an edible coating of hydroxypropyl methylcellulose and beeswax. Food Chem. 346, 128925.

Tanou, G., Minas I.S., Karagiannis E., Tsikou D., Audebert S., Papadopoulou K.K., Molassiotis A. 2015. The impact of sodium nitroprusside and ozone in kiwifruit ripening physiology: A combined gene and protein expression profiling approach. Ann. Bot. 116(4): 649-662.

Tesfay, S.Z., 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 Packag. Shelf Life 11, 40-48.

Wang, T., Wright D., Xu H., Yang Y., Zheng R., Shi J., Chen R., Wang L. 2019. Expression patterns, activities and sugar metabolism regulation of sucrose phosphate synthase, sucrose synthase, neutral invertase and soluble acid invertase in different Goji cultivars during fruit development. Russian J. Plant Physiol. 66(1): 29-40.

Wei, C., Wu H., Ma W., Wang S., Sun G. 2013. Sucrose metabolism and changes of relative enzymes in *Mangifera indica* L. 'Irwin'. Acta Hortic. 992(70): 569-574.

Zhao, Z., Xu G., Han Z., Li Q., Chen Y., Li D. 2013. Effect of ozone on the antioxidant capacity of "Q iushui" pear (*Pyrus pyrifolia* Nakai cv. Q iushui) during postharvest storage. J. Food Qual. 36(3): 190-197.

Chapter 6

Evaluating the use of ozone and moringa leaf and carboxymethyl cellulose coatings in controlling anthracnose and stem-end rot in mango fruit

Abstract

Anthracnose and stem-end rot are major postharvest diseases of mango (*Mangifera indica* L.) fruit, causing significant economic losses. This *in vitro* and *in vivo* study was conducted to evaluate the antifungal effect of ozone (O_3) and edible coatings on *Colletotrichum gloeosporioides* causing anthracnose and *Lasiodiplodia theobromae* causing stem-end rot of 'Keitt' mango fruit. The Petri dishes or mango fruit were treated with moringa leaf extract + carboxymethyl cellulose (EC) and O_3 (0.25 mg L^{-1}). In the *in vitro* study, mycelial growth inhibition of *L. theobromae* and *C. gloeosporioides* was evaluated on potato dextrose agar. In the *in vivo* study, mango fruit were coated with EC before O3 treatment. Gaseous ozone was applied intermittently for 24 or 36 hours, and control fruit were untreated. Mango fruit was artificially inoculated with *C. gloeosporioides* and *L. theobromae* at ambient temperature. The fruit was stored at 10°C and 90% relative humidity for twenty-one days followed by a seven-day shelf-life at ambient temperature. Data was collected at seven-day intervals. Parameters measured were disease incidence, firmness, mass loss (%), total flavonoid content and peroxidase activity. The EC and EC + O_3 24 h (62.35%) significantly inhibited the mycelial growth of *C. gloeosporioides* and *L. theobromae.* At the end of storage, fruit treated with EC + O_3 36 h (36.47N) were firmer compared to $O_3$36 h (32.34 N), O_3 24 h (31.72 N), and the control (23.33 N). The EC maintained mango fruit's total flavonoid content and peroxidase activity during storage. The current findings indicated that the treatment combination of EC and O_3 induces disease resistance of

anthracnose and stem-end rot in mango fruit. The mechanism of these treatments in controlling anthracnose and stem-end rot requires further investigation.

Keywords: Anthracnose, stem-end rot, mango, flavonoids, shelf-life

1 Introduction

Anthracnose caused by *Colletotrichum gloeosporioides* and stem-end rot caused by *Lasiodiplodia theobromae* are the major postharvest diseases of mango fruit, causing substantial economic losses in the South African mango industry (Sivakumar et al., 2011). These postharvest diseases occur during fruit ripening and handling chain (Sivakumar et al., 2011). The fungus *C. gloeosporioides* colonizes the fruit preharvest and remains dormant until ripening commences (Konsue et al., 2020). The increase in respiration rate and ethylene production triggers biochemical processes resulting in mango fruit ripening (Hmmam et al., 2021). The ripening process triggers black spots formation on the fruit surface, which later becomes lesions (Siddiqui and Ali, 2014; Konsue et al., 2020). Although the lesions often affect the peel; in worse scenarios, the entire whole, including the pulp, may be affected (Siddiqui and Ali, 2014).

The *L. theobromae* infection occurs preharvest, and the pathogen pierces the stem through mechanical injuries and lenticels and other natural openings (Terao et al., 2019). The fruit remains asymptomatic until postharvest when storage conditions change and ripening occurs (Galsurker et al., 2018). The symptoms include dark brown and water-soaked spots appearing at the fruit stem end, which becomes severe towards fruit senescence (Terao et al., 2019; Galsurker et al., 2020). Chemical treatment such as

fungicides has been used to control postharvest diseases. The South African mango industry has adopted hot water (25 °C, 20 seconds) and prochloraz as a postharvest treatment to prevent diseases (Sivakumar et al., 2011). There is increasing consumer demand for chemical-free treated fresh produce. Furthermore, there is increasing resistance of pathogens to fungicides and the risks of chemical residues in fresh produce (King et al., 2021). Therefore, environmentally friendly postharvest technologies such as edible coatings, hot water, UV-C and ozone (O_3) are essential to control postharvest diseases.

Ozone has high oxidation potential with antimicrobial properties against a broad spectrum of fungi, bacteria, and viruses (Contigiani et al., 2018; De Santis et al., 2021). Ozone inhibited conidia germination and caused hyphae cell death in *Penicillium citrinum*, *Fusarium graminearum* and *Fusarium verticillioides* (Savi and Scussel, 2014). Recently, Terao et al. (2019) reported that treatment combination of O_3 (3 mg O_3 L^{-1}, 5 minutes) and hot water (70 °C, 15 seconds) reduced the disease incidence of stem-end rot in papaya stored at 10°C, for eight days. Ozone has the potential to be used as a postharvest treatment to control diseases in fresh produce.

Plant-based edible coatings contain antimicrobial properties that effectively control postharvest disease (Escamilla-García et al., 2018; Khaliq et al., 2019). Studies have demonstrated that combining two or more edible coatings yields superior results in controlling postharvest diseases (Zillo et al., 2018; Kubheka et al., 2020; Shah et al., 2021). For instance, Zillo et al. (2018) reported that *Lippia sidoides* essential oil + carboxymethyl cellulose (CMC) (25%) decreased the disease severity of papaya during storage at ambient temperature for nine days. Kubheka et al. (2020) reported that gum Arabic (15%) + moringa incorporated with CMC (1%) effectively inhibited the mycelial growth of *C. gloeosporioides*. Similary, Samithri et al. (2020) observed that essential oils of Cardamom (1000 μL L^{-1}) and citronella

(750 µL L^{-1}) inhibited the mycelia growth of *L. theobromae* and *C. gloeosporioides* from 'Red Lady' papaya.

The potential use of O$_3$ in combination with edible coatings in decreasing postharvest diseases is scarce. The use of O$_3$ and EC could provide an environmentally friendly and economical postharvest technology to control postharvest diseases in fresh produce. The objective of this study was to evaluate the antifungal activity of edible coatings (moringa leaf extract and carboxymethyl cellulose) and O$_3$ on the growth of *C. gloeosporioides* and *L. theobromae* in mango fruit.

2   Materials and methods

2.1 In vitro study

The antifungal activity of ozone and edible coatings was determined as described by Tesfay et al. (2017), with minor modifications. Potato dextrose agar (PDA) media was prepared by adding distilled water into 39 g of PDA and autoclaved for 15 minutes at 121°C, cooled down, then poured into 90 mm Petri dishes. A mycelia disc (3 mm) was cut from the growing colonies (five days) of *C. gloeosporioides* and *L. theobromae* cultures and transferred into PDA plates. The mycelia disc was put in the middle of the petri dish. Four paper discs were dipped in EC and put opposite each other on the petri dish. For the control treatment, discs were submerged in sterilized distilled water. Gaseous O3 (0.25 ppm) was applied at seven-day intervals for twelve hours. Petri dishes were kept closed during O$_3$ exposure, and the experiment was replicated three times. The plates were stored at 10°C and 90% relative humidity for fourteen days and thereafter transferred to 25 °C for seven days. Radial mycelial growth was measured with a ruler.

Mycelial growth inhibition (MGI) percentage was calculated using equation 1:

$$MGI\ (\%) = \frac{dc - dt}{dc}\ x100 \qquad\qquad equation\ (1)$$

Where dc and dt are the mycelial growth diameter of the control and treated petri dish, respectively.

2.2 In vivo study

2.2.1   Fruit material

Mango fruit (*cv*. 'Keitt') were harvested from Goedgelegen Farm of Westfalia (Pty) Ltd, a commercial farm located in Tzaneen, South Africa. Fruit were transported overnight to the Postharvest Research Laboratory of the University of KwaZulu-Natal (Pietermaritzburg Campus). Fruit used in this experiment was of the same size, colour, maturity, and was also disease-free. The experiment was replicated three times, with three fruit per replicate. A total of 270 fruit was used for the experiment.

2.2.2   Artificial inoculation

Mango fruit were surface sterilized with 0.1% sodium hypochlorite for two minutes and air-dried at ambient temperature for two hours. The spore suspension was prepared as described by Ong and Ali (2015) with slight modification. Briefly, two-week-old plates of *C. gloeosporioides* and *L. theobromae* were rinsed with 6 mL of distilled water. The conidia were removed on the plate surface using a sterile rod. After that, the conidial count was adjusted to 10^5 conidia mL^{-1} using a hemocytometer. Mango fruit were soaked on the conidial suspension for five minutes and air-dried at ambient temperature for two hours. The treatments used for the investigation were:

Control

T1: O_3 (24 hours)T2: O_3 (36 hours)

T3: MLE 1% + CMC 1% (EC)

T4: EC + O_3 (24 hours)

T5: EC + O_3 (36 hours)

2.2.3 Edible coating (EC)

Moringa leaf was extracted as described by Tesfay et al. (2017) with some modification. Briefly, moringa leaf powder (100 g) was extracted with 1000 mL of ethanol (70%) at ambient temperature for twelve hours with constant shaking. The extract was evaporated using the Genevac evaporator (Genevac® EZ 2.3; IPSWICH; England, UK) for fifteen hours at 37 °C. The crude extract was suspended with distilled water and homogenized with CMC (10 g).

2.2.4 Ozone treatment

Corona discharge ozone generator (Ozone Purification Technology, Johannesburg, South Africa) was used to generate gaseous ozone. Mango fruit were treated with O_3 (0.25 mg L^{-1}) after seven days for twelve hours during cold storage. Fruit were treated at day zero and day seven, for the 24 hours treatment; and at days zero, seven and fourteen for 36 hours. Fruit were coated with EC before O_3 treatment and the control fruit was untreated. Fruit were stored at 90% relative humidity and (0.5 ± 10 °C) temperature for twenty-one days simulating shipment to European union export markets. After cold storage, the fruit were ripened for seven days at ambient temperature

2.2.5   Sample preparation

Mangoes were peeled, pulp removed from the kernel, and cut into small cubes. The peel was freeze-dried using Vir Tis BenchTop Pro freeze drier (SP Scientific, Warminster, Pennsylvania, USA). After that, samples were crushed into powder and stored at -20 ℃ until used. The experiment was replicated three times, with three fruit per replicate.

2.2.6   Disease incidence

In each treatment, nine fruit were evaluated for the DI on the peel. The fruit were classified accordingto their disease severity. Score 0: no visible decay, score 1: few (1%) scattered decay, score 2: 2-20% decay incidence, score 3: 21-50% decay incidence, and score 4: more than 50% decay incidence. The DI index was calculated as defined by Khaliq et al. (2016) using equation 2:

$$DI\,(\%) = \Sigma\ \frac{(Disease\ scale\ x\ number\ of\ fruit\ in\ each\ class)\ x100}{number\ of\ total\ fruit\ x\ highest\ disease\ scale} \qquad (2)$$

2.2.7   Fruit firmness and mass loss (%)

Fruit firmness, expressed in newton (N), was done on the opposite side of the fruit cheeks using a handheld firmness tester (Bareiss, Germany). Fruit were weighed on arrival at the laboratory and after every seven days. The same fruit were weighed throughout the storage period. Mass loss percentage was calculated using equation 3:

Weight loss (%) $= \frac{A-B}{A} x100$ (3)

Where A and B is the mass at day zero and at the end of storage, respectively.

2.2.8   Total flavonoid content

Flavonoids induce resistance in plants against fungal and pathogen attack (Sivankalyani et al., 2016; Zhu et al., 2019). Total flavonoids content was measured as described by Eghdami and Sadeghi (2010), with modification. In triplicates, peel powder (0.5 g) was extracted with 80% methanol at 40°C for sixty minutes. Samples were cooled down and centrifuged (Avanti J-265 XP, Beckaman Coulter, Indianapolis, USA) at 10 000 rpm for fifteen minutes. Deionised water (0.3 mL) was added to 0.1 mL plant extract, followed by 0.03 mL sodium nitrate 5% and incubated at 25 °C for five minutes. Aluminium chloride 10% (0.03 mL) was added and allowed to stand for five minutes. Sodium hydroxide 1 mM (0.2 mL) was added to the solution, and the reaction mixture was diluted to 1 mL with deionised water. The absorbance was measured at 510 nm using a spectrophotometer (Shimadzu Scientific Instruments INC., Columbia USA) against methanol as a blank. Results were expressed as mg GEA/gplant dry matter.

2.2.9   Determination of peroxidase activity

The peroxidase (POD) enzyme extraction was done as described by Criado et al. (2016), with modification. In triplicates, the fruit sample (1 g) was extracted with 10 mL sodium phosphate buffer (0.2 M, pH 7.0). The extract was centrifuged (Avanti J-265 XP, Beckaman Coulter, Indianapolis, USA) at 15 000 rpm for fifteen minutes at 4 °C. Enzyme extract (0.1 mL) was added to 2.7 mL sodium phosphate

buffer (0.05 M). Thereafter, 0.1 mL hydrogen peroxide (1.5% w/v) and 1% p-phenylenediamine (0.2 mL) was added. The absorbance was measured at 485 nm using a spectrophotometer (Shimadzu Scientific Instruments INC., Columbia USA). Enzyme activity was defined as an increase in absorbance at 485 nm per mg protein per minute.

2.3 Scanning electron microscopy

Scanning electron microscopy analysis of growing PDA media and mango fruit samples were performed as described by Tesfay et al. (2017). The PDA media and fruit samples were fixed in 3% glutaraldehyde buffer for three hours. Thereafter, samples were washed twice in sodium cacodylate buffer for five minutes. Subsequently, samples were dehydrated in various ethanol (10% - 100%) concentrations for ten minutes. Samples were then dried to critical drying point using a critical point dryer (Quorum K850, Quorum Technologies, United Kingdom). After drying, samples were mounted to stubs, and gold sputter coat was applied using Plus-Rotary Pumped coater Q 150R ES (Quorum Technologies, United Kingdom). The samples were viewed using a scanning electron microscope (Zeiss EVO LS15).

2.4 Statistical analysis

Data was subjected to the analysis of variance (ANOVA), using GenStat statistical software (GenStat®, 18 edition, VSN International, Hemel Hempstead, UK). Fischer's least significant difference (LSD) was used to separate means at 5% level of significance.

3 Results

3.1 In vitro antifungal assay of O_3 and EC against C. gloeosporioides and L. theobromae

The mycelial growth inhibition (%) of *C. gloeosporioides* varied between treatments (Table 6.1). The EC + O_3 (36 h) significantly ($p < 0.05$) had higher inhibition percentage compared to other treatments. There were no significant differences in the inhibition (%) of EC, EC + O_3 (24 h) and EC + O_3 (36 h). The control was not effective in inhibiting the mycelial growth of *C. gloeosporioides*.

Table 6.1: Effect of gaseous ozone and edible coatings on mycelial growth inhibition (%) of *C. gloeosporioides* and *L. theobromae* incubated at 10 °C for fourteen days and seven days at 25 °C

	MGI (%)	
Treatment	*C. gloeosporioides*	*L. theobromae*
Control	0,00[a]	0,00[a]
O_3 (24 h)	21,65[b]	62,35[bc]
O_3 (36 h)	42,43[c]	59,41[bc]
EC	61,50[d]	50,44[b]
EC + O_3 (24 h)	55,39[d]	55,88[bc]
EC + O_3 (36 h)	63,99[d]	72,35[c]

Means with the same letter indicate no significant difference between treatments. Data represent means (LSD $=10.75$, $n = 4$)

The radial mycelial growth inhibition of *L. theobromae* ranged from 0-72.35%. There were significant(p

< 0.05) differences between the treatment means with regards to the MGI (%) of *L. theobromae*. The highest percentage of mycelial growth was observed in EC + O_3 (36 h) (72.35%), whereas the control (0%) had the lowest.

