Postharvest technologies for predicting and reducing susceptibility of ‘Marsh’ grapefruit (*Citrus paradisi* Macfad.) to rind pitting disorder

By

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Declaration

I, Mawande Shinga (Stu No.: 213522957), declare that the research work reported in this thesis is my original work, except where I referenced. I further declare that this thesis has not been submitted for obtaining any qualification at any another university.

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Dedication

I dedicate this dissertation to Ethandwa, my precious little daughter, I hope she grows and sees this work. I would also like to dedicate this work to my late father, Vela Shinga. Rest in peace, my hero.

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Summary

Citrus fruit is globally one of the most important fruit due to their nutritional value and sensorial attributes, however, they were susceptible to various postharvest disorders, especially during shipping period. Therefore, the aim of this study was to determine the effect of edible coating, carboxymethyl cellulose (CMC) infused with moringa leaf extracts (MLE) on reducing postharvest physiological rind pitting disorder in 'Marsh' grapefruit (*Citrus paradisi* MacFad.). The study also reviewed the literatures on the ability of edible coatings to improve fruit quality and extend shelf life of citrus fruit. Edible coatings recently received attention due to their ability to enhance fruit quality without compromising human health. The first experimental chapter was conducted to evaluate the ability of CMC and MLE as edible coatings to control the disorder in 'Marsh' grapefruit. A total of 300 fruit (150 from outside canopy and 150 from inside canopy) were harvested from a commercial orchard at Dole Bolton Citrus Estate in Nkwalini at Showe, KwaZulu Natal, South Africa. Fruit were subjected to different treatments, control (untreated), CMC (0.5%) + MLE (10%), CMC (1%) + MLE (10%), CMC 0.5% and CMC 1%. Treatments were organised in a factorial design. Fruit were stored at 3 ± 0.5 °C and 90-95 % relative humidity (RH), for nine weeks and thereafter taken to room temperature (22 ± 2 °C) for two weeks to simulate shelf life. The physicochemical attributes (total soluble solids. titratable acidity, maturity index, fruit mass loss, fruit colour, rind dry matter) of the fruit were analysed during this period. Rind pitting as well as sensory quality was evaluated at the end of storage period. This study identified that CMC 0.5% + MLE 10% and CMC 1% + MLE 10% reduced postharvest rind pitting disorder incidence compared to CMC 0.5%, CMC 1% and the control treatment. High mass loss contributes largely to rind pitting development, however, edible coatings managed to provide semi-permeable barrier to the fruit. Uncoated fruit had high mass loss which may be due to high water loss from the rind, most probably rind cell collapsed thereby leading to visible pitting in fruit rind. Coated fruit with low rind pitting incidence had low rind dry matter (RDM) compared to uncoated fruit with high rind pitting incidence. This study reported that total soluble solids (TSS) increased with storage time, however, low rate of increase was noticed in coated fruit compared to uncoated fruit. Fruit with higher TSS at the end of storage had high rind pitting incidence compared to fruit with low TSS. Rind colour was expressed as citrus colour index (CCI). Citrus colour index was noticed to increase with storage, however, the rate of increase in coated fruit was lower than that of uncoated fruit. At the end of storage, CCI was therefore higher in uncoated fruit than coated fruit, while higher CCI was correlated with high rind pitting incidence. These physicochemical quality parameters can be used to predict rind pitting occurrence in ‘Marsh’ grapefruit. The second experimental chapter investigated rind phytochemical quality attributes that can be used as pre-symptomatic markers of rind pitting disorder in ‘Marsh’ grapefruit. Treatments used for this chapter were similar to the abovementioned. Treatments were organised in a factorial design. Visible to near infrared spectroscopy (Vis/NIRS) as a non-destructive technique was used to develop models that can assist in rind pitting disorder prediction. Partial least square (PLS) regression models were developed to predict rind phytochemical quality attributes such as ascorbic acid, phenolics, flavonoids, antioxidant capacity and activity, pigments (chlorophyll a and b, β carotene and total carotenoids) and sugars (sucrose, glucose and fructose), and these models were developed to predict rind pitting disorder of ‘Marsh’ grapefruit. Noticeably, CMC when combined with MLE were able to reduce the incidence of rind pitting disorder when compared to their counterparts. This could be due to the fact that moringa is believed to have high content of flavonoids, phenolics and antioxidants, which may be released to fruit and act as free radical scavengers from the cell matrix and protect fruit from external stress. These studies further investigated the effect of canopy position on susceptibility of rind pitting development. It was found that outside canopy (OC) fruit were more susceptible to rind pitting disorder compared to fruit from inside canopy (IC). This may be due to that OC fruit are exposed to different climate during fruit growth and development which could lead to rind quality stress and damage rind cells. Since OC fruit were more prone to disorder development than IC fruit, it would make financial sense to export fruit to the low demanding market with less penalties if fruit develop pitting prior to destination. Alternatively, fruit with higher chances of developing disorders (OC fruit) must be sent to local markets or fruit may be processed to other sellable products such as juices and dried fruits.

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CHAPTER 1

1.1. General Introduction

Citrus is one of the commonly consumed fruit in the world. It is mostly consumed as fresh fruit or juice because of its high nutritional value and special flavour (Guimarães et al., 2010; Nekvapil et al., 2018). High citrus consumption is reported to lower the risk of several human diseases such as diabetes, hypertension as well as certain types of cancers (Joshipura et al., 2001; Palou et al., 2015). In South Africa, the citrus industry is export-orientated and it is ranked as third largest exporter of citrus in the world after Spain and Turkey and ranked 13th for production among citrus producers worldwide (Edmonds, 2018). The most exported citrus species include oranges (*Citrus sinensis*), mandarins (*Citrus reticulata*), grapefruit (*Citrus paradisi*) and lemons (*Citrus limon*) (Ncama, 2016).

Citrus fruit are susceptible to micro-organism infections, physiological disorders and mechanical injuries. Different postharvest handling procedures are used to control postharvest pests and disorders. For instance, the quarantine treatment against Mediterranean fruit fly (*Ceratitis capitate)* necessitates that the fruit be stored at very low temperatures (-0.6 °C) for first two weeks then stored at 3-5 ℃ during shipping (Bassal and El-Hamahmy, 2011). However, most citrus species develop chilling injury which is exhibited by necrosis in the outer part of the peel at these temperatures (Lafuente et al., 2011). ‘Marsh’ grapefruit (*Citrus* x *paradisi* MacFad) is one of the highly susceptible cultivars to chilling injury (Ezz and Awad, 2009). Additionally, ‘Marsh’ grapefruit has also been reported to be susceptible to rind physiological disorders, which becomes visible after 4-6 weeks of harvesting (Ncama, 2016). The visibility of the symptoms occurs during commercial shipping period.

Rind pitting disorder is a non-chilling disorder that affects only the external part of the fruit without damaging the internal quality but it may result in an increased susceptibility to pathogen invasion which consequently decrease fresh market value (Agusti et al., 2001; Petracek et al., 1995). It was reported to be morphologically and casually distinct from chilling injury (Petracek et al., 1995). This disorder is characterized by cluster of collapsed oil glands that develop over the external part of the fruit. The disorder is linked to both biotic and abiotic factors at pre-harvest or during postharvest handling and storage (Alférez and Burns, 2004). The difference in water potential as well as nutrition at pre-harvest are some of the key factors influencing rind pitting. Fruit canopy position has been found to also contribute to rind physiological disorders of citrus fruit (Magwaza et al., 2013c).

To control postharvest rind pitting disorder, different postharvest treatments have been used. While some treatments have been shown to be effective, the opposite has also been reported. For instance, Petracek et al. (1998) reported that 'Marsh' grapefruit coated with shellac-based waxes had higher incidence of rind pitting. Citrus waxes often incorporate synthetic chemical fungicides to control postharvest diseases and prolong fruit shelf life. Extensive use of postharvest chemical fungicides as treatments, either alone or into conventional coatings has been reported to pose harmful threats to human and pollute environment itself (Arnon et al., 2014). Since consumers prefer less or no use of chemicals on minimally processed fruits, that brings more attention to find the naturally occurring substances as an alternative. Therefore, various postharvest approaches including modified atmosphere packaging (MAP) (Li et al., 2013), hot water (Spadoni et al., 2014), biocontrol agent application (Yao and Tian, 2005) etc have been widely reviewed in recent years to extend citrus shelf life. Among these applications, edible coating approach is a promising technique with a good safety, biodegradable, environmentally friendly and satisfying performance (Arnon et al., 2015; Dhall, 2013; Embuscado and Huber, 2009; Galus and Kadzińska, 2015; Silva-Weiss et al., 2013; Tharanathan, 2003).

Therefore, edible coatings are a potential replacement of natural waxes in citrus packing house (Valencia-Chamorro et al., 2010). Natural edible coatings are generally composed of polysaccharides, proteins or lipids (Dhall, 2013; Valencia-Chamorro et al., 2010). The ability of edible coatings to retard moisture, oxygen, aromas and soluble transport may be enhanced by mixing with additives such as antioxidants, antimicrobials, colorants, flavours and fortifying nutrients in coating formulation (Pranoto et al., 2005). Lately, most studies of edible coatings for citrus fruit focused on hydroxypropyl-methylcellulose (HPMC)/beeswax/shellac composites (Contreras-Oliva et al., 2011, 2012; Navarro-Tarazaga et al., 2007; Navarro-Tarazaga et al., 2008; Valencia-Chamorro et al., 2011). These composite coatings retained fruit quality during storage, however, they involve the use of powerful organic solvents such as ammonia to dissolve the shellac. These organic solvents are known to limit gaseous exchange and encourage anaerobic conditions and off-flavours (Contreras-Oliva et al., 2011, 2012; Navarro-Tarazaga et al., 2007). There is currently no study of carboxymethyl-cellulose (CMC) on citrus as a stand-alone coating but Arnon et al. (2014) reported CMC as a bilayer coating with chitosan on ‘Or’ and ‘Mor’ mandarins, ‘Navel’ oranges, and ‘Star Ruby’ grapefruit. Therefore, there is a need to evaluate the effect of CMC as stand-alone on postharvest citrus quality, as this coating is relatively cheap, easy to prepare and does not require the use of organic solvent. In addition, CMC provides a uniform and stable matrix and maintains high structural integrity during storage (Arnon et al., 2014).

