

Efficacy of carboxymethyl cellulose and gum arabic edible coatings in combination with moringa leaf extract in improving postharvest quality of new avocado (*Persea americana* Mill.) cultivar, ‘Maluma’

by

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(Horticulture)**

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Declaration

I, Sanele Fana Kubheka (213547223) declare that this is my original work and it is referenced where some information is taken from other sources. I did all the analyses involved and this work has never been submitted in any intention for any other degree or at other institution. I will be held responsible for plagiarism should this work or any part of it be found to belong to someone else.

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General overview

Efficacy of carboxymethyl cellulose and gum arabic edible coatings in combination with moringa leaf extract in improving postharvest quality of new avocado (*Persea americana* Mill.) cultivar, ‘Maluma’

The first chapter is general introduction outlining aim and objectives of this research. The second chapter reviews previous literature in an attempt to gain an understanding of the biological mechanism of edible coatings and their application in the fresh horticultural industry. Third chapter is investigating the effect of polysaccharide-based coatings incorporated with moringa leaf extract on ‘Maluma’ avocado postharvest quality and sensory attributes. The fourth chapter is evaluating the effect of carboxymethyl cellulose and gum arabic edible coatings incorporated with moringa leaf extract on phytochemical and antioxidants activities of ‘Maluma’ avocado fruit. The fifth chapter is effect looking at the ability of edible coating incorporated with moringa leaf extract on inhibiting growth of *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae* of avocado fruit. Overall discussions and conclusions are made in chapter six, where future research prospects are also recommended.

Publications

All chapters of this work, except chapter 1 and 6, were intended for publication and as a result, are written in the form of manuscripts. Due to the time limit, only chapter 4 is currently submitted to relevant journals but all chapters will be submitted to relevant journal, Journal of Food Packaging and Shelf Life.

Kubheka, S.F., Magwaza, L.S., Tesfay, S.Z., Mditshwa, A. Investigation the effect of carboxymethyl cellulose and gum arabic edible coatings incorporated with moringa leaf extract on phytochemical and antioxidants activities of 'Maluma' avocado fruit. *Under Review*.

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Chapter 1:

General introduction

1.1 Introduction

Avocado (*Persea americana* Mill.) is one of the most important tropical and subtropical fruit with an estimated annual production of more than 5.2 million tonnes (Fao, 2017; Acosta-Rangel *et al.*, 2018). In South Africa, 87 584 tonnes were produced during the 2015/2016 season, and more than 43% were exported (Daff, 2017) In last decade there has been an increase in the fruit consumption because of the increase in consumer awareness of the healthy dietary value of avocado, which is largely monosaturated fatty acids, amino acids, carbohydrates, proteins, vitamins and other beneficial phytochemicals (Magwaza and Tesfay, 2015; Ortiz-Viedma *et al.*, 2018). The high content of monounsaturated fatty acids has been related to the decrease in risk of cardio related disease by preserving the level of high density lipoproteins and their capacity to also act as antioxidants (Villa-Rodríguez *et al.*, 2011). Depending on cultivar fruit may vary in shape, skin colour and texture and harvest season.

Commercially, 'Hass' is the leading cultivar accounting for 98% in Mexico, 90% in California, 98% in New Zealand, 80% in Spain and 36% in South Africa (Fao, 2017). The cultivar is a hybrid combination of Guatemalan (85%) and traces of Mexican germplasm (15%). 'Hass' is known to midseason cultivar with some fruit available during off season, and in South Africa the cultivar season is from May to October. The popularity of the cultivar is mainly due to the outstanding postharvest characteristics. The thick leathery skin leads to better storage and shipping capability, less susceptibility to physical damages such as bruises and blemishes as these becomes less visible with fruit changing from green to purple-blackish upon ripening. Compared to many cultivars, 'Hass' is less susceptible to physiological disorders such as chilling injury and has a good creamy texture with a slightly nutty taste preferred by many consumers.

With challenges such as alternate bearing, susceptibility to lenticel damage and orchard chilling injury or shipping injury and season dependency, there are major economic loss associated with the cultivar and season availability. Alternate bearing results in large fruit during an 'ON' season and smaller fruit in 'OFF' season, resulting to inconsistent supply to the market. Being a seasonal cultivar, there is a gap created by the unavailability of the cultivar in the market during off season. Additionally, the cultivar also relies much on growth regulators such as uniconazole, paclobutrazol to control its vigorous growth and improving fruit size. The dependency of 'Hass' to this chemicals is unsustainable and results reduced profit margins for producers (Ernst, 2015).

Over the past decades there has been considerable research done to mitigate challenges facing 'Hass' cultivar, this includes developing 'Hass' like cultivars. Among commercially released 'Hass' like cultivars are 'Lamb Hass', 'Gem', 'Harvest', 'Carmen' and 'Maluma' (Crane *et al.*, 2013). 'Maluma' is the latest commercially released 'Hass' like cultivar, discovered by Andreis Joubert in Limpopo South Africa in the early 1990's (Joubert, 2010). The cultivar is predominantly Guatemalan with smaller Mexican genes similar to those of 'Hass'. 'Maluma' was commercially released in 2007 (Ernst, 2007). Since its release, the cultivar has grown significantly both locally and internationally, with the production now in major avocado producing countries such as Israel, Spain, New Zealand, Australia, South Africa and Chile. The cultivar owes this increase in production to its consistent high bearing pattern, higher yield per hectare, less susceptibility to orchard chilling injury and lenticel damage when compared to 'Hass'. 'Maluma' also has a better flesh to seed ratio than 'Hass', ranging between 10 – 15% compared to 20 – 25 % of 'Hass'. The cultivars was earlier reported to be an early maturing cultivar (week 12 -17) than 'Hass' (Ernst, 2007; Mhlophe and Kruger, 2012) but a recent study

by Ernst and Ernst (2015) demonstrated that the availability of 'Maluma' can be stretched up to week 29.

'Hass' market is usually at peak between week 18 – 26, mainly because the cultivar needs to be harvested before cold damage by the upcoming winter as it is susceptible to chilling injury (Ernst and Ernst, 2015). One of the options that the farmers can take, is to replace the low lying 'Hass' orchards with 'Maluma' as it is less susceptible for orchard chilling injury. These may then be harvested late, creating a better season extension supply to the market.

Although cultivars may be able to stretch the avocado season, there's still a need to maintain postharvest quality that is required by the market. Over the past years, the industry has heavily relied on refrigeration systems to maintain postharvest fruit quality during transportation periods, which is usually 3 – 4 weeks for South African avocados to reach their destined market, mainly the European Union. Avocado fruit is sensitive to low temperatures thus it is important to ensure that fruits are harvested at correct physiological maturity in order to reduce fruit susceptibility to chilling injury and other physiological disorders. Failure to harvest fruits at correct maturity results in significant economic losses as a result of development of physiological disorders (Nelson, 2010). Currently, the recommended temperature when transiting avocado for long distance is 5.5 °C, this is a compromise between cold damage and rapid fruit softening (Bower, 1988).

About more than a half a century ago, synthetic waxes and fungicides were introduced to complement cold storage. Natural and synthetic waxes such as bees wax and petroleum wax have been shown to delay ripening by reducing respiration rates and improving fruit glossiness. (Kremer-Kohne and Duvenhage, 1997). While, copper fungicides and prochloraz have been

reported to reduce fruit decay (Bill *et al.*, 2014). Besides the health concerns regarding synthetic waxes and fungicides, the major challenge is their disposal which has been shown to result in many environmental concerns such as soil and water pollution (Youssef *et al.*, 2017). The South African avocado industry is export driven, exposing the country to adhere to importing countries strict regulations for the maximum residue limits (MRL) on the skin of the fruit, and the recommended MRL for South African avocado is 2 mg kg⁻¹ MRL (Bill *et al.*, 2014).

Furthermore, the European Union is no longer accepting waxed fruit (Tesfay and Magwaza, 2017), arguably due to harmful residues and the alteration of fruit organoleptic properties by waxes. In conjunction with the recent rise in demand for organic products, South African avocado fruits are now supplied without any protective coating making them more prone to water loss and development of postharvest physiological disorders. This results in significant economic losses for the South African avocado industry as the EU is their major market. This has exerted pressure to the industry to invest in research to develop novel and environmentally friendly coatings and other postharvest treatments with low residues.

Use of plant based polymers such as gum arabica, carboxymethyl cellulose (CMC) as of edible coatings has proven to be an effective alternative to synthetic waxes, especially when incorporated with anti-microbial agents (Aloui and Khwaldia, 2016). Adetunji *et al.* (2012) demonstrated the potential of hydroxypropyl methyl-cellulose (HPMC) incorporated with moringa extract as an edible coating for oranges. Similar observations were made by Tesfay and Magwaza (2017); Tesfay *et al.* (2017) who reported that carboxy methyl cellulose CMC incorporated with moringa leaf extract can be recommended for commercial applying. While gum arabic (GA) was found to effective in delaying ripening, and maintaining organoleptic

properties on tomatoes (Ali *et al.*, 2010). Maqbool *et al.* (2011) reported GA10% combined with 0.4% cinnamon oil was the optimal concentration in controlling decay (80% and 77%) showing a synergistic effect in the reduction of *C. musae* and *C. gloeosporioides*, respectively.

The biological mechanism of edible coatings alone or in combination with plant extracts is still not well established and it is not yet clear that the performance of one coating is uniform across all fruit cultivars. Therefore, the aim of this MSc study was to evaluate the efficacy of edible coatings CMC and GA combined with moringa plant extracts on newly developed avocado cultivar 'Maluma'. To achieve this aim, the study included the following specific objectives:

1. Investigate the effect of postharvest application of polysaccharide-based coatings incorporated with Moringa leaf extract on new avocado cultivar 'Maluma'.
2. Evaluate the effect of coatings incorporated with moringa leaf extract on phytochemical status and antioxidant system in 'Maluma' avocado fruit
3. Assess the level of effectiveness of polysaccharide coating in combination with moringa leaf extract on reducing postharvest disease of avocado.

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Chapter 2: Literature review

Edible coatings and their effect on horticultural produce

Abstract

There is growing trend towards an increase in demand for environmental friendly and sustainable postharvest treatments for fresh horticultural produce. This trend is also coupled by the recent increase in demand for 'organic' fresh products. In response to these demands, fresh produce industry together with researchers in the postharvest biology and technology have identified polymers such as polysaccharides, proteins and waxes to develop what is known as edible coatings. Edible coatings have been successful in reducing mass loss, delaying senescence thus prolonging shelf life of fresh produce. In addition, edible coatings have proven to be excellent carriers of active ingredients such as colourants, antimicrobials which helps alleviate antimicrobials properties of coated produce. The application edible coatings can also provide relief to both producers and consumers as they are economical affordable compared to other techniques. This review looks at formulation of edible coatings with focused on maintenance of postharvest quality. Recent advances in application and their effect on phytochemicals and sensory properties is also discussed. Furthermore, this review makes recommendations that could of assistance in the future, whilst assisting in future research.

Keywords: Postharvest quality, Respiration, Food safety, Microorganisms, Composites.

2.1. Introduction

The evolution of postharvest handling and postharvest treatment industry is in response to the ever-growing challenges posed by modern society. Current challenges in the postharvest chain of the fresh produce industry include legislation of postharvest treatments, longer shelf life demands by markets and consumers, safer and healthier food, postharvest losses, waste and environmental concerns (Realini and Marcos, 2014; Miteluț *et al.*, 2015). According to FAO (2011), approximately one-third of the world's food is lost in the postharvest chain, which amounts to about 1.3 billion tons per year with more of these losses occurring in developing countries. Lack of infrastructure and resources has been named as one of the major contributors towards postharvest food losses in developing countries (Nkolisa *et al.*, 2018). Unexpectedly,

less than 5% of the funding aimed at agricultural research goes to postharvest systems, while the reduction of these losses has been recognised as a major component of improved food security (Nahman *et al.*, 2012). In addition, reducing postharvest losses has environmental benefits, given that each time there is a reduction of a tonne of postharvest loss, 4.2 tonnes of carbon dioxide emission is avoided (Opara and Mditshwa, 2013).

In the past years, several postharvest technologies of fresh horticultural produce such as cold storage, ultraviolet (UV) radiation (D'hallewin *et al.*, 2000), controlled atmosphere (CA) and modified atmosphere packaging (MAP) (Oliveira *et al.*, 2015) and recently ozone (Pérez *et al.*, 1999), have been used in the food packaging industry to alleviate fruit and vegetable quality deterioration and prolonging shelf life while improving physical appearance and retaining nutritional value of fresh fruits and vegetables (Mohseni *et al.*, 2014). The main challenges with these methods include their sustainability, affordability in the case of the developing countries and limited use, since the application of some of these technologies is not feasible at supermarkets and retail level. Additionally, the negative effect on both human and environmental health during production and disposal has been one of the challenges faced by the horticultural industry regarding the application of these methods.

Recently, scientific research has focused on more sustainable and environmentally friendly techniques to package and reduce the cost of food packaging. Researchers in postharvest biology and technology have identified the potential of biopolymers such as polysaccharides, proteins and some waxes to maintain postharvest quality and to prolong shelf life of fresh produce (Shapi'i and Othman, 2016; Tesfay and Magwaza, 2017; Tesfay *et al.*, 2017).

This is due to their biodegradability, renewability and their ability to act as carriers of antimicrobials. These biopolymers are developed into what is commonly known as edible coatings which are applied to mitigate the losses experienced by the fresh produce industry.

Edible coatings are defined as a thin continuous, colourless, odourless and consumable layer made of edible material applied directly over food in liquid form by different techniques such as dipping, spraying or application with brush or cotton wool (Ochoa-Reyes *et al.*, 2013). Edible coatings are prepared by mixing the edible material in solvents such as water, organic solvents and/or a combination of the two depending on the solubility and compatibility of the coating materials. Plasticisers and emulsifiers are sometimes used to overcome the brittleness

of the film, and to improve the flexibility and adhesion of the coating solution to the fruit surface. The application of edible coatings depends on both the coating material to be used and the fruit to be coated.

The formulation, source, purity and material used to develop an edible coating result in the variation on colour of the coatings, from transparent to translucent (Lamdande *et al.*, 2013). Falguera *et al.* (2011) further explained that edibility of a coating can only be achieved if all components including additives and plasticizers involved in the process of preparing the coating are acceptable for food processing. The cohesiveness of an edible coating depends on the physical and chemical interaction between the material and solvent used. The role of the material to be used in the development of a coating, solvent type, temperature, pH, and the concentration of all these components, and the level of additives, greatly influence the performance of the coating (Ncama *et al.*, 2018). Therefore, the objective of this review is to update information available on edible coatings for horticultural produce, focusing on their composition, active ingredients and the implications they have on organoleptic properties and future perspectives.

2.2. Overview and history of coatings

Biodegradable coatings have been used to prolong shelf life and maintain postharvest quality of various fresh produce. The use of edible coatings can be traced as far back as the 12th century when the Chinese used wax to reduce water loss and to improve glossiness of citrus fruit (Guilbert *et al.*, 1995). These were then followed by synthetic waxes such as polyethylene and paraffin waxes. According to Kaplan (1986), waxes such as hot melt paraffin have been used to prolong the shelf life of peach, citrus and apple fruit as early as the 1930's. A study by Erbil and Muftugil (1986), demonstrated that the use of wax emulsions on peach reduced water loss reduced oxygen transmission and maintained fruit quality. For many years coatings have been only limited to only waxes because it was believed that only waxes could retard respiration and reduce moisture loss (Park, 1999).

However, these waxes had detrimental effects on the food due to their low permeability with respect to gases. They caused surface discolouration and production of off flavours and odours (Bill *et al.*, 2014). These negative effects together with health concerns regarding the effect of

deleterious residues on food, led to some of these commercial waxes such as Avoshine[®] (Citrashine [Pty] Ltd, Johannesburg, South Africa) being banned by the European Union (EU) market. The ‘banning’ of Avoshine[®] in the avocado industry by the EU market has put pressure on researchers and markets to look for alternatives that are eco-friendly these may include carboxymethyl cellulose CMC, chitosan and gelatin (Aguilar - Méndez *et al.*, 2008; Tesfay and Magwaza, 2017; Tesfay *et al.*, 2017).

The application of polysaccharide, protein- and some lipid-based coatings has proved to be beneficial in delaying ripening of fresh horticultural produce. In addition, these coatings have an ability to carry active ingredients such as antimicrobials, antioxidants, nutraceutical and probiotics which are used to reduce the development of postharvest diseases of fruits and vegetables (Lin and Zhao, 2007). Maqbool *et al.* (2011) demonstrated that the addition of essential oils (cinnamon and lemongrass) on gum is effective against *Colletotrichum musae* and *Colletotrichum gloeosporioides*, which cause anthracnose of banana and papaya, respectively. Results such as these have made researchers focus on and study the role of various plant-based active ingredients in improving the performance of edible films.

2.3. Formulations of coatings

There are several materials that may be used by the fresh produce industry, with the limitation set on the effect of the material interaction with the fruit surface. The material used must be food safe, and compatible with additives such as antioxidants. For horticultural industry, much of the focus has been on polysaccharides, proteins, lipids and composites when developing edible coatings (Dhall, 2013).

2.3.1. Polysaccharides

Polysaccharides are the most used and well researched edible coatings. The popularity of polysaccharides as edible coatings is mainly due to their abundance in nature which makes them affordable and accessible. They are also preferred in the development of edible coatings because of their biodegradability (Khalil *et al.*, 2017). Different studies have reported that polysaccharides, besides being used to develop edible coatings have multiple uses. These include being used as drug delivery agent in the pharmaceutical industry and food thickeners

in the food industry. Essentially, they are hydrocolloids possessing high molecular weight that are soluble in water. Upon dissolving in water, they form intensive hydrogen bonds which result in a gel-like solution. Among the most popular polysaccharides used for edible coatings are cellulose and its derivatives, chitosan, starch and its derivatives and pectin (Campos *et al.*, 2011a). Due to their hydrophilic nature, the moisture barrier effect is negligible but they have selective gas permeability and can resist lipid migration (Dehghani *et al.*, 2018). Arnon *et al.* (2014) reported that polysaccharide based edible coating was able to retard respiration and improve appearance of citrus fruit. However, coating was not effective in reducing water permeability.

2.3.2. Cellulose

Cellulose is a linear polymer comprising of (1-4) linked β glucosidic unit (Fig. 1) and it is the most abundant polysaccharide found in nature (Kapelanakau *et al.*, 2014). Cellulose has been the most studied polysaccharide dating back as far as the 18th century. It is a complex carbohydrate with about 300 or more glucose units. When compared to synthetic polymers it is unique due to its distinct poly-functionality, high chain stiffness and sensitivity towards hydrolysis and oxidation forming acetyl groups. Acetyl groups play a major role in determining the chemistry of cellulose (Klem *et al.*, 2005). The acetyl groups may restrict cellulose accessibility by inhibiting productive binding resulting in an increase in diameter of the cellulose chain, thus changing its hydrophobicity (Meng and Ragauskas, 2014).

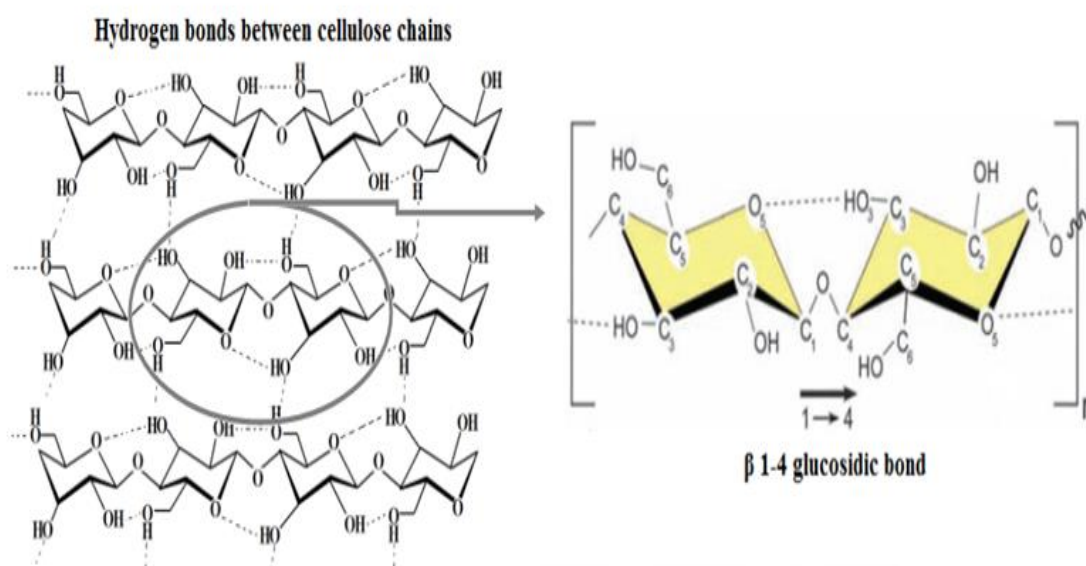


Fig. 1. The molecular structure of a single cellulose chain showing the direction of β 1-4 glucosidic with intrachain hydrogen bonding, *Dotted lines* Adapted from Poletto *et al.* (2013)

In nature, cellulose is water-insoluble, due to the hydroxy and hydroxymethyl groups which protrude from the chain, which makes them more suitable to forming hydrogen bonds with neighbouring cellulose chains (Nevell and Zeronian, 1985). The order of hydrogen bonds plays a significant role in both determining their accessibility to different catalysts and in its reactivity (Geboers *et al.*, 2011). The solubility of cellulose can be enhanced by treating it with alkali to help weaken the hydrogen bonds. Chemical modification of cellulose can be taken further by treating the swollen structure of cellulose with alkali, resulting in different chemical and physical characteristics, which then allows for various applications in different industries such as pharmaceutical, food production and others (Hamad and Hu, 2010). Methylcellulose (MC), carboxymethyl cellulose (CMC) and hydroxypropyl cellulose (HPC) are some of the common celluloses derived through etherification to improve cellulose solubility and they are the most used cellulose derivatives in food packaging (Hamad and Hu, 2010; Dhall, 2013; Khalil *et al.*, 2017).

Table 1 show the effect of various cellulose derivatives on different type of fruits. Amongst all cellulose derivatives, MC has the highest water barrier property and has good coating forming properties which results in the transparent and flexible coating (Chaple *et al.*, 2017). Vishwasrao and Ananthanarayan (2017) indicated that using a MC-palm oil (PO) edible

composite extended the shelf life of sapota fruit (*Manilkara zapota* L.) by reducing weight loss, retaining fruit firmness and delaying loss of phenolic content. Methylcellulose is combined with PO because most cellulose based coatings are highly permeable to water, thus lipids such as PO are used to improve barrier to moisture.

Table 1: Effect of postharvest application of cellulose based edible coating on fresh horticultural produce.

Type of fruit cellulose	Effect	Ref	
Methyl cellulose	‘Hass’ avocado	Coating slowed down rate of respiration, retained mass and firmness of fruit stored at 20 °C for 10 days	(Maftoonazad and Ramaswamy, 2005)
MC, CMC, HPMC	‘Berangan’ banana	HPC was the most effective coating in maintaining postharvest quality.	(Malmiri <i>et al.</i> , 2011)
CMC ‘Pro-long’	‘Julie’ mango	Pro-long (0.75%) significantly increased the storage life of mangoes, retarding ripening and reducing weight loss, without adversely affecting the sensory quality of the fruit	(Dhalla and Hanson, 1988)
HPMC + Bees wax	‘Autumn giant’ plum	Reduced mass loss and retain sensory quality attributes	(Perez - Gago <i>et al.</i> , 2003)
HPMC + essential oil	‘Formosa’ plum	Coating were effective in reducing the respiration rate, ethylene production, total weight loss, and total cell count in plums	(Choi <i>et al.</i> , 2016)
CMC + Moringa leaf extract	‘Fuerte’ and ‘Hass’ avocado	Incorporation of moringa to CMC reduced mass loss by 50%, slowed down respiration, retained firmness and delayed fruit ripening	(Tesfay and Magwaza, 2017)
CMC + essential oils	Papaya	Coatings reduced anthracnose severity in papayas and improved shelf life.	(Zillo <i>et al.</i> , 2018)

Chaple *et al.* (2017) demonstrated that MC can reduce water loss in chillies, which leads to a significant reduction in weight loss in addition, the coating also reduced skin shriveling on chillies. A similar observation was also made by Maftoonazad *et al.* (2008) who reported that in addition to maintaining postharvest fruit quality of peaches (*Prunus persica* cv 'Alberta'), MC extended fruit shelf life by 60% compared to sodium alginate with 40%. Baldwin and Wood (2006) reported that hydroxypropyl methylcellulose (HPMC) and CMC composite was better at reducing rancidity and development of off flavours while improving the glossiness of shelled pecan kernels at ambient temperature when compared to MC alone. It could be argued that this is due to that MC restricted gaseous movement, which might lead to anaerobic conditions resulting in the development of off flavours. This argument is in agreement with Arnon *et al.* (2015) who indicated that MC coating was found to impair mandarin fruit flavour.