3.2 Disease incidence of C. gloeosporioides and L. theobromae

The O_3 and EC significantly ($p < 0.05$) decreased disease incidence of anthracnose in mango fruit during storage (Table 6.2). Untreated fruit had high incidence of anthracnose compared to other treatments from day fourteen to the end of storage. At the end of storage, there were no significant differences between treatment means of O_3 (36 h), EC, and EC + O_3 (24 h).

Table 6.2: Effect of gaseous ozone and edible coatings on disease incidence (%) of 'Keitt' mango fruit inoculated with *C. gloeosporioides*, stored at 10 °C for twenty-one days and seven days shelf-life at ambient temperature

Treatment	Storage time (Days)				
	0	7	14	21	28
Control	0.00[a]	14.50[bcd]	38.78[fgh]	57.89[ijk]	97.89[m]

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O₃ (24h)	0.00ᵃ	9.37ᵃᵇᶜ	27.00ᵈᵉᶠ	46.33ᵍʰⁱ	82.67ˡ
O₃ (36 h)	0.00ᵃ	6.00ᵃᵇ	24.56ᵈᵉ	36.33ᵉᶠᵍ	70.33ᵏ
EC	0.00ᵃ	5.00ᵃᵇ	23.56ᵈᵉ	38.22ᶠᵍʰ	62.22ʲᵏ
EC + O₃ (24 h)	0.00ᵃ	8.02ᵃᵇᶜ	20.44ᶜᵈ	36.67ᵉᶠᵍ	58.00ⁱʲᵏ
EC + O₃ (36 h)	0.00ᵃ	6.36ᵃᵇ	16.89ᵇᶜᵈ	36.67ᵉᶠᵍ	50.44ʰⁱʲ

Means with the same letters within a column and between columns indicate no significant difference between treatments. Data represent means (LSD = 11.87, $n = 12$)

The EC significantly ($p < 0.05$) reduced the disease incidence of stem-end rot in mango fruit during storage (Table 6.3). At the end of storage high disease incidence was observed in untreated fruit (94.44%) compared to O₃ (24 h), EC + O₃ (24 h), O₃ (36 h), EC and EC + O₃ (36 h) which had 81.11%, 72.44%, 70.67%, 59.78% and 53.56%, respectively. At the end of storage, there were no significantdifferences between the treatment means of O₃ (36 h) and EC + O₃ (24 h).

Table 6.3: Effect of EC and O_3 on disease incidence (%) of 'Keitt' mango fruit inoculated with *L. theobromae,* stored at 10 °C for twenty-one days and seven days shelf-life at ambient temperature.

Treatments	Storage time (Days)				
	0	**7**	**14**	**21**	**28**
Control	0.00[a]	10.00[bcd]	24.13[fg]	68.33[j]	94.44l
O_3 (24h)	0.00[a]	8.50[abcd]	17.00[def]	53.33[i]	81.11[k]
O_3 (36 h)	0.00[a]	8.75[abcd]	15.71[def]	43.33[h]	70.67[j]
EC	0.00[a]	3.60[ab]	10.29[bcd]	30.22[g]	59.78[i]
EC + O_3 (24 h)	0.00[a]	3.33[ab]	12.25[bcde]	23.67[fg]	72.44[j]
EC + O_3 (36 h)	0.00[a]	4.25[abc]	13.00[cde]	20.00[ef]	53.56[i]

Means with the same letters within a column and between columns indicate no significant difference between treatments. Data represent means (LSD = 7.92, $n = 12$)

3.3 Fruit firmness

Firmness significantly ($p < 0.05$) decreased in all treatments during storage in *C. gloeosporioides* inoculated fruit (Table 6.4). The untreated fruit showed a sharp decrease in firmness compared to other treatments (Table 6.5). At the end of storage, fruit treated with EC + O_3 (36 h) (36.47 N) were firmer compared to O_3 36 h (32.34 N), O_3 24 h (31.72 N), EC (30.71 N) and the control (23.33 N). The firmness of fruit treated with O_3 (24 h), EC and O_3 (36 h) were not significantly different at the end of shelf-life.

Table 6.5: Effect of O₃ and EC on 'Keitt' mango fruit firmness inoculated with *C. gloeosporioides,* stored at 10 °C for twenty-one days and seven days shelf-life at ambient temperature.

Treatments	Storage time (Days)				
	0	**7**	**14**	**21**	**28**
Control	$50{,}03^{o}$	$41{,}69^{hij}$	$37{,}72^{defg}$	$32{,}47^{bc}$	$23{,}33^{a}$
O₃ 24 h	$50{,}78^{o}$	$46{,}19^{klm}$	$43{,}23^{ijk}$	$37{,}22^{def}$	$31{,}72^{b}$
O₃ 36 h	$48{,}86^{mno}$	$43{,}94^{ijkl}$	$41{,}48^{hi}$	$36{,}85^{def}$	$32{,}34^{bc}$
EC	$49{,}53^{no}$	$44{,}94^{jkl}$	$40{,}73^{ghi}$	$35{,}85^{d}$	$30{,}71^{b}$
EC + O₃ 24 h	$49{,}70^{no}$	$44{,}86^{jkl}$	$43{,}61^{ijkl}$	$39{,}85^{fgh}$	$34{,}89^{cd}$
EC + O₃ 36 h	$50{,}87^{o}$	$46{,}36^{lmn}$	$43{,}36^{ijk}$	$39{,}35^{efgh}$	$36{,}47^{de}$

Means with the same letters within a column and between columns indicate no significant difference between treatments. Data represent means (LSD = 2.89, $n = 9$)

The firmness of *L. theobromae* inoculated fruit followed a similar trend to that of *C. gloeosporioides*.Fruit treated with EC + O₃ (24 h) were significantly ($p < 0.05$) firmer compared to other treatments from day fourteen till the end of storage (Table 6.5). At the end of storage, there were no significant differences between the firmness of EC and untreated fruit. The high firmness was observed in EC + O₃ 24 h (33.84 N) compared to O₃ 36 h (32.72 N), O₃ 24 h (32.34 N), EC (27.33 N) and the control(25.90 N) at the end of storage.

Table 6.6: Effect of EC and O₃ of 'Keitt' mango fruit firmness inoculated with *L. theobromae,* stored at 10 °C for twenty-one days and seven days shelf-life at ambient temperature.

Treatments	Storage time (Days)				
	0	**7**	**14**	**21**	**28**
Control	48,36klm	42,69fgh	38,85de	32,59b	25,90a
O₃ 24 h	51,2m	46,86hij	42,86fgh	38,85cde	32,34b
O₃ 36 h	48,78klm	45,02ijk	41,98efgh	37,94cd	32,72b
EC	48,61klm	44,77hi	39,48def	35,47bc	27,33a
EC + O₃ 24 h	49,28lm	45,44hijk	43,23gh	40,23defg	33,84b
EC + O₃ 36 h	46,86jkl	42,94fgh	40,35defg	37,72cd	32,22b

Means with the same letters within a column and between columns indicate no significant difference between treatments. Data represent means (LSD = 1.39, $n = 9$)

3.3 Fruit mass loss

The percentage mass loss of *C. gloeosporioides* and inoculated fruit increased in all treatments during storage (Figure 6.1). Mass loss of untreated fruit was significantly ($p < 0.05$) higher than other treatments from day fourteen until the end of storage. Fruit mass loss in fruit treated with O₃ (36 h) was low

compared to other treatments from day twenty-one until the end of shelf-life. At the end of storage, untreated fruit had higher mass loss (25.70 %) compared to O_3 24 h (24.57%), EC + O_3 24 h (22.45%), EC + O_3 36 h (19.88%), EC (19.21%) and O_3 36 h (17.66%).
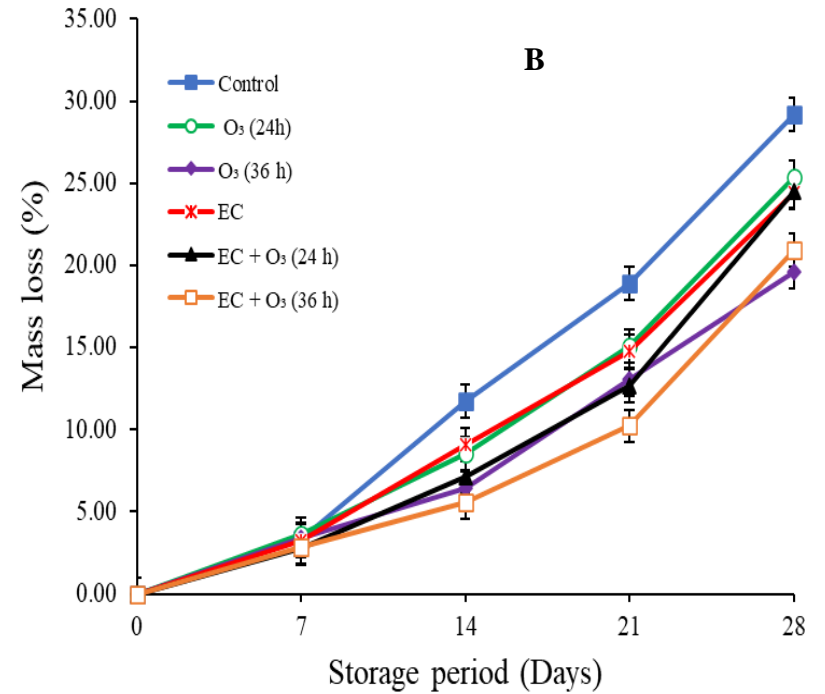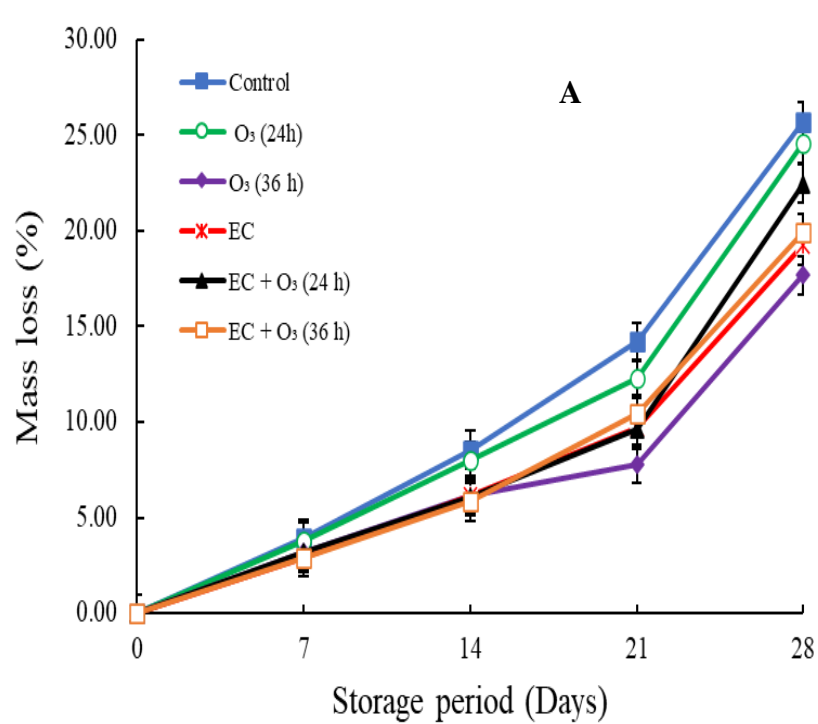
Figure 6.1: Effect of O_3 and EC on mass loss (%) in 'keitt' mango fruit inoculated with *C. gloeosporioides* (A) and *L. theobromae* (B); stored at 10 °C for 21 days and seven days shelf-life at ambient temperature (±SE, *n* = 9).

Treatments significantly ($p < 0.05$) affected the mass loss percentage of mango fruit inoculated with *L. theobromae* (Figure 1B). Mass loss (%) of untreated fruit rapidly increased during storage. On day fourteen, mass loss of EC (9.09 %) and O_3 24 h (8.56 %) was not significantly different. The mass loss of EC + O_3 24 h (12.68 %), O_3 36 h (13.05 %) and EC (14.77 %) was not significantly different at day twenty-one. At the end of storage, the highest mass loss was observed in untreated fruit compared to O_3 (24 h), EC, EC + O_3 (24 h), EC + O_3 (36 h) and O_3 (36 h).

3.5 Total flavonoid content

The total flavonoid content of *C. gloeosporioides* inoculated fruit was significantly ($p < 0.05$) affected by various treatments. The total flavonoid content decreased in all treatments during storage (Figure 6.2). High flavonoid content was observed at day fourteen in EC + O_3 36h (87.00 mg GAE/g DM). At the end of storage, O_3 24 h (66.80 mg GAE/g DM) had the highest total flavonoid content compared to EC + O_3 (24 h), O_3 (36 h), EC, EC + O_3 (36 h) and untreated fruit which had 57.50 mg GAE/g DM, 55.30 mg GAE/g DM, 54.40 mg GAE/g DM, 44.80 mg GAE/g DM and 27.80 mg GAE/g DM, respectively. There was no significant difference between treatment means of O_3 (36 h), EC and EC + O_3 (24 h) at the end of storage. The total flavonoid content of *L. theobromae* inoculated fruit increased from day zero to day fourteen, then decreased until end of the storage period. The total flavonoid content of EC treated fruit was significantly ($p < 0.05$) high compared to other treatments from day fourteen till the end of storage. At the end of storage, untreated fruit had a low total flavonoid content compared to other treatments. There were no significant differences between treatment means of O_3 (24 h) and EC + O_3 (24h).
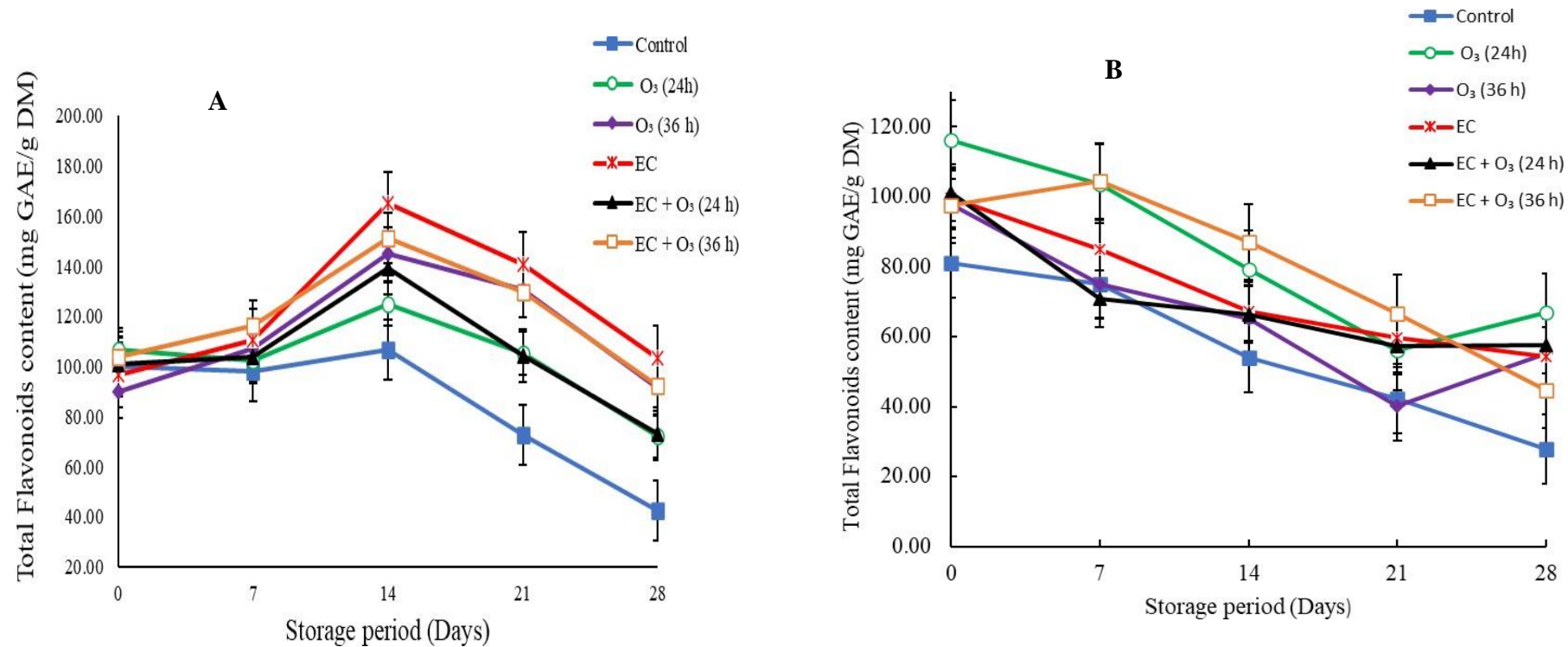
Figure 6.2: Effect of edible coatings and gaseous ozone on total flavonoid content of 'Keitt' mango fruit inoculated with

C. gloeosporioides (A),and *L. theobromae* (B) stored at 10 °C for twenty-one days and seven days shelf-life at ambient

temperature (±SE, *n* = 9)

3.6 Peroxidase activity

The POD activity of *C. gloeosporioides* inoculated fruit increased in all treatments from day zero until day fourteen then declined till the end ofstorage (Figure 6.3). On day fourteen, EC + O₃ (24 h) significantly ($p < 0.05$) had higher POD activity compared to other treatments.