Moringa *(Moringa oleifera* Lam.) is commonly grown in many tropical and subtropical regions. It is the most credible but inexpensive for not only enhancing fruit quality but also providing antimicrobial effects during postharvest storage. The literature has revealed that leaf extracts of moringa contains high antioxidant activity and exhibit antibacterial potential against many microorganisms (John et al., 2013). Although there is limited research on the use of moringa on citrus, edible coatings such as corn starch and CMC incorporated with moringa extracts have shown to reduce mass loss in citrus fruit (Adetunji et al., 2012; Yousef et al., 2015).

In most cases, citrus industries grade their fresh produce according to their external attributes such as fruit colour and presence or absence of defects (Xudong et al., 2009). In previous years, human evaluation of produce has been the major tool to quantify the quality parameters. However, this evaluation method is quite subjective which then limits accurate communication of quality parameters within the handling and value chain (Nicolaï et al., 2014). However, recently it was reported that non-destructive strategies are objective, fast, reliable and less costly (Magwaza et al., 2013d; Shiroma and Rodriguez-Saona, 2009). Non-destructive quality measurement methods are advantageous than destructive methods because they permit analysis of individual fruit, reduce waste and allow the repetitive measures of the same produce over time (Nicolai et al., 2007). As a result, visible to near infrared (Vis/NIRS) spectroscopy has become very popular and is one of the mostly used techniques for evaluating a wide range of postharvest quality assessment for both fruit and vegetables (Wedding et al., 2013). Vis/NIRS was reported to be feasible for examining citrus physicochemical attributes as discussed in a review by (Magwaza et al., 2012b) and internal biochemical attributes of citrus (Ncama, 2016). Therefore, this study was aimed at evaluating the effect of carboxyl methylcellulose (CMC) containing moringa plant leaf extracts as new postharvest organic edible coating for ‘Marsh’ grapefruit quality and predicting postharvest rind pitting disorder using Vis/NIRS.

1.2. Aims and objectives

This Masters research aimed to determine the effect of edible coatings (CMC combined with moringa leaf extract at different concentrations) in citrus industry for maintaining the quality and controlling rind pitting disorder of ‘Marsh’ grapefruit.

The specific objectives of this study were to:

i) Determine the effect of carboxymethyl-cellulose incorporated with moringa leaf extracts on postharvest physicochemical in relation to rind pitting disorder susceptibility on ‘Marsh’ grapefruit (*Citrus x paradisi* MacFad.)

ii) Predict susceptibility of rind pitting disorder in response to edible coatings using Vis/NIRS by determining the pre-symptomatic biochemical markers in ‘Marsh’ grapefruit.

1.3 References

Adetunji, C., Fawole, O., Arowora, K., Nwaubani, S., Ajayi, E., Oloke, J., Majolagbe, O., Ogundele, B., Aina, J., Adetunji, J., 2012. Quality and safety of Citrus Sinensis coated with Hydroxypropylmethylcellulose edible coatings containing Moringa oleifera extract stored at ambient temperature. Global Journal of Science Frontier Research, 2249-4626.

Agusti, M., Almela, V., Juan, M., Alferez, F., Tadeo, F., Zacarias, L., 2001. Histological and physiological characterization of rind breakdown of 'Navelate'sweet orange. Annals of Botany 88, 415-422.

Alférez, F., Burns, J.K., 2004. Postharvest peel pitting at non-chilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage. Postharvest Biology and Technology 32, 79-87.

Arnon, H., Granit, R., Porat, R., Poverenov, E., 2015. Development of polysaccharides-based edible coatings for citrus fruits: A layer-by-layer approach. Food chemistry 166, 465-472.

Arnon, H., Zaitsev, Y., Porat, R., Poverenov, E., 2014. Effects of carboxymethyl cellulose and chitosan bilayer edible coating on postharvest quality of citrus fruit. Postharvest Biology and Technology 87, 21-26.

Bassal, M., El-Hamahmy, M., 2011. Hot water dip and preconditioning treatments to reduce chilling injury and maintain postharvest quality of Navel and Valencia oranges during cold quarantine. Postharvest Biology and Technology 60, 186-191.

Contreras-Oliva, A., Rojas-Argudo, C., Pérez-Gago, M.B., 2011. Effect of solid content and composition of hydroxypropyl methylcellulose–lipid edible coatings on physicochemical, sensory and nutritional quality of 'Valencia'oranges. International journal of food science & technology 46, 2437-2445.

Contreras-Oliva, A., Rojas-Argudo, C., Pérez-Gago, M.B., 2012. Effect of solid content and composition of hydroxypropyl methylcellulose–lipid edible coatings on physico-chemical and nutritional quality of 'Oronules' mandarins. Journal of the Science of Food and Agriculture 92, 794-802.

Dhall, R., 2013. Advances in edible coatings for fresh fruits and vegetables: a review. Critical reviews in food science and nutrition 53, 435-450.

Edmonds, J., 2018. Key industry statistics for citrus growers 2018. Citrus Growers' Association of Southern Africa, 1-48.

Embuscado, M.E., Huber, K.C., 2009. Edible films and coatings for food applications. Springer.

Ezz, T.M., Awad, R.M., 2009. Relationship between mineral composition of the flavedo tissue of “Marsh” grapefruit and chilling injury during low temperature storage. Research Journal of Agriculture and Biological Sciences 5, 892-898.

Galus, S., Kadzińska, J., 2015. Food applications of emulsion-based edible films and coatings. Trends in Food Science & Technology 45, 273-283.

Guimarães, R., Barros, L., Barreira, J.C., Sousa, M.J., Carvalho, A.M., Ferreira, I.C., 2010. Targeting excessive free radicals with peels and juices of citrus fruits: grapefruit, lemon, lime and orange. Food and Chemical Toxicology 48, 99-106.

John, N.R., Gala, V.C., Sawant, C.S., 2013. Inhibitory effects of plant extracts on multi-species dental biofilm formation in-vitro. Int J Pharm Bio Sci 4, 487-495.

Joshipura, K.J., Hu, F.B., Manson, J.E., Stampfer, M.J., Rimm, E.B., Speizer, F.E., Colditz, G., Ascherio, A., Rosner, B., Spiegelman, D., 2001. The effect of fruit and vegetable intake on risk for coronary heart disease. Annals of internal medicine 134, 1106-1114.

Lafuente, M.T., Ballester, A.R., Calejero, J., González-Candelas, L., 2011. Effect of high-temperature-conditioning treatments on quality, flavonoid composition and vitamin C of cold stored ‘Fortune’mandarins. Food Chemistry 128, 1080-1086.

Li, F., Zhang, X., Song, B., Li, J., Shang, Z., Guan, J., 2013. Combined effects of 1-MCP and MAP on the fruit quality of pear (Pyrus bretschneideri Reld cv. Laiyang) during cold storage. Scientia Horticulturae 164, 544-551.

Magwaza, L.S., Opara, U.L., Cronjé, P.J., Landahl, S., Terry, L.A., Nicola ï, B.M., 2013a. Nonchilling physiological rind disorders in citrus fruit. Horticultural Reviews Volume 41, 131-176.

Magwaza, L.S., Opara, U.L., Nieuwoudt, H., Cronje, P.J., Saeys, W., Nicolaï, B., 2012. NIR spectroscopy applications for internal and external quality analysis of citrus fruit—a review. Food and Bioprocess Technology 5, 425-444.

Magwaza, L.S., Opara, U.L., Terry, L.A., Landahl, S., Cronje, P.J., Nieuwoudt, H.H., Hanssens, A., Saeys, W., Nicolaï, B.M., 2013b. Evaluation of Fourier transform-NIR spectroscopy for integrated external and internal quality assessment of Valencia oranges. Journal of Food Composition and Analysis 31, 144-154.

Navarro-Tarazaga, M.-L., Plotto, A., Goodner, K., Baldwin, E., Perez-Gago, M.-B., 2007. Effect of edible coatings on the flavor of'Valencia'oranges, HortScience. AMER SOC HORTICULTURAL SCIENCE 113 S WEST ST, STE 200, ALEXANDRIA, VA 22314-2851 USA, pp. 869-869.

Navarro-Tarazaga, M.L., Del Río, M.A., Krochta, J.M., Perez-Gago, M.B., 2008. Fatty Acid Effect on Hydroxypropyl Methylcellulose− Beeswax Edible Film Properties and Postharvest Quality of Coated 'Ortanique'Mandarins. Journal of Agricultural and Food Chemistry 56, 10689-10696.

Ncama, K., 2016. Non-destructive prediction of 'marsh'grapefruit (*Citrus x paradisi* MacFad) postharvest quality and physiological rind disorders using visible to near infrared spectroscopy.

Nekvapil, F., Brezestean, I., Barchewitz, D., Glamuzina, B., Chiş, V., Pinzaru, S.C., 2018. Citrus fruits freshness assessment using Raman spectroscopy. Food Chemistry 242, 560-567.

Nicolai, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, K.I., Lammertyn, J., 2007. Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. Postharvest Biology and Technology 46, 99-118.

Nicolaï, B.M., Bulens, I., De Baerdemaeker, J., De Ketelaere, B., Lammertyn, J., Saeys, W., Verboven, P., Hertog, M.L., 2014. Non-destructive evaluation: detection of external and internal attributes frequently associated with quality and damage, Postharvest Handling (Third Edition). Elsevier, pp. 363-385.