2.3.3. Chitosan

Chitosan, similar to cellulose, is a linear polysaccharide comprising of β -(1 \rightarrow 4)-linked 2-amino-2-deoxy-D-glucose residues (Fig. 2). It is a derivative of chitin through the deacetylation process. Chitin is the second most abundant polysaccharide found in nature after cellulose and the shellfish waste industry is the major source (Xu *et al.*, 2005). Although chitin is not soluble in most organic solvents, in nature chitosan is a weak base (pH = 6.2-7.0) that is insoluble in neutral or alkaline solutions but soluble in slightly acidic solutions (pH \leq 6) (Miteluț *et al.*, 2015; Younes and Rinaudo, 2015; Zargar *et al.*, 2015; Khlibsuwan and Pongjanyakul, 2016). The solubility of chitosan depends on the degree of deacetylation (by sodium hydroxide), molecular weight, temperature, acetyl group distribution along the chain and nature of the acid used in protonation. A study by Chien and Chou (2006) demonstrated that the molecular weight of chitosan has an effect on the performance of coatings. Their results showed that chitosan coating with lower molecular weight (92.1 kDa) was more effective in retaining Vitamin C in 'Tankan' citrus compared to higher molecular weight (357.3 kDa) based coating. One could argue that molecular weight has a bearing effect on the thickness of the coating. This argument is also supported by Park *et al.* (2002) who studied the effect of different organic acids on the characteristics of chitosan films with different molecular weight (37 kDa, 79 kDa and 92 kDa). Their results demonstrated that molecular weight, generally influenced all characteristics of coating regardless of the organic acid with exception of water vapour permeability. Acetic acid had toughest films whereas citric acid had less oxygen permeable coatings. This may suggest

that if you are looking to control oxygen transmission you must prepare coatings with citric acids.

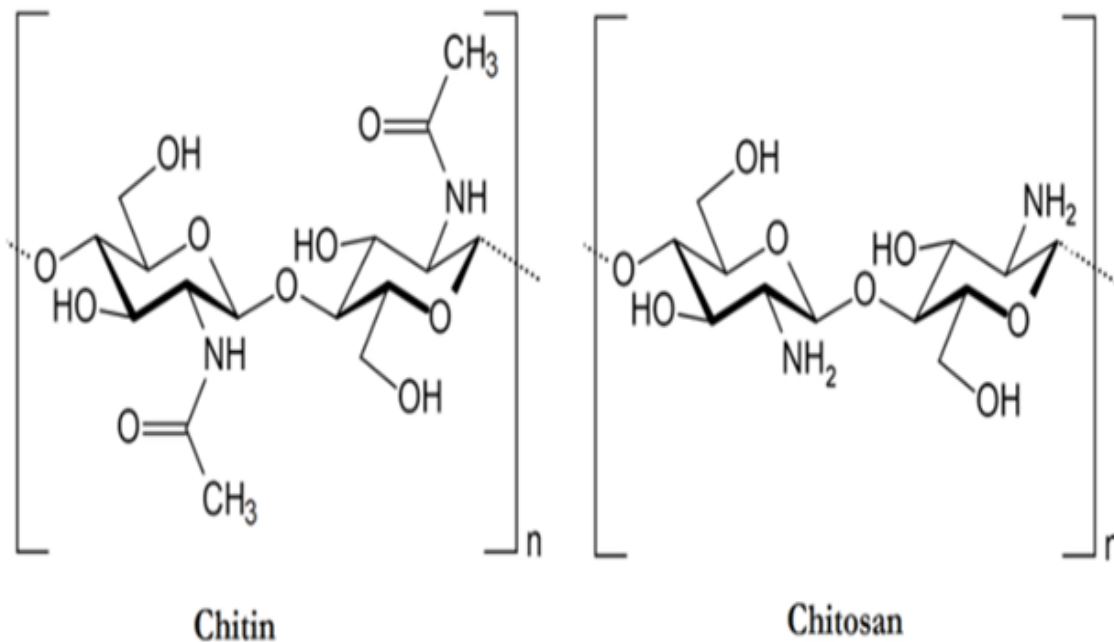


Fig. 2: Chemical of chitin and its derivative chitosan (Younes and Rinaudo, 2015)

The success of postharvest application of chitosan as an edible coating on fresh horticultural produce is well established in the literature (Table 2). A study by Kumar *et al.* (2017) reported that chitosan (2%) was able to prolong the shelf life of plum fruit 'Santa Rosa' stored $1 \pm 1^\circ\text{C}$ for 35 days. This was further linked with the ability of coatings to reduce the activity of pectin methylesterase, a cell wall degrading enzyme and maintain other fruit quality parameters such as colour and vitamin C. A study by Kaya *et al.* (2016) on the effect of chitosan with molecular weight (4.16 kDa) on prolonging the shelf life of red kiwi fruit, demonstrated that the coating was able to retain antioxidants levels but the coating was not effective against firmness loss. This might suggest that chitosan with low molecular weight is highly water permeable which may result in firmness loss.

High moisture loss is highly correlated with high respiration rate. High respiration rate results in an increase of enzyme activity, including cell wall degrading enzymes which cause loss of firmness. This hypothesis is supported by Jitareerat *et al.* (2007) who reported that high molecular weight chitosan (350 kDa) was able to reduce respiration, ethylene production and retained firmness on 'Nam Dok Mai' mango fruit. However, they reported that coating with more than 1% (w/v) concentrations resulted in poor organoleptic properties. This may be due

to the coating being too thick, reducing gas movement resulting in anaerobic conditions which cause the development of off flavours.

Table 2: Effect of postharvest application of chitosan based edible coating on fresh horticultural produce.

Fruit	Effect	
Banana	Chitosan reduced mass loss, retained vitamin c and maintained organoleptic properties of cavendish banana's	(Suseno <i>et al.</i> , 2014)
Logan	Coating reduced respiration rate and weight loss, delayed the increase in PPO activity and the changes in colour.	(Jiang and Li, 2001)
'Pearl' guava	Significantly increased activities of peroxidase superoxide dismutase, catalase and inhibited superoxide free radical production	(Hong <i>et al.</i> , 2012)
'Tainong' mango	2% chitosan significantly delayed ripening and decay in fruit stored at 5 °C, 85–90% relative humidity for 16 days.	(Zhu <i>et al.</i> , 2008)
'Palmer' mango	Coatings extended the shelf life and retained mango quality during ripening.	(Silva <i>et al.</i> , 2017)
Strawberry	Coatings were effective in reducing fungal decay (50%) and reduced mass loss and extended shelf life of strawberries.	(Hajji <i>et al.</i> , 2018)
Strawberry	Chitosan coatings reduced phenolics loss, had higher antioxidant enzyme activity and oxygen radical absorbance, maintained fruit quality.	(Wang and Gao, 2013)

Furthermore, the effect of chitosan treatment on various pathogen affecting fresh produce has been reported. Ghaouth *et al.* (1991) demonstrated that chitosan in addition to maintaining fruit quality, was effective also in inhibiting spore germination, germ tube elongation and radial growth of *Botrytis cinerea* and *Rhizopus stolonifera* and reducing decay caused by these pathogens on strawberry. This action by chitosan may not be related to defense enzymes such as chitosonase and chitinase found in berry fruit tissue. The antimicrobial activity of chitosan is a result of the protonated ammonium (NH^{3+}) which is believed to form an electrostatic interaction with negatively charged microbial cell membranes, thus provoking internal osmotic imbalances and consequently inhibit the growth of microorganisms (Goy *et al.*, 2009). There is evidence in the literature that the antimicrobial activity of chitosan is correlated with molecular weight and concentration in both *in vivo* and *in vitro* application (Bautista-Baños *et al.*, 2006). Meng *et al.* (2010) observed that low molecular weight chitosan (6 kDa) was more effective in inhibiting germ tube elongation and mycelia growth of *Alternaria kikuchiana* Tanaka and *Physalospora piricolain* pear fruit compared to high molecular weight (350 kDa). It could be argued that the antimicrobial activity and its effectiveness against postharvest diseases have to do with molecular weight. This argument was supported by Chien and Chou (2006) who reported that chitosan with low molecular weight (92.1 ± 3.3 kDa) showed an increase in microbial activity with an increase in concentration while the opposite was observed in chitosan with high molecular weight (357.3 ± 40.3 kDa).

2.3.4. Starch and derivatives

Starch is considered one of the most abundant molecules found in nature. It is the main storage reserve polysaccharide of higher plants and is it very important for humans. It can be easily extracted from roots, fruits, seeds and tubers. Starch is an affordable, biodegradable naturally occurring material that has multipurpose uses in different industries (Wang and Copeland, 2015). In the food packaging industry, starch together with its derivatives is used as an edible coatings component, plasticizer, thickener, emulsifier and stabilizer (Pająk *et al.*, 2013). There are two types of starch found in nature, namely amylose and amylopectin. Amylose is a linear polymer α -1, 4 anhydroglucose units that has excellent film-forming ability, rendering strong, isotropic, odourless, tasteless, and colourless film while amylopectin is more complex as it is highly branched with α -1, 4 chains linked by α -1, 6 glucosidic branching points occurring every 25–30 glucose units (Jiménez *et al.*, 2012). Starch is naturally a hydrophilic polymer. Different

starches and derivatives swell and dissolve in water, this increase with an increase in temperature which forces the disassociation of amylose and amylopectin (Bertuzzi *et al.*, 2007).

Starch-based edible coatings have poor mechanical properties and tend to be brittle when prepared in the absence of a plasticizer. Incorporation of plasticizers such as glycerol tween, sorbitol and others have been reported to reduce brittleness and improve mechanical properties of coatings (Farahnaky *et al.*, 2013). Laohakunjit and Noomhorm (2004) reported that 30% sorbitol increased tensile strength, reduced both the oxygen and water vapour transmission rate which improved the performance in reducing coating failures when compared to glycerol. This suggests that plasticizers chemically interact with coating material differently and that the concentration of the plasticizer has a bearing effect on the mechanical properties of starch-based edible coatings. A study conducted by Sanyang *et al.* (2015) indicated that plasticizer concentration has an effect on the elongation of sugar palm starch coating. Their results reported that increasing the concentration of glycerol (G), sorbitol (S) and glycerol – sorbitol (GS) (15% - 30%) registered significant increase in coating elongation: 26.52 – 61.63% (G), 38% – 34.5% (S) and 15.1% – 46.65 (GS) plasticizes coatings. The observed increase in coating elongation is due to plasticizers reducing intermolecular bonds, resulting in reduced rigidity thus improving coating flexibility.

There has been considerable research on the changes in fresh produce postharvest quality after the application of starch-based coatings (Table 3). Kapetanakou *et al.* (2014) studied the effect of glycerol (G), sorbitol (S) and glycerol – sorbitol (GS) plasticizers on mango kernel starch coating to be applied on fresh tomatoes. All plasticizers had a significant effect on the performance of coating applied to tomatoes, with sorbitol outperforming the other two in delaying changes in titratable acidity, soluble solids and I retaining fruit sensory quality. Kapetanakou *et al.* (2014) and Treviño - Garza *et al.* (2015) reported that pullan starch coating could prolong the shelf life of strawberry from 6 (control) to 15 days, and maintain organoleptic fruit properties. In contrast, Diab *et al.* (2001) reported that pullan starch coating accelerated ripening in ‘Hayward’ kiwi. These inconsistent results may be because of the two different respiration pattern of these two fruits. The Kiwi follows a climacteric respiration pattern while strawberry following non-climacteric respiration pattern. Climacteric fruits experience an increase in ethylene (C₂H₄) during ripening while non-climacteric exhibit no rise in C₂H₄ (Atta-Aly *et al.*, 2000). Pullan coating may have reduced gaseous movement in kiwi fruit, which

resulted in the accumulation of C₂H₄ causing the fruit to ripen. This shows that coating performance may vary with the coated produce respiration pattern.

Table 3: Effect of postharvest application of starch based edible coating on fresh horticultural produce.

Starch type	Fruit	Effect of coating on fruit	Reference
Rice starch	Plum	Coating reduced both weight loss and respiration rate and inhibiting the endogenous ethylene production when compared to the uncoated control fruit stored at room temperature	(Thakur <i>et al.</i> , 2018b)
Mango kernel starch	Tomato	Coatings reduced weight loss and restricted changes in soluble solids concentration, titratable acidity, ascorbic acid content, firmness and decay percentage compared to uncoated sample.	(Nawab <i>et al.</i> , 2017)
Potato and maize starch	Strawberry	Coatings reduced decay and extended storage life of strawberries by retarding senescence	(García <i>et al.</i> , 1998)
Cassava starch	‘Tommy Atkins’ mango	Cassava starch edible coatings and citric acid dipping promoted a decrease in respiration rate of mango slices, with values up to 41% lower than the control fruit.	(Chiumarelli <i>et al.</i> , 2010)
Cassava starch + propolis	Strawberry	Coating increased vitamin C and retained antioxidant activity in strawberry fruit.	(Thomas <i>et al.</i> , 2016)
Cassava starch + chitosan	Strawberry	Coating reduced mass loss (6%) lower yeast counts and psychrophilic microorganisms and the best appearance according to the sensory analysis.	(Campos <i>et al.</i> , 2011b)
Rice starch	Banana	Coating delayed fruit ripening.	(Thakur <i>et al.</i> , 2018a)

2.3.5. Pectin

Pectin is a biocompatible, nontoxic polysaccharide with high molecular weight. Chemically, it is composed of poly α 1–4-galacturonic acids (Fig. 3a) consisting of different degrees of methylation (DM) of carboxylic acid residues and amidated polygalacturonic acids (Fig. 3b). Esterification of galacturonic acid in methanol is used to obtain methoxylated carbon groups, while ammonia is used to convert galacturonic acid from amidated carboxyl groups (Espitia *et al.*, 2014). The degree of methylation (DM) significantly contributes to and influences the chemical properties such as gel formation, gelling temperature, gel properties and solubility of the pectin (Sriamornsak, 2003).

The degree of methylation (DM) and esterification bring up two different types of pectins; high methoxyl pectin (HMP) with DM > 50 esterified carboxyl groups and low methoxyl pectin (LMP) with DM < 50 esterified carboxyl group. Gel formation in HMP occurs in the presence of sucrose and acids, whereas LMP gels form in the presence of calcium ions (Ca^{2+}) (Zhou *et al.*, 2014). The sucrose acid mechanism followed by HMP depends on the potential of sucrose interacting hydrogen bonds and hydrophobic interactions, with the chemical structure of HMP and the conversion of carboxyl ions to unionised carboxylic groups by an acid. This results in reduced negative charges and attraction between pectin molecules and it allows pectin to remain in the dispersed state, which results in gel formation when allowed to cool off (Sriamornsak, 2003). Gelation in LMP follows what is known as ‘egg box’ method. This is when electrostatic interactions between cations especially Ca^{2+} and the negative cavities formed by the polymer chain, where the cations are inserted. Different egg boxes are stabilized by the Van der Waals force interacting with hydrogen bonds in addition to the electrostatic interaction (Espitia *et al.*, 2014).

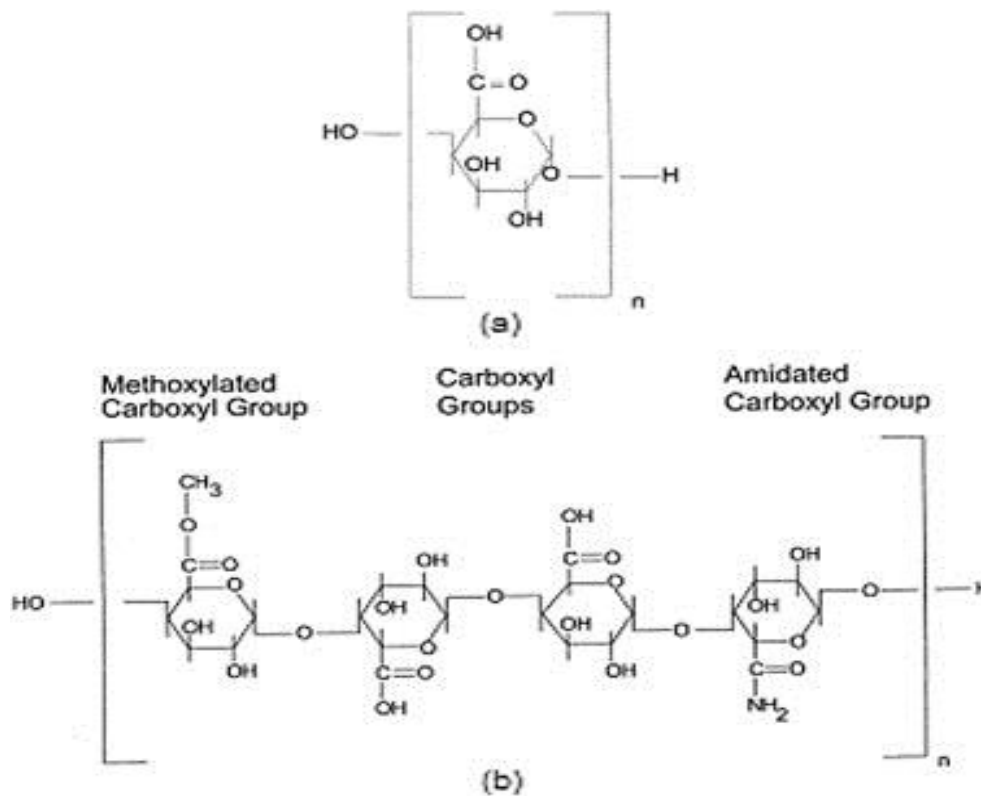


Fig. 3: Chemical structure of polygalacturonic acid (a) and representative chemical structure of pectin showing typical repeating groups (b) (Espitia *et al.*, 2014).

HMP and LMP gels are taken advantage of to develop pectin edible coatings. LMP is mostly used to develop edible coatings due to their ability to form strong gels. Calcium used during the gelation of LMP assists in reducing water permeability and improves the structural integrity of the film (Ferrari *et al.*, 2013). A similar observation was made by Park and Zhao (2006) when comparing both HMP and LMP reporting that LMP with sorbitol has better water vapour properties compared to HMP which has more elasticity.

Although pectins can be used to develop edible coatings, it is reported that the results are usually not “that” satisfactory if they are used alone i.e. for more effective results they must be incorporated with other materials, such as starch (Porta *et al.*, 2011), chitosan and sodium alginates (Galus and Lenart, 2013). Similar to other edible coating materials, pectins may also be used as carriers of antimicrobials, antioxidants, flavours and other beneficial compounds that help improve their function. Rojas-Graü *et al.* (2007) reported that Oregano essential oils in alginate-apple puree were more effective against *E. coli* O157: H7 when compared to Hermon grass, cinnamon oil and vanillin. In addition, all these additives had no negative effect

on the functionality of the coating, which is the most essential characteristic when selecting an additive.

2.4. Proteins

Films and coatings may be made from proteins of both animal and plant origin. Protein-based films and coatings are prepared from solutions comprised of three main components: protein, plasticizer and solvent due to their poor mechanical strengths (Galus and Kadzińska, 2016b). They have a good barrier to oxygen and carbon dioxide but have high water vapour permeability. The latter is due to their brittleness which results in poor surface adhesion of coatings (Mohajer *et al.*, 2017). They are grouped into two; fibrous and globular proteins. Fibrous proteins are obtained from animals. This includes casein, whey, gelatin and all these are water-insoluble. While globular proteins are obtained from plants and include soy proteins, wheat gluten and others (Dhall, 2013). Various physical properties of proteins such as milk proteins allow for effective performance of protein-based coatings as they can act as emulsifiers in coating preparations.

2.5. Gelatin

Gelatin is a soluble protein derived from hydrolysis of collagen. It is the major fibrous protein obtained from different animal body parts which include bones, skin and cartilages (Kapetanakou *et al.*, 2014). Gelatin is used in various industries including the food, pharmaceutical and cosmetic due to its gel-forming ability. Gelatin is characterized by the high presence of amino-glycine, hydroxyproline and proline content. Its properties are directly influenced by age, source and the type of collagen (Gómez-Guillén *et al.*, 2011). It may be that these properties have an influence on the amino acid composition and the molecular weight distribution. Besides the standard physiochemical properties of gelatin such as solubility, transparency, color, aroma and composition parameters, the most important properties that are of interest in the development of edible coatings are gel strength and thermal stability. These are of great importance because they determine whether gelatin can or not be used in the development of edible coating. Gelatin is high in amino acids glycine proline, and hydroxyproline content, and is soluble at a temperature close to body temperature. The cohesion of gelatin coating is also improved by the mixture of single and double unfolded chain of hydrophilic (Shit and Shah, 2014). Due to the natural brittleness of gelatin, 20-30% gelatin,

10-20% plasticizer and 30-80% water must be used when preparing coatings (Dhall, 2013). This helps to improve the flexibility of the coating and cohesion properties of the coating based on gelatin.

Over the few years, there has been a sudden exponential increase in interest in gelatin and its derived products such as edible coatings, emulsifiers and stabilizers. This coincides with the current rise in demand for organic-based product relative to synthetic ones by the consumers. Pigskin (46%) is the major source of gelatin followed by bovine hides (29%), bones (23.1%) and lastly fish (1.5%) (Gómez-Guillén *et al.*, 2011). The properties of a coating made from these sources differ. In the study by Shafiur Rahman and Al-Mahrouqi (2009) comparing gelatin from mammals and fish, they reported that fish gels had no brittleness, unlike bovine based gels. Similar results were also observed by Hanani *et al.* (2012) when comparing fish gelatin and mammalian gelatin they reported that fish gelatin had the lowest water vapour permeability for every concentration used (4-8%).

The application of gelatin and composites has shown to be effective in maintaining postharvest quality of fresh produce. A study by Poverenov *et al.* (2014) developed gelatin and gelatin – chitosan composites to maintain postharvest quality and prolong shelf life of red bell pepper. Their results indicated that gelatin could retain firmness and weight of the produce but was not effective against microbial decay. On the other hand, the composite could prolong cold storage (7 °C) by 7 days and shelf life by 16 days when compared to uncoated which lasted 12 days at storage and 5 days at shelf life. The ability of the composite coating might be due to the contribution of the antimicrobial activity of chitosan which was able to slow down decay.

2.6. Casein and whey proteins

Coatings produced from milk proteins have been used as a protective barrier on pharmaceutical products and later on fresh produce. Their functions include reducing mass loss, providing mechanical protection and improving or maintaining the sensory appeal of fresh produce. Cow milk contains about 33 g/L of proteins, with milk proteins classified into two groups or families namely casein (80% w/w) and whey (20% w/w) (Ramos *et al.*, 2012). One of the major properties that differentiate the two is that casein is insoluble while whey is soluble. The milk protein structure allows for the formation of a cohesive film due to the biopolymer-biopolymer interactions which form a continuous three-dimensional network (Khwaldia *et al.*, 2004).

Three groups namely α , β , and κ -casein combine to form casein. Due to their high nutritional value and multi-functional properties, such as an ability to act as emulsifiers, milk proteins can be used in edible film development and preparation.

Whey protein has been studied as an edible coating in minimally processed fruits and vegetables with positive results. At low relative humidity, whey proteins tend to produce flexible and translucent films around the coated subject with good respiration and aroma barrier properties (Ramos *et al.*, 2012). Whey proteins are derived from the waste stream of the cheese processing industry. They are used in the food and packaging industry because of their film-forming ability (Schmid *et al.*, 2012). Most whey proteins are obtained through the casting and drying of aqueous protein isolates, and this results in the transparent, odourless, flexible and colourless edible films (Galus and Kadzińska, 2016a). These are the major properties that allow whey proteins to be used in the development and preparation of edible coatings.

They have been tested as an edible coating on different fruits, cereals and nuts where they offered good volatile, fat, moisture and oxygen barriers (Schmid *et al.*, 2012). This provides a clear indication of whey ability to prolong the shelf life of different highly perishable fresh food products. A study conducted by Soazo *et al.* (2015) on strawberry using whey protein and 20 % beeswax in combination with pre-nitrogen freezing as an edible coating reported that this combination was able to prolong shelf life by reducing weight loss by 54 % during shelf life.

2.7. Lipids

Lipid-based edible coatings have been used as early as the 12th century to prevent moisture loss in citrus (Galus and Kadzińska, 2016b). The common used lipids to develop edible coating includes waxes (candelilla, carnauba and beeswax), triglycerides (milk fats), acetylated monoglycerides (vegetable oil) unsaturated fats and surfactants. Essentially, lipids are not polymers, they have low affinity for water and poor mechanical properties, which restrict their use in the development of edible coatings. Lipid-based edible coatings have been reported to improve fruit glossiness and retard moisture loss by reducing water permeability due to their hydrophobic nature (Baldwin *et al.*, 1997). Coatings based on lipids have poor mechanical properties and they are brittle. Plasticizers such as glycerol have been used to reduce their brittleness thus improving their flexibility (Gunaydin *et al.*, 2017).

2.8. Waxes

Beeswax, candelilla wax and carnauba wax are the most used commercial waxes in fresh horticultural produce. These waxes are generally recognised as safe (GRAS) and have been approved by the food and drug administration (FDA) for their application in fresh produce. Beeswax is derived from honey bees, while candelilla wax is derived from the candelilla plant (*Euphorbia antisyphilitica*). Carnauba wax is derived from the leaves of palm tree (*Copaernicacerifera*) and possess high boiling point (68–72.5 °C). Candelilla wax has been applied as a coating to improve gloss in citrus as early as 1974 (Lakshminarayana *et al.*, 1975). Donhowe and Fennema (1993) reported that carnauba wax has a 55 % higher water vapour permeability (WVP) and beeswax has 31% compared to candelilla wax. This low WVP of candelilla wax suggests that it has a potential to reduce WVP in coated products. Chick and Hernandez (2002) also reported that the addition of candelilla wax in casein coating resulted in a 72% decrease of WVP while 25% reduction achieved when carnauba wax was incorporated. However, Shellhammer and Krochta (1997) reported that addition of carnauba and candelilla wax in whey protein had no effect on the WVP of the coating. This suggests that the waxes interact differently with polymers.