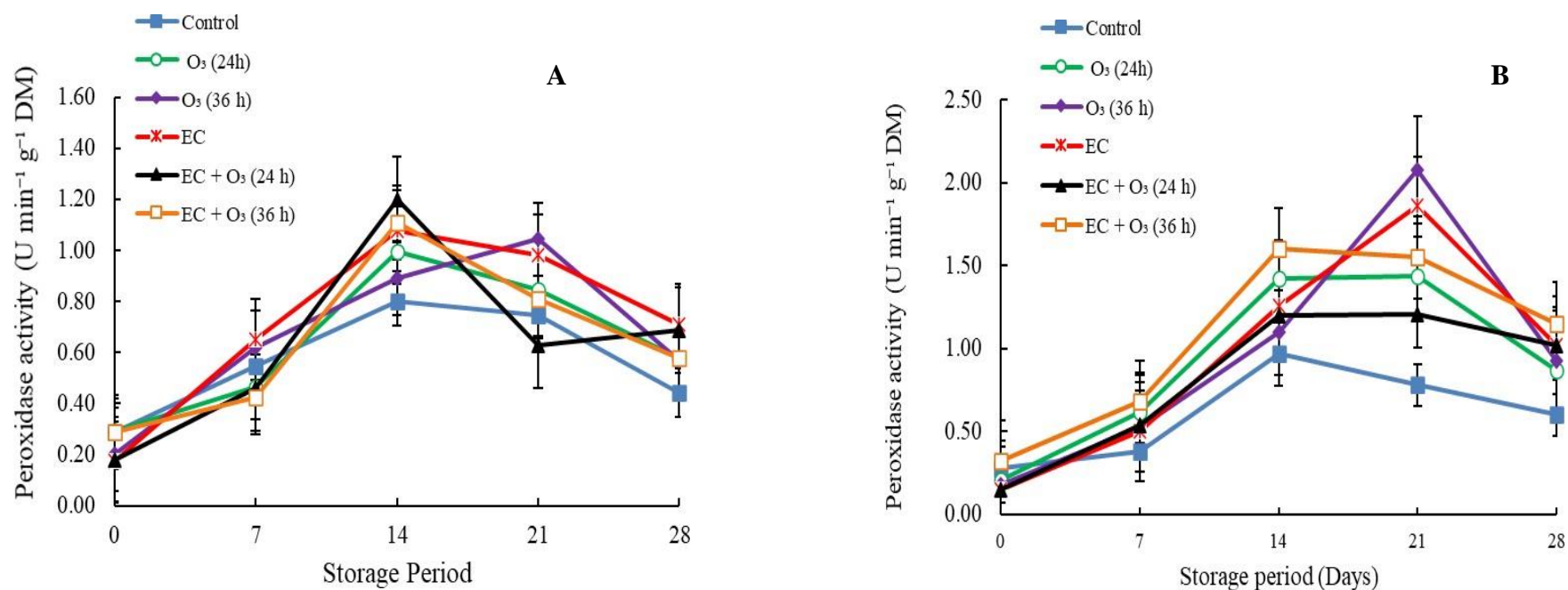**Figure 6.3**: Effect of edible coatings and gaseous ozone on peroxidase activity in *C. gloeosporioides* (A) and *L. theobromae* (B) inoculated mangofruit stored at 10 °C for twenty-one days and seven days shelf-life at ambient temperature (±SE, $n = 9$)

Fruit treated with O_3 (36 h) (1.04 U min^{-1} g^{-1} DM) had higher POD than other treatments at day twenty-

one. At the end of storage, EC and EC + O_3 (24 h) had the highest POD activity compared to EC + O_3 (36

h), O_3 (36 h), O_3 (24 h) and untreated fruit. The POD activity of *L. theobromae* inoculated mango fruit

significantly ($p < 0.05$) varied among treatments. On day twenty-one, POD activity of O_3 (36 h) (2.08 U

min^{-1} g^{-1} DM) was higher compared to EC, EC + O_3 (36 h), O_3 (24 h), EC + O_3 (24 h) and untreated fruit

which had POD of 1.55 U min^{-1} g^{-1} DM, 1.44 U min^{-1} g^{-1} DM, 1.21 U min^{-1} g^{-1} DM and 1.86 U min^{-1} g^{-1}

DM, respectively. Moreover, a notably higher POD activity was observed at the end of storage in EC + O_3

(36 h) treated fruit. There were no significant differences between the treatment means of O_3 (36 h), EC

and EC + O_3 (24 h) at the end of storage.

3.7 Scanning electron microscopy

The SEM image analysis of mango fruit showed changes on the surface during storage (Figure 6.4). The

texture of the untreated fruit was coarse and showed damage to the cell structures. This showed injury on

the fruit peel caused by anthracnose during storage. Fruit treated with edible coatings and gaseous ozone

was smooth and had few cracks on the surface. Current findings show that edible coatings formed a

protective barrier protecting the fruit from the injury caused by anthracnose. The image analysis of *L.

theobromae* inoculated varied among the treatments (Figure 6.5). The stem-end rot caused severe damage

on the peel of untreated fruit compared to other treatments. The fruit treated with O_3 showed few cracks

and minor damage on the surface. The fruit treated with edible coatings and gaseous ozone were smooth

and showed minimal injury on the peel surface.
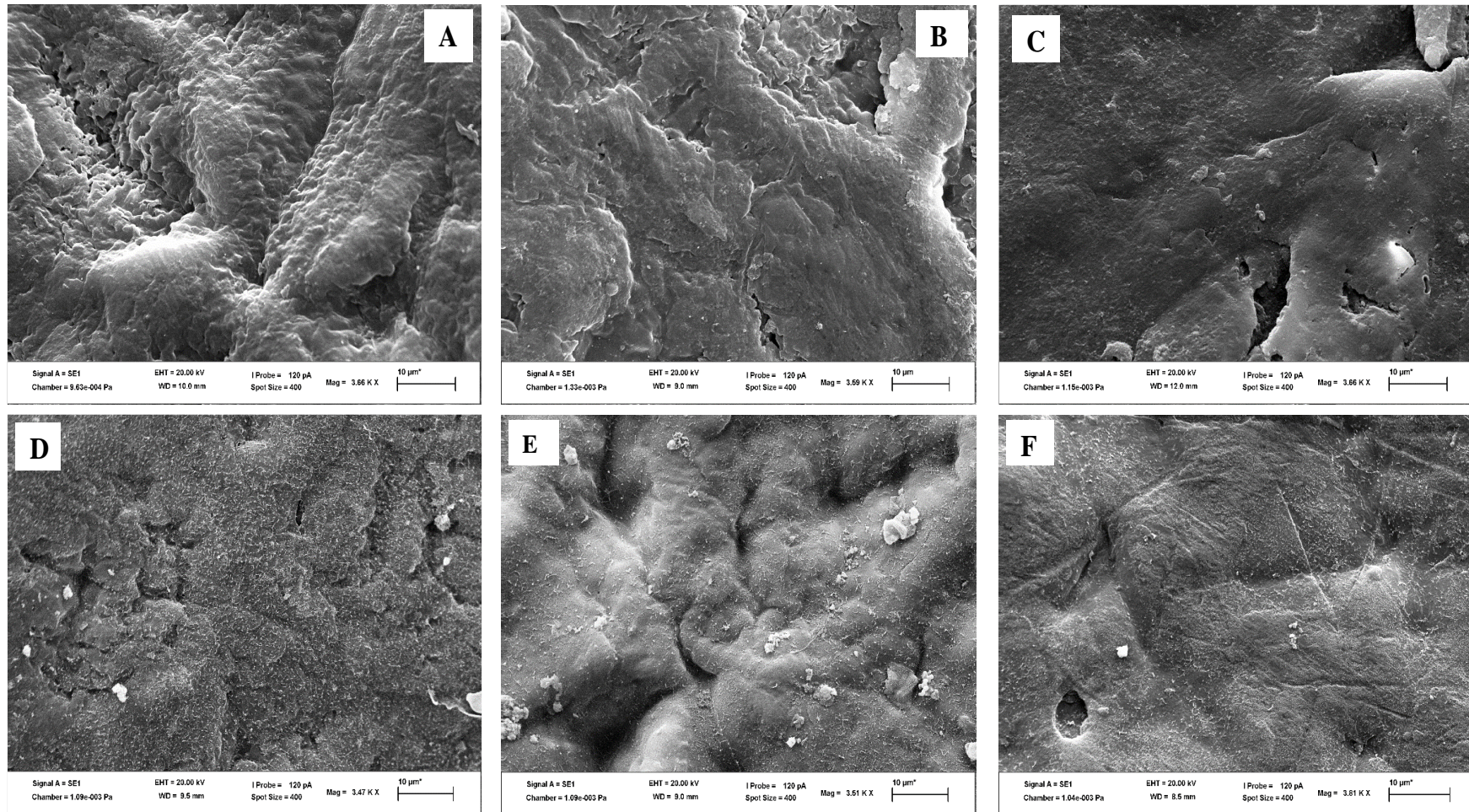
Figure 6.4: Scanning electron microscopy micrographs of 'Keitt' mango fruit inoculated with *C. gloeosporioides* in control, O_3 24 h (A), O_3 36 h (B), EC (D), EC+O_3 24 h (E), EC+O_3 36 h (F). Fruit were stored at 10°C for twenty-one days and seven days shelf-life at ambient temperature

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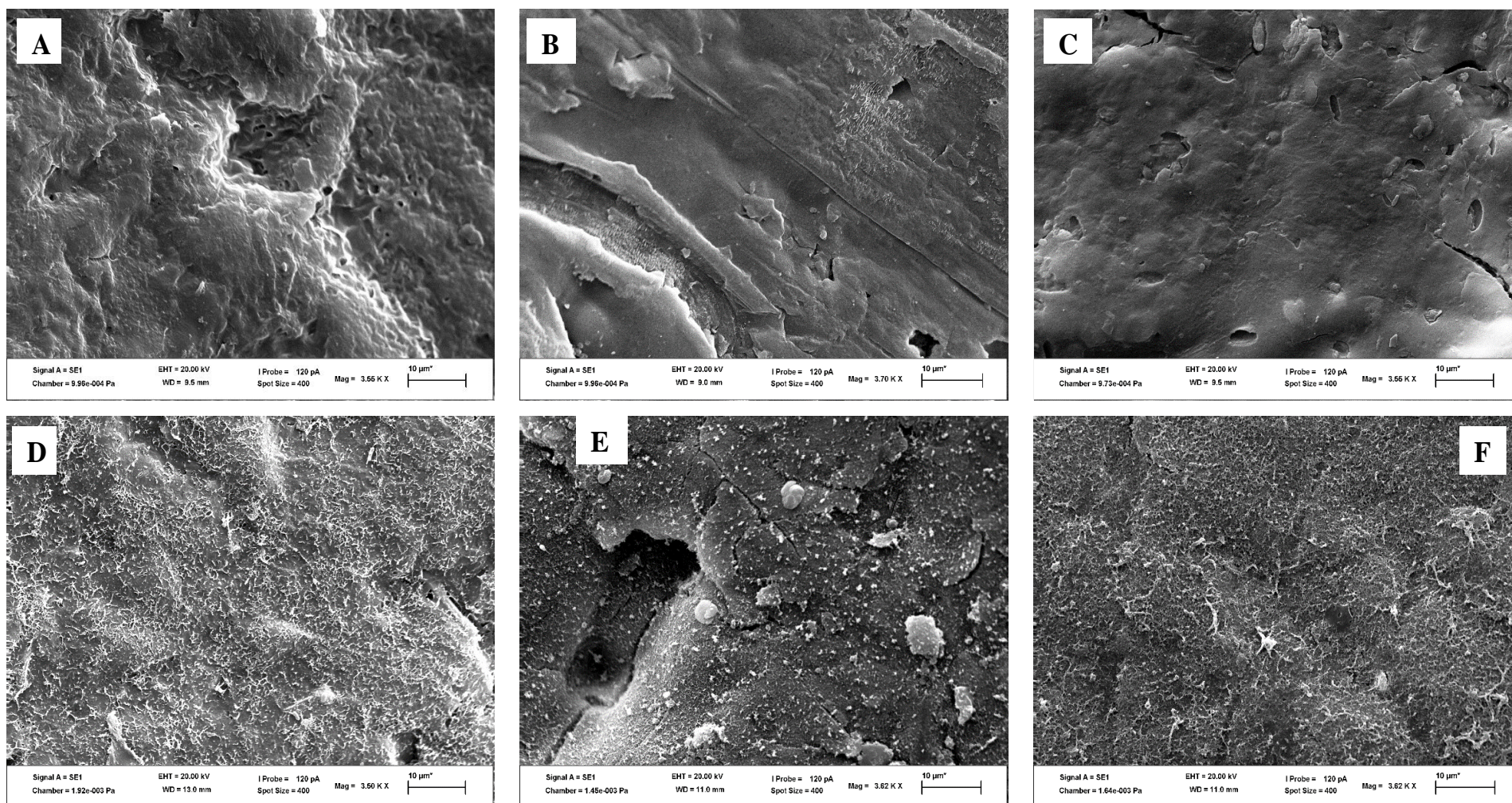
Figure 6.5: Scanning electron micrographs of 'Keitt' mango inoculated with *L. theobromae* in control (A), O_3 24 h (B), O_3 36 h (C), EC (D), EC +O_3 24 h (E), EC + O_3 36 h (F) at the end of storage. Fruit were stored at 10 °C for twenty-one days and seven days shelf-life at ambient temperature

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3.8 Shelf-life

The shelf-life of mango fruit inoculated with *C. gloeosporioides* was significantly ($p < 0.05$ affected by various treatments. Untreated fruit ripened faster compared to other treatments. The longest ripening time was observed in EC + O$_3$ (24 h) and EC + O$_3$ (36 h) which was six and seven days, respectively. Notably, fruit treated with EC took only five days to ripen.
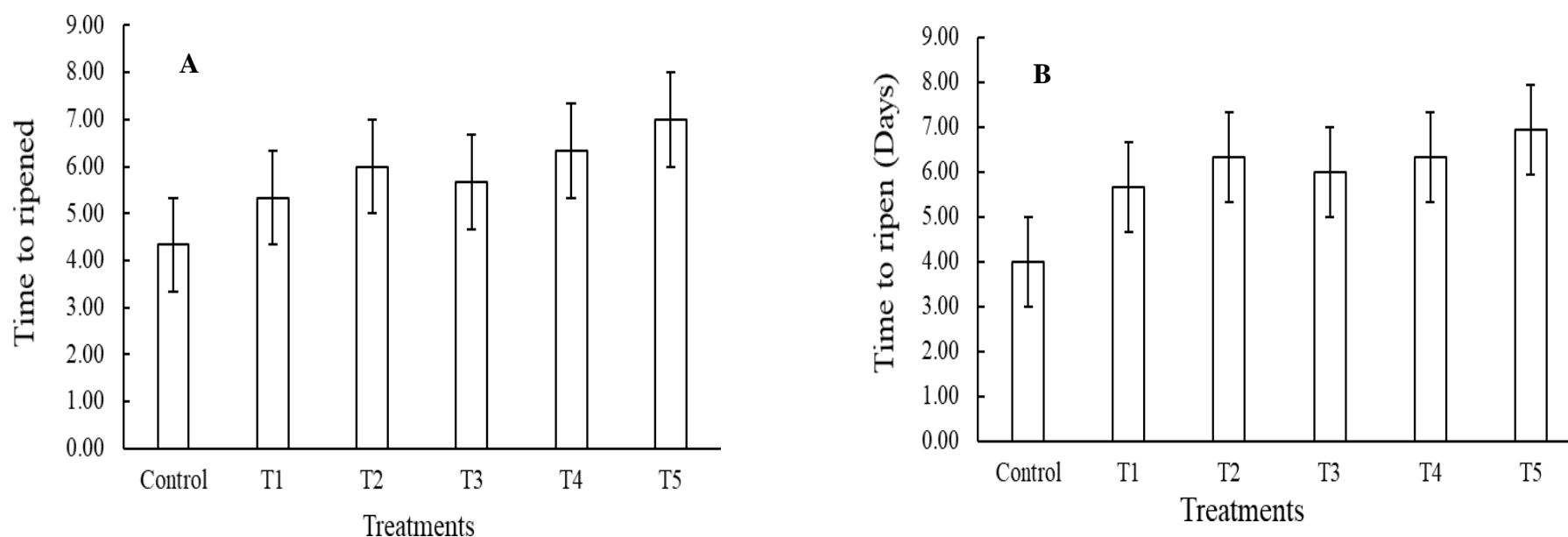
Figure 6.6: Effect of edible coatings and gaseous ozone on shelf-life of mango fruit inoculated with *C. gloeosporioides* (A) and *L. theobromae* (B),stored at ambient temperature for seven days (±SE, $n = 12$)

The shelf-life of mango fruit inoculated with *L. theobromae* significantly varied between treatments (Figure 6.6). The longest time to ripen was observed in fruit treated with EC + O$_3$ (36 h) and EC, which was seven and six days, respectively. The untreated fruit took only four days to ripen. There were no significant differences between the shelf-life of O$_3$ (24 h) and EC + O$_3$ (24 h).