Palou, L., Valencia-Chamorro, S.A., Pérez-Gago, M.B., 2015. Antifungal edible coatings for fresh citrus fruit: a review. Coatings 5, 962-986.

Petracek, P.D., Dou, H., Pao, S., 1998. The influence of applied waxes on postharvest physiological behavior and pitting of grapefruit. Postharvest Biology and Technology 14, 99-106.

Petracek, P.D., Wardowski, W.F., Brown, G.E., 1995. Pitting of grapefruit that resembles chilling injury. HortScience 30, 1422-1426.

Pranoto, Y., Rakshit, S., Salokhe, V., 2005. Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. LWT-Food Science and Technology 38, 859-865.

Shiroma, C., Rodriguez-Saona, L., 2009. Application of NIR and MIR spectroscopy in quality control of potato chips. Journal of Food Composition and Analysis 22, 596-605.

Silva-Weiss, A., Ihl, M., Sobral, P., Gómez-Guillén, M., Bifani, V., 2013. Natural additives in bioactive edible films and coatings: functionality and applications in foods. Food Engineering Reviews 5, 200-216.

Spadoni, A., Guidarelli, M., Sanzani, S.M., Ippolito, A., Mari, M., 2014. Influence of hot water treatment on brown rot of peach and rapid fruit response to heat stress. Postharvest Biology and Technology 94, 66-73.

Tharanathan, R., 2003. Biodegradable films and composite coatings: past, present and future. Trends in Food Science and Technology 14, 71-78.

Valencia-Chamorro, S.A., Palou, L., Del Rio, M.A., Pérez-Gago, M.B., 2011. Performance of hydroxypropyl methylcellulose (HPMC)-lipid edible coatings with antifungal food additives during cold storage of ‘Clemenules’ mandarins. LWT-Food Science and Technology 44, 2342-2348.

Valencia-Chamorro, S.A., Pérez-Gago, M.B., Del Río, M.A., Palou, L., 2010. Effect of Antifungal Hydroxypropyl Methylcellulose-Lipid Edible Composite Coatings on Penicillium Decay Development and Postharvest Quality of Cold-Stored “Ortanique” Mandarins. Journal of Food Science 75, S418-S426.

Wedding, B., Wright, C., Grauf, S., White, R., Tilse, B., Gadek, P., 2013. Effects of seasonal variability on FT-NIR prediction of dry matter content for whole Hass avocado fruit. Postharvest Biology and Technology 75, 9-16.

Xudong, S., Hailiang, Z., Yande, L., 2009. Nondestructive assessment of quality of Nanfeng mandarin fruit by a portable near infrared spectroscopy. International Journal of Agricultural and Biological Engineering 2, 65-71.

Yao, H., Tian, S., 2005. Effects of a biocontrol agent and methyl jasmonate on postharvest diseases of peach fruit and the possible mechanisms involved. Journal of Applied Microbiology 98, 941-950.

Yousef, A.R., El-Moniem, E.A., Saleh, M., 2015. The effect of some natural products on storability and fruit properties of Fuerte avocado. International Journal of Chemical Sciences and Research 8, 1454-1462.

CHAPTER 2

Effect of edible coatings on postharvest quality of citrus fruit: a review

2.1. Abstract

Citrus is one of the most important world fruit crops due to their nutritional value and special value. However, they are susceptible to postharvest diseases and physiological rind disorders. Fruit diseases and disorders are the major cause of postharvest loss. Good external appearance such as colour, gloss, absence of defects is important for consumer perception. Effective postharvest treatments are thus required to control the decay and prolong the fruit shelf life. The objective of this review is to highlight the effectiveness of edible coatings in extending and enhancing postharvest citrus fruit quality. Commonly, harvested citrus is washed mainly with fungicides then dipped in chemical synthetic coatings which may impose danger to human health. Recently, edible coatings have received attention due to their ability to prolong shelf life and maintain postharvest quality. Biodegradability, biocompatibility and non-toxicity add to their advantage over chemicals. Some of the edible coatings such as chitosan, carboxyl methylcellulose (CMC), aloe vera and Arabic gum have gained popularity due to their ability to improve fruit quality and prevent decay during postharvest storage. CMC was reported to retain firmness, total soluble solids (TSS) and ascorbic acid in mandarins while reducing weight loss. Chitosan was reported to retain firmness in grapefruit and oranges, retain TSS in tangerine and also reported to be highly effective in fruit disease inhibition. Other edible coatings such as gum arabic, coconut oil, beeswax and shellac were reported to improve sweet oranges, lime fruit and ‘Valencia’ oranges respectively. It was concluded that edible coatings application improves citrus fruit quality and therefore can be recommended for future use in citrus industries.

Keywords: Postharvest diseases, physiological rind disorders, citrus fruit quality, shelf-life, synthetic coatings, edible coatings.

2.2. Introduction

Botanically, citrus fruit is classified as a hesperidium berry, it comprises of two structurally distinct parts; the pericarp and endocarp (Magwaza et al., 2013b). This *genus* is comprised of several species and varieties such as mandarins, oranges, lemons, grapefruits, pummelos, citrons, limes, kumquats and different hybrids (Lado et al., 2018). Citrus fruit is the rich source of essential nutrients such as vitamin C, minerals, bioactive compounds and dietary fibre (Contreras-Oliva et al., 2012). Khalid et al. (2012) reported that fruit surface appearance highly influences the marketability of citrus fruit, therefore fruit must be free from both physical blemishes and physiological disorders.

To consumers, good external appearance such colour, gloss, absence of defects is the most important attribute since it attracts buyers and therefore induces fruit market value (Xudong et al., 2009). The accumulation of pathogenic micro-organisms at postharvest poses a threat to consumer health (Dhall, 2013; Suseno et al., 2014). Moreover, tropical and sub-tropical fruit often present a greater problem in storage and transportation than temperate fruit because of their high perishability (Mitra, 1997). To ensure that good fruit quality is delivered to consumers, postharvest handling system must be carefully managed (Mitra, 1997). Temperature management is the most important environmental factor used to maintain the quality of fresh horticultural produce after harvest. Low-temperature storage reduces respiration and ethylene production rate, water loss, pathogen growth, and decay incidence (Dhall, 2013; Kader, 2003; Thompson et al., 2002) but it might be insufficient to protect the fruit from decay in some cases (Perez-Gago et al., 2002). Additionally, storing fruit in low temperatures may lead to physiological damage such as chilling injury (Perez-Gago et al., 2002).

Commercially, to reduce microbial load on the whole and minimally processed fruit, it must be washed with tap or chlorinated water (Garcia et al., 2003). Chlorination effectiveness is limited because it may result in the formation of harmful by-products like trihalomethanes and halo acetic acids which may have detrimental effects to consumers and the environment (Akbas and Ölmez, 2007; Han et al., 2002). Synthetic fungicides such as copper oxychloride and copper hydroxide are common pre-harvest treatments used to control fruit diseases and stem-end rot while prochloraz is used as postharvest treatment (Pérez-Jiménez, 2008). Unfortunately, these fungicides are not effective anymore due to pathogen resistance (Horvitz and Cantalejo, 2014). Moreover, health concerns regarding the residues on chemically treated fruit and the undesirable effect on the environment are a serious issue (Tesfay et al., 2017). Palou et al. (2002) also reported that there is growing pressure due to health and environmental concerns related to synthetic coatings. These synthetic postharvest treatments may induce off-flavours and negatively change the taste of treated fruit (Hassenberg et al., 2008).

This situation has forced food scientists to find safe and environmentally friendly techniques for disease management and postharvest quality maintenance (Sivakumar et al., 2005). Environmental friendly, safe and economically acceptable alternative methods such as the application of inorganic salts, sodium bicarbonate or ammonium carbonate, alone or in combination with biocontrol agents and fruit coatings have been shown to control post-harvest fruit decay (Sivakumar et al., 2002). Graham (1997) reported that ozone (O_3) can be used as an antimicrobial agent in fruit. Xu (1999) also reported that at present, O_3 is being used in more than 30 different industries because of its high oxidant capacity and its effectiveness over a much wider spectrum of microorganisms compared to chlorine and other disinfectants.

In general, natural edible coatings comprises of polysaccharides, proteins or lipids (Valencia-Chamorro et al., 2010). Most evaluations of edible coating have largely focused on hydroxyl-propyl methylcellulose (HPMC)/beeswax/shellac composites (Navarro-Tarazaga et al., 2008; Valencia-Chamorro et al., 2011). All these composite coatings maintain fruit quality during postharvest storage, but they require the use of powerful organic solvents that will dissolve the shellac because it blocks the exchange of gases and promotes the occurrence of anaerobic conditions and off-flavours (Navarro-Tarazaga et al., 2007). Coatings with beeswax often appear impervious (Arnon et al., 2014) because of an increase in beeswax micro-particles that may produce hydrophobicity of coating dispersion (Dickinson, 2010) that lead to lower water vapour permeability of coatings (Peressini et al., 2003).

Recently, edible coatings have received attention from the researchers due to their ability to prolong shelf life and maintain postharvest quality (Krochta, 1996). Edible coatings such as chitosan have been reported to control decay in fruit by inhibiting fungal growth and triggering defensive mechanisms against infections (Hernández-Muñoz et al., 2008). Studies based on citrus edible coatings tend to focus on lipid-containing composites which are excellent water barriers (Dhall, 2013). However, there is a disadvantage of lipid-based coatings because of their less permeability to gases and that might cause CO_2 and ethanol accumulation and lead to off-flavours (Hagenmaier, 2002) and may also injure the coated fruit (Perez-Gago et al., 2002; Valencia-Chamorro et al., 2010). The objective of this article is to review the literature on the effect of edible coatings to extend the shelf life and enhance postharvest fruit quality of citrus fruits. Research gaps which warrant further investigation are also highlighted.