2.9. Effect of edible coating on fruit quality

2.6.1 Appearance

Physical or visual appearance is one of the key quality attributes that consumers consider first before deciding to purchase fresh produce. It is imperative that the selected material help improve the visual appearance of the coated product, as this may enhance product attractiveness to consumers. It has been reported that a variety of coating materials enhances and maintain the visual appearance of various fruits. Arnon *et al.* (2015) showed that coating citrus fruits with polysaccharide based edible coating improved the glossiness of the fruit. A similar observation was made by Gol *et al.* (2015a) based on the sensory evaluation of carambola (*Averrhoa carambola* L.) fruits coated with 0.3% chitosan and 1% Gum arabic based edible coating. A study conducted by (Ragunathan *et al.*) on the use chitosan in combination with silver nanoparticles showed that chitosan silver nanocomposites have the ability to retain tomato physical appearance when stored at room temperature for 28 days.

Colour and colour changes in fruits, may also indicate quality and maturity stage in fresh horticultural produce. Certain fruits such as dark-skinned avocado and strawberry change colour as they ripen. For example, in 'dark skinned' avocados, the green colour retention by edible coating may indicate an increase in postharvest life of the fruit. Studies by Saucedo-Pompa *et al.* (2009) demonstrated that in addition to prolonging shelf life, candelilla wax coating improved lightness (L^* values) in 'Hass' avocado fruit. L^* value is related to luminosity, and it is a good indicator of colour changes in fresh produce which are usually caused by oxidative reactions and produce aging (Rocha and Morais, 2003). Similarly, Hernández-Muñoz *et al.* (2008) reported that chitosan coating maintained the L^* value, chroma and hue angle compared to uncoated strawberry fruit. It could be argued that coatings greatly influence respiration and mass loss. This argument is in agreement with Holcroft and Kader (1999) who indicated that higher respiration in strawberry triggers the synthesis of anthocyanin which is mainly responsible for colour in fruits and that higher respiration rates results in fruit browning as a result of oxidative reactions.

2.6.2 Organoleptic properties

While edible coatings can improve physical appearance, and improve the shelf life of various fruits, one must be careful of their interaction and effect on sensory parameters such as aroma and flavour as they are as important as visual appearance. There is evidence in the literature that polysaccharides-based coatings have a less negative effect on the organoleptic properties of coated product compared to proteins and lipids. Studies by Velickova *et al.* (2013) demonstrated that, based on luminosity, aroma, sweetness, aftertaste, strawberry 'Camarosa' fruit coated with chitosan were more acceptable and received higher hedonic scored compared to the wax coated fruit. They further noted that fruit coated with wax developed an untypical alcoholic aroma. This may suggest that the wax coating resulted in anaerobic conditions, causing such off flavour. This suggestion is in agreement with Hagenmaier and Shaw (1992) who indicated that waxes low gas permeability, results in an inhibition of oxygen (O_2) and carbon dioxide (CO_2) exchange which causes anaerobic conditions within the coated product.

The success of the postharvest application of the edible coating on retaining or improving organoleptic properties of fresh produce can be aligned to their ability to reduce the fluctuations in pH, sugar/ acid ratio and retaining fruit volatiles by through their gas permeability. No *et al.* (2007) suggested that polysaccharides retain flavour and aroma in horticultural produce by

slowing down the conversion of organic acids into aldehydes, alcohols and ketones. Furthermore, Stec *et al.* (1989) demonstrated that these organic compounds significantly influence both flavour and aroma in fruit. Ali *et al.* (2011) studied the effect of chitosan concentration on the physicochemical characteristics of 'Eksotika II' papaya fruit and reported that chitosan 1.5% was the best concentration as it attained high scores by the panellist in all tested parameters (taste, peel and pulp colour, texture and flavour) after 5 weeks of cold storage. Fruit treated with 0.5% and the control both, however, both ripened and decomposed after 3 weeks. Fruit coated with 2% retained firmness better than other treatments, thus they did not ripen even after 5 weeks and they were discarded. This may be due to coating being too thick and blocking all pores thus inhibiting the metabolic processes associated with ripening.

2.6.3 Phyto-nutrients

2.6.3.1 Vitamin C

Vitamin C is a naturally occurring water-soluble antioxidant that is known to reduce or prevent the damage caused by the reactive oxygen species produced in many fresh produce such as citrus, guava, peach, avocado, strawberry and others (Lester, 2006). Ascorbic acid includes crucial compounds that have biological activity of L-ascorbic acid (AsA) plus its oxidation product L-dehydroascorbic acid (DHA) (Mditshwa *et al.*, 2017). Vitamin C is essential in human health as humans cannot synthesise vitamin C due to the lack of L-gulonolactone oxidase, an enzyme responsible for vitamin C synthesis. Consequently, humans rely on dietary sources, mainly vegetables and fruits for vitamin C (Zhang *et al.*, 2018). Health benefits of vitamin C include the prevention of scurvy, a reduction in the risk of cardiovascular diseases, a reduction in different forms of cancer, a reduction in the risk of atherosclerosis (Naidu, 2003).

Most fruits are consumed fresh or recently dried, this allows for a greater intake of vitamin C. Vitamin C is sensitive and can be easily lost if exposed to different storage conditions and adverse handling. Vitamin C can be used as a quality indicator in most citrus fruits. This is mainly since ascorbic acid is a cofactor enzyme. It plays a huge role in wounding response, photoprotection and cell expansion and division (Conklin, 2001). This is in agreement with Yan *et al.* (2017) who reported that an application of ascorbic acid (1-3%) was effective in controlling browning and reduced pathogen development in apples. Like other antioxidants, AsA also plays a huge role in providing fruits with protection against oxidative stress.

Retention of Vitamin C by fruits, can improve quality retention and can provide the much-needed protection against the oxidative stress and pathogen attack. Table 4 shows the effect of different coatings on Vitamin C level in various fruits. A study by Drevinskas *et al.* (2017) demonstrated that in addition to fruit mass and phenolic content retention, chitosan was able to retain Vitamin C of Kiwifruit ('Anyksta', 'Sentiabrskaya' and 'VIR2'). They also noted that the molecular weight of chitosan had an effect on the performance of the coating on Vitamin C. Medium molecular weight (83 ± 6.5 kDa) was more effective in comparison to lower (6.7 ± 2.1 kDa) and higher (253.9 ± 38.4 kDa) molecular. Regardless of concentration, chitosan retained Vitamin C in 'Paluma' guava fruit stored at 25 °C for 4 days (Silva *et al.*, 2018). Lo'ay and Taher (2018) studied the effect of edible coatings chitosan/PVP blending with salicylic acid on biochemical fruit skin browning incidence and shelf life of guava fruits cv. 'Banati'. All the treatments significantly retained Vitamin C content on the fruit stored for fifteen days at 27 ± 1 °C and relative humidity of $48 \pm 2\%$. This was linked with the ability of the coating to reduce enzyme activity of polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) which are responsible for fruit browning.

Table 4: The effect of edible coatings on vitamin C of different fresh horticultural produce.

Coating	Fruit	Effect of coating on Vitamin C	References
Chitosan	Tankan	Chitosan-based edible coating retained Vitamin C with lower molecular weight (91 kDa) more effective compared to higher molecular weight (357.3 kDa).	(Chien and Chou, 2006)
Paraffin wax, beeswax and soybean oil; carboxymethyl cellulose	Peach and Pear	The coating retained Vitamin C during storage.	(Toğrul and Arslan, 2004)
γ -Irradiation Combined with Waxing	‘Tanaka’ lime	Without radiation Waxing results in loss of Vitamin C.	(Mahrouz <i>et al.</i> , 2002)
Carnauba wax	‘Cat hoa loc’ mango	Wax had no effect on Vitamin C level	(Hoa and Ducamp, 2008)
Shea butter wax	Pawpaw	Shea butter wax had better retention of Vitamin C stored at 10 ± 1 °C compared to room temperature	(Adetuyi <i>et al.</i> , 2008)
Chitosan	‘pearl’ guava	Retarded loss of Vitamin C during storage at 11 °C and 90–95% RH	(Hong <i>et al.</i> , 2012)
Chitosan	‘Paluma’ guava	Coating delayed Vitamin C biosynthesis with 3% outperforming 1% and 2% levels of chitosan	(Silva <i>et al.</i> , 2018)
Chitosan	Banana	2% Chitosan with 80% degree of deacetylation (DD) was the most efficient coating in reducing Vitamin loss in banana compared to those of 70% DD	(Wang and Gao, 2013)

2.6.3.2 Total phenolics

Phenolic compounds are plant-based substances which have an aromatic ring with one or more hydroxyl group. It is estimated that there are about 8000 plant phenolic compounds which about a half of these being flavonoids (Marinova *et al.*, 2005). Flavonoids belong to the polyphenol compound group and possess both a diverse chemical structure and diverse characteristics. Phenolic compounds possess a wide variety of biochemical functions and activities such as to act as antioxidants and their ability to modify gene expression (Sulaiman and Balachandran, 2012). Studies by Aliyu *et al.* (2009) and Okpuzor *et al.* (2009) demonstrated that phenolics are the largest phytochemical group that contribute to the total antioxidants in different plant products. Being the largest group of phenolics, flavonoids play a huge role in their antioxidant activity, this is due to their ability to scavenge free radicals such as super oxide and hydroxyl radicals (Kähkönen *et al.*, 1999). Due to their antioxidant activity, dietary consumption of fruit rich in flavonoids or any other phenolic compounds are beneficial as they can protect against heart diseases.

The use of surface coatings has been reported to be effective in extending shelf life and retaining the level of phenolics of berry fruit (Table 5) (Chiabrando and Giacalone, 2015). This can be related to the ability of surface coating in reducing respiration in coated products. Application of chitosan 2 % on 'Santa Rosa' plums stored at 1 ± 1 °C and 90 ± 5 RH, maintained total phenols with coated fruit retaining higher levels (~38%) (Kumar *et al.*, 2017). A study conducted by Jongsri *et al.* (2016) on the effect of the molecular weight of chitosan on postharvest and physicochemical properties on 'Nam Dok Mai' mangoes. Their findings demonstrated the ability of chitosan coating to reduce the production of reactive oxygen species by inducing oxygen scavenging species and in increasing phenolic compounds. They further reported that high molecular chitosan (360 kDa) was more effective in delaying ripening and reducing ethylene production when compared to low (40 kDa) and medium (270 kDa). Cellulose derivatives and waxes provide a gas barrier which retards the autoxidation of antioxidants including phenolics in the presence of O₂ (Hoa *et al.*, 2002). Baldwin *et al.* (1999) reported that cellulose-based edible coating (Nature seal) had lower O₂ compared to carnauba wax (Tropical fruit coating) at 30 °C and 60% RH but at cold storage (15 °C and 99 % RH and room temperature (21 ± 1 °C and 56 % RH) the two coatings have similar O₂ and CO₂ levels. This proves that both relative humidity and temperature have an influence on the coating performance. A similar observation was also made by Saberi *et al.* (2018) who demonstrated

that pea starch with gua gum (PSGG) and PSGG gum-shellac composite had no effect on total phenolics at early storage but with increased storage time the composite retained more phenols.

Table 5: The effect of various coating on total phenolic content.

Coating	Fruit	Effect of coating on total phenolic content	Reference
Chitosan	Strawberry	Coating maintained higher levels of phenolics in strawberry stored at 5 °C and 10 °C	(Wang and Gao, 2013)
Methyl cellulose - limonene	Strawberry	Coating retained total phenolic compared to control	(Dhital <i>et al.</i> , 2017)
Guar gum (GG) and ginseng extract (GSE)	Sweet cherry	Coating increased total phenolic content by 40% and 68% respectively	(Dong and Wang, 2018)
Chitosan	Litchi	Coating delayed the decrease in total phenolic	(Jiang <i>et al.</i> , 2005)
Chitosan	Loquat	Coating maintained antioxidant capacity in fruits during cold storage	(Ghasemnezhad <i>et al.</i> , 2011)
Starch	Strawberry	The coating had no significant effect on total phenolics of strawberry	(Garcia <i>et al.</i> , 1998)
Arabic gum, sodium caseinate	‘Baruipur’	Coating maintained the decrease in total phenol content compared to uncoated	(Murmu and Mishra, 2018)

Different factors such as cultivar, maturity, pre-harvest and postharvest handling may affect the antioxidant activity of phenolics. A study by Ayala-Zavala *et al.* (2004) demonstrated that storage has a significant effect on phenolics in strawberry fruit when stored at 0, 5 and 10 °C for 13 days. Other postharvest methods that have been used to retain the levels of phenolics include control atmosphere. But recently the attention has turned to the use of edible coatings to prolong shelf life other than the effect of coating on phytonutrients. Seeram *et al.* (2006) studied the effect of berry extract in inhibiting growth and stimulate apoptosis of human cancer cells. They observed that anthocyanins, flavanols, ellagitannins, gallotannins, proanthocyanins, and phenolic acids are the major phenolics in six popular consumed berries (black raspberry, blueberry, cranberry, red raspberry and strawberry). They further reported that blackberry and strawberry extracts were the most significant against cancer.

2.6.3.3 Total antioxidants

Antioxidants prevent or retard oxidative processes in fruits. Due to the different measurement methods used to determine antioxidants in fresh fruits. Separately it is feasible that their role and effectiveness can be additive hence total antioxidants activity or total antioxidant capacity in some cases total antioxidant power is measured (Erel, 2004). It could be argued that due to the diversity in the fruit matrix, it makes it difficult to quantify different antioxidant using a similar method. This argument would in agreement with De Souza *et al.* (2014) who demonstrated that a method used in the determination of total antioxidants has an effect on the level of antioxidants. They used 3 methods namely 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), β -carotene and 2,2-diphenyl-1-picrylhydrazyl (DPPH) in different berries and the measured total antioxidants differed with each method.

The effect of edible coating on the TAC levels of fruit has been reported (Table 6). A study by Chiabrando and Giacalone (2015) demonstrated that edible coating based on sodium alginate and chitosan retained TAC in blueberry fruits stored at 0 °C in the dark for 45 days. Chitosan was the most effective coating as it had the highest total antioxidant capacity (15.22 mmol Fe²⁺/kg FW) after storage compared to alginate (14.66 mmol Fe²⁺/kg FW) and composite (14.93 mmol Fe²⁺/kg FW). It can be argued that chitosan retained more TAC due to its antimicrobial properties. Similarly, Wang and Gao (2013) found that chitosan edible coatings reinforced the microbial defense mechanism of strawberry fruit which improved resistance against pathogen attack and retained TAC. Gol *et al.* (2015b) studied the effect of chitosan

(CH1% and 1.5%), alginate (Al 1 % and 1.5%), and carboxymethyl cellulose (CMC 1% and 1.5%) edible coatings on their ability to preserve the postharvest quality of Indian blackberry. All coatings significantly maintained higher TAC compared to uncoated fruit after 12 days of storage but by the end of storage, Ch 1.5% and CMC 1.5% concentration maintained higher levels of TAC compared to all other coatings. Based on this report, it could be argued that the response of fruits in relation to coating thickness is not homogenous. The variation in fruit response may be linked to the different gas permeability of strawberry fruit, which significantly influences the internal oxygen (O₂) concentration. High internal O₂ may lead to oxidation of antioxidants, therefore, reducing the TAC. In this context, Cisneros- Zevallos and Krochta (2003) studied the effect of Hydroxypropyl methylcellulose (HPMC) coating thickness on Fuji apples. They reported that increasing time to dry or coating thickness reduced internal oxygen in 'Fuji' apples.

Table 6: Effect of postharvest application of the edible coating on total antioxidant capacity on fresh horticultural produce.

Coating	Fruit	Effect of coating on total antioxidant capacity (TAC)	Reference
Chitosan	Sweet cherry	Coating delayed changes in total antioxidant capacity compared to control	(Petriccione <i>et al.</i> , 2015)
Rice starch (RS)	Plum	RS coating retained TAC but there was no significant difference between coated and control	(Thakur <i>et al.</i> , 2018b)
Chitosan	Guava	Chitosan coating induced antioxidant activities which resulted in higher TAC in coated fruit	(Hong <i>et al.</i> , 2012)
Hydroxypropyl methylcellulose (HPMC)–beeswax (BW)	Mandarin	The coating had no effect on TAC	(Contreras- Oliva <i>et al.</i> , 2012)
Pea starch and guar gum	‘Valencia’ oranges	Coating maintained TAC with the DPPH method having high TAC compared to FRAP method after 4 weeks at 20 °C	(Saber <i>et al.</i> , 2018)

Shellac, gelatin and persian gum	‘Valencia’ oranges	Coatings retained TAC, with shellac-based coating being the most effective treatment compared to gelatin and persian gum	(Khorram <i>et al.</i> , 2017)
Chitosan	‘Darasht’ apricot	TAC was induced by chitosan coatings and maintained total phenolics	(Ghasemnezhad and Shiri, 2010)
Semperfresh™, Chitosan, calcium caseinate and odium alginate	‘Duke’ and ‘Elliot’ blueberries	The chitosan-based coating was the most effective coating in maintaining TAC levels on both ventilated and non-ventilated containers	(Duan <i>et al.</i> , 2011)

2.6.4 Anti-Microbial activity

Antimicrobial agents expand the functionality of edible coating by providing protection against microbial spoilage while maintaining produce quality and safety for consumption. Plant-based essential oils (Sánchez-González *et al.*, 2011), organic acids (Ayranci and Tunc, 2004) and polymers such as chitosan (Kaya *et al.*, 2016) have been used as agents against microorganism in horticultural produce. The mode of action by these active agents differs as they interact differently with the coated produce. Most of the activity of these microbial is believed to be related to the protonated membrane of fruits and they enter through cytoplasm diffusion (Dhall, 2013). Incorporation of edible coatings with antimicrobial agents allows for a specific control of pathogenetic microorganism when applied (Henriques *et al.*, 2016). Table 7 shows the effects of incorporating active agents against various microorganisms. Consideration should be made when one selects a microbial agent as they are host specific, as this will influence the activity and performance of the selected antimicrobial agent. Additionally, the interaction of the antimicrobial agent with the edible coating should not negatively affect the function and properties of the coatings, but rather improve their performance (Rojas-Graü *et al.*, 2009).

There has been considerable research on different active agents and their effect on various microorganisms. Du *et al.* (2011) reported that the use of apple skin polyphenols (1.5 w/w) was effective in controlling *Listeria monocytogenes*, this is due to the action of phenolics compounds contained in apple skin. High phenolic compounds have been reported to inhibit activation of pathogen enzymes and other fruit deterioration enzymes such as polyphenol oxidases (Shapi'i and Othman, 2016). Study conducted by Wang *et al.* (2011) on chitosan-corn starch, gelatin-carrot puree films combined with oregano oil, carvacrol, cinnamaldehyde, and citral, were effective against *Staphylococcus aureus*, with the first three recommended for preparation of antimicrobial edible films for food applications. Importantly, the presence of these antimicrobials did not influence the properties of the films. This is essentially recommended when antimicrobials are to be used in addition to the films as the most important part of the films are its properties. The use of plant-based antimicrobials is also in line with the recent pressure exerted by consumers regarding the use of synthetic food additives. While antimicrobial can be effective in controlling pathogenic microorganisms, they must also be coupled with proper fruit handling during both harvest and postharvest stages as this can help reduce pathogen infection.

Table 7: Antimicrobial activity of coating incorporated with active agents

Coating material	Antimicrobial agent (s)	Evaluated microorganism	Effect of active agent	Reference
Whey protein isolate	Oregano, rosemary and garlic essential oils	<i>Staphylococcus aureus</i> , <i>Salmonella enteritidis</i> , <i>Listeria monocytogenes</i> , <i>Lactobacillus plantarum</i> and <i>Escherichia coli O157:H7</i>	Oregano oil was the effective agent compared to the other agents	(Seydim and Sarikus, 2006)
Chitosan	Lemon essential oil	<i>Botrytis cinerea</i>	Lemon essential oil enhanced the antimicrobial activity of chitosan	(Perdones <i>et al.</i> , 2012)
Candelilla wax	<i>Bacillus subtilis</i>	<i>Rhizopus stolonifer</i>	Incorporation of coating with <i>Bacillus subtilis</i> significantly reduced <i>R. stolonifer</i> severity index	(Oregel-Zamudio <i>et al.</i> , 2017)
Chitosan	Aloe vera	<i>Botrytis cinerea</i>	Addition of Aloe Vera had no effect on <i>Botrytis cinerea</i>	(Vieira <i>et al.</i> , 2016)
Gum arabic	Chitosan	<i>Colletotrichum musae</i>	Addition of chitosan suppressed microbial growth by 100% and reduced decay by 80%	(Maqbool <i>et al.</i> , 2011)

Carboxyl methylcellulose	Moringa leaf and seed extracts	<i>C. gloeosporioides</i> and <i>A. alternata</i>	Moringa ethanolic extracts had higher antimicrobial activity and were more effective in inhibiting postharvest diseases compared to methanolic extracts (Tesfay <i>et al.</i> , 2017)
Chitosan and locust bean gum (LGB)	Pomegranate peel extract	<i>Penicillium digitatum</i>	Chitosan and LBG incorporated with the peel extract reduced disease incidence by 95% and 75 % respectively. (Kharchoufi <i>et al.</i> , 2018)

2.7 Conclusion and future research aspects

Food is lost throughout the food chain, with most losses experienced during the postharvest chain. In developing countries, food losses cannot be separated from the socio-economic factors such as lack of capital, wars and lack of education amongst others. Technical limitations such as harvesting techniques, infrastructure and cooling and storage facilities all contribute towards the food losses experienced by developing countries. The losses experienced by these countries are of huge concern as most of their economies are dependent on agriculture. Thus, it is important to develop methods that are economically affordable, sustainable and can preserve produce shelf life.

Edible coating is one of the recent affordable and sustainable methods that can be used to reduce postharvest losses experienced by the fresh produce industry. They are also in line with recent demand for 'organic' food as they are based on environmentally friendly materials, with an advantage to act as carriers for antimicrobials and other active ingredients. Increased focus on the ability of the edible coating to retard respiration, mass loss, water loss and to prolong shelf life. These applications have only been tested in the laboratory in many cases. While this is a good step, it is now time that their application is also tested by industries. This will allow checking to check their impact and effectiveness on a commercial scale. There has been no report on equipment that can be used to apply these coatings. This can be related to the fact that the preparation of coating is material and product specific, thus the equipment to be used to commercially prepare and apply them might differ.

There has been little information on the use of edible coatings as ripening agents, on fruits with uneven ripening sequence such as sweet peppers. The unacceptability of synthetic waxes and synthetic postharvest treatments such as Avoshine® by European market has caused major economic losses. This puts more urgency on the development of a postharvest treatment that can help prolong shelf life and maintain fruit quality during the postharvest chain.

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Chapter 3:

Evaluating the efficacy of gum arabic and carboxymethyl cellulose incorporated with moringa leaf extract as a novel postharvest edible coating for ‘Maluma’ avocado

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Abstract

This study investigated the effectiveness of edible coatings such as gum arabic (GA), carboxymethyl cellulose (CMC) as well as moringa (M) leaf extract on maintaining the postharvest organoleptic quality properties of ‘Maluma’ avocado fruit. Harvested fruit were dipped into the following treatments: GA 10%, GA 15%, GA 10% + M, GA 15% + M and CMC 1% + M while uncoated fruit served as control. Fruit were stored at cold storage (5.5 °C), for 3 weeks, and moved to ambient conditions (21 ± °C) for 1 week. Sampling was done on a seven-day interval and sensory evaluation was done after 4 weeks in storage. Quality parameters evaluated included mass loss, firmness loss and colour changes (L*, a*, b*). Sensory quality attributes, namely; taste, colour, mouthfeel, odour and overall acceptability were evaluated. Edible coatings and storage time had a significant ($p < 0.001$) effect on fruit quality. Fruit coated with GA 15% + M had lower mass loss (3.66%) and retained firmness (62.37 N), L* (30.85), a* (-2.33) and b* (7.14) compared to other treatments. However, the results also revealed that GA15% + M (1.85 and 1.61) had a negative effect on sensory quality as it attained the lowest scores for taste and overall acceptability compared to CMC + M (4.37 and 4.66) and GA 10% + M (3.9 and 3.98) which had the higher sensory scores respectively. Pearson correlation showed that firmness loss and mass loss had a strong correlation ($r = 89$), while taste and overall acceptability had an intermediate correlation ($r = 65$), except for the correlation between odour and taste which was strongly positive ($r = 71$).

Keywords: *Persea americana*, Sensory evaluation, Storage time, Postharvest quality, Respiration

3.1 Introduction

Avocado (*Persea americana* Mill.) is one of the most important tropical fruit which has high nutritional and therapeutic value (Maria Do Socorro *et al.*, 2010). The past few decades have seen a significant rise in the demand and consumption of avocado fruit which is linked to the health benefits associated with the consumption of the fruit and its derived products (Fulgoni *et al.*, 2013). The fruit is a rich source of monosaturated fatty acids, Vitamin B6 and E, ascorbic acid β – carotene, potassium, and magnesium and other phytochemical content (Orhevba and Jinadu, 2011; Magwaza and Tesfay, 2015). Consumption of avocado and its products has previously been reported to have anti-cancer properties and to reduce cardiovascular diseases and diabetes (Wang *et al.*, 2015). This is linked mainly to the high levels of monounsaturated fatty acids which maintain normal serum total cholesterol (Dreher and Davenport, 2013), high antioxidant and phytochemicals found in the fruit (Fulgoni *et al.*, 2013).

Avocado fruit is highly perishable mainly due to its characteristically high metabolic rates, resulting in short postharvest life. High metabolic rates are associated with high respiration rates and high endogenous production of ethylene, increase in ethylene accelerates fruit ripening. (Maftoonazad and Ramaswamy, 2005; Tesfay and Magwaza, 2017). Avocado is also considered to have high mass loss, which can have negative consequence on fruit quality leading to significant economic loss as the value of fresh produce is often determined by its mass (Teskay and Magwaza, 2017). Postharvest losses of fresh produce do not only result in major economic losses but have a negative impact on climate and environment, as for every tonne of food wasted about 4.2 tonnes of carbon dioxide is emitted (Quested *et al.*, 2011; Opara and Mditshwa 2013).