4   Discussion

The in vitro study revealed that fungi causing anthracnose and stem-end rot have various sensitivity to O$_3$ and EC. The present study results are comparable to those of Alvindia and Mangoba (2020), who reported that Moringa oleifera extracts (2.5 g/L) inhibited mycelial growth and spore germination of *C. gloeosporioides* during incubation at 25 °C for seven days. Similarly, Samithri et al. (2020) reported that citronella essential oil (1000 µL L^{-1}) inhibited the mycelial growth of *L. theobromae* and *C. gloeosporioides*. The reduced mycelial growth could be attributed to the phytochemicals such as stigmasterol, quercetin and kaempferol present in moringa (Tesfay et al., 2017). Savi and Scussel (2014) reported that ozone (60 µmol/mol for 120 minutes) inhibited mycelial growth and spore germination of *Penicillium citrinum*, *Fusarium graminearum*, and *Aspergillus parasiticu*. Ozone forms relative oxygen species (ROS) within the cell interior, which induce cell death (Ong and Ali, 2015). The reduced mycelial growth could also be attributed to O$_3$ damaging the mitochondria of fungal spores (Ong and Ali, 2015).

The treatment combination of EC and O$_3$ effectively delayed the onset of stem-end rot and anthracnose symptoms. Current results are comparable to those reported by Khaliq et al. (2019), where 0.1% *Aloe vera* + garlic oil reduced the disease incidence of anthracnose in banana during storage. Gutiérrez-Martínez et al. (2017) reported that 1% chitosan decrease the disease incidence of anthracnose in mango

and banana during storage.

Firmness is important because consumers buy fresh produce based on appearance. The present study agrees with Kubheka et al. (2020), who reported that CMC 1% + moringa maintained firmness in 'Maluma' avocado during storage. Similarly, Shezi et al. (2020) observed high firmness in 'Hass' avocado coated with CMC 1% + Moringa 2% during storage at 5.5 ℃ for twenty-eight days. Fruit softening occurs due to the modification of cell wall structures. Panahirad et al. (2015) observed low enzyme activity of polygalacturonase in 'Golden Drop' plum coated with pectin 0.5% + CMC 1.5% during storage at 19 ℃ for ten days. It can be speculated that the modified atmosphere created by edible coating delayed the degradation of cell walls and starch by polygalacturonase, thus retaining fruit firmness. Zhang et al. (2021) observed high firmness in cantaloupe melon treated with O_3 (1 ppm for 5 hours, seven-day intervals) during storage.

Previous studies revealed that incorporating O_3 with other postharvest treatments such as edible coating, hot water and chemical treatments yielded superior results. For instance, a recent study by Bambalele et al. (2019) reported that MLE+CMC+ O_3 (36 h) maintained firmness in 'Keitt' mango during storage. Panou et al. (2021) observed high firmness in 'Carbarosa' strawberries treated with O_3 (1 ppm) + hot water (55 ℃, 15 seconds) during storage at 1 ℃ for fifteen days. Ozone modifies cell wall degrading genes such as beta-D-galactosidase, polygalacturonase and expansin (Minas et al., 2018). The retained firmness could be attributed to O_3 altering turgor loss, cell walls, and enhancing beta-D-galactosidase, thus inhibiting the accumulation of polygalacturonase genes (Contigiani et al., 2018; Minas et al., 2018).

Mass loss occurs during postharvest storage due to increased transpiration, ethylene production and respiration rate. Plant-based edible coatings alter the fruit exterior, reducing moisture loss and respiration

rate (Hmmam et al., 2021). Bambalele et al. (2019) reported that MLE + CMC (1%) decreased mass loss in 'Keitt' mango during storage. Paico et al. (2018) reported that O_3 was ineffective in delaying mass loss in 'Nam Dok Mai' mango during storage. Silva Neto et al. (2019) reported that ozone (1.5 ppm) decreased daily mass loss in 'Sunrise Solo' papaya during storage at ambient temperature for fourteen days. Ozone congeals and strengthens the fruit cuticle, thus decreasing moisture loss (Contigiani et al., 2018).

Flavonoids are plant phytochemicals with antioxidant activities. Kumar et al. (2021) reported that chitosan 2% + pullulan 2% preserved the flavonoid content in 'Safeda' mango. Sousa et al. (2021) reported that hydroxypropyl methylcellulose 10% + beewax 10% decreased the flavonoid content of 'Palmer' mango during storage. The resistance to *C. gloeosporioides* in mango is due to flavonoid accumulation and is correlated to the fruit colour (Sivankalyani et al., 2016). The current results confirm those of Chen et al. (2019), where O_3 (5 ppm, 10 hours) maintained the flavonoid content in 'Jing Tao Xiang' strawberries during storage. Zhang et al. (2021) reported that O_3 (1 ppm for 5 hours, at seven-day intervals) decreased the accumulation of flavonoid content of the peel in cantaloupe melon during storage at 4 °C for thirty days. Tzortzakis et al. (2013) reported that O_3 (0.05 µmol mol^{-1}, for one week) upregulated proteins associated with flavonoid biosynthesis proteins such as dihydroflavonol-4-reductase and farnesyl pyrophosphate synthase in 'Carousel' tomato inoculated with *Botrytis cinerea*. The high flavonoid content could be attributed to O_3 oxidizing anthocyanins into flavonoids. Chen et al. (2019), reported that O_3 (5 ppm, for 10 hours) upregulated proteins involved in phenylpropanoid metabolism such as CHS and CHI in 'Jing Tao Xiang' strawberries. The current results suggest that O_3 could induce secondary metabolites as a defence mechanism against anthracnose and stem-end rot.

The POD is an enzyme that accumulates during plant growth till senescence. Ong et al. (2014) reported that O_3 (5 μL L^{-1}) induced the POD activity in 'Sekaki' papaya. Recently, Terao et al. (2019) reported that aqueous O_3 (3 mg L^{-1}) did not affect POD activity in 'THB' papaya during storage at 10 ℃ for eight days. The increased POD activity in O_3 treated fruit could be attributed to disease resistance. This hypothesis could be supported by Zhu et al. (2019) who reported that O_3 (2.5 μg L^{-1}, 24 hours) increased the POD gene expression in Satsuma mandarin. The POD gene expression is positively correlated to phenolic and flavonoid content (Zhu et al., 2019). Results of the present study indicate that O3 increases POD activity and flavonoid content, resulting in enhanced defence resistance against fungal attacks in mango fruit. Additionally, the increased POD activity could be responsible for maintaining the membrane integrity of mango fruit.

Shah et al. (2021) reported that chitosan 1% + thyme oil 0.1% increased the POD activity of 'Chaunsa' mango inoculated with *C. gloeosporioides* during storage. The POD enzyme scavenges the ROS during oxidative stress (Kumari et al., 2021). Moreover, the POD enzyme is known to delay the accumulation of hydrogen peroxide (Jongsri et al., 2017). The increased enzyme activity of POD could increase mango fruit resistance against *C. gloeosporioides* and *L. theobromae*. The high POD enzyme activity was observed in EC + O_3 (24 h) in fruit inoculated with *C. gloeosporioides* and *L. theobromae*. The enhanced enzyme activity coincides with the increased total flavonoid content, fruit firmness and decreased mass loss. Therefore, it could be argued that the defence resistance against fungal attacks involves enhancing the POD enzyme activity and flavonoid content.

The shelf-life of fresh produce is when they are of good quality and acceptable to consumers. Silva Neto et al. (2019) reported that O_3 (1.5 ppm) enhanced the shelf-life of 'Sunrise Solo' papaya. Similarly, Terao et al. (2019) reported that ozonated water (3 mg L^{-1}) increased the shelf-life of 'THB' papaya inoculated with *L. theobromae*. Vilaplana et al. (2020) reported that hot water (49°C) + chitosan (20 g^{-1}) increased the shelf-life of papaya inoculated with *Colletotrichum fructicola*. Current results suggest that EC and EC+O_3 (24 h) effectively extended shelf-life of mango fruit.

5   Conclusion

This study revealed that the treatment combination of ozone and EC effectively controlled stem-end rot and anthracnose in mango fruit. The EC+O_3 (24 h) and EC delayed mass loss accumulation, maintained firmness, flavonoid content, and POD activity. The current study provides an understanding of the mechanism of ozone in controlling some of the common and devastating mango postharvest diseases. The increased POD and flavonoid content were associated with the delayed disease incidence of anthracnose and stem-end rot. The in vitro study revealed that treatment combination of O_3 and EC yielded similar results. Therefore, the treatment combination of O_3 (24 h) and EC should be considered in controlling postharvest diseases. Further research is required to gain understanding of the EC+ O_3 mode of action in controlling mango postharvest diseases.

6 References

Alvindia, D.d.G., Mangoba, M.A.A. 2020. Biological activities of *Moringa oleifera* Lam. against anthracnose of mango caused by *Colletotrichum gloeosporioides* Penz. Arch. Phytopathol. Plant Protect. 53, 659-672.

Bambalele, N., Mditshwa, A., Tesfay, S., Magwaza, L. 2019. Moringa leaf extract infused into carboxymethyl cellulose edible coating combined with gaseous ozone as postharvest treatments maintain quality and extend shelf life of mango fruit. II International Symposium on Moringa 1306, pp. 225-232.

Chen, C., Zhang, H., Dong, C., Ji, H., Zhang, X., Li, L., Ban, Z., Zhang, N., Xue, W. 2019. Effect of ozone treatment on the phenylpropanoid biosynthesis of postharvest strawberries. RSC Advances 9, 25429-25438.doi:10.1039/c9ra03988k.

Contigiani, E.V., Jaramillo-Sánchez, G., Castro, M.A., Gómez, P.L., Alzamora, S.M. 2018. Postharvest quality of strawberry fruit (*Fragaria x ananassa* Duch cv. Albion) as affected by ozone

washing: fungal spoilage, mechanical properties, and structure. Food Biopr. Technol. 11, 1639-1650.

De Santis, D., Garzoli, S., Vettraino, A.M. 2021. Effect of gaseous ozone treatment on the aroma and clove rot by *Fusarium proliferatum* during garlic postharvest storage. Heliyon 7, e06634.

Escamilla-García, M., Rodríguez-Hernández, M., Hernández-Hernández, H., Delgado-Sánchez, L., García-Almendárez, B., Amaro-Reyes, A., Regalado-González, C. 2018. Effect of an edible coating based on chitosan and oxidized starch on shelf life of *Carica papaya* L., and its physicochemical and antimicrobial properties. Coatings 8, 318.doi:10.3390/coatings8090318.

Galsurker, O., Diskin, S., Duanis-Assaf, D., Doron-Faigenboim, A., Maurer, D., Feygenberg, O., Alkan, N. 2020. Harvesting mango fruit with a short stem-end altered endophytic microbiome and reduce stem-end rot. Microorganisms 8, 558.

Galsurker, O., Diskin, S., Maurer, D., Feygenberg, O., Alkan, N, 2018. Fruit stem-end rot. Horticulturae 4, 50.doi:10.3390/horticulturae4040050

Gutiérrez-Martínez, P., Bautista-Baños, S., Berúmen-Varela, G., Ramos-Guerrero, A., Hernández-Ibañez, A.M. 2017. In vitro response of *Colletotrichum* to chitosan. Effect on incidence and quality on tropical fruit. Enzymatic expression in mango. Acta Agronómica 66, 282-289

Hmmam, I., Zaid, N.m., Mamdouh, B., Abdallatif, A., Abd-Elfattah, M., Ali, M. 2021. Storage behavior of "Seddik" mango fruit coated with CMC and guar gum-based silver nanoparticles. Horticulturae 7, 44.doi:10.3390/horticulturae7030044.

Jongsri, P., Rojsitthisak, P., Wangsomboondee, T., Seraypheap, K. 2017. Influence of chitosan coating combined with spermidine on anthracnose disease and qualities of 'Nam Dok Mai' mango after harvest. Sci. Hortic. 224, 180-187.

Khaliq, G., Abbas, H.T., Ali, I., Waseem, M. 2019. *Aloe vera* gel enriched with garlic essential oil effectively controls anthracnose disease and maintains postharvest quality of banana fruit during storage. Hortic. Environ. Biotechnol. 60, 659-669.

King, K.M., Bucur, D.E., Ritchie, F., Hawkins, N.J., Kaczmarek, A.M., Duan, Y., Kildea, S., West, J.S., Fraaije, B.A. 2021. Fungicide resistance status and chemical control options for the brassica pathogen *Pyrenopeziza brassicae*. Plant Pathol. 70(9): 2086-2103.

Konsue, W., Dethoup, T., Limtong, S. 2020. Biological control of fruit rot and anthracnose of postharvest mango by antagonistic yeasts from economic crops leaves. Microorganisms 8, 317. Kubheka, S.F., Tesfay, S.Z., Mditshwa, A., 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, N., Pratibha, Neeraj, Petkoska, A.T., Al-Hilifi, S.A., Fawole, O.A. 2021. Effect of chitosan–pullulan composite edible coating functionalized with pomegranate peel extract on the shelf life of mango (*Mangifera indica*). Coatings 11, 764. doi:10.3390/coatings11070764.

Kumari, S., Singh, A., Kumar, A., Singh, K., Bains, G., 2021. Evaluating the efficacy of chitosan and salicylic acid on photosynthetic pigments and antioxidant enzymes towards resistance of mango malformation. Sci. Hortic. 285, 110160.

Minas, I.S., Tanou, G., Krokida, A., Karagiannis, E., Belghazi, M., Vasilakakis, M., Papadopoulou, K.K., Molassiotis, A 2018. Ozone-induced inhibition of kiwifruit ripening is amplified by 1-methylcyclopropene and reversed by exogenous ethylene. BMC Plant Biol. 18, 1-19.

Ong, M.K., Ali, A. 2015. Antifungal action of ozone against *Colletotrichum gloeosporioides* and control of papaya anthracnose. Postharvest Biol. Technol. 100, 113- 119. doi:10.1016/j.postharvbio.2014.09.023.

Ong, M.K., Ali, A., Alderson, P.G., 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. Sci. Hortic. 179, 163-169.

Paico, M.J.P., Jittanit, W., Jarupan, L., Chonhenchob, V. 2018. Effect of ozone on prolonging the shelflife of mango and broccoli during cold storage and distribution. The 21st IAPRI World Conference on Packaging.

Panahirad, S., Mahna, N., Rahim Naghshiband-Hassani, B., Ghanbarzadeh, F.Z.-N. 2015. Plum shelflife enhancement by edible coating based on pectin and carboxymethyl cellulose. J. Biod. Environ. Sci. 7, 423-430.

Panou, A.A., Akrida-Demertzi, K., Demertzis, P., Riganakos, K.A. 2021. Effect of gaseous ozone andheat treatment on quality and shelf life of fresh strawberries during cold storage. Int. J. Fruit Sci. 21, 218-231.

Samithri, Y., Karunanayake, K., Kulasinghe, A. 2020. In vitro study of selected essential oils against *Colletotrichum* sp. and *Lasiodiplodia* sp. causing postharvest diseases in papaya. Ceylon J. Sci. 16(49): 389-396

Savi, G.D., Scussel, V.M. 2014. Effects of ozone gas exposure on toxigenic fungi species from *Fusarium, Aspergillus*, and *Penicillium* genera. Ozone Sci. Eng. 36, 144-152.