2.3. Edible coatings: An overview

Edible coatings are thin layers of edible material which can be consumed and applied to the surface of fresh horticultural products as a replacement or sometimes in addition to natural protective waxy coatings and to provide a barrier to moisture, oxygen and movement of solutes (Bourtoom, 2008; Dhall, 2013; Pavlath and Orts, 2009). Edible coatings are commercially applied to citrus fruit to enhance gloss, control loss of fruit weight and to carry postharvest fungicides (Grant and Burns, 1994; Petracek et al., 1998). Fungicides are commonly mixed with citrus coatings for simplicity and economy, although the effectiveness of decay control has previously been reported to be reduced when fungicides are dissolved into the coatings (Grant and Burns, 1994). Wax coatings are reported to restrict the exchange of gases (CO_2 and O_2) through the fruit surface, this often leads to anaerobic conditions being initiated in the fruit internal atmosphere resulting to the accumulation of ethanol and off-flavours (Baldwin et al., 1995; Hagenmaier, 2000; Hagenmaier and Shaw, 1992; Porat et al., 2005).

Human health has been a concern lately, therefore the use of edible films and coatings in fruit industries for protection and preservation has increased, and edible coatings are more advantageous than synthetic materials because of their edibility, biocompatibility, biodegradability and environmentally friendly, non-toxicity, non-polluting and aesthetic appearance (Han et al., 2002; Tharanathan, 2003; Valencia-Chamorro et al., 2010). They serve as a barrier to moisture, CO_2 and O_2, reducing volatile loss and they also carry functional additives (Olivas and Barbosa-Cánovas, 2005), so that they satisfy customer demands (Pavlath and Orts, 2009). Edible coatings have been used in citrus fruit such as fresh oranges (Baldwin et al., 1995), tangerines (Chien et al., 2007; Xu et al., 2018), mandarins (Arnon et al., 2014; Chien and Chou, 2006; Navarro-Tarazaga et al., 2008; Rojas-Argudo et al., 2009), 'Navel' (Arnon et al., 2014), grapefruit (Arnon et al., 2014; Petracek et al., 1998; Rojas-Argudo et al., 2009).

Applying coatings in a fresh horticultural product has been reported to be effective in prolonging the shelf life of coated produce (Toğrul and Arslan, 2004). Although coatings have shown potential in extending shelf life of different agricultural products (Baldwin et al., 2011), they are also used for different purposes within food system though, consumers might not be aware (Pavlath and Orts, 2009). Commercially, applied coatings are mostly based on oxidised polyethylene, organic solvents and different surfactants and stabilizers (Porat et al., 2005). Lately, studies are focusing on edible coatings with antimicrobial properties, it is believed that due to their lag phase extension and growth rate reduction of pathogens and spoilage microorganism in the fruit, postharvest life of fruit is prolonged (Quintavalla and Vicini, 2002).

Other studies approach a bilayer coating strategy where two or more coatings are mixed or applied both on the same fruit, the approach is done on the deposition of oppositely charged polyelectrolytes (Arnon et al., 2015). For instance, layer-by-layer edible coatings were applied as alginate and chitosan in melons (Poverenov et al., 2014), other multi-layered edible coatings which were prepared with chitosan and pectin and were shown to prolong the shelf life of papaya (Brasil et al., 2012). Layer by layer technique was also done by Arnon et al. (2015) where polysaccharide coatings were prepared, Carboxymethylcellulose (CMC) and chitosan coatings. Carboxymethylcellulose represent negatively charged carboxylic groups and chitosan represent the positively charged ammonium group so that they will interact perfectly. Carboxymethylcellulose was applied as an inner layer due to the fact that when chitosan was applied directly to the citrus surface it affect gas exchange processes, therefore, it was alternatively applied as a second external layer and it was reported to improve gloss and firmness of mandarins (Arnon et al., 2015). Figure 2.1 demonstrates how the layer-by-layer method of CMC and chitosan in mandarin works. Step 1 involves CMC edible coating while step 2 involves chitosan edible coating.

Figure 2. 1: The layer-by-layer edible coatings method of mandarin fruit (Arnon et al., 2015).

Edible coatings can be applied with some additives such as antioxidants, antimicrobials, colorants, flavours, fortifying nutrients and spice in film formulation to improve the ability of edible coatings to retard moisture, O_2, off-flavours and solutes transport (Diab et al., 2001; Olivas and Barbosa-Cánovas, 2005; Pranoto et al., 2005). The concentration, chemical structure, dispersion degree in the coating and interaction degree with the polymer of these additives would determine their influence on a product (Kester and Fennema, 1986). These mentioned additives have an ability to improve fruit nutritionally, which often result in an increased market value (Mchugh and Senesi, 2000). These additives would be important since edible coatings are made from different materials such as lipids, resins, polysaccharides and proteins, therefore they pose different capabilities towards their application on fruit and vegetables (Baldwin, 1994). Edible coatings can carry and transfer active ingredients and antimicrobial compounds that will enhance the shelf life of a product and also reduces the pathogen risk occurrence on the fruit surface (Oussalah et al., 2004; Ponce et al., 2008; Pranoto et al., 2005). Therefore, edible coated fruit would have quality and shelf life improved (Rooney, 1995).

It is significant to ensure the degree of temperature and the percentage level of relative humidity as it determines the permeability of oxygen in edible coatings. Several edible coatings have widely been assessed in improving the quality of citrus and it storability, considering well temperature and relative humidity (Navarro-Tarazaga et al., 2008; Perez-Gago et al., 2002; Valencia-Chamorro et al., 2011; Valencia-Chamorro et al., 2010). Chitosan, carboxyl methylcellulose, aloe vera and gum arabic are some of the edible coatings that have gained popularity due to their ability to improve the fruit quality and prevent decay during postharvest storage (Cháfer et al., 2012; Maqbool et al., 2010; Navarro et al., 2011).

There is growing scientific evidence suggesting that aloe vera gel has enormous antifungal activity against various pathogenic fungi (Aburjai and Natsheh, 2003). Studies have shown that aloe vera can positively affect the concentration of bioactive compounds of the treated produce (He et al., 2005), thereby ensuring the biological integrity, sensorial stability and the final quality of a product. Edible coatings can be safely used to improve food appearance and conservation since they retard food deterioration and enhance the quality. Moreover, edible coatings have advantages over synthetic coatings due to their natural biocide activity, biodegradability and being environmentally friendly.

2.4. Physicochemical quality

Physicochemical attributes such as firmness, weight loss, total soluble solids and colour play an important role in the marketability of citrus fruit (Qin et al., 2015). Therefore, in this section, the effect of different edible coatings on physicochemical qualities of citrus will be reviewed. Table 1 below shows the effect of different edible coatings on the physicochemical quality of citrus fruit.

Table 2. 1: The effect of various edible coatings on citrus physicochemical attributes.

Physicochemical attributes	Fruit	Edible coatings	Response to application	References
Firmness	‘Ortanique’ Mandarins	HPMC	Maintained	Valencia-Chamorro et al. (2010)
	‘Star Ruby’ grapefruit	CMC/Chitosan bilayer	Slightly decreased	Arnon et al. (2014)
	‘Navel’ oranges	CMC/Chitosan bilayer	Significantly decreased	Arnon et al. (2014)
	Sweet oranges	HPMC and HPMCME	Retained	Adetunji et al. (2012)
	‘Rishon’&‘Michal’Mandarins	CMC	Retained	Arnon et al. (2015)
Colour	'Delta Valencia' orange	Carnauba-based wax	No significant difference	Pereira et al. (2013)
	‘Rishon’& ‘Michal’ Mandarins	CMC-Chitosan	Change delayed	Arnon et al. (2015)
TSS	‘Murcott’ tangor tangerine	Chitosan	Retained	Chien et al. (2007)
	‘Hort.ex Tanaka’ Tangerine	CS + MMT	Retained	Xu et al. (2018)
	Mandarin (Kinnow fruit)	CMC and Guargum-based silver nanoparticle coatings	Increased	Shah et al. (2015b)
TA	'Delta Valencia' orange	Carnauba-based wax	No significant difference	Pereira et al. (2013)
	Hort. ex Tanaka’ Tangerine	CS/MMT	Sharp rise then decreases	Xu et al. (2018)
	‘Kagzi’ lime fruit	Coconut oil and mustard oil coatings	Acidity increased	
Weight loss	Mandarins	HPMC and CMC/Chitosan bilayer	Reduced significantly	(Arnon et al., 2014; Valencia-Chamorro et al., 2010)
	Oranges and grapefruit	Commercial wax	Reduced significantly	Arnon et al. (2014)
		CMC/ Chitosan bilayer	No significant difference	
	Valencia’ orange	HPMC-based coatings + BW⁄shellac ratio	Reduced significantly	Contreras-Oliva et al. (2011)

HPMC-. Hydroxyl-propyl methylcellulose, **HPMCM**E-hydroxy-propyl methylcellulose with Moringa Extract, **CMC**-Carboxymethyl Cellulose, **CS**-Chitosan, **MMT**-Montmorillonite , **BW**-beeswax

2.4.1. Firmness

Firmness is an important quality attribute that directly influences the decision by consumers to purchase fresh fruit (Lu, 2004). Previous research has shown that edible coatings have an influence on fruit firmness during cold chain and postharvest handling (Table 2.1). Some edible coatings are capable of reducing the softening rate and therefore prolonging shelf life (Tesfay and Magwaza, 2017). For instance, shellac edible coatings were reported to retain firmness in ‘Valencia’ orange (Khorram et al., 2017). Among three different concentrations of shellac coatings (i.e. 9, 10 and 11%), the highest concentration had higher firmness retention while the untreated fruit had the lowest fruit firmness. Deterioration of texture is mainly caused by depolymerisation of pectin, associated with pectin esterase, pectin lyase and polygalactronase activities (Khorram et al., 2017). During storage of citrus fruit, coatings that provide a modified atmosphere within the fruit would be able to decrease these enzyme activities and consequently result in firmness retention (Maftoonazad and Ramaswamy, 2005).