Previous studies have demonstrated the effectiveness of low temperature during storage and transportation on maintaining the quality and extending the shelf-life of avocado fruit (Zauberman and Jobin-Decor, 1995; Woolf *et al.*, 2003; Bill *et al.*, 2014; Glowacz *et al.*, 2017). However, avocado fruit are sensitive to low temperature thus long-term cold storage may result in chilling injury (CI), which causes mesocarp discolouration and pulp browning (Kruger,

2013). The potential incidence of mesocarp discolouration and pulp browning (which are symptoms of CI) increase with storage time and fruit maturity (Pesis *et al.*, 2002).

Controlled atmosphere (CA) (Alamar *et al.*, 2017), modified atmosphere packaging (MAP) (Meir *et al.*, 1997), 1-Methylcyclopropene (1-MCP) (HersHKovitz *et al.*, 2005) and surface coatings (Zhang *et al.*, 2014) have been reported to be effective in prolonging the shelf-life of avocado fruit. All these techniques result in the modification of atmosphere (increased carbon dioxide and low oxygen levels) within the product, thus reducing respiration and fruit ripening rate. However, CA, MAP and 1-MCP have been associated with either negative environmental impact, high cost, development of physiological disorders or human health concerns (Watkins, 2006; Bill *et al.*, 2014; Ncama *et al.*, 2018).

Edible coatings have recently emerged as an innovative, effective and sustainable technique for prolonging the postharvest life of fresh horticultural produce. Edible coatings are biodegradable soluble formations that are applied on fruit surface and can be consumed with the coated product (Embuscado and Huber, 2009; Zhang *et al.*, 2014). Coatings must form a continuous film around the coated surface for better functionality. Coatings have been reported to improve the postharvest life of fresh produce and maintain postharvest quality by acting as a barrier against gases, moisture, and solute movement (Park, 1999; Ncama *et al.*, 2018). This is achieved by the semi-permeable layer formed by coating on the fruit surface. Importantly, edible coatings can also act as carriers for active ingredients such as colourants, antimicrobials and flavours, amongst others that can enhance their functionality (Azarakhsh *et al.*, 2014).

Among edible coatings, carboxymethyl cellulose (CMC) and gum arabic (GA) have been studied as postharvest treatments for fresh produce. Reduced moisture loss, gaseous movement and decay incidence coupled with high firmness and ascorbic acid retention have been reported in CMC treated strawberries (Hussain *et al.*, 2012). A delay in colour development and superior organoleptic properties has been reported in tomatoes coated with gum arabic, compared to the control treatment (Ali *et al.* (2010).

Moringa olifera is known for its high phytochemical content which can be used in the pharmaceutical, agricultural and food industries. Leaves, seeds, flowers and bark have all been reported to have a high content of proteins, β -carotene, vitamins, phenolics, flavonoids fatty acids and other bioactive compounds (Tsfay *et al.*, 2016; Saucedo-Pompa *et al.*, 2018).

Various studies have demonstrated the potential of moringa leaves as a functional additive for food products and food application (Adetunji *et al.*, 2012). Moringa leaves are rich in phenolic acids, flavonoids, glucosinolates and isothiocyanates, and hot water or methanolic extracts are reported to have high chlorogenic acid, quercetin and kaempferol content (Cuellar-Nuñez *et al.*, 2018). This has shown the potential of moringa leaf extract to be used as an antimicrobial and antioxidant agent in the food industry.

Incorporation of moringa extracts into edible coatings have been reported to enhance the functionality of coatings (Tesfay *et al.*, 2017). Adetunji *et al.* (2012) demonstrated that the addition of moringa leaf extract to hydroxypropyl methylcellulose (HPMC) enhanced the performance of the coating. The coating with moringa extract was more effective in extending the shelf-life of sweet oranges compared to the coating without the extract. Similar observations were made by Tesfay and Magwaza (2017), who demonstrated that the incorporation of moringa leaf extracts with CMC significantly reduced respiration, retained firmness and delayed ripening in ‘Fuerte’ and ‘Hass’ avocados.

Previous studies on avocado fruit has looked at postharvest quality using quantitative methods. However, current trends in postharvest technology are not focusing only on instrumental methods, but rather integrating both quantitative and qualitative methods. Therefore, the aim of this study was to investigate the effect of polysaccharide-based coatings incorporated with moringa leaf extract on ‘Maluma’ avocado postharvest quality and sensory attributes.

3.2 Materials and methods

3.2.1 Fruit samples, treatment and storage

A total of 180 avocado fruit (‘Maluma’ cultivar) used in this study were procured from a commercial orchard at ZZ2 (Pty) LTD in Limpopo Province, South Africa. Using dry matter as maturity index, ‘Maluma’ avocado fruit were harvested at commercial maturity where the mean dry matter content was 30% (Magwaza and Tesfay, 2015). Harvested fruit were packed in open display boxes and transported overnight in a ventilated vehicle to the Postharvest Laboratory of the University of KwaZulu-Natal, Pietermaritzburg campus.

Upon arriving at the laboratory, fruit were washed with distilled water. They were then assigned to six postharvest treatments: Control (untreated), GA 10%, GA 15%, GA 10% + moringa 10%, GA 15% + moringa + 10% and CMC 1% + moringa 10%. Each treatment had 3 replicates of 10 fruit per replicate. Fruit were dipped in treatment solutions for one minute, and air dried on a laboratory bench at room temperature (21 ± 1 °C) for 30 – 45 minutes. After drying, fruit were packed into commercial boxes and transferred to a cold room which had a delivery air temperature set at 5.5 °C and relative humidity (RH) set at $90 \pm 2\%$ for 3 weeks, simulating shipping conditions. After 3 weeks of cold storage, fruit were transferred to ambient conditions (21 ± 1 °C, RH 60 %) for 7 days, simulating ripening and retail conditions. Samples for biochemical analysis were taken in from fruit per replication (3 per treatment) on a seven-day interval from day 0 to the last day of the experiment. All the samples were stored at -26 °C prior to freeze-drying using a Virtis Benchtop freeze drier system (ES Model, SP industries Inc., Warmister, USA) for 5 days a 0.015 kPa and -75 °C. Samples were thereafter ground into powder using pestle and mortar. The ground samples were stored at -26 °C until all biochemical analysis was conducted.

3.2.2 Postharvest quality measurements

3.2.2.1 Mass loss

The fruit mass was measured using a RADWAG (Wagi Electronic Inc, Poland) digital balance (± 0.01 g) and was reported as percentage loss in moisture using Eq. 1.

$$\text{Weight loss \%} = [(W_i - W_f) \div W_i 100] \quad 1$$

Where:

W_i = Weight of fruit before post-harvest storage

W_f = Weight of fruit at a specific ripening day

3.2.2.2 Firmness

Firmness was determined using a hand-held densimeter (Bareiss, Germany) based on the method by (Tesfay and Magwaza, 2017). Four readings were taken at an equatorial region of

avocado opposite sides and the average was recorded. The hand-held densimeter measures fruit firmness by means of a metal ball (diameter 5mm) that is pressed onto the fruit. The scale ranges from 100 (hard) to 0 (soft) (Köhne *et al.*, 1998).

3.2.2.3 Peel colour

For each replicate, four individual fruit were marked on the equatorial region (3 regions per fruit) and the colour was recorded on the same spot every time sampling was done. Colour was determined according to Mcguire (1992) using the hunter lab system with Minolta Chroma Meter CR-2000 (Chroma Meter, Konica Minolta Sensing, INC., Japan). The Chroma meter was calibrated with a white standard tile ($Y = 87.0$, $X = 0.3146$ and $y = 0.3215$) prior fruit scanning at 30 min intervals. Values were recorded as L^* (white =100, black =0), a^* (green-red) and b^* (yellow-blue scale).

3.2.3 Sensory evaluation

Sensory evaluation was carried out according to Arpaia *et al.* (2015) with slight modifications. Sensory panellists were 37 students at the University of KwaZulu-Natal, Pietermaritzburg campus. The majority of the panellists had never performed a sensory evaluation of avocado in the past. Each panellist was provided training on the meaning of the sensory characteristic and on how to use the hedonic scale. Carrot and water were used to cleanse the palate after each sample was evaluated. Panellists rated each sample for overall liking using a 5-point hedonic scale where 1 = dislike extremely and 5 = like extremely. All samples were rated for the degree of colour, taste, mouthfeel, odour and overall acceptability.

3.2.4 Statistical analysis

The collected data were subjected to the analysis of variance (ANOVA) using GenStat statistical software (GenStat[®], 18.1 edition, VSN International, UK) 17. Mean separation was performed using Fischer's least significant difference (LSD) at 5% level of significance. Standard error values were calculated when a significant standard deviation was found at $p \leq 0.05$ between individual values. The Pearson correlation coefficient between the parameters was established using Statistical Analysis System 9.3 (SAS Institute Inc., Cary, NC, USA).

3.3 Results and discussions

3.3.1 Fruit mass loss

The postharvest mass loss in fresh horticultural produce such as fruit and vegetables results in loss of quality, freshness and subsequently economic loss, as their market value is mainly determined by their mass (Maalekuu *et al.*, 2006). Reducing mass loss does not only ensure good economic gains but plays a significant role in improving the shelf life of fresh produce. Mass loss with storage time is shown on Fig. 1. In this study, mass loss was significantly ($p \leq 0.01$) influenced by the interaction between storage time and coatings. The significance of this interaction could be explained by the rapid mass loss in control fruit with storage time contrary to the relative low mass loss in coated fruits. These results are in agreement with Tesfay and Magwaza (2017); Tesfay *et al.* (2017) who demonstrated that polysaccharide coatings such as chitosan and carboxymethyl cellulose was able to retard mass loss in avocados. Also, Al-Juhaimi *et al.* (2012) reported that gum arabic novel edible coating was able to reduce mass loss in cucumbers. The higher postharvest loss in uncoated fruit could also be closely related to the destruction of fruit natural skin wax that occurred when washing them.

GA 15% + moringa (M) (3.66%) was the most effective treatment in reducing mass loss, followed by CMC + M (6.19%) and GA 10% + M (8.30%). The observed variation in mass loss for gum arabic coatings could be explained by the difference in thickness and rheological properties of coatings. On our results, it can clearly be seen that GA 15% with or without moringa outperformed GA 10% throughout the study. The correlation between coating thickness and mass loss is contributed by the coating closing stomatal openings in the fruit resulting in reduced solute movement. GA 10% (10.53%) and GA 15% (9.01%) without moringa extract were found to be less effective in reducing mass loss compared to those incorporated with moringa.

For both uncoated and coated fruit, the highest mass loss occurred during the last week (week 4) of storage period. This is consistent with previous reports that mass loss is predominantly driven by temperature and vapour pressure difference between the fruit surface and the

environment (Bower, 2005; Thakur *et al.*, 2018), thus major mass loss occurred at ambient conditions compared to cold storage. Moisture loss through the fruit surface is a natural aspect of fruit metabolic aspect process that occur through stomatal openings and skin cracks. Tesfay *et al.* (2017) further explained that loss of membrane integrity in avocado fruit result in an increase in stomatal opening which subsequently increases mass loss. Loss of membrane integrity is correlated to membrane permeability, which subsequently increases metabolic activities such lipid peroxidation resulting in an increased mass loss (Song *et al.*, 2009).

The lower mass loss observed during cold storage at 5.5 °C compared to mass loss at ambient (21 ± 1 °C), is due to the correlation between metabolic activities and vapour pressure gradient with temperature. Major mass loss from fresh produce is mainly due to vapour pressure differences which influence the rate of transpiration (Magwaza *et al.*, 2013). Although, the loss of carbon dioxide from respiration process also accounts for mass loss (Ali *et al.*, 2010). Downward movement of solutes in a water pressure gradient is the major cause of mass loss in fruit during storage (Magwaza *et al.*, 2013). The vapour pressure deficit between the fruit and surrounding environment is higher at higher temperatures, promoting excessive moisture loss from the avocado. The reduction in mass loss observed in coated fruit is due to the semi-permeable layer created by these coating, which results in a modified atmosphere between fruit and the environment (Park, 1999).

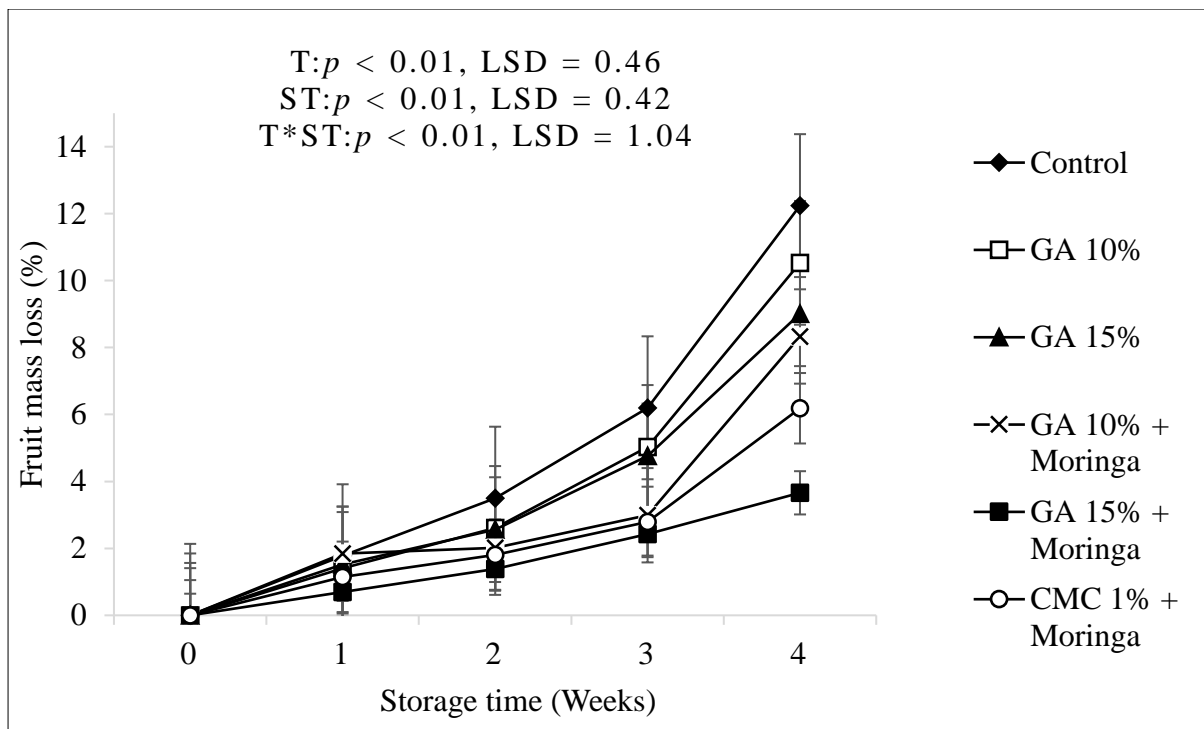


Fig. 1: Effect of edible coatings on mass loss of ‘Maluma’ avocados stored at 5.5 °C for 3 weeks, then transferred to ambient temperature ($21 \pm 1^\circ\text{C}$) for one week. Vertical bars represent standard error (SE) at $n = 4$. T = Treatment, ST: Storage time

3.3.2 Firmness

The firmness of ‘Maluma’ avocado fruit were significantly ($p \leq 0.01$) affected by the interaction between storage time and coatings. This could be explained by the sharp decline of firmness in control fruit in comparison to the steady decline in coated fruit. Loss of firmness gradually increased with storage time for both coated and control fruit (Fig. 2). At the end of storage time, uncoated fruit clearly had the lowest firmness (22.20 N). On the other hand, GA 15% + moringa (M) (62.37 N), CMC + M (59.93) and GA 10% + M (59.48 N) maintained higher firmness throughout the study. A similar trend was also observed in mass loss, where minimal change was observed in fruit coated with the above-mentioned coatings. It can be argued that the restriction of moisture loss was the major factor why coatings retained higher firmness than uncoated fruit. This argument is in agreement with Aguirre-Joya *et al.* (2017) who demonstrated that moisture loss is not only related to mass loss, but also correlates with fruit softening.

Avocado softening is a result of the loss of membrane integrity caused by mass loss and enzymatic activity that hydrolyses the cell wall structure and solute leakage (Pesis *et al.*, 1978). Avocado membrane structure is made up of mainly cellulose, hemicellulose, as well as pectins, hydrolysis and depolymerization of these structures by enzymes such as polygalacturonase and pectin methylesterase results in fruit softening (Bower and Cutting, 1988). The activity of these enzymes has been reported to be affected by the presence of carbon dioxide (CO₂) and oxygen (O₂). Dhalsamant *et al.* (2017) reported that the use of modified atmosphere packaging with O₂ (2%) and CO₂ (10%) delayed ripening and softening in mango fruit. In this study, it could be argued that coating fruit with GA 15% + M, CMC + M and GA 10% + M resulted in a modified atmosphere with reduced O₂ and increased CO₂ levels, causing in a reduction of enzymatic activities in coated compared to uncoated avocados. In agreement with these findings is Bill *et al.* (2014) who reported that chitosan and aloe vera based coatings in combination with thyme oil retained ‘Hass’ avocado firmness.

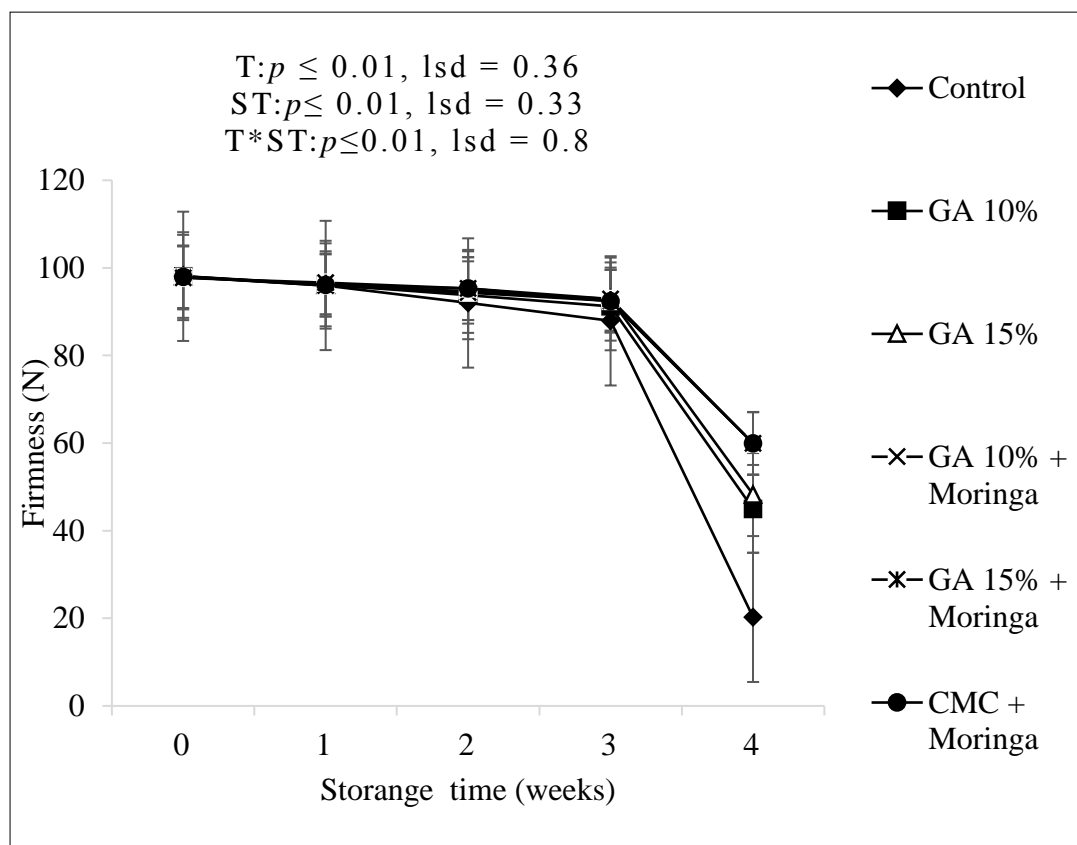


Fig. 2: Effect of edible coatings on ‘Maluma’ avocado firmness stored at 5.5 °C for 3 weeks, then transferred to ambient temperature (21 ± 1°C) for one week. Vertical bars represent standard error (SE) at $n = 4$. T = Treatment, ST: Storage time

3.3.3 Colour

Colour is the primary and most used perception parameter in determining the quality of fresh horticultural produce. This is correlated with the visual changes fresh produce undergo during development to maturity thus the influence on consumer decision (Iglesias *et al.*, 2008). For avocado fruit, skin colour is not usually a good indicator of fruit maturity or stage of ripeness as some cultivars do not undergo any colour changes. The exception being ‘black skinned’ cultivars such as ‘Maluma’ which may undergo skin colour changes during both maturity and ripening (Magwaza and Tesfay, 2015). In this study L^* , a^* and b^* were the observed colour parameters.

Changes in L^* , a^* and b^* values of avocado skin colour are shown in Table 1. this present study, an interaction between storage condition and storage time ($p < 0.001$) had a significant effect fruit lightness. This interaction could be explained by the rapid decline in L^* values in uncoated fruit with increasing storage. Fruit coated with GA 15% + M was the most effective coating in improving fruit lightness followed by CMC + M, GA 10% + M, GA 15% and GA 10% in comparison to control. By the end of the experiment a similar trend was observed as fruit coated with GA 15% + M, as they experienced the least decrease in lightness. Even though there was a loss of fruit lightness, it was more dominant on uncoated fruit, as by the end of the storage period the L^* value was 23.18. This can suggest that coatings have a positive effect on the reduction of changes in fruit lightness.

The observed rapid loss of fruit lightness in uncoated fruit is due mainly to the microstructural changes in the fruit membrane as a result of high respiration, and the loss of membrane integrity which increase with fruit senescence. This observation can be supported by Corey and Schlimme (1988) who alluded that the sharp decrease of fruit lightness is mainly due to the structural and quantitative changes in the cuticle that takes place during fruit ripening. This study further confirmed that incorporation of active ingredients can improve the performance of coatings.

As shown in Table 1, at the beginning of the study, the a^* value was more negative indicating that fruit were green. In this study, an interaction between edible coatings and storage time had a significant ($p < 0.001$) effect on the increase in a^* values. This interaction could be explained

by the rapid increase in a^* values of control fruit with storage time. This increase common in dark-skinned avocados as this indicates the degradation of chlorophyll and synthesis of anthocyanins which increase with avocado ripening (Cox *et al.*, 2004). This increase was dominant in uncoated fruit compared to coated fruit. This result corroborates the findings of Adjouman *et al.* (2018) who reported that polysaccharides coatings delayed a^* value increase in tomatoes compared to control.

There were significant differences in performance amongst edible coatings, with GA 15% + M being the most effective coating followed by CMC + and GA 10% + M. The ability of these coatings to retain avocado green colour suggest that coatings were able to delay fruit ripening. This can be supported by the findings of Jeong *et al.* (2003); Cox *et al.* (2004) who reported that any postharvest treatment that can delay colour change from green to dark purple can prolong the shelf life of dark-skinned cultivars.

Changes in b^* values were significantly ($p < 0.001$) influenced by the interaction between edible coatings and storage time. The significance of this interaction can be explained by the sharp decline in b^* values in control fruit with storage time. Similar results were also reported by Maftoonazad and Ramaswamy (2005) who demonstrated that edible coatings based on methylcellulose retained b^* values in comparison to untreated fruit. Changes in b^* values ranged from 14.08 - 3.19, 14.08 - 3.75, 14.08 - 4.13, 14.08 - 5.48, 14.08 - 7.17 and 14.08 - 5.53 for control, GA 10%, GA 15%, GA 10% + M, GA 15% + M and CMC + M, respectively. According to Maftoonazad *et al.* (2007), reduction in b^* values in avocado is an indication of a decrease in yellowness and increase towards a darker chroma.