Shah, S., Hashmi, M.S., Qazi, I.M., Durrani, Y., Sarkhosh, A., Hussain, I., Brecht, J.K. 2021. Pre-storage chitosan-thyme oil coating control anthracnose in mango fruit. Sci. Hortic. 284, 110139.

Shezi, S., Tesfay, S.Z., Magwaza, L.S., Mditshwa, A., Motsa, N.M. 2020. Gaseous ozone and plant extract-based edible coatings as postharvest treatments for 'Hass' avocado (*Persea americana* Mill.) fruit. Acta Hortic. 10.17660/ActaHortic.2020.1275.8, 53-60. doi:10.17660/ActaHortic.2020.1275.8.

Siddiqui, Y., Ali, A. 2014. *Colletotrichum gloeosporioides* (Anthracnose), Postharvest Decay. Elsevier, pp. 337-371.

Silva Neto, O.P.d., Pinto, E.V.d.S., Ootani, M.A., Silva Junior, J.L.d., Lima, J.L.d.S.B., Sousa, A.E.D.d. 2019. Ozone slows down anthracnose and increases shelf life of papaya fruits. Revista Brasileira de Fruticultura 41. doi:10.1590/0100-29452019439.

Sivakumar, D., Jiang, Y., Yahia, E.M., 2011. Maintaining mango (*Mangifera indica* L.) fruit quality during the export chain. Food Res. Inte. 44, 1254-1263.

Sivankalyani, V., Feygenberg, O., Diskin, S., Wright, B., Alkan, N. 2016. Increased anthocyanin and flavonoids in mango fruit peel are associated with cold and pathogen resistance. Postharvest Biol. Technol. 111, 132-139. doi:10.1016/j.postharvbio.2015.08.001.

Sousa, F.F., Pinsetta Junior, J.S., Oliveira, K., Rodrigues, E.C.N., Andrade, J.P., Mattiuz, B.H. 2021. Conservation of 'Palmer' mango with an edible coating of hydroxypropyl methylcellulose and beeswax. Food Chem. 346, 128925. doi:10.1016/j.foodchem.2020.128925.

Terao, D., de Lima Nechet, K., Frighetto, R.T.S., Sasaki, F.F.C. 2019. Ozonated water combined withheat treatment to control the stem-end rot of papaya. Sci. Hortic. 257, 108722.

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

Tzortzakis, N., Taybi, T., Antony, E., Singleton, I., Borland, A., Barnes, J. 2013. Profiling shifts in protein complement in tomato fruit induced by atmospheric ozone-enrichment and/or wound-inoculation with *Botrytis cinerea*. Postharvest Biol. Technol. 78, 67-75.

Vilaplana, R., Chicaiza, G., Vaca, C., Valencia-Chamorro, S. 2020. Combination of hot watertreatment and chitosan coating to control anthracnose in papaya (*Carica papaya* L.) during the postharvest period. Crop Prot. 128, 105007.

Zhang, X., Tang, N., Zhang, H., Chen, C., Li, L., Dong, C., Cheng, Y. 2021. Comparative transcriptomic analysis of cantaloupe melon under cold storage with ozone treatment. FoodRes. Int. 140, 109993.

Zhu, X., Jiang, J., Yin, C., Li, G., Jiang, Y., Shan, Y. 2019. Effect of ozone treatment on flavonoid accumulation of Satsuma mandarin (*Citrus unshiu* Marc.) during ambient storage. Biomolecules 9, 821.

Zillo, R.R., da Silva, P.P.M., de Oliveira, J., da Glória, E.M., Spoto, M.H.F. 2018. Carboxymethylcellulose coating associated with essential oil can increase papaya shelf life. Sci. Hortic. 239, 70-77.

Chapter 7

Impact of gaseous ozone and CMC-moringa edible coating on organic volatile compounds of'Keitt' mango fruit during storage

Abstract

Volatile organic compounds contribute to the attractive taste and aroma of mango fruit. This study was conducted to evaluate the effect of edible coatings (EC) and ozone (O_3) on volatile compounds of mango fruit during storage. 'Keitt' mango fruit were treated with EC before exposure to O_3 (0.25 mg L^{-1}), during cold storage. The fruit were stored for twenty-one days at 10 °C and ripened at ambient temperature for seven days. The fruit were treated with O_3 for 24 or 36 hours at seven-day intervals, and control fruit were untreated. Volatile compounds were extracted utilizing the headspace solid- phase microextraction method. There were thirty volatile compounds detected in mango fruit during storage. These compounds were classified as esters, alcohols, ketones, aldehydes, terpenes, and carbonyl compounds. On day fourteen, EC + O_3 (36 h) had a higher 2-decenal content compared to other treatments. At the end of the cold storage, the highest hexanal content was observed in untreated fruit (1003.40 mg/g) compared to the EC, EC + O_3 (24 h), EC + O_3 (36 h), O_3 (36 h) and O_3 (24 h) which were 881.30 mg/g, 718.08 mg/g, 620.40 mg/g, 472.70 mg/g and 432.22 mg/g, respectively. The highest α-pinene content was observed in O_3 (24 h) at the end of storage. Octanal content rapidly decreased in fruit treated with O_3 (24 h), O_3 (36 h), EC + O_3 (24 h), and the control after cold storage. The trans-2-octenol content significantly (p < 0.05) increased from the beginning of storage until day fourteen then decreased till the end of storage in all treatments. In the current study, fruit treated with EC had the highest content of volatile compounds. The current results indicate that ozone can preserve the flavor and quality of

mango fruit.

Keywords: Cold storage, mango, volatile compounds, ozone, and postharvest

1 Introduction

Mango is one of important fruit due to its nutritional value and aroma. Fruit and vegetables contain volatiles such as allicin, cavracol, borneol, and allyl isothiocyanate, which have antimicrobial properties (Goff and Klee, 2006). The compounds have anti-malarial, immune booster, and anticarcinogenic properties that are beneficial to human health (Goff and Klee, 2006; Ayseli and Ayseli, 2016). Volatile organic compounds contribute to the mango aroma and taste (Liu et al., 2020). Biochemical changes during mango fruit ripening lead to color changes from green to orange-yellow, increased sweetness, taste, and aroma (Cuevas-Glory et al., 2020). Numerous volatile compounds have been discovered and studied in mango fruit, including monoterpenes, esters, sesquiterpenes, aldehydes, and ketones (Lehner and Siegmund, 2020; Liu et al., 2020). Bonneau et al. (2016) reported that β- caryophyllene, limonene, γ-butyrolactone, β-myrcene, 3-methylbutyl, and δ-3-carene are the significant compounds contributing to 'Kent' mango aroma. Recently, Cuevas-Glory et al. (2020) identified new odour active compounds such as α-phellandrene, dodecanal, β-pinene, α-humulene, and γ-terpinene that contribute to 'Ataulfo' mango. The volatile compounds in mango fruit are influenced by harvest maturity, genotype, postharvest treatments, and preharvest factors (Singh and Saini, 2014; Liu et al., 2020; Thiruchelvam et al., 2020).

Mango fruit is highly perishable, rapidly decreasing in quality, taste, and flavor (Bonneau et al., 2016). Postharvest technologies such as plant extract edible coatings, ozone, and chemical treatments are utilized to enhance mango fruit quality (Río Segade et al., 2018; Bambalele et al., 2021). The plant-extract edible layer contains natural antioxidants and antimicrobial properties that preserve fruit quality

(Ncama et al., 2018). Plant-extract edible coatings are known to increase the volatile compounds and improve the fruit flavor. For instance, Aghofack-Nguemezi et al. (2019) observed high levels of 6-ethyl-3,5-heptadien-2-ol, 2,3-butanediol, phenylethanol, and 2-furanmethanol in 'Balkonstar' tomatoes treated with cocoa leaf or coffee hull extracts. Edible coatings have also been shown to effectively reduce fermentation compounds in papaya fruit. For instance, Escamilla-García et al. (2018) reported that starch-chitosan (1%) coating decreased the accumulation of acetic acid, methyl acetate, and ethyl butanoate in 'Maradol' papaya stored for ten days at ambient temperature.

Ozone (O_3) has been approved as a food additive and disinfectant during postharvest storage (De Santis et al.,2021). Ozone is continuously produced onsite, has no chemical residue, making it safe for consumers (Mustapha and Zhou, 2021). Mustapha and Zhou (2021) reported that aqueous O_3 (0.2 mg/L) reduced the hexenal compound in 'Jutou' cherry tomatoes. Similarly, ozone (60 µL/L, 48 hours) treatment preserved α-terpineol, linalool, and cis-furan linalool oxide in 'Moscato Bianco' grapes (Río Segade et al., 2018). The short-term O_3 (12 mL min^{-1}) treatment decreased off-flavors and volatile compounds such as linalool, terpinene-4-ol, and α-terpineol in orange juice (Alves Filho et al., 2019). Preserving volatile compounds is vital to enhance the postharvest fruit quality. Continuous exposure and high ozone concentration on fruit can lead to off-flavors. Therefore, the objective of this study was to compare changes in mango fruit volatile compounds as affected by edible coatings (MLE and CMC) and O_3 treatments during cold storage and fruit ripening; to determine the treatment combination that will enhance fruit quality.

2 Materials and methods

2.1 Fruit material

Mango fruit (*cv*. 'Keitt') were harvested from Goedgelegen Farm of Westfalia (Pty) Ltd, a commercial farm located in Tzaneen, South Africa. Fruit were transported within 24 hours to the Postharvest Research Laboratory of the University of KwaZulu-Natal (Pietermaritzburg Campus). The fruit used in this experiment were of the same physiological maturity, size and had no visible disease or mechanical injury. The fruit were allocated to various treatments as follows:

Control: untreated

T1: O_3 (24 hours)

T2: O_3 (36 hours)

T3: EC (MLE 1% + CMC 1%)

T4: EC+O_3 (24 hours)

T5: EC+O_3 (36 hours)

1.1 Edible coating

Moringa leaf was extracted as described by Tesfay and Magwaza (2017), with some modification. Briefly, 100 g of moringa leaf powder was extracted with 70% ethanol (1000 mL) for twelve hours at room temperature with constant agitation. The extract was evaporated at 37 °C using a Genevac evaporator (Genevac® EZ 2.3; IPSWICH; England). The crude extract was suspended with 1000 mL distilled water and incorporated to 10 g CMC.

1.2 Ozone treatment

Gaseous ozone was generated using the corona discharge ozone generator (Ozone Purification Technology, Johannesburg, South Africa). Ozone treatment (0.25 mg L^{-1}) of mango fruit was done in cold storage for twelve hours at seven-day intervals. For the 24 hour treatment, fruit were exposed to O_3 at days zero and seven, whereas for 36 hours, it were days zero, seven, and fourteen. Fruit were coated with EC before O_3 treatment. The control fruit were untreated, and data were collected after every seven days. Fruit were stored at 90% relative humidity and 10 °C ($\pm$0.5) temperature for twenty-one days, mimicking shipment to export markets. After cold storage, fruit were transferred to shelf-life at ambient temperature for seven days.

1.3 Sample preparation

Mangoes were peeled, pulp removed from the kernel, and cut into small cubes. The peel was freeze-dried using Vir Tis BenchTop Pro freeze drier (SP Scientific, Warminster, Pennsylvania, USA). Thereafter, samples were crushed into powder and stored at -20°C until used. The experiment was replicated three times, with three fruit per replicate. In triplicates, sample powder (500 mg) was weighed into SPME vials, and Anisole d8 was added. Volatile buffer (5 mL) and 3 mL of sodium chloride 20% kept at 25 °C was added to the sample and vortexed for 60 seconds.

1.4 Extraction and gas chromatographic analysis of volatile compounds

Volatile compounds were analyzed as described by Liu et al. (2020), with modification. Volatile compounds were extracted utilizing the headspace solid-phase microextraction (SPME) method. A SPME vial was calibrated at 250 rpm for ten minutes in the CTC autosampler incubator (70°C). Thereafter, divinylbenzene/-carboxen/-polydimethylsiloxane (DVB/CAR/PDMS) 50/30 m fiber was exposed to the sample headspace at 50°C for twenty minutes. The desorption of the volatile compounds from the coated fiber was executed for ten minutes in the injector port of the gas chromatography-mass spectrometry (GC-MS). Quantification of the volatile compounds was conducted using Agilent 6890 N (Agilent, Palo Alto, CA) on a gas chromatograph, incorporated with an Agilent mass spectrometer detector Agilent 5975 MS (Agilent, Palo Alto, CA). The GC-MS system was furnished with a polar Zebron ZB-Wax capillary column from Phenomenex with 0.25 µm film thickness, length of 30 m, and 250 µm internal diameter. The 1 mL min^{-1} helium was utilized as the carrier gas for the analysis with 250°C injector temperature. The oven program was five minutes at 40°C, ultimately raised up to 7 min^{-1} at 240°C (five minutes, holding time). The MSD was operated in full scan mode, with temperatures 150°C and 230°C for the quadrupole and ion source, respectively. The transfer line was kept at 250°C, and compounds were distinguished by comparing the retention time (RI) with NIST05 or WHILEY275 mass spectral libraries.

1.5 Statistical analysis

Data was subjected to the analysis of variance (ANOVA), using GenStat statistical software (GenStat®, 18 edition, VSN International, UK). Fischer's least significant difference (LSD) was used to separate means at 5 % level of significance.

2   Results

There were thirty volatile compounds detected in mango fruit during storage (Table 7.1). The compounds were classified into esters, alcohols, ketones, aldehydes, terpenes, and carbonyl compounds. The aldehyde compounds were the most identified in 'Keitt' mango fruit, while esters and alcohols were the lowest. Volatile compounds that significantly responded to treatments are discussed in this chapter.

Table 7.7: Volatile compounds detected in mango fruit during storage at 10 °C for twenty-one days and seven days shelf-life at ambient temperature.

Compound

Terpenes
α-carene
α-pinene
D-limonene
Pinane
Caryophyllene
Humulene

Aldehydes
Hexanal
Cis-Hept-2-enal
trans,trans-2,4 heptadienal
trans,trans-2,4 heptadienal (01)
E-Nonenal
α-cyclocitral
2-undecenal
trans-2-octenal
trans-2-decenal
cis-hept-2-enal

Carbonyl compounds
Heptanal
Octanal
1-octen-3-one
Nonanal
Decanal

Ketones
6-methyl-5-hepten-2-one
6,10-dimethyl-undeca-5,9-dien-2-one
3-methyl-hepta-1,6-dien-3-ol

Alcohol
1-octene-3-ol
1-octanol
trans-2-octenol

Esters
Butyl_isobutyrate
n-butyl_butyrate

Other

2.1 Aldehydes

The aldehydes of mango fruit were significantly ($p < 0.05$) affected by various treatments during storage. On day fourteen, EC + O_3 (36 h) had the highest 2-decenal content compared to other treatments (Table 7.2). There were no significant differences between treatment means of O_3 (24 h), EC, and untreated fruit. At the end of storage, the EC had the high 2-decenal content compared to EC+ O_3 (36 h), O_3 (24 h), EC + O_3 (24 h), and untreated fruit. The hexanal content increased from day zero to day fourteen and then decreased until storage. On day seven, highest hexanal content was observed in the untreated fruit. There were no significant differences between treatment means of O3 (24 h), EC, and EC+ O_3 (36 h) at day fourteen. At the end of cold storage, the highest hexanal content was observed in untreated fruit (1003.40 mg/g) compared to the EC, EC + O_3 (24 h), EC + O_3 (36 h), O_3 (36 h) and O_3 (24 h) which were 881.30 mg/g, 718.08 mg/g, 620.40 mg/g, 472.70 mg/g and 432.22 mg/g, respectively. The treatment means of O_3 (24 h), O_3 (36 h), and EC were not significantly different at the end of the storage period. The α-cyclocitral content gradually increased during storage. The EC (1080.80 mg/g) had the highest α-cyclocitral content compared to other treatments. On day fourteen, there were no significant differences between treatment means of O3 (24 h), O3 (36 h), EC+ O_3 (24 h), EC + O_3 (36 h), and untreated fruit. At the end of the storage period, the highest α-cyclocitral content was observed in the untreated fruit.