A study by Valencia-Chamorro et al. (2010) reported that mandarins coated with hydroxypropyl methylcellulose (HPMC) lipid edible composite coatings containing potassium sorbate (PS), sodium propionate (SP) and sodium benzoate (SB) or their mixtures as food preservatives had significantly lower firmness than uncoated fruit samples after storage for 6 weeks at 5 °C and 1 week at 20 °C. Edible coating with the mixture of SB + SP was reported to retain firmness, this can be linked to the lower weight loss observed in these fruit samples. A significant correlation between fruit firmness and weight loss was observed in a study by Navarro-Tarazaga et al. (2008) where HPMC-BW (beeswax) containing oleic acid (OA) at a ratio of BW/OA 1:0.5 was applied on ‘Ortanique’ mandarins. However, there was no correlation between fruit firmness and weight loss reported on Valencia oranges coated with the same coatings (Valencia-Chamorro et al., 2009). This shows that citrus species respond differently to edible coatings, and proper evaluation of each coating should be carried out before its adoption for commercial use.

2.4.2. Total soluble solids and titratable acidity

Total soluble solids (TSS) in fruit juice contains sugars, small acid quantities, vitamins, minerals, as well as some soluble pectins (Teerachaichayut and Ho, 2017). Titratable acidity (TA) comprises of components such as organic acids which appear as substrates of enzymatic reactions during respiration (Hazrati et al., 2017). The content of TA is one of the important quality parameters of citrus fruit, both TSS and TA affect the internal quality and eating experience of various fruit (Sdiri et al., 2012). TSS and TA contents in fruit juice may change depending on the conditions under which fruit are subjected (Ladanyia and Ladaniya, 2010).

The effect of edible coatings on TSS and TA content of citrus fruit has been studied (Table 2.1). For instance, Xu et al. (2018) evaluated the effect of chitosan (CS) and chitosan + montmorillonite (CS + MMT) coatings on tangerine fruit stored at 10 °C for 11 days. Their study revealed that TSS in both treated and untreated samples increased for the first days of storage then decreased as time continues. The results showed that treated samples had lower TSS than control at the end of storage (Xu et al., 2018).

Lime fruit (*Citrus aurantifolia* Swingle) coated with $CaCl_2$ (1%) and (2%), $KMnO_4$ (1%), coconut oil (100%), mustard oil (100%), sesamum oil (100%) and castor oil (100%) edible coatings showed TSS content increased at a slow rate as compared to uncoated fruit during storage period at 25-30 °C and 60-70% relative humidity (RH) for 18 days (Bisen et al., 2012). It was reported that TSS increases with increasing storage period (Sindhu and Singhrot, 1996). Ismail et al. (2010) reported that the increase in TSS content during storage time may be attributed to the acid hydrolysis and polysaccharide deposition with storage period.

Titratable acidity (TA) has also been demonstrated to be influenced by edible coatings. For instance, Bisen et al. (2012) found that lime fruit treated with coconut oil and castor oil coated fruit had higher concentrations of TA compared to untreated fruit. Their findings were comparable to those reported in ‘Kinnow’ mandarins (Sharma and Sandhooja, 1991). The higher acidity in coated fruit could be linked to lower oxygen availability to fruit at the end of storage (Bisen et al., 2012). Some coatings have been shown to have a marginal effect on TA concentration. For example, mandarin fruit coated with chitosan coating had similar TA content with the uncoated fruit at the end of storage (El Guilli et al., 2016).

2.4.3. Mass loss

In citrus fruit, water is generally lost from the rind and very rare from the pulp (Alquezar et al., 2010). One of the main causes of fruit weight loss is transpiration where water moves out and results in wilted rind and shriveled appearance (Wills and Golding, 2016), it was reported in citrus (Adetunji et al., 2012). Since most fruit are sold by weight, therefore, it is important to minimize the mass loss in fresh produce (Khout et al., 2007).

A couple of edible coatings are known to reduce mass loss in citrus fruit (Petracek et al., 1998). Studies by Khorram et al. (2017) have shown that shellac coatings at different concentrations (9, 10 and 11%) managed to reduce mass loss in ‘Valencia’ oranges compared to untreated fruit after 60 days at 5 °C. It was reported that shellac coatings when compared to gelatin and Persian gum, reduce mass loss because of their hydrophobic nature (Khorram et al., 2017). Additionally, protein and polysaccharide-based coatings are known for not forming effective water vapour barriers (Dhall, 2013). Their results correspond with the findings Perez-Gago et al. (2002) which reported that coatings comprising of various types of lipids were able to reduce the mass loss of treated ‘Fortune’ mandarins. The effect of two edible coatings, hydroxypropyl methylcellulose containing moringa leaf extract (HPMCME) and hydroxypropyl methylcellulose without moringa leaf extract (HPMC) has been studied in oranges (Adetunji et al., 2012). The findings concluded that both HPMCME and HPMC significantly reduce mass loss. Toğrul and Arslan (2004) indicated that the regular structure and array of the hydroxyl group of cellulose derivatives attract each other to form a strong hydrogen bond with water molecules of fruit and environment. Moreover, edible composite coatings based on HPMC and lipids, such as beeswax, carnauba wax maintain postharvest quality of citrus fruit by reducing loss of mass and keeping firmness and sensory quality of treated fruit (Navarro-Tarazaga et al., 2008; Perez-Gago et al., 2002).

2.4.4. Fruit colour

Fruit colour is one of the most important quality attributes that is evaluated by consumers before they purchase the fruit (Opara and Pathare, 2014; Rehman et al., 2018). Well coloured citrus fruit is more attractive to buyers than green fruit (Mditshwa et al., 2017). Improving citrus glossiness and appearance is one of the main purposes of applying coatings on the fruit (Arnon et al., 2014). Chitosan was reportedly to have no much impact on fruit colour where mandarin treated samples were compared with untreated samples (El Guilli et al., 2016). Figure 2.2 shows the effect of commercial wax and edible coatings (chitosan and CMC bilayer) on citrus gloss and colour. These attributes were evaluated after four weeks of cold storage and one week of shelf life under 20 °C temperature conditions (Arnon et al., 2014).

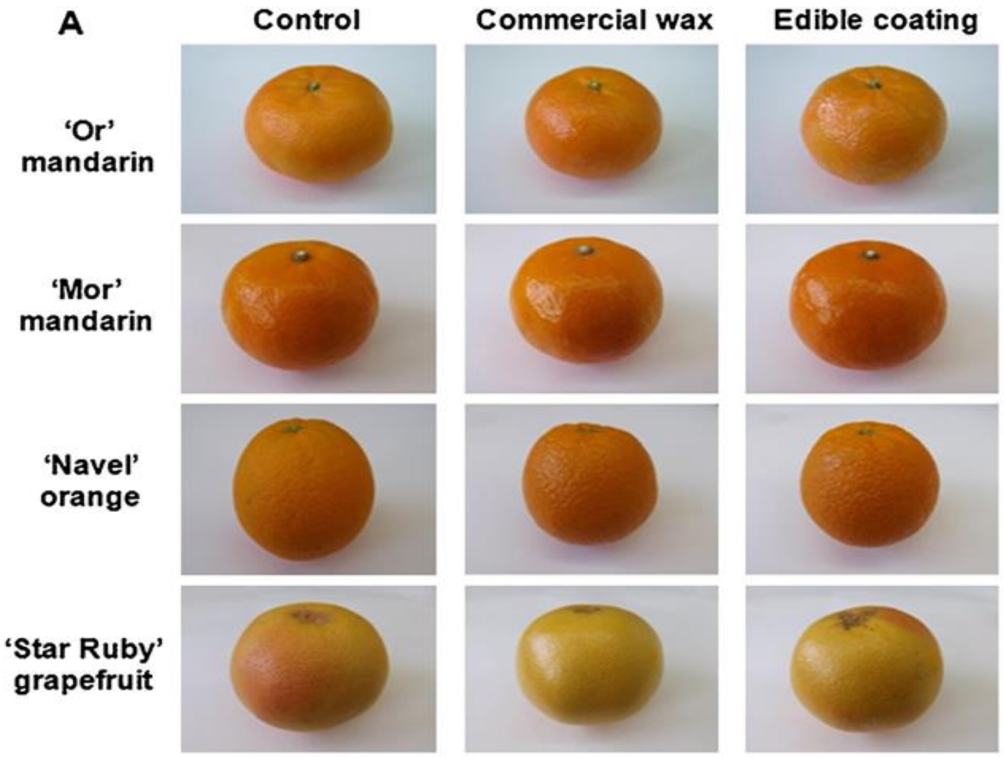


Figure 2. 2: Shows the efficacy of commercial wax compared to polysaccharide based edible coatings comprising CMC/chitosan bilayer on preserving colour and glossiness on different citrus cultivars (Arnon et al., 2014).

This study reported that both CMC/chitosan bilayer and commercial wax had a positive effect on citrus gloss. Untreated citrus fruit was not attractive to buyers due to its low glossiness unlike commercial wax and CMC/chitosan bilayer coating which had a significantly improved citrus gloss and overall appearance including fruit colour.

2.5. Sensory quality

Sensory attributes are one of the most important determinants of quality. Attributes such as taste, aroma, appearance (glossiness) are often performed by trained and consumer panels because they give the most accurate feedback to acquire information on human taste and aroma perception (Beullens et al., 2006; Beullens et al., 2008; Rudnitskaya et al., 2006). Consumers mostly use visual, olfactory and touch senses to evaluate fruit maturity (Ladaniya, 2008). Instruments may be used to measure fruit quality, however, consumers contribute a lot in judging of fruit quality by using their own senses towards their purchase, therefore sensory evaluation is significant in deciding the fruit marketability (Ladaniya, 2008). The flavour may have a large influence on product acceptability (Barrett et al., 2010).