Table 1: Effect of edible coatings on colour parameters (L*, a*, b*) of ‘Maluma’ avocado fruit stored in cold storage for 3 weeks (5.5 °C) then stored at ambient conditions (21 ± 1 °C) for one week. Values and standard error in the table represents means of 12 fruit

L*	0	1	2	3	4	P value	LSD	CV%
Control	30.45 ± 0.39 hij	29.56 ± 0.11 defg	29.02 ± 0.03 cde	28.29 ± 0.12 bc	23.18 ± 0.10 a			
GA 10%	31.55 ± 0.13 lmn	31.13 ± 0.11 jkl	29.56 ± 0.15 defg	28.89 ± 0.24 bcd	28.24 ± 0.19 b			
GA 15%	31.96 ± 0.19 mno	31.53 ± 0.31 lmn	30.53 ± 0.42 hijk	29.87 ± 0.28 fgh	28.24 ± 0.37 b	0.001	0.57	1.1
GA 10% + M	31.59 ± 0.26 lmn	31.23 ± 0.28 klm	30.31 ± 0.74 ghi	29.69 ± 0.83 efg	29.11 ± 0.48 de			
GA 15% + M	33.39 ± 0.32 q	33.19 ± 0.27 pq	32.57 ± 0.33 op	31.95 ± 0.30 mno	30.85 ± 0.82 ijkl			
CMC + M	32.45 ± 0.06 op	32.01 ± 0.13 no	31.49 ± 0.26 lmn	30.98 ± 0.11 ijkl	29.23 ± 0.15 def			
a*								
Control	-10.33 ± 0.02 m	-9.21 ± 0.06 ijk	-8.46 ± 0.44 fg	-7.23 ± 0.13 ce	1.30 ± 0.03 d			
GA 10%	-10.34 ± 0.01 m	-9.35 ± 0.04 ijk	-8.89 ± 0.18 ghi	- 7.99 ± 0.21 f	0.23 ± 0.60 c			
GA 15%	-10.34 ± 0.01 m	-9.50 ± 0.05 jkl	-9.01 ± 0.14 hij	- 8.68 ± 0.17 gh	0.08 ± 0.57 c	0.001	0.38	3.1
GA 10% + M	-10.32 ± 0.02 m	-9.86 ± 0.03 lm	-9.49 ± 0.17 jkl	- 9.05 ± 0.06 hij	-1.18 ± 0.39 b			

GA 15% + M	-10.33 ± 0.01 m	-10.13 ± 0.07 m	-9.90 ± 0.03 lm	-9.61 ± 0.03 kl	-2.33 ± 0.40 a			
CMC + M	-10.34 ± 0.01 m	-9.96 ± 0.10 lm	-9.55 ± 0.01 kl	-9.16 ± 0.02 hijk	-1.26 ± 0.45 b			
<hr/>								
b*								
Control	14.08 ± 0.02 m	12.56 ± 0.09 h	11.18 ± 0.03 f	9.98 ± 0.03 e	3.19 ± 0.05 a			
GA 10%	14.08 ± 0.01 m	12.79 ± 0.29 hi	12.02 ± 0.53 g	10.79 ± 0.52 f	3.75 ± 0.33 b			
GA 15%	14.08 ± 0.01 m	13.30 ± 0.16 jk	12.50 ± 0.14 h	11.88 ± 0.10 g	4.13 ± 0.17 b	0.001	0.34	1.8
GA 10% + M	14.08 ± 0.02 m	13.54 ± 0.11 kl	13.21 ± 0.06 ijk	12.70 ± 0.18 h	5.48 ± 0.23 c			
GA 15% + M	14.08 ± 0.02 m	13.86 ± 0.01 lm	13.59 ± 0.02 kl	12.93 ± 0.10 hij	7.14 ± 0.50 d			
CMC + M	14.08 ± 0.01 m	13.56 ± 0.1 kl	13.17 ± 0.04 ijk	12.82 ± 0.19 hi	5.53 ± 0.24 c			

CV%, coefficient of variation; LSD, least significant difference; *p* value presents the overall statistics between coatings and storage time; data reported with letters show the significance of difference if different from each other based on Fisher's analysis.

3.3.4 Sensory evaluation

Consumer acceptability of produce coated with surface coating is still a major concern, as coatings may change the organoleptic properties of coated produce (Guerreiro *et al.*, 2015). This makes sensory evaluation an important factor in the development of edible coatings. Fig. 4 shows the sensory evaluation scores of colour, taste, mouthfeel, odour and overall acceptability of each treatment. Statistical analysis demonstrated that the edible coating significantly ($p \leq 0.01$) influenced all tested parameters.

The sensory quality varied among fruit coated with GA 10%, GA 15%, GA 10% + M, GA 15% + M and CMC + M. However, fruit coated with CMC + M attained high scores in colour, taste, and mouthfeel, had no off flavours and had the highest overall acceptability compared to all other treatments. While GA 15% + M was the most effective coating in reducing mass and firmness loss and in retaining fruit colour, it was the least effective treatment in sensory fruit quality as the treatment attained lower scores during sensory evaluation. This might suggest that the coating was too thick to allow for gaseous movement resulting in the development of anaerobic conditions. This argument is supported by Ali *et al.* (2010) who reported that an increase in gum arabic concentration to 15% and 20% resulted in tomatoes developing poor pulp colour, inferior texture and off flavours.

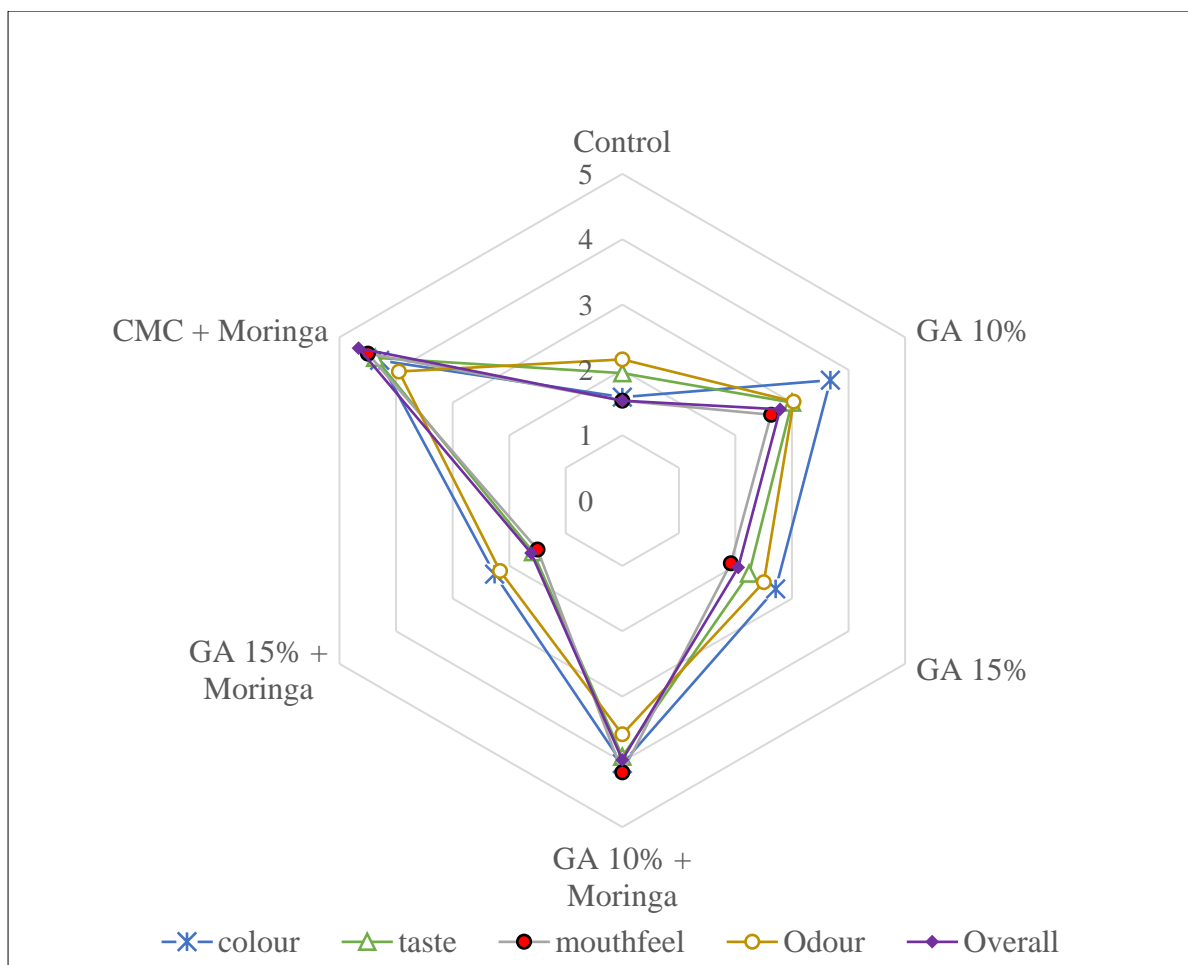


Fig. 3: Sensory evaluation scores of colour, taste, mouthfeel, odour and overall acceptability of ‘Maluma’ avocados treated with different edible coatings after 3 weeks at cold storage (5.5 °C) and further stored a week at ambient conditions (21 ± 1 °C). Mean of 37 panellists for each sample using the following hedonic scale 1 = Disliked extremely; 2 = disliked slightly; 3 = neutral; 4 = liked slightly; 5 = liked extremely.

3.3.5 Pearson correlation

Correlation coefficients (r) describing the relationship between studied parameters, both instrumental and sensory quality are shown in table 2. A strong negative correlation between firmness and mass ($r = -89, p < 0.0001$) was observed. This is in agreement with Tesfay and Magwaza (2017); Tesfay *et al.* (2017) who demonstrated that mass loss and firmness are inversely related, while Maftoonazad and Ramaswamy (2005) reported that a^* has a positive relationship with mass loss. Strong correlation between L^* and b^* ($r = 87, p < 0.00001$) was observed while correlation between L^* and a^* ($r = 66, p < 0.0001$) was positive but moderate. Strong correlation between taste and mass loss ($r = 77, p < 0.00001$) while correlation between

taste and firmness was intermediate ($r = 0.65, p < 0.0005$). Among sensory attributes correlation was intermediate but positive. Correlation revealed an intermediate relationship between taste and overall acceptability ($r = 67, p < 00001$).

Table 2: Pearson correlations among physical qualities and sensory quality of avocado fruit

	F	M	L	a	b	Cr	T	MF	O	OA
F	1									
M	-0.89***	1								
L*	0.88***	-0.88***	1							
a*	0.79***	0.62**	0.66***	1						
b*	0.82***	-0.92***	0.87***	0.71***	1					
Cr	0.66***	0.76***	0.75***	0.51*	0.67***	1				
T	0.65**	0.77***	0.73***	0.59**	0.74***	0.65***	1			
MF	0.41*	0.65***	0.65**	0.35ns	0.58**	0.56***	0.57***	1		
O	0.66***	0.79***	0.77***	0.61**	0.75***	0.63***	0.71***	0.59***	1	
OA	0.44*	0.57***	0.55**	0.32ns	0.55**	0.65***	0.67***	0.67***	0.72***	1

***, **, *Significance at $p < 0.0001$, $p < 0.0005$, and $p < 0.01$, respectively; ns, not significant.

F= Firmness, Cr = Colour, T = Taste, MF = Mouth feel, O = Odour, OA = Overall acceptability

3.4 Conclusion

Overall, the study demonstrated that edible coatings incorporated with moringa leaf extract reduced mass loss, retained firmness and delayed colour changes in 'Maluma' avocado compared to the control. The results showed that GA 15% + M was the most effective treatment in reducing mass loss and firmness loss as well as delaying colour changes. However, sensory evaluation showed that GA 15% +M had a negative impact on sensory quality attributes, while CMC + M and GA 10% + M maintained the sensory quality of avocado fruit during storage. This study shows that edible coatings containing moringa leaf extract could be useful in the avocado industry for maintaining postharvest quality and extending the shelf-life.

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Chapter 4:

Investigation the effect of carboxymethyl cellulose and gum arabic edible coatings incorporated with moringa leaf extract on phytochemical and antioxidants activities of ‘Maluma’ avocado fruit

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Abstract

This study evaluated the efficacy of Carboxymethyl cellulose (CMC) and gum arabic (GA) edible coatings incorporated with moringa (M) leaf extract on phytochemical and antioxidants contents of avocado fruit. ‘Maluma’ avocados harvested at commercial maturity were dipped into the following treatments: GA 10%, GA 15%, GA 10% + M, GA 15% + M and CMC 1% + M while uncoated fruit served as control. Fruit were thereafter stored at 5.5 °C for 3 weeks and later at 21°C for further one week, with sampling performed on weekly basis. The changes in the concentrations of total phenolic (TPC), flavonoids, vitamin C, polyphenol activity (PPO), D-mannoheptulose as well as perseitol were evaluated. The antioxidant activity was determined using 2, 2- diphenyl-1-picryl hydrazyl (DPPH) and ferric reducing ability of plasma (FRAP) assays. Pearson correlation coefficient was used to correlate relationship between all the measured phytochemical and antioxidant attributes. The findings showed that the edible coatings significantly ($p \leq 0.01$) reduced PPO activity, reduced accumulation of TPC, flavonoids and retained vitamin C, perseitol and maintained high antioxidant activity. Notably, GA 15% + M was the most effective treatment followed by CMC + M and GA 15% + M in retaining antioxidant levels in avocado fruit. Pearson correlation showed that vitamin C had a strong positive relationship ($r = 98$) with both DPPH and FRAP assay, while D-mannoheptulose and perseitol had strong negative relationship with TPC ($r = -73$ and -69), flavonoids ($r = -91$ and -85), PPO ($r = -91$ and -89), respectively. Moreover, DPPH and FRAP

had a strong correlation ($r = 96$). The CMC incorporated with moringa leaf extract could be potentially used as an organic edible coating for avocado.

Keywords: *Persea americana*, ripening, reactive oxygen species, phenylalanine ammonia-lyase, postharvest physiology

4.1 Introduction

Avocado (*Persea americana* Mill.) is regarded as one of the most nutrient-rich tropical and subtropical fruit. Thus, there has been a significant increase in both production and consumption of the fruit over the last decade. This is not only attributed by the pleasant sensory attributes but also mainly due to the health benefits associated with the consumption of avocado fruit and its derived products. These health benefits include prevention of non-communicable diseases such as cancer (Rodríguez-Carpena *et al.*, 2011), cardiovascular disease and diabetes (Lu *et al.*, 2009; Magwaza and Tesfay, 2015). These health benefits are mainly contributed by the high level of unsaturated fatty acids, low cholesterol levels (Zhang *et al.*, 2013), proteins, antioxidants, minerals, and other phytochemicals found in avocado fruit (Villa-Rodríguez *et al.*, 2011).

Vitamin C, phenolic compounds are among phytochemicals with antioxidant activity found in the flesh of avocado fruit. Phenols have been reported to increase with fruit ripening mainly attributed by the increase in phenylalanine ammonia-lyase (PAL) activity. PAL is an enzyme initiating the synthesis of phenols, while the decline in phenols is attributed by polyphenol oxidase (PPO) which catalyses the oxidation of phenolic compounds into quinones that polymerise into brown pigments (Gómez-López, 2002) or a decrease in tannins (Stafford and Lester, 1980). Also, high PAL activity is associated with the accumulation of volatile compounds, anthocyanins and carotenoids (Cheng and Breen, 1991). Besides vitamin and phenolic compounds, avocados have antioxidant enzymes such superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) in which all plays a critical role in scavenging reactive oxygen species (ROS) such as hydroxyl radicals (OH^\cdot) and hydrogen peroxide (H_2O_2) (Tefay, 2009; Tesfay *et al.*, 2010; Rodríguez-Carpena *et al.*, 2011).

In addition, avocado fruit have been reported to have high concentration of carbon seven (C7) carbohydrates (D-mannoheptulose and its reduced polyform perseitol) (Liu *et al.*, 2002;

Tesfay, 2009; Tesfay *et al.*, 2012), which has been reported to be efficient scavengers of free radicals (Liu *et al.*, 1999; Tesfay *et al.*, 2010). The toxicity of ROS is due to their oxidative reactions with various cell defense components resulting in lipid peroxidation, protein break down, cell wall degradation and inactivation of enzymes (Barka, 2001; Bertling *et al.*, 2007). Under normal metabolism, antioxidants provide adequate cellular protection against ROS. But when stress is exerted on the fruit ROS overcomes the defense mechanism provided by antioxidant systems resulting in oxidative stress.

Fruit ripening is described as an oxidative phenomenon and in the case of climacteric fruit such as avocados, the increase in respiration during climacteric peak results in accumulation of ethylene and over production of ROS (Rosalie *et al.*, 2015). Moreover, accumulation of ROS results decrease in C7 sugars, increase antioxidant activity, activation of antioxidant enzymes and also the increase in activity of cell degrading enzymes such as in phenylalanine ammonia-lyase (PAL), cellulose, amongst the few (Bower and Cutting, 1988; Goulao and Oliveira, 2008; Tesfay and Magwaza, 2017). As a result of high activity of cell degrading enzymes fruit quality deteriorates at a faster rate. Therefore, there must a balance between ROS production and their regulation by antioxidant systems in order to reduce oxidative stress linked with fruit ripening.

Over the years it has demonstrated that any postharvest treatment that can retard respiration has a potential of maintaining the antioxidant levels and retard enzyme responsible for the production of free radicals. The development of sustainable and consumer-friendly postharvest treatments to maintain postharvest quality of fresh horticultural products has become an important issue amongst food scientists and postharvest technologists. Edible coatings are ecological friendly substitutes applied on fruit surface to reduce respiration, gaseous movement, water loss and oxidation processes (Alvarez *et al.*, 2018). Studies by Maftoonazad and Ramaswamy (2005); Bill *et al.* (2014); Tesfay *et al.* (2017) demonstrated the effectiveness of edible coatings in reducing antioxidants loss by inhibiting respiration rates as well as the activity antioxidant-degrading enzymes such as polyphenol oxidase. In addition, edible coatings can act as carriers of active ingredients such as anti-browning agents, nutrients, colourants and antimicrobial compounds that can assist maintain and prolong postharvest fruit quality and reduce the risk of pathogen infection (Zúñiga *et al.*, 2012).

Among edible coatings, carboxymethyl cellulose (CMC) and gum arabic (GA) have been reported to retain antioxidants of various fruits. In fact, Gol *et al.* (2015a) reported that 1% GA

retained total phenols, vitamin C and maintained sensory fruit quality of carambola fruit compared to control. On the other hand, CMC has been reported to retain C7 sugars, delay PPO activity and lipid peroxidation (Tesfay and Magwaza, 2017). In addition, the ability of edible coatings to be carriers of active ingredients has been reported to improve their performance in maintaining antioxidants levels and in some cases, increase antioxidants levels (Aloui *et al.*, 2014; Tesfay *et al.*, 2017; Nair *et al.*, 2018). Ponce *et al.* (2008) demonstrated that incorporation of rosemary and olive into chitosan edible coating improved antioxidant content of squash and prevented browning reaction associated with quality loss.

Previous studies by Tesfay *et al.* (2017); Adetunji *et al.* (2013) have demonstrated the effect of CMC and moringa leaf extract on fruit quality and physiological response to postharvest treatment. Moreover, postharvest treatment may be cultivar specific. Moreover, the impact of these edible coatings on phytochemical and antioxidant attributes during storage and shelf-life remains unknown. Therefore, this study was conducted to investigate the effect of carboxymethyl cellulose (CMC) and gum arabic (GA) incorporated with moringa leaf extract on phytochemical contents and antioxidant activities of 'Maluma' avocado.

4.2 Materials and methods

A total of 180 avocado fruit (cv. 'Maluma') used in this study were procured from a commercial orchard at ZZ2 (Pty) LTD in Limpopo Province, South Africa. Using dry matter as maturity index (DM), 'Maluma' avocado fruit were harvested at commercial maturity where the mean DM content was 30%. Harvested fruit were packed in open display boxes and transported overnight in a ventilated vehicle to the Postharvest Laboratory of the University of KwaZulu-Natal, Pietermaritzburg campus.

Upon arriving at the laboratory, fruit were washed with distilled water. Fruit were then assigned to six postharvest treatments, namely, control (untreated), GA 10%, GA 15%, GA 10% + moringa 10%, GA 15% + moringa + 10% and CMC 1% + moringa 10%. Each treatment had 3 replicates of 10 fruit per replicate. Fruit were dipped in treatment solutions for one minute, and air dried on a laboratory bench at room temperature (21 ± 1 °C) for 30 – 45 minutes. After drying, fruit were packed into commercial boxes and transferred into a cold room which had a delivery air temperature set at 5.5 °C and relative humidity (RH) set at $90 \pm 2\%$ for 3 weeks,

simulating shipping conditions. After 3 weeks of cold storage, fruit were transferred to ambient conditions (21 ± 1 °C, RH 60%) for 7 days, simulating ripening and retail conditions. Samples for analysis (mesocarp) were taken in four fruits per replicate on a seven days interval. They were stored at -26 °C prior to freeze-drying using a Virtis Benchtop freeze drier system (ES Model, SP industries Inc., Warmister, USA) for 5 days a 0.015 kPa and -75 °C. Samples were thereafter ground into powder using pestle and mortar. The ground samples were stored at -26 °C until all biochemical analysis was conducted.

4.3 Measurements of anti-oxidant levels

4.3.1 Total phenolic content

Total phenolics (TP) were extracted according to Mokrani and Madani (2016) with slight modification. Briefly, 0.2 g ground avocado sample was extracted with 10 ml ethanol (v/v). The solution was kept at room temperature with constant vortexing and centrifuged at 10 000 rpm for 15 minutes at 4 °C to obtain clear extracts. The clear extracts were collected and stored at -26 °C for further analysis of flavonoids.

Total phenolics were analysed spectrophotometrically using the modified Folin-Ciocalteu method (Marinova *et al.*, 2005). Aliquot (1 ml) was added into 5 ml of distilled water and 1 ml of Folin-Ciocalteu reagent and the solution was allowed to stand for 5 minutes. Thereafter, 10 ml of 7% (w/v) of sodium carbonate (Na_2CO_3) was added and the volume was topped to 25 ml with distilled water. The sample were then placed in the dark at room temperature for 2 hrs. The absorbance was measured at 725 nm using UV-1800 Spectrophotometer (Shimadzu Scientific Instruments INC., Columbia, USA) against ethanol as a blank. TP was measured using gallic acid and expressed as mg GAE/g DM.

4.3.2 Flavonoids

Total flavonoid content was determined by following the method of Atanassova *et al.* (2011) with slight modification. Briefly, plant extract (0.1 ml) was added to 0.3ml distilled water, followed by the addition of 0.003 ml of 5% (w/v) sodium nitrate (NaNO_2) and the solution was allowed to stand for 5 min at room temperature. Thereafter, 0.003 ml of 10% (w/v) of

aluminium chloride (AlCl_3), on the sixth minute 0.2 ml of 1 mM of sodium hydroxide (NaOH) and the total volume was made up to 10 ml with distilled water. Absorbance was measured at 510 nm using UV-1800 Spectrophotometer (Shimadzu 52 /Scientific Instruments INC., Columbia, USA) against ethanol as a blank. The results were expressed as mg GAE/g DM.

4.3.3 Vitamin C

Vitamin C was determined using 2, 6 dichloro-Indophenol (DCPIP) according to (Boonkasem *et al.*, 2015). Briefly, 0.5 mg of freeze-dried mesocarp powder was extracted using 10 ml of 3% (w/v) metaphosphoric acid, followed by vortexing for 1 minute. The extracts were centrifuged at 10 000 rpm for 15 minutes at 4 °C to obtain clear extracts. Thereafter, 1 ml of the extract was added into 3 ml of 0.2 mM DCPIP and read at 515 nm. The results were expressed in mg ascorbic acid per 100g dry weight (mg AsA/100 g DW).

4.3.4 Carbohydrates

To extract soluble sugars, freeze-dried mesocarp powder (0.1 g) was added to 10 mL of 80% (v/v) ethanol and homogenised for 1 min. The mixture was then placed for 60 min in a water bath at 80 °C. Subsequently, the mixture was stored in a refrigerator at 4 °C overnight to facilitate the release of soluble sugars. The mixture was then centrifuged at 4 °C for 15 minutes at 12000g. The supernatant was filtered through glass wool and the filtrate taken for drying under vacuum in a GenVac® concentrator (SP Scientific, Genevac LTD., Suffolk, UK). Dehydrated samples were re-constituted using 2 mL ultra-pure water and filtered through a 0.45 µm nylon syringe filter into an HPLC vial. Sugars were analyzed using an isocratic HPLC system equipped with a refractive index detector, according to Tesfay *et al.* (2010). Sample extracts were injected into a Rezex RCM monosaccharide Ca^+ (8%) column of 7.8 mm diameter × 300 mm (Phenomenex, Torrance, CA, USA) with a Carbo- Ca^{2+} guard column of 3 mm × 4 mm (Phenomenex). The column temperature was kept at 80 ° C using a thermoregulated column compartment. The mobile phase was ultra-pure water at a flow rate of 0.6 mL/min. The concentration and the presence of individual sugars (mannoheptulose, and perseitol) were determined by matching peak areas of samples with the peak areas and concentration of the standard curves (0.05–1.25 mg/L; $R^2 = 0.99$).

4.3.5 Polyphenol oxidase

The extraction of polyphenol oxidase (PPO) was conducted according to a protocol by Bi *et al.* (2015) with slight modifications. Briefly, 1 g freeze-dried mesocarp powder was homogenised in 10 ml of 0.1 M sodium phosphate buffer (pH 6.5) containing 1% (w/v) polyvinyl polypyrrolidone (PVPP).