Table 7.2: Effect of edible coatings and gaseous ozone on aldehydes of 'Keitt' mango fruit stored at 10 °C for twenty-one days andseven days shelf-life at ambient temperature

Storage time (Day)	Treatments					
	2-decenal					
	Control	O₃ (24 h)	O₃ (36 h)	EC	EC + O₃ (24 h)	EC + O₃ (36 h)
0	142.44ᵃᵇ	179.40ᵃᵇ	140.29ᵃ	120.57ᵃ	188.08ᵃᵇ	139.73ᵃ
7	341.16ᶜ	172.55ᵃ	95.28ᵃ	51.89ᵃ	55.92ᵃ	78.66ᵃ
14	163.90ᵃᵇ	121.25ᵃᵇ	308.84ᶜ	169.79ᵃᵇ	450.56ᶜ	734.03ᵈ
21	310.17ᶜ	119.03ᵃᵇ	64.13ᵃ	106.86ᵃᵇ	68.18ᵃ	137.55ᵃᵇ
28	147.65ᵃᵇ	177.78ᵃᵇ	148.78ᵃ	349.02ᶜ	178.78ᵃ	284.60ᵇᶜ
	Hexanal					
0	237.10ᵃᵇ	553.70ᵃᵇᶜ	461.41ᵃᵇ	542.17ᵇᶜᵈ	549.90ᵇᶜᵈ	362.90ᵃᵇ
7	1415.00ᵍ	755.63ᶜᵈ	632.12ᶜᵈ	565.66ᵇᶜᵈ	316.05ᵃᵇ	655.88ᵇᶜᵈ
14	1178.90ᶠᵍ	543.73ᵃᵇ	893.81ᵈᵉ	611.10ᵃᵇᶜ	1215.63ᶠᵍ	886.04ᶜᵈᵉ
21	1003.40ᵈᶠ	432.22ᵃᵇ	472.70ᵃᵇ	881.30ᶜᵈᵉ	718.08ᶜᵈᵉ	620.40ᶜᵈ
28	403.00ᵃᵇ	358.40ᵃᵇ	196.50ᵃ	607.50ᶜᵈ	263.50ᵃᵇ	373.40ᵃᵇ

	α-cyclocitral					
0	327.77[a]	346.09[ab]	474.00[ab]	316.55[a]	382.50[ab]	440.60[ab]
7	502.09[ab]	717.40[bcd]	435.44[ab]	1080.80[cde]	457.90[ab]	778.00[cd]
14	411.73[ab]	463.13[ab]	564.20[ab]	393.81[ab]	734.60[cd]	631.90[bc]
21	704.31[cd]	389.60[ab]	520.70[abc]	1093.99[def]	718.03[cde]	916.00[cde]
28	1956.00[h]	1407.60[fg]	1298.20[ef]	1398.70[def]	1602.60[fg]	1190.90[e]

Means sharing the same letter between rows and within the columns are not significantly different (LSD for 2-decenal, hexanal and α-cyclocitral = 115.30, 228.00, and 268.80, respectively; $n = 9$)

2.2 Terpenes

The α-carene content significantly *($p < 0.05$)* increased from the beginning until day fourteen, thereafter it started to decrease (Table 7.3). The EC +O_3 (36 h) had a high α-carene content compared to other treatments. However, there were no significant differences between the treatment means of EC, EC + O_3 (24 h), and EC + O_3 (36 h) at day fourteen. The α-carene content decreased rapidly after cold storage in all treatments. The α-pinene content followed a similar trend to that of α-carene during the storage time. However, a decrease was observed in O_3 (36 h), EC, EC + O_3 (36 h) after day seven of cold storage. The highest α-pinene content was observed in O_3 (24 h) at the end of storage. There were no significant differences between the treatment means of O_3 (36 h), EC + O_3 (24 h), and the untreated fruit at the end of storage.

A significant ($p < 0.05$) decrease in D-limonene content was observed from the beginning of storage until day fourteen in all treatments. The D-limonene content increased rapidly from day twenty-one till the end of the storage period in all treatments. On day fourteen, there was no significant difference between treatment means of O_3 (24 h), O_3 (36 h), EC, EC + O_3 (24 h), and untreated fruit. The O_3 (24 h) has low d-limonene compared to other treatments at the end of storage time.

Table 7.3: Effect of gaseous ozone and edible coatings on terpenes of 'Keitt' mango stored at 10 °C for twenty-one days and seven daysshelf-life at ambient temperature

Storage time (Days)	Treatments					
	α-carene					
	Control	O₃ (24 h)	O₃ (36 h)	EC	EC + O₃ (24 h)	EC + O₃ (36 h)
0	129.10^a	279.40^a	267.32^a	197.00^a	211.00a^b	208.00^a
7	643.71^a	1426.00^b	1629.00^b	3976.00^g	2871.19^{cdef}	2659.33^{cdef}
14	908.01^a	2261.00^c	1291.17^b	2334.20^{cd}	1042.11^{ab}	2831.56^{bcd}
21	887.00^a	1816.48^b	1902.40^b	5464.00^h	4894.00^{gh}	2937.00^{cd}
28	616.00^a	802.00^a	441.45^a	253.50^a	546.71^a	474.61^a

α-pinene

0	190.90[ab]	149.80[a]	204.20[abc]	182.08[ab]	410.00[abc]	378.10[ab]
7	277.25[abc]	443.22[abc]	528.94[abc]	892.65[cd]	830.10[cd]	633.40[ab]
14	287.60[abc]	724.10[bc]	417.08[abc]	604.70[abc]	308.82[abc]	1099.01[cd]
21	299.88[abc]	555.50[abc]	553.44[abc]	1252.30[d]	1215.01[d]	712.10b[cd]
28	1414.00[d]	2001.07[e]	1366.00[d]	609.00[bc]	1461.80[cd]	989.90[bc]
			D-limonene			
0	6207.38[de]	2132.00[b]	8742.00[ef]	3847.00[bc]	7693.00[def]	4910.00[cd]
7	858.01[a]	1049.15[a]	1033.30[a]	2508.64[abc]	1237.00[a]	1632.41[a]
14	753.00[a]	1645.70[a]	1673.00[a]	3123.00[bc]	1685.73[a]	1715.22[ab]
21	2661.55[ab]	3245.00[bc]	2938.47[abc]	4847.08[def]	4080.58[cde]	2710.00[ab]
28	4324.00[def]	2313.00[ab]	2605.11[ab]	3079.04[bcd]	3141.00[bcd]	2783.35[b]

Means sharing the same letter between rows and within the columns are not significantly different (LSD for α-carene,

α-pinene andD-limonene = 1060.00, 415.20, and 1999.60, respectively; $n = 9$)

2.3 Ketones

In all treatments, 6-methyl-5-hepten-2-one content increased till day seven then decreased until day twenty-one (Table 7.4). A significant ($p < 0.05$) increase in 6-methyl-5-hepten-2-one content was observed after cold storage in all treatments. On day fourteen, O_3 (36) had a high 6-methyl-5-hepten-2-one content compared to other treatments. The fruit treated with EC (2042.42 mg/g) had the highest 6-methyl-5-hepten-2-one content compared to EC + O_3 (24 h), EC + O_3 (36 h), and the control which had 1819 mg/g, 1619.17 mg/g, 1404.08 mg/g, respectively. At day fourteen, untreated fruit (189.09 mg/g) had the highest 6,10-dimethyl undeca 5,9 dien-2-one content compared to other treatments. There were no significant differences between treatment means of O_3 (24 h), O_3 (36 h), and untreated fruit at the end of the cold storage period. The 6,10-dimethyl undeca 5,9 dien-2-one content increased in all treatments after cold storage.

Table 7.8: Effect of gaseous ozone and EC on ketones of 'Keitt' mango fruit stored at 10 °C for twenty-one days and seven days shelf-life at ambient temperatures.

Storage time (Days)	Treatments					
	6-methyl-5-hepten-2-one					
	Control	O₃ (24 h)	O₃ (36 h)	EC	EC + O₃ (24 h)	EC + O₃ (36 h)
0	787.00a	1080.01abc	829.99ab	945.30ab	1280.15bcd	607.52ab
7	1432.00cd	2348.00e	1731.81cd	1752.88cd	1353.21bcd	1684.00cd
14	1020.10ab	693.00ab	1118.77ab	631.23ab	847.11ab	1107.00ab
21	1293.25bc	769.60ab	855.00ab	1720.00cd	863.37ab	1563.32cd
28	1404.08cd	1385.66cd	1045.33ab	2042.42e	1819.00cd	1619.71cd
	6,10-dimethyl undeca 5,9 dien-2-one					
0	96.68a	185.67b	149.90ab	165.24ab	163.24ab	134.67ab
7	179.60ab	270.74cd	186.64ab	231.00bc	162.25ab	173.42ab
14	189.09b	147.45ab	154.36ab	127.93a	144.61ab	180.46b
21	223.82bcd	172.78b	146.19ab	317.66cd	198.11b	311.13cd
28	513.28ef	453.34e	337.40bcd	406.41cd	413.12cde	327.10cd

Means sharing the same letter between rows and within the columns are not significantly different (LSD for 6-methyl-5-hepten-2-one, and 6,10-dimethyl undeca 5,9 dien-2-one = 403.40 and 69.29, respectively; $n = 9$).

2.4 Carbonyl compounds

The carbonyl compounds were significantly ($p < 0.05$) affected by the various treatments during the storage time (Table 7.5). The octanal content increased in fruit treated with EC and EC + O_3 (36 h) from day fourteen until the end of the storage period. Octanal content rapidly decreased in fruit treated with O_3 (24 h), O_3 (36 h), EC + O_3 (24 h), and the control after cold storage. On day twenty-one, untreated fruit had high octanal content compared to other treatments. There were no significant differences between treatment means of O_3 (24 h), O_3 (36 h) EC + O_3 (24 h), and untreated fruit at the end of the storage period. A significant decrease in 1-octen-3-one content was observed in all treatments at day seven. On day fourteen, fruit treated with O_3 (36 h) had the highest 1-octen-3-one content compared to other treatments. Fruit treated with O_3 (24 h), O_3 (36 h), and EC + O_3 (24 h) were not significantly different at the end of the storage period. The highest 1-octen-3-one content was observed in EC + O_3 (36 h) at the end of the storage period. The decanal content decreased from day fourteen until the end of the storage period in untreated fruit. The decanal content increased in O_3 (24 h), EC, and O_3 (36 h) from day twenty-one till the end of the storage period. At the end of the storage period, there were no significant differences between the treatment means of O_3 (24 h), O_3 (36 h), and EC + O_3 (24 h) at the end of the storage period.

Table 7.9: Effect of EC and gaseous ozone on Carbonyl compounds of 'Keitt' mango fruit stored at 10 °C for twenty-one days and seven days shelf-life at ambient temperature.

Storage time (Days)	Treatments					
	Octanal					
	Control	O$_3$ (24 h)	O$_3$ (36 h)	EC	EC + O$_3$ (24 h)	EC + O$_3$ (36 h)
0	624.50ab	1785.60cd	2011.70de	1319.80bcd	1472.10cd	597.71abc
7	463.30a	251.10a	150.88a	105.44a	179.80a	144.50a
14	517.67ab	144.90a	265.70a	155.00a	523.60ab	324.60ab
21	2387.00e	1099.40bc	817.36bcd	728.06bcd	510.54ab	932.20bcd
28	847.90bcd	806.00b	328.63ab	1937.10de	440.00ab	1228.10cd
	1-Octen-3-one					
0	391.14bc	492.17c	309.61bc	423.56bc	341.65bc	408.32bc
7	164.60abc	223.29ab	230.77ab	111.63a	89.96ab	164.18ab
14	291.11abc	529.78c	606.84d	546.66c	429.04bc	488.74bc
21	151.03ab	81.18a	56.99a	83.14a	50.30a	87.46a
28	453.79cd	358.27bc	466.15bc	641.70de	366.41ab	676.89e
	Decanal					
0	109.80a	185.22a	182.80a	140.30a	205.80ab	112.80a
7	323.90bc	240.01ab	161.42a	137.00a	160.60a	160.90ab
14	575.20c	157.00a	252.71ab	179.49a	493.50bc	375.70bc

21	243.30ab	133.10a	111.09a	169.38a	120.00a	143.60a
28	215.50ab	203.10ab	119.60a	316.20bc	139.33a	226.43ab

Means sharing the same letter between rows and within the columns are not significantly different (LSD for octanal, 1-octen-3-one, and decanal = 601.80, 191.75, respectively; $n= 9$).

2.5 Alcohols

The trans-2-octenol content significantly ($p < 0.05$) increased from the beginning of storage until day fourteen then decreased till the end of storage (Table 7.6). This behaviour was observed in fruit treated with O_3 and untreated fruit. Fruit treated with EC+ O_3 showed a decrease in trans-2-octenol content throughout the storage period. However, an increase of trans-2-octenol content in these treatments was observed only on day fourteen. The trans-2-octenol content increased in fruit treated with EC from day fourteen until the end of the storage period. At the end of the storage period, fruit treated with EC had the highest trans-2-octenol content compared to EC + O_3 (36 h), control, O_3 (24 h), and O_3 (36 h).

Table 7.6: Effect of edible coatings and gaseous ozone on trans-2-octenol of 'Keitt' mango fruit stored at 10 °C for twenty-one days andseven days shelf-life at ambient temperature

Storage time (Days)	Treatments					
	trans-2-octenol					
	Control	O₃ (24 h)	O₃ (36 h)	EC	EC + O₃ (24 h)	EC + O₃ (36 h)
0	775.47ᵃ	1007.22ᵃ	845.00ᵃ	832.20ᵃ	1782.09ᵇ	1551.00ᵃᵇ
7	2252.00ᶜ	2038.10ᵇᶜ	1257.00ᵃᵇ	1267.41ᵃᵇ	835.71ᵃᵇ	1359.48ᵃᵇ
14	4009.00ᵈ	881.05ᵃᵇ	1852.00ᵃᵇ	1001.00ᵃᵇ	2638.32ᶜ	2322.00ᵇᶜ
21	1616.03ᵃᵇ	920.80ᵃᵇ	660.00ᵃᵇ	1072.00ᵃᵇ	682.80ᵃᵇ	1100.35ᵃᵇ
28	749.33ᵃᵇ	452.50ᵃ	306.00ᵃ	1491.56ᵇ	280.13ᵃ	771.00ᵃᵇ

Means sharing the same letter between rows and within the columns are not significantly different (LSD = 763.30, $n = 9$)

4   Discussion

The aldehyde compounds give the fruit a sweet, green, and citrusy smell (Bonneau et al., 2016; Sunget al., 2019). Dang et al. (2008) observed a high concentration of hexanal and hexadecanal in 'Kensington Pride' mango coated with mango carnauba during storage. Wu et al. (2019a) reported that ozone (4 mg m⁻³) treatment decreased the aldehyde content such as hexanal, 2-heptenal, and 2- octenal in 'Red' pitaya fruit during storage. Aldehydes contributed 33% to the total volatile compounds of mango fruit. The combination of aldehydes, ketones, and acetals contributes to a balanced aroma infruits (Wei et al., 2019). In the present study, fruit treated with EC had a high content of aldehydes. The enhanced aldehydes suggest that the edible coating creates a modified atmosphere within the fruit,thus delaying biosynthesis of the compound and senescence.

Terpenes have a strong odor and consist of monoterpenes and sesquiterpenes (Wu et al., 2019a). In the present study, six terpenes were identified; they included pinane, humulene, α-carene, caryophyllene, and α-pinene. Wu et al. (2019b) reported that ozone increased the content of α-pinene, α- caryophyllene, germacrene, and β-myrcene in Satsuma mandarin during storage. Dang et al. (2008) reported that mango carnauba coating maintained monoterpenes including δ-car-3-ene, γ-terpinene, and α-pinene in 'Kensington Pride' mango during storage. Terpenes are synthesized through the isoprenoid pathway and contribute to the sweet, green, and citrusy aroma (Bonneau et al., 2016; Sunget al., 2019). Terpenes are a substantial variety of organic compounds that contain antioxidant properties and increase during fruit ripening (Wu et al., 2019a). The current results suggest that O_3 could induce a defense mechanism during oxidative stress.

Ketones are the least abundant class of volatile compounds in mango fruit. Singh and Saini (2014)

reported that ketones contribute 5-11% to the total quantity of volatile compounds in 'Chausa' mango. Ketones contribute to off-flavors in the fruit and increase during fruit senescence. Wu et al. (2019a) reported that O_3 (4 mg m^{-3}) decreased the content of 3,5-di-tert-butylbenzoquinone, α-ionone, and β-ionone in red pitaya fruit during storage. In the current study, fruit treated with O_3 (36 h) had low ketone content. The reduced ketones suggest that treating fruit with O_3 for 36 hours could contribute to pleasing fruit aroma and taste. The fruit treated with EC and the control had high ketone content. This indicates that EC created anaerobic respiration leading to increased fruit ripening and poor aroma.