One of the core reasons for coating fruit is to improve gloss and attractiveness of the produce (Arnon et al., 2014). Edible coatings reduce moisture loss, control surface dehydration and discoloration, delay the surface whitening and enhance the glossiness of fruit surfaces (Lin and Zhao, 2007). However, hydroxypropyl methylcellulose, beeswax coatings were reported to have no significant effect on fruit appearance (Contreras-Oliva et al., 2011). Carboxymethyl cellulose coatings were reported to preserve high structural integrity and provided an even matrix during storage yet conveyed little gloss (Arnon et al., 2014). Glycerol was reported to improve the transparency of edible coatings and enhanced the citrus fruit appearance since it provides their surface with glossy appearance (Lee et al., 2002).

2.6. Nutritional quality

Fruit are major sources of macro (fibre and carbohydrate) and micronutrients (vitamin A, B complex, C and E minerals) (Barrett et al., 2010). The nutritional quality of citrus juice is primarily related to the content of vitamin C (Zerdin et al., 2003), additionally ascorbic acid is the major antioxidant compound in citrus fruits (Gardner et al., 2000). Nutritional quality is also composed of phenolic compounds, antioxidants and carotenoids, organic acids, minerals, total sugars. Storage conditions have been reported to affect postharvest quality and nutritional properties of citrus fruit (Mditshwa et al., 2017), yet postharvest treatments may inversely influence fruit nutritional quality (Lee and Kader, 2000).

2.6.1 Vitamins

To date, vitamin content has gained a great interest to food scientist and technologist as it plays a vital role in human health (Guerreiro et al., 2017; Mditshwa et al., 2017). In citrus fruit, one of the most important nutrients is vitamin C (Contreras-Oliva et al., 2011) and it plays an important role in superoxide radical suppression since it is a water-soluble antioxidant in fruit (Kaur and Kapoor, 2001). Vitamin C in fruit is utilized by many oxidation reactions of postharvest physiological activities, free radicals then accumulate in fruit consequently cell aging accelerate (Xu et al., 2018). Some postharvest treatments have been reported to decrease vitamin C content in fruit because of the solubility of water and thermos-sensibility of vitamin C (Mditshwa et al., 2017). It is important to retain ascorbic acid content in citrus fruit for consumer health. Edible coatings have been reported to influence the concentrations of certain vitamins in citrus fruit (Machado et al., 2012).

Findings by Toğrul and Arslan (2004) showed that mandarin fruit coated with carboxymethyl cellulose had delayed ascorbic acid loss compared to uncoated fruit after storage. Similarly, Chien and Chou (2006) also showed that chitosan coating resulted in higher vitamin C retention in ’Tankan’ citrus fruit. The study also investigated the effect of low molecular weight chitosan (LMWC, with Mw of 92.1 kDa) and high molecular weight chitosan (HMLW, Mw with 357.3 kDa) on ‘Murcott tangor’ quality, it was reported that LMWC was more effective on retaining vitamin C as compared to HMLW, this also applied irrespective of concentration contained by both coatings (Chien and Chou, 2006).

Carnauba wax supplemented with grapefruit seed extract was used to treat ‘Satsuma’ mandarin fruit, it displayed to be good in retaining vitamin C during cold storage (Shin et al., 1998). Citrus fruit coated Arabic gum were compared with uncoated fruit and it was concluded that coated fruit had higher vitamin C unlike uncoated sample (Eskandari et al., 2014). The effect of Arabic gum on retaining high vitamin C level is said to be due to its secondary compounds displaying antioxidant properties which also hinder vitamin C oxidation (Ali et al., 2009). A study by Bisen et al. (2012) reported that a pure coconut oil coating was able to retain a maximum ascorbic acid of ‘Kagzi’ lime fruit at 49.9 mg/10 mg which were stored for 18 days, it was marked as significant treatment as compared to control treatment. This high ascorbic acid content was suggested to be caused by metabolic variations and increment of acidity percentage under pure coconut oil coating. While low ascorbic acid in control was possibly in line with a conversion of ascorbic acid to dehydro-ascorbic acid in the enzyme presence called ascorbinase. The results found in this study are in agreement with (El-Monem et al., 2003) in apples and (Jadhao et al., 2008) in Kagzi lime fruit. Similarly, Xu et al. (2018) reported that ascorbic acid content of tangerine was increased by chitosan (CS) and chitosan/montmorillonite (CS/MMT) coatings. Overall, the ascorbic acid content was higher in the fruit coated with CS, CS/MMT (0.5%) and CS/MMT (1%) compared to untreated fruit (Xu et al., 2018). These findings were best explained by the gas barrier of coatings which decreased the potential autoxidation of ascorbic acid in the presence of O_2. On the other hand, some studies have shown that edible coatings have a marginal effect on the concentration of vitamins during storage and shelf-life. A study by ContrerasOliva et al. (2011) reported that ‘Valencia’ oranges coated with HPMC, BW and shellac had no significant difference in ascorbic acid. Also, Pereira et al. (2013) reported that carnauba-based wax applied to ‘Delta Valencia’ oranges which were stored at 7 °C and 85+2% RH for 28 days had no effect on vitamin C. It could be argued that some coatings are not dense enough to inhibit the autoxidation of ascorbic acid during storage.

2.6.2. Phenolics and antioxidants

Phenolic and antioxidant compounds play a significant role in nutritional and sensory quality of numerous fruit (Sandhu and Gu, 2010), they contribute to fruit quality prior to colour, taste, aroma and flavor (Tomás-Barberán and Espín, 2001). However, there are some phenolic compounds that impart bitterness taste in fruit such as tannins (Tomás-Barberán and Espín, 2001). Citrus fruit are rich in bioactive phenolic compounds (anthocyanins, flavolols, isoflavones and catechins), with strong antioxidant capacity (Mditshwa et al., 2017). Antioxidants are secondary plant metabolites which are a diverse group of naturally produced chemicals with no primary functions in plant cell growth. They are produced by plant in response to the exterior environment and commonly play a critical role in regulating the function of physiological and metabolic reactions against stress (Brandt and Mølgaard, 2001). Citrus rinds are naturally rich in non-volatile organic acids including phenolic compounds (Benavente-García et al., 1997; Manthey, 2004), additionally, these compounds are the most significant in responsible for the antioxidant activities of the fruit.

A study on the effect of HPMC, beeswax and shellac coatings on the nutritional quality of ‘Valencia’ oranges by Contreras-Oliva et al. (2011) reported that total phenolic compounds of ‘Valencia’ oranges were not affected during storage at 5 °C. Similarly, a study on the effect of carboxymethyl cellulose coating from sugar beet pulp on mandarin fruit reported that the treatment had no effect on total antioxidant content (Toğrul and Arslan, 2004). Importantly, the study concluded that applying this coating on mandarins before cold storage could be successful in prolonging shelf life and enhancing quality and also controlling postharvest decay.

2.7. Edible coatings on fruit diseases and disorders

Citrus fruit is highly appreciated in the international fruit market because of its commercial value. However, citrus fruit are susceptible to micro-organism infections such as blue mould, green mould, stem end rot and physiological disorders such rind pitting disorder, rind breakdown, rind staining (Arnon et al., 2015; Chien and Chou, 2006; Xu et al., 2018). These fruit diseases and disorders are the major cause of fresh produce loss every year (Janisiewicz and Korsten, 2002). Effective postharvest treatments are thus required in order to control the decay and prolong the shelf life of fruit (Chien and Chou, 2006). Figure 2.3 represents two of many postharvest physiological disorders in citrus fruit.

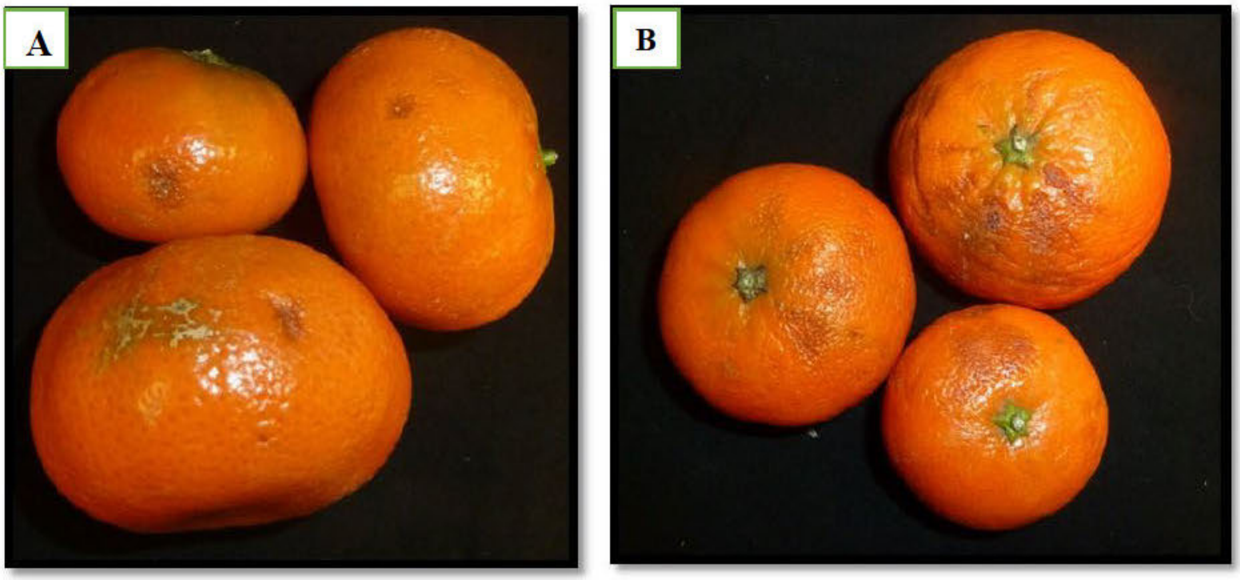


Figure 2. 3: 'Nadorcott' mandarin with rind pitting (A) and rind staining (B) disorders developed during the postharvest time (Cronjé, 2012).