The homogenates were then allowed to stand for 3 min in water bath at 25 °C then centrifuged (Centrifuge 5810R, Eppendorf AG, Germany) at 10 000 g at 4 °C for 15 minutes. The supernatants were then used as enzyme source. Reaction mixture was composed by mixing 1.5 ml of 40 mM 4-methyl-catechol and 2.3 ml of 0.1 M sodium phosphate buffer (pH 6.5) without PVPP, the mixture was allowed to stand for 5 minutes in water bath at 25 °C. Then, 0.2 ml of enzyme extract was added, and absorbance was read immediately and after 3 minutes of reaction at 420 nm using UV-1800 Spectrophotometer (Shimadzu Scientific Instruments INC., Columbia, USA). PPO activity was expressed as U/kg DM determined using equation 1, where one unit (U) of PPO activity was defined as an increase of 0.01 units of absorbance per min.

$$PPO \text{ activity (U/kgDM)} = (0.01(Abs_{3 \text{ min}} - Abs_{0 \text{ min}})/min)/(g \text{ DM})$$

eq. (1)

4.3.6 DPPH

The hydrophilic extracts were obtained according to a protocol by Kalita *et al.* (2013) with slight modifications. About 0.5 g of mesocarp powder was homogenised in 10 ml of 80% (v/v) methanol using an Ultra Turrax digital homogenizer (IKA, model T 25 D). The mix was then sonicated for 30 minutes and centrifuged (Centrifuge 5810R, Eppendorf AG, Germany) at 10 000 rpm for 15 minutes at 4 °C. The aliquots were collected and stored at -26 °C for analysis by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. The assay was prepared according to Genskowsky *et al.* (2015) with slight modifications. DPPH (1 mM) methanolic stock solution was prepared. About 10 ml of the stock solution was diluted to produce 0.1 mM of DPPH working solution. Aliquot (200 µL) was added to test tube containing 800 µL of 80 % methanol, then 1 ml of 0.1 mM DPPH solution was added. Blank solution was prepared by omitting aliquot from the reacting solution. The test tubes were covered with aluminium foil and placed in the dark for 30 min at room temperature and thereafter absorbance was measured at 517 nm

using UV-1800 Spectrophotometer (Shimadzu/Scientific Instruments INC., Columbia, USA) against methanol as a blank. Activity of antioxidants was determined as percentage of radical scavenging activity (% RSA) as per equation 2.

$$\%RSA = \frac{Absblank - Absextract}{Absblank} * 100 \quad \text{eq. (2)}$$

4.3.7 FRAP

The samples were extracted according to Thaipong *et al.* (2006), with slight modifications. Briefly, 70:29.5: 0.5 (v/v) ethanol: water (HPLC grade): hydrochloric acid was used as an extraction solvent. Each sample of 0.5 mg was weighed into test tube and 3 ml of 70% ethanol was added. The mixture was then placed in an incubator shaker at temperature 35 °C for 90 minutes. Samples were then allowed to cool down then centrifuged at 3200 rpm for 20 minutes to obtain a clean solution which was then filtered using 0.2 µm filter. Before analysis samples were diluted (1:19) using de-ionised water.

The FRAP assay was carried out according to Wang *et al.* (2016) with slight modifications of. To prepare the FRAP reagent, a solution of 300 mM sodium acetate (pH 3.6), 10 mM 2, 4, 6-tripyridyl-2-triazine (TPTZ) and 20 mM ferric chloride (10:1:1, v/v/v) was made. An aliquot of 50 µl was added to 3.6 ml of working FRAP reagent and mixed thoroughly. After the reaction had been left at 37 °C for 10 min, the absorbance at 593 nm was determined. Calibration was based on concentrations of ferrous ion from 2 mM using freshly prepared ammonium ferrous sulphate. Results were reported as µmol Fe²⁺ g⁻¹ FW, using ferric chloride standard curve obtained from concentration ranging between 0 to 70 mg/ml with R² = 0.981

4.3.8 Statistical analysis

The collected data were subjected to the analysis of variance (ANOVA) using GenStat statistical software (GenStat®, 18.1 edition, VSN International, UK) 17. Mean separation was performed using Fischer's least significant difference (LSD) at 5% level of significance. Standard error values were calculated when a significant standard deviation was found at $p \leq 0.05$ between individual values. The Pearson correlation coefficient between the parameters was established using Statistical Analysis System 9.3 (SAS Institute Inc., Cary, NC, USA).

4.4 Results and discussion

4.4.1 Total phenolic content

Phenolics play a key role in fruit quality as they have effect on quality attributes such as colour, taste, flavour and are also major players in fruit defence system against reactive oxygen species and any stress that can be exerted on the fruit (Tomás- Barberán and Espín, 2001). In the current study, an interaction between edible coatings and storage period had a significant effect ($p \leq 0.001$) on total phenolic accumulation (Fig. 1). This interaction could be explained by the rapid phenolic accumulation in control fruit. Unlike control, edible coatings significantly delayed accumulation of total phenols in avocado fruit. The initial concentration of total phenols was 0.40 GAE/g, after week 1 in cold storage, there were no significant changes in phenolic concentration in all fruit, however, significant change between coated and control fruit was observed from week 2 onwards. For both coated and control fruit, greatest total phenolic increase was observed during the last week (week 4) of storage period.

This increase could be associated with high metabolic activity experienced by fruit at ambient condition as compared to cold storage. This corresponds with the findings of Kassim *et al.* (2013) who explained that avocado fruit stored under cold storage display low rate of metabolic activity compared to those stored under ambient conditions.

At the end of the storage period, TPC in avocado fruit was 0.83 GAE/g, 0.96 GAE/g, 0.63 GAE/g, 0.59, GAE/g, 0.52 GAE/g and 0.59 GAE/g for control, GA 10%, GA 15%, GA 10% + M, GA 15% + M and CMC + M, respectively. The ability of coatings to minimise accumulation of total phenols could be attributed to modified atmospheric condition within the fruit created by the semi-permeable layer of edible coating, reducing diffusion of oxygen into the fruit. This could be corroborated by the finding of Kim *et al.* (2007) who demonstrated that low oxygen or increased carbon dioxide resulted in a decrease of total phenols in Tommy Atkins' mango fruit stored under controlled atmospheres.

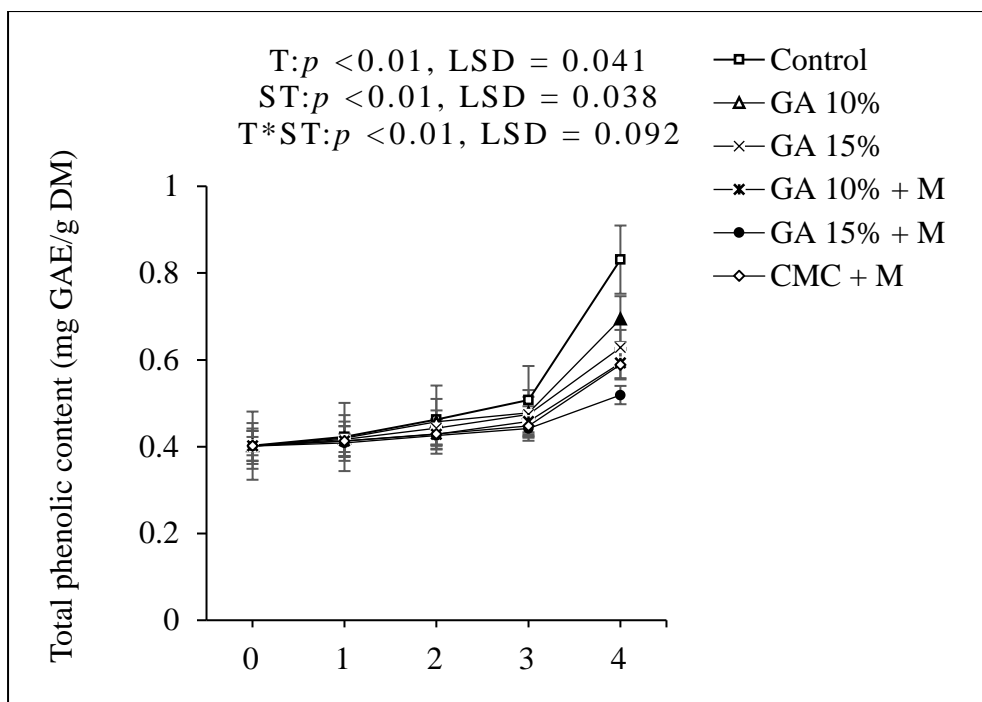


Fig. 1: Effect of edible coatings on total phenolic content of the mesocarp of ‘Maluma’ avocados stored at 5.5 °C for 3 weeks and at ambient conditions for 1 week. Vertical bars represent standard error (SE) at $n = 4$. T = Treatment, ST = Storage time.

PAL is an enzyme involved in the biosynthetic pathway of phenolic compounds (Cheng and Breen, 1991). The delay of accumulation of TPC in coated fruit could be an indication that coatings were able to retard respiration which increases with PAL activity. The effectiveness of edible coatings to retard phenolic accumulation in fruits has previously been reported. Gol *et al.* (2015b) reported that edible coatings based on chitosan (CH), alginate (AL), and carboxymethyl cellulose (CMC) reduced accumulation of total phenol in blackberry stored for 12 days at 10 ± 1 °C. Similar observation were made Dhital *et al.* (2017) who reported that methyl cellulose- limonene edible coating was able to delay changes in phenolic content of ‘Chandler’ strawberry fruit. Based on these findings, it could be hypothesized that edible coatings are not PAL inhibitors but have the ability to reduce gas permeability in turn delay changes in phenolic content.

4.4.2 Flavonoids

Flavonoids are secondary metabolites that have various important function in fresh produce such as colour, and aroma development, importantly, and they also participate in protection against biotic and abiotic stress. The interaction between coatings and storage time significantly

($p \leq 0.001$) affected flavonoid accumulation in 'Maluma' avocado. Fig. 2 represents the accumulation of flavonoids in avocado during storage period. The significant interaction could be attributed to the rapid increase in flavonoids in control fruit, contrary to the steady slow increase in coated fruit. This higher accumulation of flavonoid in control fruit may be due to oxidative stress as result of high respiration rate. This was corroborated by Nair *et al.* (2018) who explained that increase in respiration rate cause oxidative stress resulting to an the increase in PAL activity

In this study, GA 15% + M (0.31 GAE/g) significantly delayed the increase of flavonoid concentration, followed by CMC + M (0.35 GAE/g), GA 10 + M (0.36 GAE/g), GA 15% (0.40 GAE/g) and GA 10% (0.40 GAE/g). The variation in performance of gum arabic coatings could be due to the difference in coating thickness. Jiang and Li (2001) demonstrated that an increase in chitosan concentration from 0.5% to 2% improved that ability of coatings to retard respiration and subsequently maintained fruit quality. This could be attributed to the closure of stomatal openings and lenticels resulting in reduced accumulation of flavonoids. Our results demonstrated that incorporation of moringa leaf extract improved the performance, as GA without moringa leaf extract accumulated higher flavonoids as compared to those with moringa leaf extract.

Results of edible coatings observed in this study were in agreement with those of Zhang and Quantick (1997) who indicated that edible coatings based on chitosan can delay accumulation of flavonoids in litchi fruit stored at 4 °C for 30 days. Also, Nair *et al.* (2018) demonstrated that addition of pomegranate peel extract to chitosan and alginate improved the ability of coatings to retain total flavonoids and other antioxidants of guava fruit.

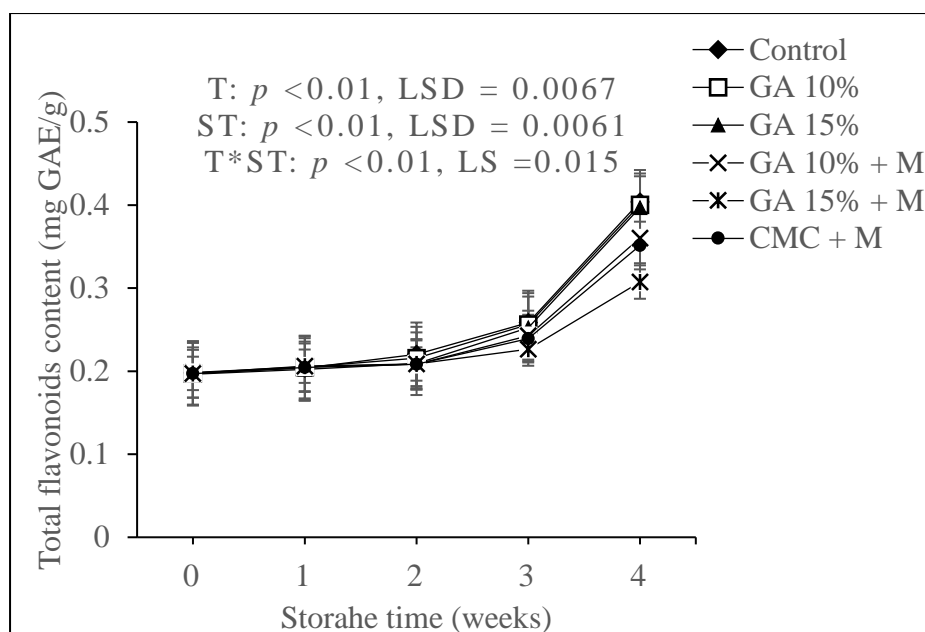


Fig. 2: Effect of edible coatings on flavonoids accumulation in the mesocarp of avocado stored at 5.5 °C for 3 weeks, then stored at ambient conditions for a further one week. Vertical bars represent standard error (SE) at $n = 4$. T = Treatment, ST = Storage time.

4.4.3 Vitamin C

Changes in vitamin C concentrations during storage are shown in Fig. 3. In this study, the interaction between storage period and edible coatings had a significant effect ($p \leq 0.001$) on vitamin C concentration. The interaction could be attributed to the rapid decline of vitamin C in control fruit in comparison to the steady decline in coated fruit. Edible coatings had a positive effect on vitamin C retention compared to the control treatment. Amongst edible coatings, GA 15% + M (18%) was the most effective treatment in retaining vitamin C followed by CMC + M (27%), GA 10% + M (31), GA 15% (34%) and GA 10% (40%) compared to the control treatment which lost more than 50% of its vitamin C content. The variation in vitamin C retention by the gum arabic coating could be attributed to the difference in gas permeability. A study by Ali *et al.* (2010) demonstrated that gum arabic (20%) was the most effective treatment in retaining vitamin C of tomato compared to 10% and 15%. Moreover, our findings are in agreement with Adetunji *et al.* (2013) who reported that incorporation of moringa leaf extract into polysaccharide coating retained high vitamin C content.

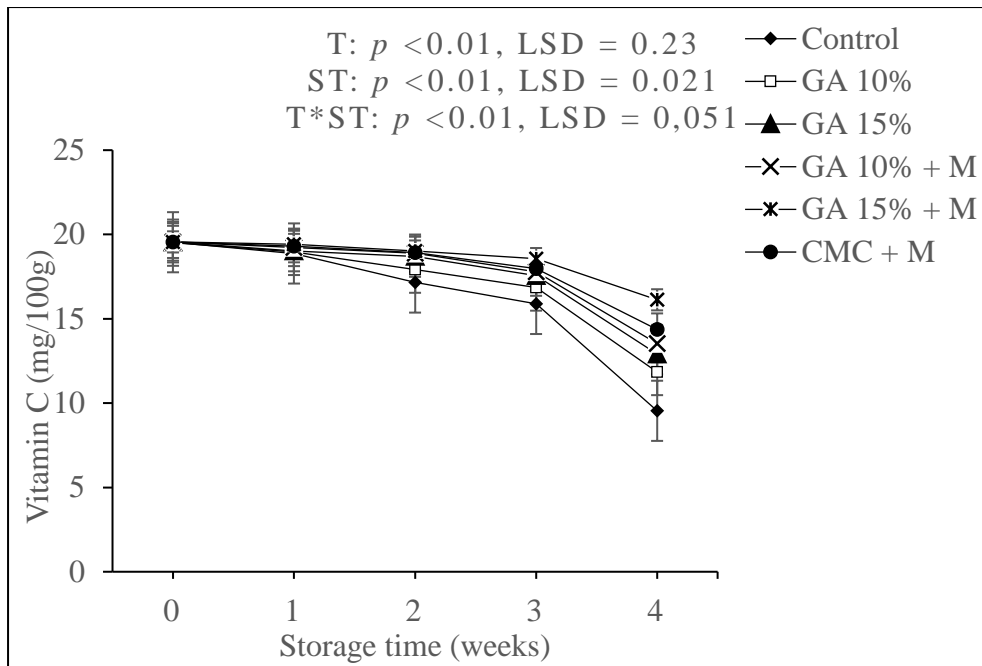


Fig. 3: Effect of edible coatings on vitamin C concentration of the mesocarp of ‘Maluma’ avocado stored at 5.5 °C for 3 weeks followed by 1 week at ambient conditions. Vertical bars represent standard error (SE) at n = 4. T = Treatment, ST = Storage time

The retention of vitamin C by coatings could be attributed to their ability to reduce diffusion of oxygen (Azarakhsh *et al.*, 2014), inhibit ripening rate (Tesfay *et al.*, 2017) as well as to reduce the activity of ascorbic acid degrading enzymes such as peroxidase (Ayranci and Tunc, 2003). These results are in agreement with those of Pagliarulo *et al.* (2016) who reported that chitosan incorporated with peony extract was able to retain vitamin C in strawberry fruit

4.4.4 Radical scavenging activity

Avocado exhibit high good antioxidant activity due to high level of phenols and the presence of carbon seven (C7) sugars (Tesfay *et al.*, 2010). The results on the effect of edible coatings and storage time on scavenging activity are presented in Fig. 4. The interaction between edible coatings and storage period significantly ($p \leq 0.01$) affected the scavenging activity of the fruit. The scavenging activity was highest in control fruit during the whole storage period while it varied significantly ($p \leq 0.01$) amongst coatings. The highest scavenging activity was observed during ambient storage conditions (week 4). This rapid increase in RSA at ambient conditions

could be linked to the high metabolic activity rates at such conditions. Rodríguez-Carpena *et al.* (2011) reported that antioxidant activity increases in avocado during ripening.

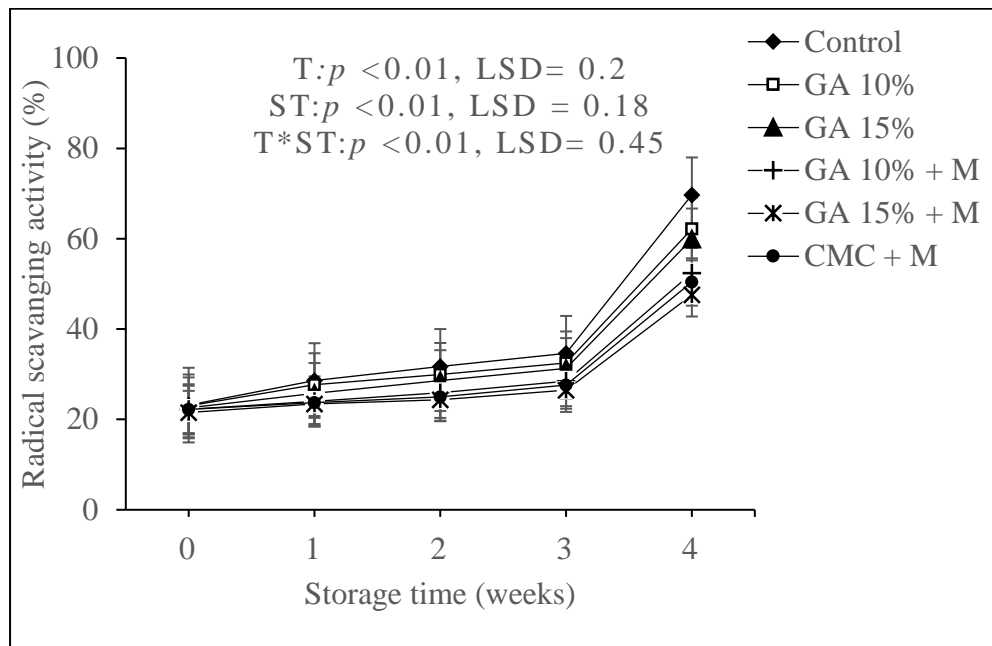


Fig. 4: Effect of edible coatings on radical scavenging activity of the mesocarp of ‘Maluma’ avocado stored at 5.5 °C for 3 weeks followed by 1 week at ambient conditions. Vertical bars represent standard error (SE) at $n = 4$. T = Treatment, ST = Storage time.

Radical scavenging activity of coated fruit was much lower compared to control treatment. The results corroborated the findings of Ali *et al.* (2013) and Ayranci and Tunc (2004) who reported that edible coatings can reduce RSA. The slow increase in RSA of coated avocado fruit could be related to delay in phenol accumulation by edible coatings. Moreover, the study supports the findings of Nair *et al.* (2018) who demonstrated that incorporation of pomegranate peel extract improved the reduced the scavenging activity of chitosan and alginate-based coatings.

4.4.5 Ferric reducing power

The FRAP assay measures the reducing potential of antioxidants reacting with ferric tripyridyltriazine (Fe^{3+} -TPTZ) producing coloured ferrous tripyridyltriazine (Fe^{2+} -TPTZ). As shown in Fig. 5, the reducing potential of avocado fruit had similar trend in antioxidant measured using DPPH assay. The interaction between edible coatings and storage time significantly ($p \leq 0.01$) influenced the antioxidant activity of avocado. After harvest (week 0)

the FRAP value was 1.63 μmol , however, this increased with storage time for all fruit but was prominent in control fruit.

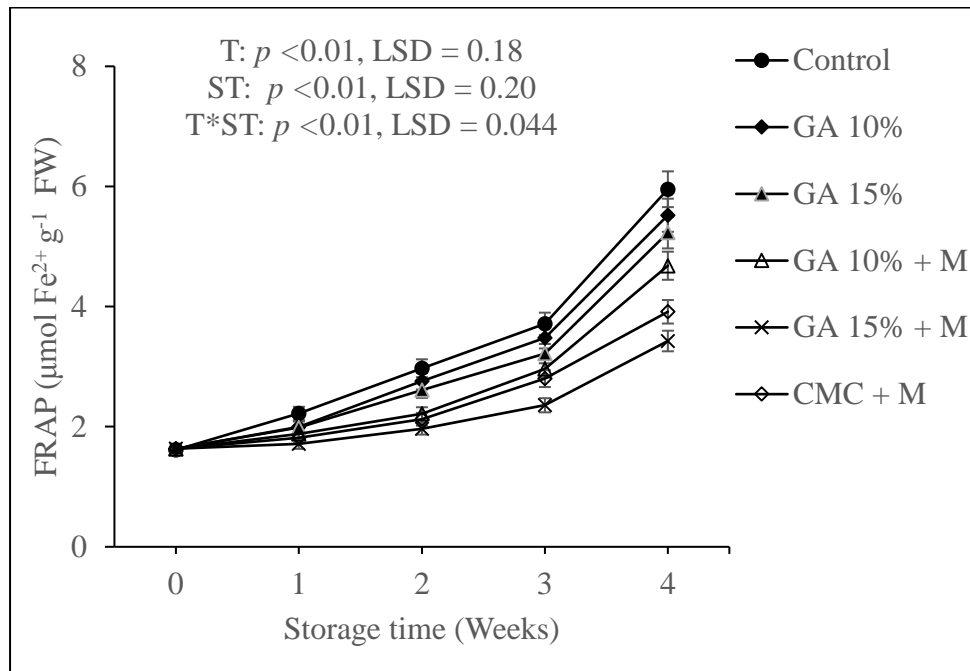


Fig. 5: Effect of edible coatings on the ferric reducing antioxidant activity of the mesocarp of ‘Maluma’ avocados stored at 5.5 °C for 3 weeks followed by 1 week at ambient conditions. Vertical bars represent standard error (SE) at $n = 4$. T = Treatment, ST = Storage time

There was a significant variation ($p \leq 0.01$) in the performance of edible coatings, with GA 15% + M (3.43 μmol) being the most effective treatment in delaying increase in FRAP values of ‘Maluma’ avocado during storage followed by CMC + M (3.91 μmol), GA 10% + M (4.68 μmol), GA 15% (5.23 μmol), GA 10% (5.53 μmol). Similar changes in total phenolics, flavonoids or vitamin C could explain the phenomenon on antioxidant activity. Edible coatings creates modified atmosphere with the fruit, resulting in reduced metabolic rate of fresh horticultural produce thus minimising the synthesis of phenolic and flavonoids (Gonzalez-Aguilar *et al.*, 2010). Aloui *et al.* (2014) demonstrated that alginate coatings enriched with grape seed extract was able to retain antioxidants in table grapes.

4.4.6 Polyphenol oxidase (PPO) activity

Soliva-Fortuny *et al.* (2002) and Quevedo *et al.* (2011) reported that PPO activity plays a role in the oxidation of polyphenolic substrates responsible for enzymatic browning in various fruits and vegetables. This enzymatic browning does not only lead to colour alteration but also result

in antioxidant degradation, organoleptic and nutritional losses (Zhang *et al.*, 2017; Tinello and Lante, 2018). Fig. 6 shows the effect of edible coating on PPO activity with storage time. PPO activity was significantly affected by coatings ($p \leq 0.01$) as well as storage time ($p \leq 0.01$) and the interaction between storage time and coating were significant ($p \leq 0.01$). The significant interaction between these two factors indicates that the differences in PPO activity caused by coatings were dependent on storage time. In this study, PPO activity increased gradually with storage period dominating over control fruit. The degree by which PPO activity increased varied in coated fruit and storage period. PPO activity was low during the cold storage period (week 0 to week 3) but increased sharply at ambient storage (week 4). This sharp rise in PPO activity can be attributed to fruit ripening at ambient conditions. During ripening, avocado fruit is characterised by a dramatic increase in ethylene, loss of firmness and increase in oxygen availability which all promote the activity of PPO (Tesfay and Magwaza, 2017).

Throughout storage, fruit treated with edible coatings had lower PPO activity compared to the uncoated fruit. Among coatings, GA 15 + M (0.44 U/kg) was the most effective coating as it had the least PPO activity compared to control. Among coatings, GA 15 + M (0.44 U/kg) was the most effective as it had the least PPO activity by the end of the storage period than CMC + M (0.49 U/kg), GA 10% + M (0.52 U/kg), GA 15% (0.61 U/kg) and GA 10% (0.71 U/kg) while control recorded PPO activity of 0.78 (U/kg). These results support the findings of Jiang *et al.* (2005) who demonstrated that chitosan based coating reduced PPO activity of litchi fruit stored at 2 °C for 20 days then moved to 25 °C for 18 hours. Similarly, Tesfay and Magwaza (2017) reported that the incorporation of moringa leaf extract into reduce PPO activity in 'Fuerte' and 'Hass' avocado fruit.