Grosso et al. (2018) reported that carboxymethyl cellulose (0.5%) coating was ineffective in preserving the nonanal compounds in 'Chandler' walnut during storage. Current results revealed that the treatment combination of O_3 and EC (24 h) decreased the carbonyl compounds of mango fruit during storage. The decline in carbonyl compounds could be attributed to the modified atmosphere caused by EC and oxidizing potential of O_3, which causes anaerobic conditions thus increasing fermentation and off-flavors of mango fruit. The EC and O_3 concentration should not alter the nutritional quality and taste of the fruit. Therefore, it is vital to develop a suitable EC and O_3 mixture that will ensure superior biochemical properties of mango fruit.

The alcohol compounds were poorly represented in mango fruit. The alcohol compounds detected in the current study include 1-octanol, trans-2-octenol, and 1-octene-3-ol. Segade et al. (2017) reported that O_3 (30 µL/L) enhanced the alcohol content in 'Moscato Bianco' grapes during storage. Escamilla-García et al. (2018) observed an enhanced alcohol content in 'Maradol' papaya coated with chitosan (1%) during storage. Alcohols and carbonyl compounds are synthesized through the lipoxygenase pathway (Zhu et al., 2018). The enzyme alcohols dehydrogenase and hydroperoxide lyase enzymes catalyze fatty acids into

volatile aroma compounds such as (Z)-hexenyl acetate, 2-butenoic acid, and γ-octalactone (Sung et al., 2019; Lehner and Siegmund, 2020).

5   Conclusion

This is the first study to reveal the combined effect of EC and O_3 on volatile compounds of mango fruit. Fruit treated with O_3 (36 h) enhanced the volatile compounds and delayed senescence in mango fruit. Current results suggest that EC + O_3 (36 h) induced anaerobic conditions, thus decreasing the volatile compounds during storage. It is essential to select edible coatings, ozone exposure time, and concentration that will not cause anaerobic respiration inside the fruit. Therefore, the current treatment combination of O_3 and EC requires further investigation to develop suitable postharvest technology.

6 References

Aghofack-Nguemezi, J., Hoffmann, T., Schwab, W. 2019. Effects of bio-based coatings on the ripening and quality attributes of tomato (*Solanum lycopersicum*) fruits. J. Sci. Food Agric. 99, 1842-1849. doi:10.1002/jsfa.9378.

Alves Filho, E., Rodrigues, T., Fernandes, F., de Brito, E., Cullen, P., Frias, J., Bourke, P., Cavalcante,R., Almeida, F., Rodrigues, S. 2019. An untargeted chemometric evaluation of plasma and ozone processing effect on volatile compounds in orange juice. Innov. Food Sci. Emerg. Technol.53, 63-69.

Ayseli, M.T., Ayseli, Y.İ. 2016. Flavors of the future: Health benefits of flavor precursors and volatile compounds in plant foods. Trends Food Sci. Technol. 48, 69-77.

Bambalele, N.L., Mditshwa, A., Magwaza, L.S., Tesfay, S.Z. 2021. The effect of gaseous ozone and moringa leaf–carboxymethyl cellulose edible coating on antioxidant activity and biochemical properties of 'Keitt' mango fruit. Coatings 11, 1406.

Bonneau, A., Boulanger, R., Lebrun, M., Maraval, I., Gunata, Z. 2016. Aroma compounds in fresh and dried mango fruit (*Mangifera indica* L. cv. Kent): impact of drying on volatile composition. Int. J. Food Sci. Technol. 51, 789-800.

Cuevas-Glory, L.F., Sauri-Duch, E., Sosa-Moguel, O., Pino, J.A. 2020. Characterization of odor-active compounds in mango 'Ataulfo' (*Mangifera indica* L.) fruit. Chemical Papers 74, 4025- 4032. doi:10.1007/s11696-020-01217-y.

Dang, K.T., Singh, Z., Swinny, E.E. 2008. Edible coatings influence fruit ripening, quality, and aroma biosynthesis in mango fruit. J. Agric. Food Chem. 56, 1361-1370.

De Santis, D., Garzoli, S., Vettraino, A.M. 2021. Effect of gaseous ozone treatment on the aroma and clove rot by *Fusarium proliferatum* during garlic postharvest storage. Heliyon 7, e0663.

Escamilla-García, M., Rodríguez-Hernández, M., Hernández-Hernández, H., Delgado-Sánchez, L., García-Almendárez, B., Amaro-Reyes, A., Regalado-González, C. 2018. Effect of an edible coating based on chitosan and oxidized starch on shelf life of *Carica papaya* L., and its physicochemical and antimicrobial properties. Coatings 8, 318. doi:10.3390/coatings8090318.

Goff, S.A., Klee, H.J. 2006. Plant volatile compounds: sensory cues for health and nutritional value? Science 311, 815-819.

Grosso, A.L., Asensio, C.M., Nepote, V., Grosso, N. 2018. Quality preservation of walnut kernels using edible coatings. Grasas Aceites 69 (4) 69(4): e281. https://doi.org/10.3989/gya.0350181.

Lehner, T.B., Siegmund, B. 2020. The impact of ventilation during postharvest ripening on the development of flavour compounds and sensory quality of mangoes (*Mangifera indica* L.) cv. Kent. Food Chem. 320, 126608. doi:10.1016/j.foodchem.2020.126608.

Liu, H., An, K., Su, S., Yu, Y., Wu, J., Xiao, G., Xu, Y. 2020. Aromatic characterization of mangoes (*Mangifera indica* L.) using solid phase extraction coupled with gas chromatography-mass spectrometry and olfactometry and sensory analyses. Foods 9. doi:10.3390/foods9010075

Mustapha, A.T., Zhou, C. 2021. Novel assisted/unassisted ultrasound treatment: Effect on respiration rate, ethylene production, enzymes activity, volatile composition, and odor of cherry tomato. LWT, 111779.

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

Río Segade, S., Vilanova, M., Pollon, M., Giacosa, S., Torchio, F., Rolle, L. 2018. Grape VOCs response to postharvest short-term ozone treatments. Front. Plant Sci. 9, 1826.

Segade, S.R., Vilanova, M., Giacosa, S., Perrone, I., Chitarra, W., Pollon, M., Torchio, F., Boccacci, P., Gambino, G., Gerbi, V. 2017. Ozone improves the aromatic fingerprint of white grapes. Sci. Rep. 7, 1-16.

Singh, S.P., Saini, M.K. 2014. Postharvest vapour heat treatment as a phytosanitary measure influences the aroma volatiles profile of mango fruit. Food Chem. 164, 387-395.

Sung, J., Suh, J.H., Chambers, A.H., Crane, J., Wang, Y. 2019. Relationship between sensory attributes and chemical composition of different mango cultivars. J. Agric. Food Chem. 67, 5177- 5188. doi:10.1021/acs.jafc.9b01018.

Tesfay, S.Z., 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 Packag. Shelf Life 11, 40-48.

Thiruchelvam, T., Landahl, S., Terry, L.A. 2020. Temporal variation of volatile compounds from Sri Lankan mango (*Mangifera indica* L.) fruit during ripening. J. Agric. Food Res. 2, 100053. doi:10.1016/j.jafr.2020.100053.

Wei, Z., Liu, X., Huang, Y., Lu, J., Zhang, Y. 2019. Volatile aroma compounds in wines from Chinese wild/hybrid species. J. Food Biochem. 43, e12684.

Wu, Q., Zhang, Z., Zhu, H., Li, T., Zhu, X., Gao, H., Yun, Z., Jiang, Y. 2019a. Comparative volatile compounds and primary metabolites profiling of pitaya fruit peel after ozone treatment. J. Sci. Food Agric. 99, 2610-2621. doi:10.1002/jsfa.9479.

Wu, Q., Zhu, X., Gao, H., Zhang, Z., Zhu, H., Duan, X., Qu, H., Yun, Z., Jiang, Y. 2019b. Comparative profiling of primary metabolites and volatile compounds in Satsuma mandarin peel after ozone treatment. Postharvest Biol. Technol. 153, 1-12. doi:10.1016/j.postharvbio.2019.03.008.

Zhu, X., Luo, J., Li, Q., Li, J., Liu, T., Wang, R., Chen, W., Li, X. 2018. Low temperature storage reduces aroma-related volatiles production during shelf-life of banana fruit mainly by regulating key genes involved in volatile biosynthetic pathways. Postharvest Biol. Technol. 146, 68-78. doi:10.1016/j.postharvbio.2018.08.015.

Chapter 8

General discussion and conclusion

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1 Introduction

Mango is an essential subtropical fruit due to its economic and nutritional value. The world's annual mango production is estimated at 51 million tons (Lebaka et al., 2021). Mango production iscontinuously growing, with India, China, Thailand, and Indonesia leading the world production (Lebaka et al., 2021). South Africa's annual mango production is 84 000 tons and is mainly planted inLimpopo, Mpumalanga, and KwaZulu-Natal (NAMAC, 2020). The demand for fresh mangoes is continuing to increase and is projected to grow at an annual rate of 6.4% by 2025 (NAMAC, 2020). The delicious flavor and aroma make the fruit more attractive to consumers. The major problem affecting mango production is fruit postharvest disease and fruit softening (Sivakumar et al., 2011).

Postharvest technologies, including chemical and non-chemical treatments, have been used to preserve mango quality during storage. Chemical treatments include nitric oxide, 1-methylcyclopropene, salicylic acid, and fungicides such as prochloraz are used in the mango industry to maintain fruit firmness and decrease postharvest diseases (Junmatong et al., 2015; Razzaq et al., 2016; Vázquez- Celestino et al., 2016). The postharvest chemical treatments pose risks to the environment and health of the consumers. The chemical residues may end up in the fruit pulp, causing harm to the health of the consumers (Swart and Broekhuizen, 2004; Mutengwe et al., 2016). Therefore, there is a need for environmentally friendly postharvest technologies such as hot water, ozone, edible coatings, and UV-C.

Edible coatings such as *Aloe vera*, gum arabic, chitosan, carboxymethyl cellulose, and moringa leaf extracthave been evaluated as postharvest treatments of fruits and vegetables (Awad et al., 2017; Gava et al.,2018; MG et al., 2019; Daisy et al., 2020). Findings revealed that edible coatings preserved antioxidant activity, maintained firmness, decreased respiration, mass loss, TSS accumulation, and controlled postharvest diseases during storage (Escamilla-García et al., 2018; Grosso et al., 2018; Kumari et al., 2021). Research has shown that combining two or more edible coatings improved the preservation offruit quality and controlled postharvest diseases (Kubheka et al., 2020; Shezi et al., 2020; Ncama et al., 2021). For instance, gum arabic 15% + moringa and CMC 1% inhibited mycelial growth of *C. gloeosporioides* on 'Maluma' avocado fruit (Kubheka et al., 2020).

Ozone has high oxidizing potential and antimicrobial properties effective against a broad spectrum of viruses, bacteria, and fungi (Savi and Scussel, 2014; De Santis et al., 2021). Ozone preserves fruit quality by decreasing the respiration rate, thus delaying biochemical changes involved in ripening (Terao et al., 2019). Ozone oxidizes ethylene in fruit and vegetable surroundings during storage (Aslam et al., 2020). The ozone reduces disease incidence and severity of stem-end rot, anthracnose, grey mould, and Fusarium stalk rot (Tzortzakis et al., 2013; Savi and Scussel, 2014; Contigiani et al.,2018).

Ozone is non-chemical, has no residues, and an environmentally friendly postharvest treatment that can preserve mango fruit quality. There is limited data on the treatment combination of gaseous ozone and edible coatings. The combined treatment can provide a non-chemical and cost-efficient postharvest technology for the mango industry to adopt. Thus, this research examined the potential of gaseous ozone and edible coatings on postharvest quality and shelf-life of 'Keitt' mango fruit. Moreover, the possible mechanisms of action employed by these treatments to retain fruit quality was also investigated

2 Postharvest technologies of mangoes

The objective of Chapter two was to review literature on the use of chemical and non-chemical treatments on postharvest quality of mango fruit. The literature review showed that nitric oxide, 1-methylcyclopropene, and salicylic acid are the most commonly used chemical treatments for fresh mangoes (Eccher Zerbini et al., 2015; Junmatong et al., 2015; Sudheeran et al., 2018). Findings indicate that these treatments decreased respiration rate, mass loss, total soluble solids accumulation, and maintained firmness in mango fruit. Edible coatings are environmentally friendly postharvest technology used to preserve the quality of horticultural crops. The efficacy of edible coatings is influenced by the concentration of the coating, properties of the coating, and the application method used. The high concentration of edible coatings has been reported to decrease the sensory quality of mango fruit (Wang et al., 2007; MG et al., 2019). Edible coatings such as carboxymethyl cellulose have poor microbial properties and cannot control certain postharvest diseases (Salinas-Roca et al., 2018). However, incorporating two or more edible coatings has effectively maintained fruit quality and prevented postharvest diseases in mango fruit (Srivastava and Bala, 2016).

Heat treatment such as hot water and hot air has been used to sanitize mango fruit during storage. These treatments are influenced by the temperature used, cultivar, and maturity stage of the fruit (Sivakumar and Fallik, 2013; Fallik and Ilic, 2019). Storage technologies such as controlled atmosphere, modified atmosphere packaging, and low oxygen storage have been used in mango fruit (de Almeida Teixeira and Durigan, 2011; Ramayya et al., 2012; Sumual et al., 2017). Findings reveal that an adequate level of CO_2 and O_2 must be used to preserve fruit quality. The review also showed that postharvest treatments can compromise quality. For instance, storing fruit under low oxygen levels can lead to discoloration, poor

aroma, and off-flavors (Ullah et al., 2012; Costa et al., 2018).

The review also revealed that there are emerging technologies such as pulsed electric field and ozone. These treatments have antimicrobial properties against a broad spectrum of fungi and bacteria (Kumar et al., 2015; Silva Neto et al., 2019). However, there is limited data available on ozone as a postharvest treatment of mango fruit. Moreover, the combined effect of ozone and edible coatings remains unclear. Therefore, the effect of non-chemical technologies, specifically ozone and edible coatings, should be thoroughly researched in order to develop evidence-based postharvest protocols for the mango industry.

3   Physicochemical, antioxidant, and phytochemical attributes of mangoes

The screening study (Chapter three) revealed that ozone application time is essential to enhance mango fruit quality. The ozone (O_3) was applied intermittently during cold storage at days 0,7,14, or 21. Ozone application at the pre-climacteric stage preserved carotenoids content, firmness, reduced mass loss (ML), total soluble solids (TSS) accumulation, and decay incidence. This could be attributed to ozone decreasing respiration rate and oxidizing ethylene, which delays biochemical changes in fruit ripening (Zhang et al., 2021). Gaseous ozone application for twelve hours was ineffective in maintaining the quality of mango fruit. Gaseous ozone application at Days 0, 7 &14 and Days 0, 7, 14 & 21 yielded similar results with regards to firmness, mass loss, TSS, decay incidence, and shelf-life. The delayed decay incidence observed in ozone 36 and 48 hours could be attributed to the antimicrobial effect against fungi and viruses (Tzortzakis et al., 2013; Chen et al., 2019; Terao et al., 2019). The ozone application at Days 0 & 7 and Days 0, 7 &14 was selected to be incorporated with edible coatings and used throughout the study.

The study on edible coatings (EC) incorporated with gaseous ozone is discussed in Chapter four.

Findings revealed that EC and EC + O_3 (36 h) effectively enhanced fruit quality and extended the shelf-life of mango fruit. The EC extended shelf-life by three days compared to the control. The sensory study revealed that fruit treated with EC and EC +O_3 (24 h) were the most preferred by consumers. These treatments had high scores for sweetness (5.66-7.13), color (5.66-5.76), firmness (4.85-5.29), and overall liking (15.69-17.85). The enhanced sensory quality in coated fruit could be attributed to the phytochemicals such as phenolic compounds infusing into the fruit. Phenolic compounds are reported to influence the fruit aroma and taste (Forney, 2015; Ayseli and Ayseli, 2016). The poor sensory of EC +O_3 (36 h) indicates that the treatment created anaerobic conditions which produced off-flavors. Furthermore, increasing ozone could induce phenylalanine ammonia-lyase (PAL) activity, thus increasing the polyphenoloxidase (PPO) enzyme activity resulting in brown discoloration and poor fruit quality and flavor (Ong et al., 2014).