Both rinds pitting and rind staining are postharvest physiological disorders, they affect various citrus cultivars and adversely decrease their market value (Palou et al., 2015). In addition, these disorders occur in flavedo (outer skin) of fruit with a random pattern of sunken areas and when they become severe, oil glands collapse. Edible coatings have reported as new alternatives in controlling postharvest pathogenic diseases and postharvest disorders (Table 2). Edible coatings have been reported to minimise disease occurrence and avoid a negative effect on human health (Bautista-Baños et al., 2006; Chien and Chou, 2006). Chitosan coatings and its derivatives have been reported as potential, eco-friendly alternatives to the use of synthetic fungicides. Among edible coatings, chitosan was reported to be the best coating for controlling postharvest fungal infection diseases (Bautista-Baños et al., 2006). Numerous studies have reported that chitosan controls postharvest rots effectively during storage, it slows down the infection process and delays the onset of infection (Bautista-Baños et al., 2006). Chitosan coating directly interacts with fungi morphological growth and may also stimulate the defense mechanism of a plant/fruit (Chien and Chou, 2006).

To control postharvest physiological rind disorders and diseases, different postharvest treatments have been used. For instance, Zeng et al. (2010) coated undamaged navel oranges with chitosan (2%) after fruit were inoculated with either *P. italicum* or *P. digitatum* and stored at 20 °C with RH of 85-95%. Treated fruit had reduced incidence of disease and lesion diameter compared to untreated fruit. Furthermore, the in vitro and in vivo development of *P. digitatum* showed that chitosan was able to reduce fungal growth in the plates compared to control, notably, increasing the chitosan concentration resulted to higher inhibition of fungal growth although inhibition percentage never reached 100% (El Guilli et al., 2016). While some treatments have been shown to be effective, the opposite has also been reported. For instance, Petracek et al. (1998) reported that 'Marsh' grapefruit coated with shellac-based waxes had a higher incidence of rind pitting. Citrus waxes are often incorporated with synthetic chemical fungicides to control postharvest diseases.

Since consumers prefer less or no use of chemicals on minimally processed fruits, that brings more attention to find the naturally occurring substances as an alternative. Therefore, various postharvest approaches including modified atmosphere packaging (MAP) (Li et al., 2013), hot water (Spadoni et al., 2014), biocontrol agent application (Yao and Tian, 2005) have been widely reviewed in recent years to extend citrus shelf life. Among these applications, the approach of using edible coating is a promising technique with a good safety, biodegradable, environmentally friendly and satisfying performance (Arnon et al., 2015; Dhall, 2013; Embuscado and Huber, 2009; Galus and Kadzińska, 2015; Silva-Weiss et al., 2013; Tharanathan, 2003).

Table 2. 2: The effect of various edible coatings on microbial attributes of citrus fruit.

Postharvest citrus diseases	**Citrus fruit**	**Coatings with concentrations**	**Response**	**References**
Penicillium digitatum and Penicilium italicum	‘Navel’ orange, Lime	Chitosan (2,4,6 and 8 g/L)	Inhibited	El-Mohamedy et al. (2015)
Colletotrichum gloeosporoides	‘Jincheng 447’ orange	Oligochitosan	Inhibited	Deng et al. (2015)
Guignardia citricarpa	‘Valencia' and 'Pera Rio' oranges	Chitosan, chitosan + TBZ (2%)	Inhibited	Rappussi et al. (2009, 2011)
Geotrichum citri-aurantii	Lime	Chitosan (2,4,6 and 8 g/L)	Inhibited	Faten (2010)
P.digitatum,P.italicum, Botrydiplodia lecanidion and Botrytis cinerea	‘Murcott’ tangor	Chitosan (HMW) 0,05%,0,1% and 0,2%	Inhibited	Chien et al. (2007)
P.italicum,B.lecanidion and B.cinerea	‘Murcott’ tangor	Chitosan (LMW) 0,05%,0,1% and 0,2%	Inhibited	Chien et al. (2007)
P.digitatum	‘Eureka’ lemon	Glycolchitosan (0,2%)	Inhibited	El-Ghaouth et al. (2000)
P.italicum	‘Navel powell’ orange	Chitosan (2%)	Inhibited	Panebianco et al. (2014)
P.digitatum	‘Valencia’ orange	Chitosan (0,5%)	Inhibited	Cháfer et al. (2012)

Keys: **HMW**- High molecular weight, **LMW**- Low molecular weight

2.8. Conclusion

The effect of edible coatings such as CMC, CS, MMT, HPMC, shellac, beeswax, gum arabic and aloe vera was studied in this review. The review has shown that the application of edible coatings maintains and improves citrus quality during storage and shelf life. This suggests that edible coatings can be recommended for commercial use by citrus industries due to their biodegradability, biocompatibility, and non-toxicity, non-polluting. Most coatings also serve as a barrier to moisture, CO_2 and O_2 which subsequently reduce the loss of volatile compounds, reduce respiration rate and mass loss and they also carry functional additives; therefore, they are beneficial to the industries compared to synthetic coatings.

Edible coatings cooperated with plant extracts have been an interesting topic lately, it is encouraged to use extracts from commonly used plants in order to gain consumer trust. Plant extracts has been promising to be an effective method for controlling plant pathogens and are generally considered to be safe. Moringa plant extracts have gained significant interest among food researchers and postharvest scientists. Currently, there is a little information on the use of moringa plant extracts as an inhibitor of citrus postharvest diseases and disorder as well as their ability to improve quality. Further research aimed at understanding the effect of edible coatings incorporated with plant extracts such as moringa is thus warranted.

2.9. References

Aburjai, T., Natsheh, F.M., 2003. Plants used in cosmetics. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 17, 987-1000.

Adetunji, C., Fawole, O., Arowora, K., Nwaubani, S., Ajayi, E., Oloke, J., Majolagbe, O., Ogundele, B., Aina, J., Adetunji, J., 2012. Quality and safety of Citrus Sinensis coated with Hydroxypropylmethylcellulose edible coatings containing Moringa oleifera extract stored at ambient temperature. Global Journal of Science Frontier Research, 2249-4626.

Akbas, M.Y., Ölmez, H., 2007. Effectiveness of organic acid, ozonated water and chlorine dippings on microbial reduction and storage quality of fresh-cut iceberg lettuce. Journal of the Science of Food and Agriculture 87, 2609-2616.

Ali, B.H., Ziada, A., Blunden, G., 2009. Biological effects of gum arabic: a review of some recent research. Food and Chemical Toxicology 47, 1-8.

Alquezar, B., Mesejo, C., Alferez, F., Agustí, M., Zacarias, L., 2010. Morphological and ultrastructural changes in peel of ‘Navelate’oranges in relation to variations in relative humidity during postharvest storage and development of peel pitting. Postharvest Biology and Technology 56, 163-170.

Arnon, H., Granit, R., Porat, R., Poverenov, E., 2015. Development of polysaccharides-based edible coatings for citrus fruits: A layer-by-layer approach. Food Chemistry 166, 465-472.

Arnon, H., Zaitsev, Y., Porat, R., Poverenov, E., 2014. Effects of carboxymethyl cellulose and chitosan bilayer edible coating on postharvest quality of citrus fruit. Postharvest Biology and Technology 87, 21-26.

Baldwin, E.A., 1994. Edible coatings for fresh fruits and vegetables: Past, present, and future. Edible coatings and films to improve food quality 1, 25.

Baldwin, E.A., Hagenmaier, R., Bai, J., 2011. Edible coatings and films to improve food quality. CRC Press.

Baldwin, E.A., Nisperos-Carriedo, M., Shaw, P.E., Burns, J.K., 1995. Effect of coatings and prolonged storage conditions on fresh orange flavor volatiles, degrees Brix, and ascorbic acid levels. Journal of Agricultural and Food Chemistry 43, 1321-1331.

Barrett, D.M., Beaulieu, J.C., Shewfelt, R., 2010. Color, flavor, texture, and nutritional quality of fresh-cut fruits and vegetables: desirable levels, instrumental and sensory measurement, and the effects of processing. Critical Reviews in Food Science and Nutrition 50, 369-389.

Bautista-Baños, S., Hernandez-Lauzardo, A.N., Velazquez-Del Valle, M.G., Hernández-López, M., Barka, E.A., Bosquez-Molina, E., Wilson, C., 2006. Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. Crop Protection 25, 108-118.

Benavente-García, O., Castillo, J., Marin, F.R., Ortuño, A., Del Río, J.A., 1997. Uses and properties of citrus flavonoids. Journal of Agricultural and Food Chemistry 45, 4505-4515.

Beullens, K., Kirsanov, D., Irudayaraj, J., Rudnitskaya, A., Legin, A., Nicolaï, B.M., Lammertyn, J., 2006. The electronic tongue and ATR–FTIR for rapid detection of sugars and acids in tomatoes. Sensors and Actuators B: Chemical 116, 107-115.

Beullens, K., Mészáros, P., Vermeir, S., Kirsanov, D., Legin, A., Buysens, S., Cap, N., Nicolaï, B.M., Lammertyn, J., 2008. Analysis of tomato taste using two types of electronic tongues. Sensors and Actuators B: Chemical 131, 10-17.

Bisen, A., Pandey, S.K., Patel, N., 2012. Effect of skin coatings on prolonging shelf life of kagzi lime fruits (Citrus aurantifolia Swingle). Journal of Food Science and Technology 49, 753-759.

Bourtoom, T., 2008. Edible films and coatings: characteristics and properties. International Food Research Journal 15, 237-248.

Brandt, K., Mølgaard, J.P., 2001. Organic agriculture: does it enhance or reduce the nutritional value of plant foods? Journal of the Science of Food and Agriculture 81, 924-931.

Brasil, I., Gomes, C., Puerta-Gomez, A., Castell-Perez, M., Moreira, R., 2012. Polysaccharide-based multilayered antimicrobial edible coating enhances quality of fresh-cut papaya. LWT-Food Science and Technology 47, 39-45.