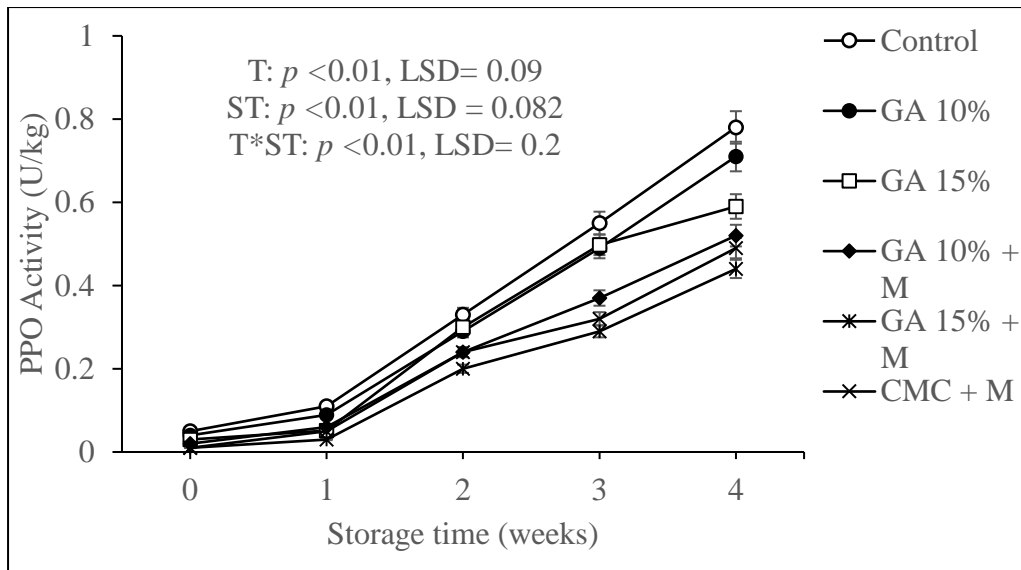


Fig. 6: Effect of edible coatings on polyphenol oxidase (PPO) activity of the mesocarp of ‘Maluma’ avocados stored at 5.5 °C for 3 weeks followed by 1 week at ambient conditions. Vertical bars represent standard error (SE) at $n = 4$. T = Treatment, ST = Storage time.

One of the major challenges compromising the postharvest quality of avocado fruit is enzymatic browning. Enzymatic browning is catalysed by PPO and it is very difficult to control due to its resistance to treatments (Gómez-López, 2002). Weemaes *et al.* (1998) reported that PPO activity of avocado is 30 times higher than that of apples. The ability of coatings to slow the increase of PPO activity suggest that coatings were able to maintain membrane integrity and regulate oxygen (O_2) diffusion. The low level of O_2 reduces the enzymatic browning and suppresses PAL activity and decrease the accumulation of phenols (Cheng *et al.*, 2009).

4.4.7 C7 sugars

The storage of carbohydrates and their use is one of the most significant aspect determining fruit quality and subsequently postharvest life as they drive and fuel metabolic activities during storage and postharvest handling. In avocado fruit, carbon seven sugars (C7) such as D-mannoheptulose and its reduced form polyol, perseitol are found in large amounts. In this study, an interaction between storage time and edible coatings had a significant ($p \leq 0.01$) effect on changes in both C7 sugars. At harvest, the concentration of C7 sugars was 12.01 g/kg and 6.24 g/kg for D-mannoheptulose and perseitol, respectively. At the end of storage time, GA 15% + M was the most effective treatment in retaining both D-mannoheptulose (4.90 g/kg) and perseitol (3.67 g/kg), while control had the lowest concentration for both C7 sugars. The high

reduction of C7 sugars in control fruit compared to relatively low declined in coated fruit could explain the interaction. Our results corroborates with those reported by Obianom *et al.* (2018) who reported that chitosan edible coating retained C7 sugars in ‘Hass’ avocado fruit.

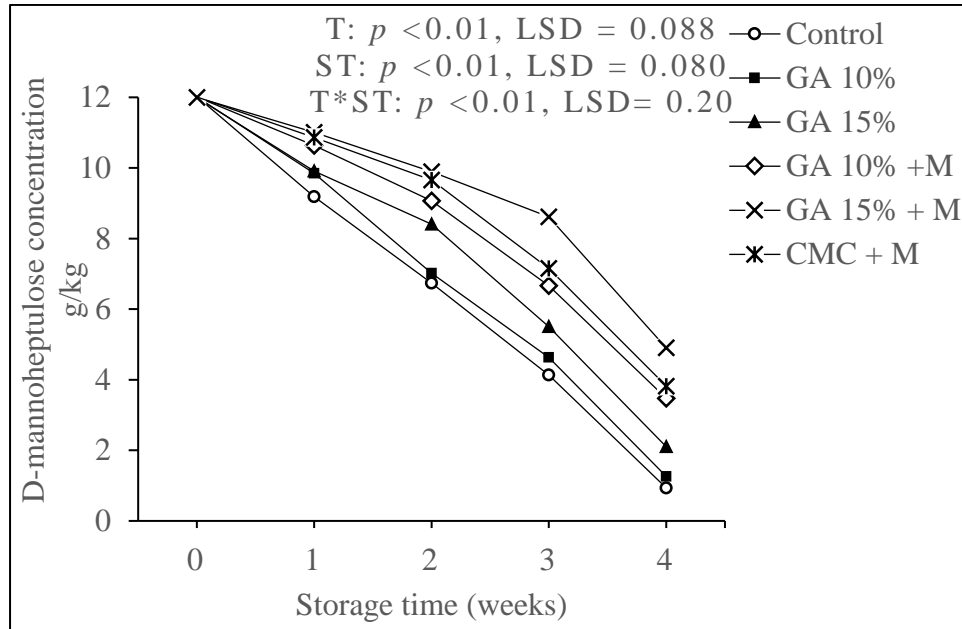


Fig. 7: Effect of edible coatings on D-mannoheptulose concentrations of the mesocarp of ‘Maluma’ avocados stored at 5.5 °C for 3 weeks followed by 1 week at ambient conditions. Vertical bars represent standard error (SE) at $n = 4$. T = Treatment, ST = Storage time

The observed slow decline in C7 sugars during cold storage corroborates the finding of Blakey *et al.* (2014) who explained that low temperatures slows down metabolic activity and delay ripening in avocado fruit thus retaining C7 sugars. Carbon seven sugars decline has been reported to decline with fruit ripening (Liu *et al.*, 1999; Tesfay, 2009; Tesfay *et al.*, 2010). Liu *et al.* (2002) reported that a correlation exists between induction of avocado ripening and D-mannoheptulose and perseitol decline. They further demonstrated that an increase in respiration rate and ethylene production associated with onset of avocado ripening, were not initiated until C7 level; sugars dropped to below 20 mg/g. This suggest that any postharvest treatment that can retain C7 sugar levels in avocado fruit has the ability to retain antioxidants levels and delay ripening. Studies by Tesfay and Magwaza (2017) and Obianom *et al.* (2018) reported that polysaccharide based coating retained C7 sugars in avocado thus delaying fruit ripening. Apart from delaying ripening, C7 sugars have been reported to be efficient scavengers of free radicals (Liu *et al.*, 1999). Bertling *et al.* (2007) reported that the deterioration of postharvest quality in

‘Hass’ avocado can be attributed to the decline in C7 sugars that can counteract the oxidative stress and slow tissue browning.

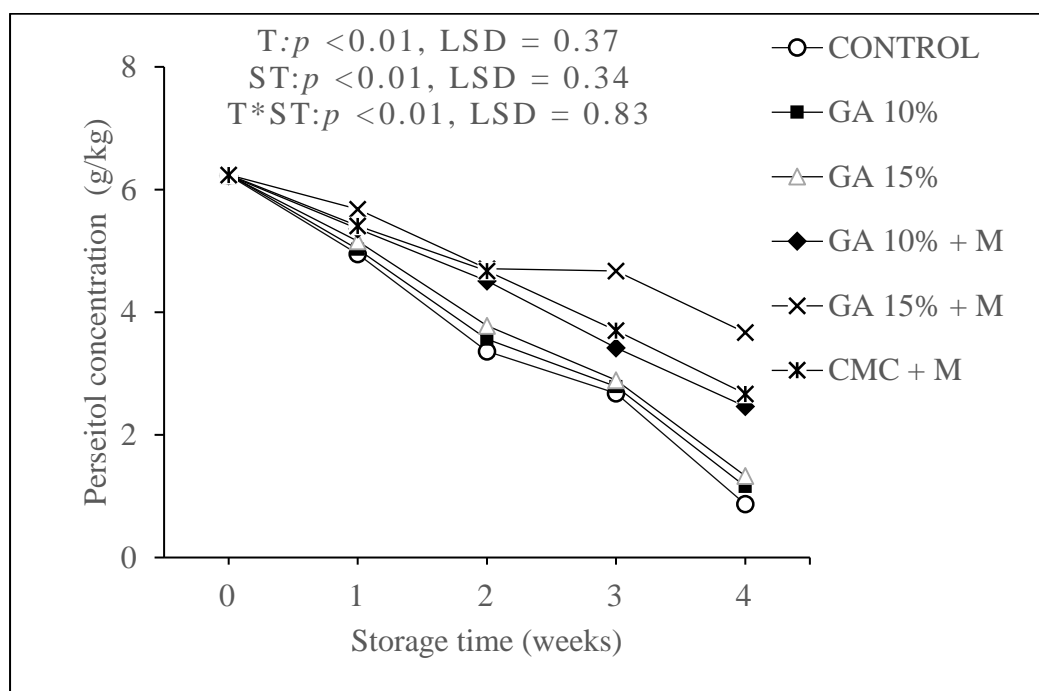


Fig. 8: Effect of edible coatings on perseitol concentrations of the mesocarp of ‘Maluma’ avocados stored at 5.5 °C for 3 weeks followed by 1 week at ambient conditions. Vertical bars represent standard error (SE) at $n = 4$. T = Treatment, ST = Storage time

4.4.8 Correlations

A strong correlation among all phytochemicals and antioxidant activity was observed (Table 1). The correlation test showed a positive but moderate relationship between TPC and PPO ($r = 69, p < 0.05$). A strong negative correlation was observed between TPC, flavonoids, PPO with respect to both C7 sugars. This allude to the findings of Tesfay *et al.* (2011) and Liu *et al.* (1999) of reported that mannoheptulose and perseitol can act as antioxidants and play a significant role in avocado ripening. It is interesting to note that DPPH and FRAP correlated well ($r = 96, p \leq 0.001$), given that the functioning of both methods is different, as DPPH is responsible for RSA while FRAP Fe^{3+} is reduced to Fe^{2+} (Genskowsky *et al.*, 2015). The restriction in loss of vitamin C was found to be strongly correlated ($r = 98, p \leq 0.001$) with both DPPH and FRAP. This is in agreement with Proteggente *et al.* (2002) who reported that vitamin C play a significant role in total antioxidant capacity of regularly consumed fruit and vegetables. As the fruit undergoes ripening, antioxidants capacity declines as results of the loss

of phytochemicals. Further, these changes contribute to various metabolic reactions leading to oxidative changes and senescence.

Table 1: Pearson’s correlation coefficients (r) among investigated parameters of ‘Maluma’ avocados stored at 5.5 °C for 3 weeks followed by 1 week at ambient conditions

	TPC	Flav	Vitamin C	DPPH	FRAP	PPO	D-manno	Perseitol
TPC	1.00							
Flav	0.80**	1.00						
Vitamin C	-0.83**	-0.97**	1.00					
DPPH	0.79*	0.96**	-0.98**	1.00				
FRAP	0.79*	0.96**	-0.98**	0.96**	1.00			
PPO	0.69*	0.84*	-0.85*	0.82**	0.89*	1.00		
D-manno	-0.73*	-0.91*	0.92*	-0.90*	-0.97**	-0.91**	1.00	
Perseitol	-0.69*	-0.85*	0.87*	-0.84*	-0.93**	-0.89*	0.96*	1.00

* $p < 0.001$; ** $p < 0.05$, TPC = Total phenolic content, Flav = Flavonoids, PPO =Polyphenol oxidase, D-manno = D –mannoheptulose

3.5 Conclusion

The results from this study confirms that edible coatings are effective in retaining antioxidants levels of ‘Maluma’ avocado. The results showed that edible coatings incorporated with moringa leaf extract reduced the loss of antioxidants and phytochemicals. The study demonstrated the potential of GA 10% + M and CMC 1% + M 10% as an organic postharvest treatment for ‘Maluma’ avocado. However, GA 15% + M was the most effective treatment in retaining all antioxidants but in the previous chapter it was demonstrated that GA 15% + M resulted in development of off-flavours in the same fruit. Further research is still needed to

determine a much effective concentration of gum arabic, before it could be recommended for commercial application.

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Chapter 5:

Antifungal effect of gum arabic incorporated with moringa leaf extract against *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae* in avocado fruit

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Abstract

Avocado fruit has high economic value; however, major postharvest losses are encountered throughout the supply chain mostly due to anthracnose disease and stem-end rot caused by *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae*, respectively. Increase in consumer concern regarding food safety and demand for organic foods make it necessary for researchers develop environmental friendly alternative products in a quest to reduce postharvest decay and losses. This study investigated the effect of gum arabic (GA) and moringa leaf extract (M) as well as their combination against the mycelial growth of avocado postharvest pathogens that cause fruit decay. The antifungal effect of GA 10%, GA 15%, GA 10% + M, GA 15% + M and M were tested against isolates of these pathogens isolated from infected avocado. Moringa leaf extract, GA 10% + M and GA 15% + M inhibited radial mycelial growth of *C. gloeosporioides* by 30%, 28% and 33%, respectively. Additionally, all treatments showed little or zero inhibition against *L. theobromae* with GA 15% + M being the most effective treatment against mycelial growth of this pathogen showing a 6.5% inhibition. Gum arabic edible coating alone had little inhibition effect against *C. gloeosporioides* and had zero inhibition against *L. theobromae*. The results demonstrated the potential of GA 15% + M in controlling both pathogens. However, there is still a need to test the treatments in infected fruit and also find ways of improving the efficacy of this treatment against *L. theobromae*.

Keywords: Postharvest disease, *In vitro*, postharvest quality, avocado, decay, moringa, gum Arabic

5.1 Introduction

Avocado (*Persea americana* Mill.) fruit is among the few exceptional fruit, as it does not ripen while still attached to the tree. As such, avocado fruit are picked once matured in order to withstand postharvest handling conditions, especially long distance shipping (Magwaza and Tesfay, 2015). While unripe fruit usually do not show any symptoms of postharvest disorders, this becomes more visible with fruit ripening. The rate of postharvest disorders increases with fruit ripening (Hopkirk *et al.*, 1994). This can be attributed to the high rate of postharvest metabolic activities of avocado fruit.

Fungal pathogens are the most common causes of postharvest diseases and decay in avocado fruit. Anthracnose which is caused by *Colletotrichum gloeosporioides* Penz. and Sacc and pathogens such as *Lasiodiplodia theobromae*, *Botryosphaeria dothiorella*, *Dothiorella dominicana* and *Phomopsis* spp. that results in stem end rot are the major postharvest pathogens of avocado (Bill *et al.*, 2014). All these pathogens negatively affect fruit quality, marketability, nutritional value and shelf life of avocado fruit, thus causing major economic losses for the avocado industry (Tesfay *et al.*, 2017). Postharvest losses due to anthracnose can increase up to 80% (Bosse *et al.*, 2012). During fruit development, these pathogens remain dormant, this can be attributed to the presence of inhibiting diene in fruit skin, which disappears during fruit ripening (Prusky *et al.*, 1991; Bosse *et al.*, 2012).

Over the years, synthetic fungicides have been applied to reduce decay in various fruit (Oregel-Zamudio *et al.*, 2017). For instance, in avocado, copper-based fungicides such as copper hydrochloride and copper oxychloride have been extensively used to control anthracnose, whilst stem-end rot has been effectively controlled by postharvest application of prochloraz (Bill *et al.*, 2014; Tesfay *et al.*, 2017). However, with persistent use of these chemical treatments, pathogens develop resistance against fungicides. In addition, the increase in human health concerns regarding chemical residues on treated fruit and the negative impact on the environment has resulted in synthetic fungicides becoming unfavourable (Maqbool *et al.*, 2010).

Currently, in a quest to reduce postharvest losses, the use of edible coatings as postharvest treatment has gained interest amongst food scientists, postharvest technologists and physiologists. Edible coatings are natural biodegradable food safe substitutes applied on produce surface known for reducing mass loss, solute and gaseous movement and oxidation of fresh produce. Gum arabic is amongst polysaccharides that have been reported to maintain the postharvest quality of horticultural produce (Ali *et al.*, 2010). Bilawal *et al.* (2017) demonstrated that gum arabic based edible coatings reduced postharvest losses and improved the shelf life of guava fruit. However, gum arabic as a standalone treatment is not effective in reducing fruit decay resulting from postharvest pathogens and diseases (Bill *et al.*, 2014).

Various studies have demonstrated that plant extracts exhibit good activity against different fungal pathogens (Şesan *et al.*, 2015; Bhutia *et al.*, 2016; Onaran and Yanar, 2016). The antimicrobial activity of plant extracts against various pathogens can be attributed to the high concentration of phytochemicals and antioxidant capacity, however, the actual mode of action in a pathogen's growth and development has not been documented (Onaran and Yanar, 2016). Gatto *et al.* (2016) reported that *Orobanche crenata* and *Sanguisorba minor* plant extracts at different levels inhibited decay of sweet cherry stored at 0 ± 1 °C for 21 days and 7 days at ambient by 79% and 80%, respectively. Similarly, Nicosia *et al.* (2016) demonstrated that pomegranate plant extract was effective in inhibiting spore germination of *Botrytis cinerea*, *Penicillium digitatum* and *Penicillium expansum*. Likewise, Tesfay *et al.* (2017) demonstrated that ethanolic moringa leaf extracts were more effective in inhibiting mycelial growth of *C. gloeosporioides* and *A. alternate* in comparison to methanolic extracts.

Due to its film-forming properties, gum arabic has a potential to act as a carrier of active ingredients that can improve its microbial activity. Mustafa *et al.* (2018) reported that incorporation of dried *Garcinia atroviridis* crude extract (15 mg/mL) into gum arabic (10%) exhibited similar effectiveness comparable to commercial fungicide (Mancozeb at 3.2 mg/mL) against *C. gloeosporioides* mycelial growth. Moringa leaf extract has been reported to have antimicrobial properties and has also been identified as a novel coating active agent (Verma *et al.*, 2009). *Geotrichum candidum*, *Mucor micheli*, *Rhizopus stolonifer* mycelial growth was inhibited by the application of moringa leaf extract (Chiejina and Onaebi, 2016). Moyo *et al.* (2012) reported that acetone extract of moringa leaves showed antimicrobial activity against *Escherichia coli*, *Enterobacter cloace*, *Proteus vulgaris*, *Staphylococcus aureus* and *Micrococcus kristinae*, however, the extract did not exhibit any antimicrobial activity against

Streptococcus faecalis, *Bacillus pumilus*, *Klebsiella pneumonia*, *Bacillus cereus* and *Pseudomonas aeruginosa*.

Therefore, the aim of this study was to investigate the effect of gum arabic coating as a standalone treatment or in combination with moringa leaf extract on *in vitro* radial mycelial growth of *C. gleosporioides* and *L. theobromae*.

5.2 Materials and method

5.2.1 Media preparation and pathogen isolation

Approximately 19.8 g of Potato dextrose agar (PDA) was weighed and poured in 500 ml of distilled water. The PDA media was autoclaved for 15 min at 121 °C and cooled to 50 °C in a water bath. Prepared media was supplemented with 100 mg of chloramphenicol dissolved in 20 ml of ethanol and poured into 90 mm Petri dishes. Pathogen isolation was done according to Xoca-Orozco *et al.* (2017), with slight modifications. Briefly, pieces (5 mm) showing symptoms of *C. gleosporioides* and *L. theobromae*, were aseptically isolated from infected avocado fruit showing symptoms of either anthracnose and stem-end rot. Both pathogens were identified on basis of their cultural and morphological characteristics such as colour, hyphae orientation and spore shape using a light microscope. The spore suspensions of each isolate were prepared aseptically using distilled water, a drop of distilled water was placed on a microscope slide then covered with coverslip slip. Identification was done at 10X magnification and images were taken at 40X magnification.

5.2.2 Isolation and identification of the pathogen causing avocado anthracnose and stem-end rot

C. gleosporioides F, which causes anthracnose and *L. theobromae* which causes fruit stem end rot were the dominant pathogens observed from the isolates. *C. gleosporioides* culture was identified on a basis of white mycelium, greyish colony, short hyphae on the edge of the growth and yellowing at the bottom of the petri dish (Fig. 1). *L. theobromae* cultures, on the other hand, initially appeared whitish, they later turned black greyish, under light microscope septate hyphae with single or two ovoid to ellipsoidal conidia (Fig. 2).

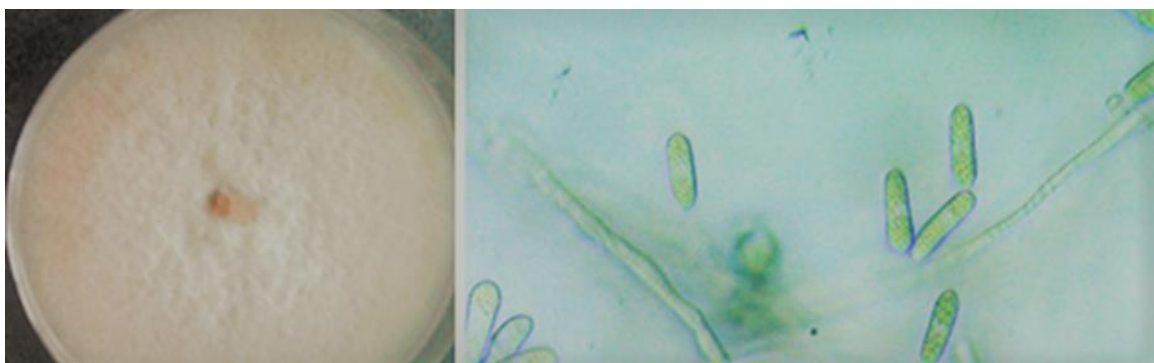


Fig. 1: Morphological features of seven days old pure cultures of isolates *C. gleosporioides* observed under a light microscope at 40X magnification.



Fig. 2: Morphological features of seven days old pure cultures of isolates *L. theobromae* observed under a light microscope at 40X magnification.

5.2.3 Plant extracts and gum arabic preparation

Moringa leaf was extracted as described by Tesfay *et al.* (2017), with slight modification. Briefly, 100 g of moringa leaf powder was dissolved in 1 L of 70% ethanol for 4 hours with constant agitation at 4 °C. The extract was concentrated in a rotary vapour and about 20 ml of distilled water was added three times each time volume was reduced in a rotary evaporator. The extract was kept in cold storage for amending growing media and for preparation of gum arabic coatings for in-vitro screening. Gum arabic solutions (alone) were prepared by dissolving 20 g and 30 g in 200 mL of distilled water and for the combination 20 g and 30 g of gum arabic powder was dissolved in 200 mL of moringa extract.

5.2.4 *In vitro* evaluation of the fungicidal activity of gum arabic and moringa leaf extract

The effect of gum arabic, moringa leaf extract and gum arabic in moringa leaf extract on the radial growth of *C. gleosporioides* and *L. theobromae* were studied using PDA. The PDA media were separately amended with GA 10% (alone), GA 15% (alone), moringa leaf extract 10% (alone) or gum arabic in moringa leaf extract. The amended PDA was poured into petri dishes (20 ml/ plate) and for control treatment, amendment of PDA was achieved by adding equal amounts of sterilized distilled water to the PDA. Discs of mycelium (5 mm diameter) were removed from the edge of 7-days old cultures of the isolates, transferred and placed at the centre of the amended PDA media. The inoculated Petri dish plates were then incubated at 28 °C for 10 days. Radial growth of cultures was measured using a ruler. The mean growth values were converted into inhibition percentage of mycelial growth in relation to control treatment according to Eq. 1.

$$GI \% = \left(\frac{x-y}{x} \right) * 100 \quad 1$$

GI% = Growth inhibition percentage

X = mycelial growth diameter in control

Y = mycelial growth diameter in treatment

5.3 Data analysis

The collected data were subjected to the analysis of variance (ANOVA) using GenStat statistical software (GenStat®, 18.1 edition, VSN International, UK). Mean separation was performed using Fischer's least significant difference (LSD) at 5% level of significance. Standard error values were calculated where a significant standard deviation was found at $p \leq 0.05$ between individual values.

5.4 Results and discussion

5.4.1 *In vitro* screening of moringa plant extracts against the isolates

The effect of gum arabic, moringa leaf extract or gum arabic incorporated with moringa leaf extract on radial mycelial growth of *C. gleosporioides* after 10 days during *in vitro* experiment is illustrated on fig. 2 and 3. It can be observed from Fig.1 and Table 1 that gum arabic edible

coatings as a standalone treatment showed a significantly lower ($p \leq 0.5$) inhibition effect on the radial mycelial growth of *C. gloeosporioides* than the application of gum arabic edible coating in moringa leaf extract. As standalone treatments, GA 10% and GA 15% had more or less similar mycelial growth as the control treatment during the 10 day incubation period, with GA 15% showing little inhibition to the mycelial growth of the pathogen. Our results are in agreement with Maqbool *et al.* (2011); Cheong and Zahid (2014) who reported that gum arabic had zero inhibition against anthracnose of banana and papaya respectively.