In Chapter five, the study on edible coatings and gaseous ozone on biochemical properties of mango fruit revealed that EC + O_3 (36 h) maintained the antioxidants 1.1-Diphenyl-2-picrylhydrazy (DPPH) and Ferric Reducing Antioxidant Power Assay (FRAP) during storage. At the end of storage, fruit treated with EC and EC + O_3 (36 h) delayed the accumulation of malondialdehyde (MDA) content by 27.81 and 15.35%, respectively, compared to the control treatment. Current findings indicate that the treatment combination of EC and O_3 forms a modified atmosphere, thus decreasing water loss. Furthermore, gaseous ozone changed the lipid membrane, thus reducing oxidative stress and membrane injury (Carbone and Mencarelli, 2015). The fruit treated with EC delayed ascorbic acid loss by 90.08% compared to the control at the end of the storage period. These findings indicate that edible coatings decrease gaseous permeability leading to delayed oxidation of ascorbic acid by the ascorbic oxidase enzyme. The fruit treated with O_3 (36 h) enhanced the accumulation of total sugars. The total sugars increased from

1.27 mg/mL at day zero to 10.60 mg/mL at the end of the storage period. Current results indicate that fruit continued to ripen postharvest and increased in sweetness.

4   Postharvest diseases and volatile compounds

Chapter six discusses the control of postharvest diseases with edible coatings and gaseous ozone. Findings revealed that edible coatings decreased the mycelial growth of *Lasiodiplodia theobromae* and *Colletotrichum gloeosporioides* by 50.44% and 61.50%, respectively. The disease incidence of anthracnose was reduced by 63.98%, 51.18%, 44.56%, 32.77%, and 16.83% in EC + O_3 (36 h), EC +O_3 (24 h), EC, O_3 (36 h), O_3 (24 h), respectively. The disease incidence of stem-end rot decreased by 54.24%, 44.95%, 28.79%, 26.37%, and 15.19% in EC + O_3 (36 h), EC, O_3 (36 h), EC +O_3 (24 h), and O_3 (24 h), respectively. Current findings indicate that the treatment combination of EC and O_3 (36 h) effectively controlled the anthracnose and stem-end rot in mango fruit. The reduced postharvest diseases in mango could be due to the high oxidation of ozone (2.07 volts) and antimicrobial properties of moringa (Tesfay et al., 2017; Aslam et al., 2020). The treatments of EC + O_3 (24 h) and EC+ O_3 (36h) had similar results of firmness in both *L. theobromae*, and *C. gloeosporioides* inoculated fruit. A similar trend was observed in peroxidase activity (POD) and shelf-life. Ozone breaks down to form reactive oxygen species (ROS) within the cell interior, which causes fungal cell death (Ong and Ali, 2015). The findings of POD and flavonoids indicate that ozone induces an increase in flavonoids content and enzyme activity of POD as a defense mechanism during the fungal attack in mango fruit.

The study on the effect of gaseous ozone and edible coatings on volatile compounds of mango fruit during storage is discussed in Chapter seven.

The findings revealed that aldehydes (33%) were the most abundant compounds compared to terpenes, carbonyl compounds, ketones, alcohols, and esters which were 20%, 17%, 10%, 10%, and 7%, respectively. The major volatile compounds in mango include terpenes, aldehydes, alcohols, and esters (Singh and Saini, 2014; Liu et al., 2020). There were no significant differences between treatment means of EC +O_3 (24 h) and EC + O_3 (36 h) in the content of aldehydes, ketones, alcohols, and terpenes. Furthermore, an increase in these treatments resulted in higher content of ketones, alcohols, and aldehydes during storage. Aldehydes and terpens are the significant compounds contributing to 28-44% of aroma in mango fruit (Liu et al., 2020). The findings indicate that EC+ O_3 (36 h) induced anaerobic conditions, thus diminishing fruit aroma.

5   Conclusion

The screening study provided an understanding of ozone's application and exposure time that can effectively preserve mango fruit quality. From this study, it can be concluded that increasing ozone exposure time did not produce the best results. Furthermore, the ozone application time is vital to delay fruit senescence and extend shelf-life. This study is the first to provide an understanding and the mode of action of the treatment combination of gaseous ozone and edible coatings as postharvest treatments of mango fruit. The sensory study showed that fruit treated with EC and EC + O_3 (24 h) were sweet, firm, and had attractive color and aroma. The shelf-life was extended by three days in fruit treated with EC + O_3 (36 h) and edible coatings. Treatment combination of ozone and edible coatings enhanced the nutritional quality of mango fruit. These treatments preserved the loss of phenolic compounds, ascorbic acid, total sugars, and flavonoids content. Treatment combination of gaseous ozone and edible coatings can potentially control

postharvest diseases of mango fruit. The mechanism of controlling anthracnose and stem-end rot is associated with phenolic compounds, flavonoids content, and initiating the enzyme activity of POD and MDA. Volatile compounds of mango fruit were decreased by EC+ O_3 (36 h) during storage. This treatment reduced the aroma and flavor of the fruit, which are the most critical characteristics of mango. Therefore, it can be concluded that EC + O_3 (24 h) should be considered as postharvest treatment of mango fruit.

6   Recommendations

- Timing of the ozone treatment is vital to enhance fruit quality; therefore, we recommend that ozone application should be done at Days 0 and 7.

- In the current study, ozone application was applied during the European markets exports simulation; therefore, safety and training protocols should be developed.

- Developing an ozone generator that will administer ozone in route during cold storage could be expensive. The use of aqueous ozone at the packhouse before shipping the fruit should be investigated.

- The treatment combination of gaseous ozone and edible coatings has a chance to be introduced to the food industry. Therefore the treatment combination of EC + O_3 (24 h) is recommended as postharvest treatment of mango fruit.

- The treatment combination of EC+O_3 (24 h) requires further investigation to develop postharvest technologies and protocols.

References

Aslam, R., Alam, M.S., Saeed, P.A. 2020. Sanitization potential of ozone and its role in postharvest quality management of fruits and vegetables. Food Eng. Rev.12, 48-67.

Awad, M.A., Al-Qurashi, A.D., Mohamed, S.A., 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. J. Food Sci. Technol. 54, 4139-4148.

Ayseli, M.T., Ayseli, Y.İ. 2016. Flavors of the future: Health benefits of flavor precursors and volatile compounds in plant foods. Tren. Food Sci. Technol. 48, 69-77.

Carbone, K., Mencarelli, F. 2015. Influence of short-term postharvest ozone treatments in nitrogen or air atmosphere on the metabolic response of white wine grapes. Food Biopr. Technol. 8,1739-1749.

Chen, C., Zhang, H., Dong, C., Ji, H., Zhang, X., Li, L., Ban, Z., Zhang, N., Xue, W. 2019. Effect of ozone treatment on the phenylpropanoid biosynthesis of postharvest strawberries. RSC Advances 9, 25429-25438. doi:10.1039/c9ra03988k.

Contigiani, E.V., Jaramillo-Sánchez, G., Castro, M.A., Gómez, P.L., Alzamora, S.M. 2018. Postharvest quality of strawberry fruit (*Fragaria x ananassa* Duch cv. Albion) as affected by ozone washing: fungal spoilage, mechanical properties, and structure. Food Biopr. Technol. 11, 1639-1650.

Costa, J.D.D.S., Figueiredo Neto, A., Almeida, F.D.A.C., Costa, M.D.S. 2018. Conservation of 'Tommy Atkins' mangoes stored under passive modified atmosphere. Revista Caatinga 31, 117- 125. doi:10.1590/1983-21252018v31n114rc.

Daisy, L.L., Nduko, J.M., Joseph, W.M., Richard, S.M. 2020. Effect of edible gum Arabic coating on the shelf life and quality of mangoes (*Mangifera indica*) during storage. J. Food Sci. Technol. 57, 79-85. doi:10.1007/s13197-019-04032-w.

de Almeida Teixeira, G.H., Durigan, J.F. 2011. Storage of 'Palmer' mangoes in low-oxygen atmospheres. Fruits 66, 279-289.

De Santis, D., Garzoli, S., Vettraino, A.M. 2021. Effect of gaseous ozone treatment on the aroma and clove rot by *Fusarium proliferatum* during garlic postharvest storage. Heliyon 7, e06634

Eccher Zerbini, P., Vanoli, M., Rizzolo, A., Grassi, M., Pimentel, R.M.d.A., Spinelli, L., Torricelli, A. 2015. Optical properties, ethylene production and softening in mango fruit. Postharvest Biol. Technol. 101, 58-65. doi:10.1016/j.postharvbio.2014.11.008.

Escamilla-García, M., Rodríguez-Hernández, M., Hernández-Hernández, H., Delgado-Sánchez, L., García-Almendárez, B., Amaro-Reyes, A., Regalado-González, C. 2018. Effect of an edible coating based on chitosan and oxidized starch on shelf life of *Carica papaya* L., and its physicochemical and antimicrobial properties. Coatings 8, 318. doi:10.3390/coatings8090318.

Fallik, E., Ilic, Z. 2019. Positive and negative effects of heat treatment on the incidence of physiological disorders in fresh produce. *In:* Postharvest Physiological Disorders in Fruits and Vegetables; Tonetto de Freitas, S., Pareek, S., Eds, 111-126.

Forney, C. 2015. Physiology and biochemistry of aroma and off-odors in fresh-cut products. III International Conference on Fresh-Cut Produce: Maintaining Quality and Safety 1141, pp. 35-46.

Gava, C.A.T., de Castro, A.P.C., Pereira, C.A., Fernandes-Júnior, P.I. 2018. Isolation of fruit colonizer yeasts and screening against mango decay caused by multiple pathogens. Biol. Contr.117, 137-146.

Grosso, A.L., Asensio, C.M., Nepote, V., Grosso, N. 2018. Quality preservation of walnut kernels using edible coatings. Grasas Aceites 69(4): e281. https://doi.org/10.3989/gya.0350181

Junmatong, C., Faiyue, B., Rotarayanont, S., Uthaibutra, J., Boonyakiat, D., Saengnil, K. 2015. Cold storage in salicylic acid increases enzymatic and non-enzymatic antioxidants of Nam Dok Mai No. 4 mango fruit. Sci. Asia 41, 12-21.

Kubheka, S.F., Tesfay, S.Z., Mditshwa, A., 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, R., Bawa, A., Kathiravan, T., Nadanasabapathi, S. 2015. Optimization of pulsed electric field parameters for mango nectar processing using response surface methodology. Int. Food Res. J. 22, 1353.

Kumari, S., Singh, A., Kumar, A., Singh, K., Bains, G. 2021. Evaluating the efficacy of chitosan and salicylic acid on photosynthetic pigments and antioxidant enzymes towards resistance of mango malformation. Sci. Hortic. 285, 110160.

Lebaka, V.R., Wee, Y.-J., Ye, W., Korivi, M. 2021. Nutritional composition and bioactive compounds in three different parts of mango fruit. Int. J. Environ. Res. Publ. Heal. 18, 741.

Liu, H., An, K., Su, S., Yu, Y., Wu, J., Xiao, G., Xu, Y. 2020. Aromatic characterization of mangoes (*Mangifera indica* L.) using solid phase extraction coupled with gas chromatography-mass spectrometry and olfactometry and sensory analyses. Foods 9. doi:10.3390/foods9010075.

MG, A.E.-G., Zaki, Z.A., Ekbal, Z. 2019. Effect of some postharvest treatments on quality of" Alphonse" mango fruits during cold storage. Middle East J. 8, 1067-1079.

Mutengwe, M., Chidamba, L., Korsten, L. 2016. Pesticide residue monitoring on South African fresh produce exported over a 6-year period. J. Food Prot. 79, 1759-1766.

Ncama, K., Magwaza, L.S., Mditshwa, A., Tesfay, S.Z., Mbili, N.C. 2021. The efficacy of moringa leaf extract as an active ingredient in organic coating of 'Marsh' grapefruit. Acta Hortic. 1306, 241-248.doi:10.17660/ActaHortic.2021.1306.30.

Ong, M.K., Ali, A. 2015. Antifungal action of ozone against *Colletotrichum gloeosporioides* and control of papaya anthracnose. Postharvest Biol. Technol. 100, 113- 119. doi:10.1016/j.postharvbio.2014.09.023.

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

Ramayya, N., Niranjan, K., Duncan, E. 2012. Effects of modified atmosphere packaging on quality of 'Alphonso' mangoes. J. Food Sci. Technol. 49, 721-728. doi:10.1007/s13197-010-0215-x.

Razzaq, K., Singh, Z., Khan, A.S., Khan, S.A.K.U., Ullah, S. 2016. Role of 1-MCP in regulating 'Kensington Pride' mango fruit softening and ripening. Plant Gro. Regul. 78, 401-411.

Salinas-Roca, B., Guerreiro, A., Welti-Chanes, J., Antunes, M.D., Martín-Belloso, O. 2018. Improving quality of fresh-cut mango using polysaccharide-based edible coatings. Int. J. Food Sci. Technol. 53, 938-945.

Savi, G.D., Scussel, V.M. 2014. Effects of ozone gas exposure on toxigenic fungi species from *Fusarium, Aspergillus*, and *Penicillium* genera. Ozone: Science & Engineering 36, 144-152. Shezi,

S., Tesfay, S.Z., Magwaza, L.S., Mditshwa, A., Motsa, N.M. 2020. Gaseous ozone and plant extract-based edible coatings as postharvest treatments for 'Hass' avocado (*Persea americana* Mill.) fruit. Acta Hortic. 1275, 53-60. doi:10.17660/ActaHortic.2020.1275.8

Silva Neto, O.P.d., Pinto, E.V.d.S., Ootani, M.A., Silva Junior, J.L.d., Lima, J.L.d.S.B., Sousa, A.E.D.d. 2019. Ozone slows down anthracnose and increases shelf life of papaya fruits. Revista Brasileira de Fruticultura 41. doi:10.1590/0100-29452019439.

Singh, S.P., Saini, M.K. 2014. Postharvest vapour heat treatment as a phytosanitary measure influences the aroma volatiles profile of mango fruit. Food Chem. 164, 387-395.

Sivakumar, D., Fallik, E. 2013. Influence of heat treatments on quality retention of fresh and fresh-cut produce. Food Rev. Int. 29, 294-320. doi:10.1080/87559129.2013.790048.

Sivakumar, D., Jiang, Y., Yahia, E.M. 2011. Maintaining mango (*Mangifera indica* L.) fruit quality during the export chain. Food Res. Int. 44, 1254-1263.

Srivastava, S., Bala, K.L. 2016. Effect of arabic gum-carboxymethylcellulose edible coatings on shelf life of button mushroom (*Agaricus bisporus*). Int. J. Res. Eng. Technol. 5, 484-494.

Sudheeran, P.K., Feygenberg, O., Maurer, D., Alkan, N. 2018. Improved cold tolerance of mango fruit with enhanced anthocyanin and flavonoid contents. Molecules 23(7): 1832. doi:10.3390/molecules23071832.

Sumual, M., Singh, Z., Singh, S., Tan, S. 2017. Fruit ripening and quality of 'Kensington Pride' mangoes following the controlled atmosphere storage. Jurnal Teknologi Pertanian (Agricultural Technology Journal 8(1): 17-27.

Swart, S., Broekhuizen, W.V. 2004. Evaluation of post-harvest disease control with regards to the new

maximum residue level of five ppm for prochloraz on mangoes in the European Union. South African Mango Growers' Association 24, 73-77.

Terao, D., de Lima Nechet, K., Frighetto, R.T.S., Sasaki, F.F.C. 2019. Ozonated water combined withheat treatment to control the stem-end rot of papaya. Sci. Hortic. 257, 108722.

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

Tzortzakis, N., Taybi, T., Antony, E., Singleton, I., Borland, A., Barnes, J. 2013. Profiling shifts in protein complement in tomato fruit induced by atmospheric ozone-enrichment and/or wound-inoculation with *Botrytis cinerea*. Postharvest Biol. Technol. 78, 67-75.

Ullah, H., Ahmad, S., Amjad, M., Khan, M.A. 2012. Response of mango cultivars to modified atmosphere storage at an ambient temperature cv. Alphanso and Chounsa. Pak. J. Agri. Sci. 49, 323-329.

Vázquez-Celestino, D., Ramos-Sotelo, H., Rivera-Pastrana, D.M., Vázquez-Barrios, M.E., Mercado-Silva, E.M. 2016. Effects of waxing, microperforated polyethylene bag, 1-methylcyclopropene and nitric oxide on firmness and shrivel and weight loss of 'Manila' mango fruit during ripening. Postharvest Biol. Technol. 111, 398-405.

Wang, J., Wang, B., Jiang, W., Zhao, Y. 2007. Quality and shelf life of mango (*Mangifera indica* L. cv. Tainong') coated by using chitosan and polyphenols. Food Sci. Technol. Int.13, 317-322.

Zhang, X., Tang, N., Zhang, H., Chen, C., Li, L., Dong, C., Cheng, Y. 2021. Comparative transcriptomic analysis of cantaloupe melon under cold storage with ozone treatment. FoodResearch International 140, 109993.