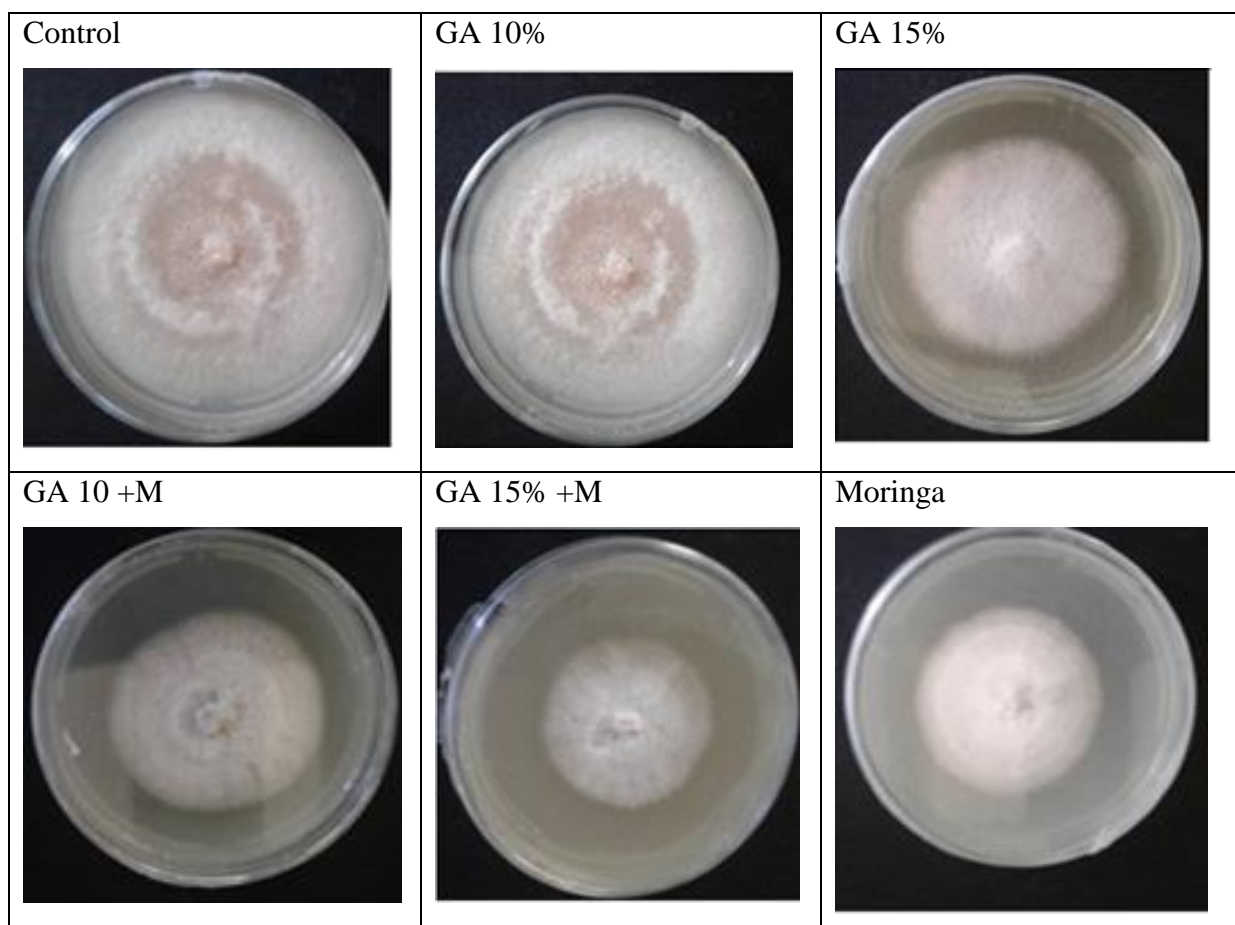


Fig. 2 Effect of gum arabic edible coating alone or in combination with moringa leaf extract on mycelial growth of *C. gloeosporioides* after 10 days incubation at 25 °C.

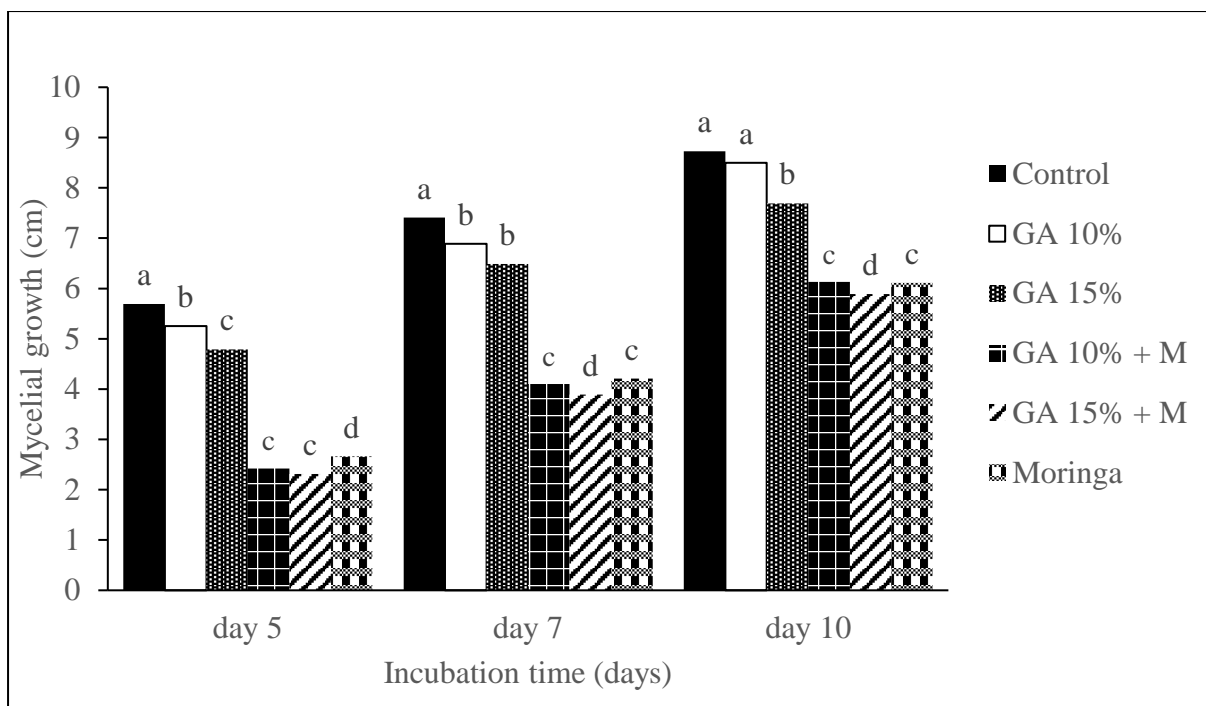


Fig. 3: Effect of gum arabic coatings, moringa leaf extract or the incorporation of gum arabic into moringa extract on mycelial growth rate of *C. gloeosporioides* incubated at 28 °C for 10 days. Means followed by the same letter within a clustered column are not significantly different according to Fisher's least significant difference test at $p \leq 0.05$.

As a standalone treatment, moringa leaf extract significantly ($p \leq 0.05$) inhibited (30%) radial mycelial growth of the pathogen as the combination of gum arabic edible coatings with moringa leaf extract. However, a maximum inhibition (33%) in mycelial growth was observed in GA 15% + M. It is evident from our results that the incorporation of moringa leaf extract with edible coatings improved the antimicrobial activity. This can be corroborated by the finding of Maqbool *et al.* (2010) and Ali *et al.* (2016) who reported that incorporation of plant-based active ingredients such as essential oils and plant extracts significantly improves the antimicrobial activity of edible coatings against postharvest pathogen that causes major economic losses. In fact, Tesfay *et al.* (2017) demonstrated that moringa leaf extract inhibited the growth of *C. gloeosporioides* during *in vitro*. Similarly, Chiejina and Onaebi (2016) reported that ethanolic moringa leaf extract showed 100% inhibition of *Geotrichum candidum* and significantly reduced mycelial growth of *Mucor micheli* and *Rhizopus stolonifera*. The inhibitory effect of plant extract on mycelial growth of various pathogens has largely been attributed to the high phytochemical constituents which includes phenols, alkaloids, tannis among the few (Anyasor *et al.*, 2011; Tesfay *et al.*, 2017).

Fig. 4 and 5 illustrates the effect of gum arabic edible coatings, moringa leaf extracts as standalone treatment or gum arabic in moringa leaf extract on radial mycelial growth of *Lasiodiplodia theobromae* after 10 days. Mycelial growth inhibition percentages are shown in Table 1. In this study, only GA 15% + M had a significant ($p \leq 0.05$) inhibition (6.5%) effect on the radial growth of *L. theobromae* compared to other treatments. Gum arabic edible coatings irrespective of concentration had zero inhibition on radial mycelial growth. This shows that gum arabic edible coatings had similar mycelial growth as the control treatment. As a standalone treatment, moringa leaf extract had little inhibition in mycelial growth. The ability of GA 15% + M also suggest that incorporation of moringa enhance the antimicrobial activity of the coating.

Similar results have also been reported by Ali *et al.* (2015), who demonstrated that chitosan and essential oil as standalone coatings were not effective against *Colletotrichum capsici* in vivo, however, their combination was effective. These results indicates that there is a certain level of *L. theobromae* sensitivity towards the concentration of plant extracts. Alam *et al.* (2017) demonstrated that ethanolic moringa extract (50 mg/mL) reduced *L. theobromae* disease severity of mango by 77.7% after 9 days of incubation at 25 °C.

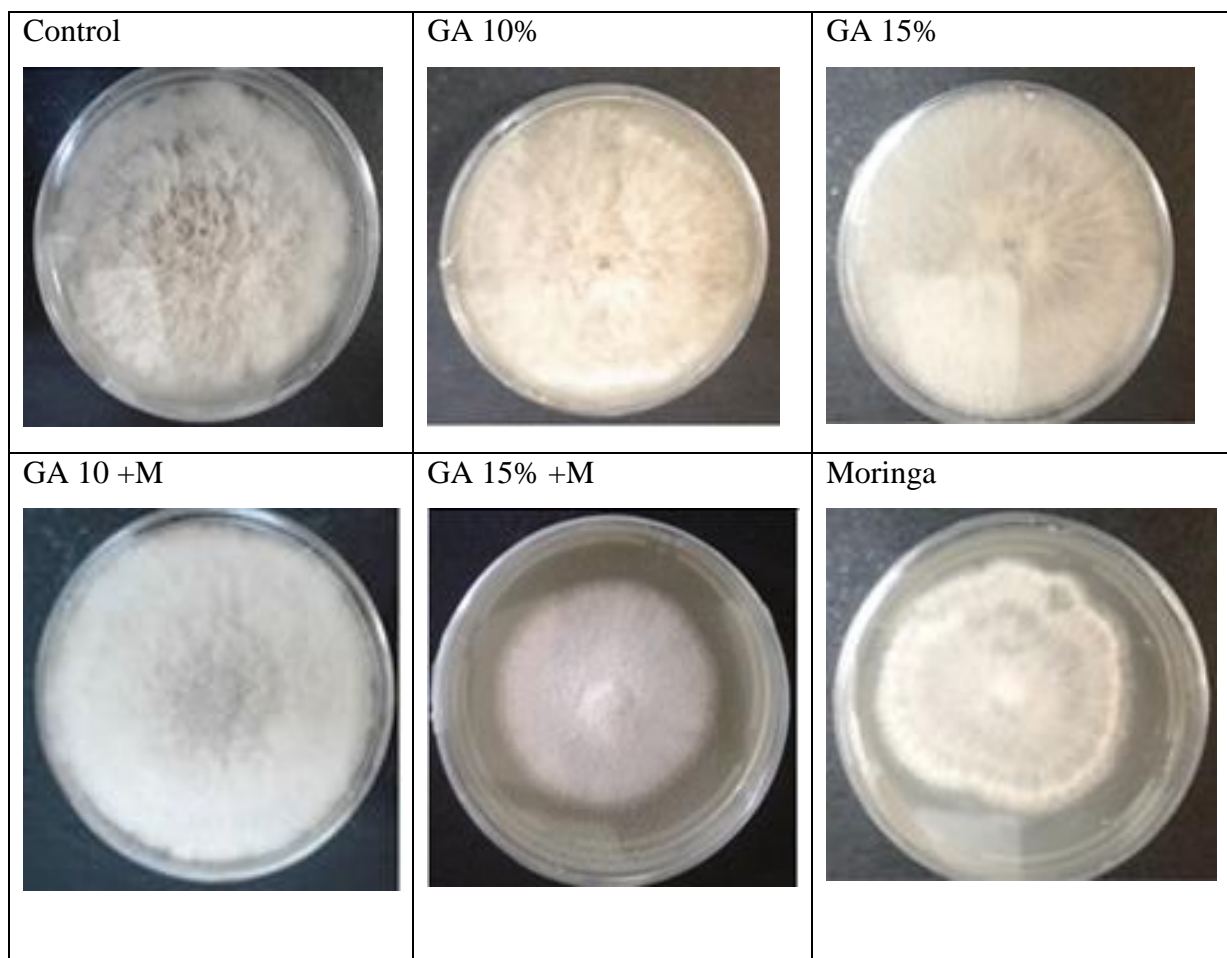


Fig. 4 Effect of gum arabic edible coating alone or in combination with moringa leaf extract on mycelial growth of *L. theobromae* after 10 days incubation at 25 °C.

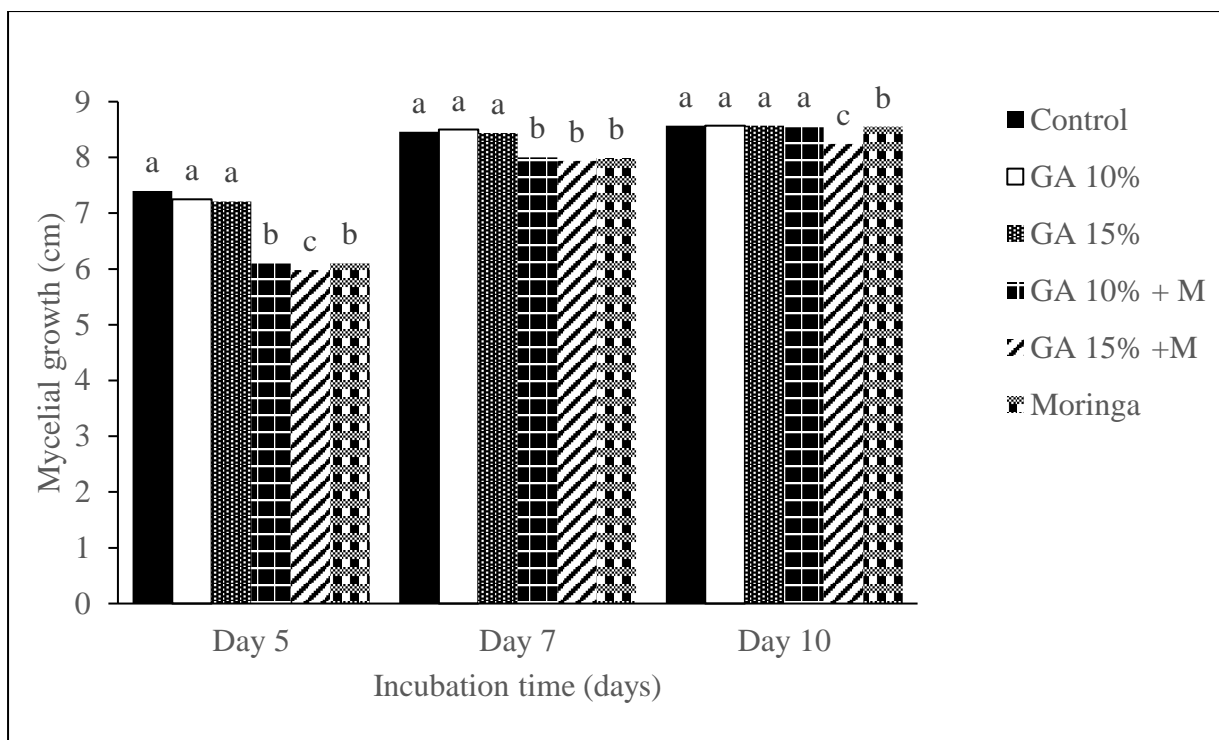


Fig. 5 Effect of gum arabic coatings, moringa leaf extract or the incorporation of gum arabic into moringa extract on the mycelial growth rate of *L. theobromae* incubated at 28 °C for 10 days. Means followed by the same letter within clustered column are not significantly different according to Fisher's least significant difference test at $p \leq 0.05$

Table 1: Mycelial growth inhibition percentage of the isolates by gum arabic edible coatings alone or in combination with moringa leaf extracts after 10 days of inoculation.

Pathogen	Treatment	Inhibition (%)
<i>C. gloesporioides</i>	GA 10%	2.6
	GA 15%	11
	GA 10 + M	28
	GA 15% + M	33
	Moringa (M)	30
$p \leq 0.05$, LSD = 0.48, CV 1.8		
<i>L. theobromae</i>	GA 10%	0
	GA 15%	0
	GA 10 + M	0
	GA 15% + M	6.5
	Moringa (M)	5
$p \leq 0.05$, LSD = 0.12		

5.5 Conclusion

The antimicrobial properties present in plants provides the much needed ecological alternative to limit the use of synthetic fungicides. However, challenges such as inconsistent results and legislation still remain a challenge in commercial application of plant-based biocontrol of postharvest diseases. In this study, incorporation of moringa leaf extract improved the antimicrobial activity of gum arabic edible coating against both *C. gloesporioides* and *L. theobromae* *in vitro*. Overall GA 15% + M was the most effective treatment in inhibiting the growth of both *C. gloesporioides* (33%) and *L. theobromae* (6.5%). However, further research is still needed to evaluate the effect of these treatments *in vivo*.

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Chapter 6:

Chapter 6: General discussion, conclusions and recommendations

6.1. Introduction

South Africa exports approximately 50 tonnes of avocado fruit, making it the second largest producer and exporter in Africa after Kenya (FAO, 2016). The avocado industry has been dominated by the 'Hass' cultivar, as it accounts for about more than 80% of the global production. However, challenges such as alternate bearing, high susceptibility to chilling injury and season availability there are major economic losses associated with the cultivar especially in South Africa. This results in an economic gap that is left untapped during the off-season period. In an effort to reduce this gap, considerable research has been done to extend the 'Hass' season, this includes modifying the existing cultivar characteristics and development of 'Hass' like cultivars. Amongst commercially available 'Hass' like cultivars includes 'Lamb Hass', 'Carmen' and recently 'Maluma'. 'Maluma' is believed to be best long-term solution, thanks to its characteristics which are believed to be superior to 'Hass'. Since its commercial release in 2007, the cultivar has grown significantly both locally and globally. This mainly due to its characteristics such as consistent bearing pattern, higher yield per hectare, less susceptibility to chilling injury amongst the few. The 'Hass' demand is usually at peak during the winter season in South Africa, this results in an untapped market by the South African producers as they must harvest prior to the winter months to avoid fruit damage by frost. However, with the newly 'Maluma' comes the opportunity for producers to tap into this market by replacing low yielding 'Hass' orchards with 'Maluma'.

Cultivars maybe able to extend season availability but there's still a need to maintain postharvest quality to ensure good economic gains. High demand for organic foods, environmental challenges and human health concerns have driven the postharvest biology and technology to look for postharvest treatments that are environmental friendly and not harmful to human health. Amongst innovative techniques that fit this description are edible coatings. Edible coatings are prepared from natural polymers such as polysaccharides, proteins and waxes. Literature indicated that edible coatings can be used to maintain postharvest quality and, in some cases, prolong shelf life of fresh horticultural produce. The ability of edible coating to effectively maintain postharvest quality can be attributed to the modified atmosphere

created by the semi-permeable layer created by edible coating (Park, 1999). This semi-permeable layer regulates gas and solute movement resulting in reduced respiration rates and moisture loss. In addition, edible coatings can act as carriers of active ingredients, which help improve their antimicrobial activity thus improving their performance against postharvest disease that causes fruit decay. Therefore, the aim of this MSc study was to evaluate the efficacy of edible coatings CMC and GA combined with moringa plant extracts on newly developed avocado cultivar 'Maluma'.

6.2. Evaluating the efficacy of gum arabic and carboxymethyl cellulose incorporated with moringa leaf extract as a novel postharvest edible coating for 'Maluma' avocado

Most previous studies have focused only on the effect of edible coatings on conventional postharvest quality of avocado fruit (Maftoonazad and Ramaswamy, 2005; Tesfay and Magwaza, 2017), other than integrating conventional parameters (mass loss, firmness and skin colour) with organoleptic properties. Chapter 3 investigated the effect of edible CMC and GA (alone) or in combination with moringa leaf extract on both conventional postharvest quality attributes and organoleptic properties intergratively on 'Maluma' avocados. The results demonstrated that GA 15%, GA 10%, CMC in combination with moringa leaf extract effectively maintained conventional quality of avocado fruits. This can be attributed to the ability of coatings to reduce mass loss, minimise decline in fruit firmness, and delay fruit skin colour changes. Considering that consumer acceptability of coatings is greatly influence by the effect of the coating on the organoleptic properties rather than conventional properties. Sensory evaluation was done performed with an attempt to evaluate the effect of edible coatings on fruit organoleptic properties. The results showed that coatings significantly influenced organoleptic properties of avocado. There was a variation in coating performance with CMC incorporated with moringa leaf extract being the outstanding treatment as it attained high scores for all tested attributes.

Considering that GA 15% was the most effective treatment in delaying change in conational quality parameters, it was the least performing treatment on organoleptic properties. This shows the importance of integrating both conventional and organoleptic qualities when evaluating the effect of postharvest treatment of horticultural produce such as avocado. Correlation analysis showed that organoleptic properties are highly correlated to fruit firmness.

While correlation amongst organoleptic properties was intermediate suggesting that they do not influence each other. However, the results can be improved in the future by increasing the sample size and number of panellist for higher chances of obtaining a high quality results with smaller margin of error. We continued to look at how do these coatings affect phytochemical and antioxidants properties in chapter 4.

6.3. Investigation the effect of carboxymethyl cellulose and gum arabic edible coatings incorporated with moringa leaf extract on phytochemical and antioxidants activities of ‘Maluma’ avocado fruit

Effect of CMC and GA incorporated with moringa leaf extract on phytochemical contents and antioxidant activities of ‘Maluma’ avocado was evaluated. The results demonstrated that edible coatings were able to delay changes in phytochemicals and antioxidants levels. Amongst evaluated phytochemicals include; Carbon seven (C7) sugars, vitamin C, total phenols, flavonoids, and antioxidant activity. Phytochemicals plays a significant role in fruit defence system against pathogen infection, oxidative stress and other plant related stress. C7 sugars have long been reported to play a significant role in avocado fruit ripening (Tesfay *et al.*, 2010; Tesfay *et al.*, 2012). In fact Liu *et al.* (2002); Blakey *et al.* (2012) reported that ripening in avocado commences once there is decline in C7 sugars. In this study, edible coatings (GA 15% + M, CMC + M, GA 10% + M) significantly delayed changes in C7 sugars during storage. In addition, these coatings had a similar effect on vitamin C levels, total phenols and total flavonoids, polyphenol oxidase activity. Higher vitamin C level retention by fruit during ripening results in higher intake by consumers, thus it is important to retain vitamin C as human cannot synthesise their own vitamin C.

Phenols are among the most abundant phytochemical found in fruit in large quantities, this due to their diversity (Levén *et al.*, 2012). Accumulation of phenols such as flavonoids in fruit is essential for fruit ripening however, as the fruit losses its membrane oxidation catalysed by PPO results in tissue discoloration results in brownish fruit colour (Luh and PHITHAKPOL, 1972). The ability of coatings to reduce the activity indicates that the loss of phenols was suppressed. The ability of coatings can be associated with modified atmosphere created by the semi-permeable layer Our results indicates that this layer reduced the intake of oxygen (O₂). Presence of O₂ in high concentration results in oxidation of these phytochemicals (Wang *et*

al., 2005). Correlational analysis demonstrated that most phytochemicals strongly correlate with C7 sugars, showing the significance of C7 sugars in fruit ripening and defence system.

6.4. Antifungal effect of gum arabic incorporated with moringa leaf extract against *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae* in avocado fruit

The effect of GA (alone) or in combination with moringa leaf extract was evaluated against of *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae* Chapter 4. It is evidently from our results that GA 15 + M was the effective treatment inhibiting mycelial growth of *C. gloeosporioides*, a pathogen causing fruit rot during storage. However, all coating showed little or zero inhibiting properties on mycelial growth of *L. theobromae*, a pathogen causing stem end rot of avocado. These fungal pathogens are amongst the most common postharvest causing on avocado, with symptoms starting to show during postharvest storage. Significant quality loss and subsequently economic losses can be caused by these pathogen if no control measure is put in place. The scenario on the little inhibition shown by treatments on *L. theobromae* can be attributed to various factors, such as the extraction method used, concentration of moringa extract amongst the few. Tesfay *et al.* (2017) showed that different extraction methods have different effect on postharvest disease. Future studies needs evaluate the effect of this coatings on infected fruits to test their potential for commercial application.

6.5. Conclusion

Although this results looks promising for commercial application. However, we only looks at one extraction method and 2 pathogens on *in vivo* it is important for future studies to also look at *in vivo* application of these treatments. Tesfay *et al.* (2017) reported that ethanolic moringa extract was effective inhibiting growth of *C. gloeosporioides*, and *A. alternata* it could be assumed that since the disease were similar the effect of our treatment would have been the same on *A. alternata*. In future one needs to look at the effect of these treatments on respiration rates, pulp colour, pH, lipid peroxidation, different plant extraction method considering that previous work by Tesfay and Magwaza (2017) demonstrated that CMC in combination with moringa leaf extract had an effect on some these parameters. Considering that these treatments were tested on controlled laboratory environment, this now need to be tested at commercial level to see their response where not everything is controlled.

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