Cháfer, M., Sánchez-González, L., González-Martínez, C., Chiralt, A., 2012. Fungal decay and shelf life of oranges coated with chitosan and bergamot, thyme, and tea tree essential oils. Journal of food science 77, E182-E187.

Chien, P.-J., Sheu, F., Lin, H.-R., 2007. Coating citrus (Murcott tangor) fruit with low molecular weight chitosan increases postharvest quality and shelf life. Food chemistry 100, 1160-1164.

Chien, P.J., Chou, C.C., 2006. Antifungal activity of chitosan and its application to control post-harvest quality and fungal rotting of Tankan citrus fruit (*Citrus tankan Hayata*). Journal of the Science of Food and Agriculture 86, 1964-1969.

Contreras-Oliva, A., Rojas-Argudo, C., Pérez-Gago, M.B., 2011. Effect of solid content and composition of hydroxypropyl methylcellulose–lipid edible coatings on physicochemical, sensory and nutritional quality of ‘Valencia’oranges. International Journal of Food Science & Technology 46, 2437-2445.

Contreras-Oliva, A., Rojas-Argudo, C., Pérez-Gago, M.B., 2012. Effect of solid content and composition of hydroxypropyl methylcellulose–lipid edible coatings on physico-chemical and nutritional quality of ‘Oronules’ mandarins. Journal of the Science of Food and Agriculture 92, 794-802.

Cronjé, P., 2012. Postharvest rind disorders of 'Nadorcott'mandarin are affected by rootstock in addition to postharvest treatments, II All Africa Horticulture Congress 1007, pp. 111-117.

Deng, L., Zeng, K., Zhou, Y., Huang, Y., 2015. Effects of postharvest oligochitosan treatment on anthracnose disease in citrus (*Citrus sinensis L. Osbeck*) fruit. European Food Research and Technology 240, 795-804.

Dhall, R., 2013. Advances in edible coatings for fresh fruits and vegetables: A review. Critical reviews in food science and nutrition 53, 435-450.

Diab, T., Biliaderis, C.G., Gerasopoulos, D., Sfakiotakis, E., 2001. Physicochemical properties and application of pullulan edible films and coatings in fruit preservation. Journal of the Science of Food and Agriculture 81, 988-1000.

Dickinson, E., 2010. Food emulsions and foams: stabilization by particles. Current Opinion in Colloid & Interface Science 15, 40-49.

El-Ghaouth, A., Smilanick, J.L., Wilson, C.L., 2000. Enhancement of the performance of Candida saitoana by the addition of glycolchitosan for the control of postharvest decay of apple and citrus fruit. Postharvest Biology and Technology 19, 103-110.

El-Mohamedy, R., El-Gamal, N.G., Bakeer, A.R.T., 2015. Application of chitosan and essential oils as alternatives fungicides to control green and blue moulds of citrus fruits. Int. J. Curr. Microbiol. Appl. Sci 4, 629-643.

El-Monem, E., Mostafa, A., El-Maged, M., 2003. Effect of harvest treatments on storage behaviour of guava fruits cv. Sardar. J Maharashtra Agric Univ 26, 297-300.

El Guilli, M., Hamza, A., Clément, C., Ibriz, M., Ait Barka, E., 2016. Effectiveness of postharvest treatment with chitosan to control citrus green mold. Agriculture 6, 12.

Embuscado, M.E., Huber, K.C., 2009. Edible films and coatings for food applications. Springer.

Eskandari, A., Heidari, M., Daneshvar, M.H., Taheri, S., 2014. Studying effects of edible coatings of Arabic Gum and olive oil on the storage life and maintain quality of postharvest

Sweet Lemon (*Citrus Lemontta*). International Journal of Agriculture and Crop Sciences 7, 207.

Faten, M., 2010. Combination between citral and chitosan for controlling sour rot disease of lime fruits. Research Journal of Agriculture and Biological Sciences 6, 744-749.

Galus, S., Kadzińska, J., 2015. Food applications of emulsion-based edible films and coatings. Trends in Food Science & Technology 45, 273-283.

Garcia, A., Mount, J., Davidson, P., 2003. Ozone and chlorine treatment of minimally processed lettuce. Journal of Food Science 68, 2747-2751.

Gardner, P.T., White, T.A., McPhail, D.B., Duthie, G.G., 2000. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. Food chemistry 68, 471-474.

Graham, D.M., 1997. Use of ozone for food processing. Food technology (USA).

Grant, L.A., Burns, J., 1994. Application of coatings. Edible Coatings and Films to Improve Food Quality. Technomic Publishing Company, Lancaster, 189-200.

Guerreiro, A.C., Gago, C.M., Faleiro, M.L., Miguel, M.G., Antunes, M.D., 2017. The effect of edible coatings on the nutritional quality of 'Bravo de Esmolfe'fresh-cut apple through shelf-life. LWT-Food Science and Technology 75, 210-219.

Hagenmaier, R., 2002. The flavor of mandarin hybrids with different coatings. Postharvest Biology and Technology 24, 79-87.

Hagenmaier, R.D., 2000. Evaluation of a polyethylene–candelilla coating for 'Valencia'oranges. Postharvest Biology and Technology 19, 147-154.

Hagenmaier, R.D., Shaw, P.E., 1992. Gas permeability of fruit coating waxes. Journal of the American Society for Horticultural Science 117, 105-109.

Han, Y., Floros, J., Linton, R., Nielsen, S., Nelson, P., 2002. Response surface modeling for the inactivation of Escherichia coli O157: H7 on green peppers (*Capsicum annuum*) by ozone gas treatment. Journal of Food Science 67, 1188-1193.

Hassenberg, K., Frohling, A., Geyer, M., Schluter, O., Herppich, W., 2008. Ozonated wash water for inhibition of Pectobacterium carotovorum on carrots and the effect on the physiological behaviour of produce. European Journal of Horticultural Science 73, 37.

Hazrati, S., Kashkooli, A.B., Habibzadeh, F., Tahmasebi-Sarvestani, Z., Sadeghi, A.R., 2017. Evaluation of aloe vera gel as an alternative edible coating for peach fruits during cold storage period. Gesunde Pflanzen 69, 131-137.

He, Q., Changhong, L., Kojo, E., Tian, Z., 2005. Quality and safety assurance in the processing of Aloe vera gel juice. Food Control 16, 95-104.

Hernández-Muñoz, P., Almenar, E., Del Valle, V., Velez, D., Gavara, R., 2008. Effect of chitosan coating combined with postharvest calcium treatment on strawberry (*Fragaria*× *ananassa*) quality during refrigerated storage. Food Chemistry 110, 428-435.

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

Ismail, O.M., El-Moniem, E., Abd-Allah, A., Rl-Naggar, M., 2010. Influence of some post-harvest treatments on guava fruits. Agric. Biol. JN Am 1, 1309-1318.

Jadhao, S., Borkar, P., Borkar, S., Bakane, P., Murumkar, R., 2008. Effect of different treatments and packaging materials on biochemical changes during storage of kagzi lime. Asian Journal of Bio Science 3, 247-250.

Janisiewicz, W.J., Korsten, L., 2002. Biological control of postharvest diseases of fruits. Annual review of phytopathology 40, 411-441.

Kader, A.A., 2003. A perspective on postharvest horticulture (1978-2003). HortScience 38, 1004-1008.

Kaur, C., Kapoor, H.C., 2001. Antioxidants in fruits and vegetables–the millennium’s health. International Journal of Food Science & Technology 36, 703-725.

Kester, J.J., Fennema, O., 1986. Edible films and coatings: A review. Food Technology (USA).

Khalid, S., Malik, A.U., Saleem, B.A., Khan, A.S., Khalid, M.S., Amin, M., 2012. Tree age and canopy position affect rind quality, fruit quality and rind nutrient content of ‘Kinnow’mandarin (*Citrus nobilis Lour*× *Citrus deliciosa Tenora*). Scientia Horticulturae 135, 137-144.

Khorram, F., Ramezanian, A., Hosseini, S.M.H., 2017. Shellac, gelatin and Persian gum as alternative coating for orange fruit. Scientia Horticulturae 225, 22-28.

Khout, M., Ritenour, M., Salvatoe, J., 2007. BASF Freshseal® CHC helps keep packed tomatoes firmer and fresher longer, Proceedings of Florida State Horticultural Society, pp. 217-221.

Krochta, J.M., 1996. Edible composite moisture-barrier films. Blakistone B (Ed.), 38-54.

Ladaniya, M., 2008. Fruit quality control, evaluation and analysis. Citrus fruit: biology, technology and evaluation, 475-500.

Ladanyia, M., Ladaniya, M., 2010. Citrus fruit: biology, technology and evaluation. Academic press.

Lado, J., Gambetta, G., Zacarias, L., 2018. Key determinants of citrus fruit quality: Metabolites and main changes during maturation. Scientia Horticulturae 233, 238-248.

Lee, S.K., Kader, A.A., 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest biology and technology 20, 207-220.

Lee, S.Y., Dangaran, K., Krochta, J., 2002. Gloss stability of whey-protein/plasticizer coating formulations on chocolate surface. Journal of Food Science 67, 1121-1125.

Li, F., Zhang, X., Song, B., Li, J., Shang, Z., Guan, J., 2013. Combined effects of 1-MCP and MAP on the fruit quality of pear (*Pyrus bretschneideri Reld cv. Laiyang*) during cold storage. Scientia Horticulturae 164, 544-551.

Lin, D., Zhao, Y., 2007. Innovations in the development and application of edible coatings for fresh and minimally processed fruits and vegetables. Comprehensive Reviews in Food Science and Food Safety 6, 60-75.

Lu, R., 2004. Multispectral imaging for predicting firmness and soluble solids content of apple fruit. Postharvest Biology and Technology 31, 147-157.

Machado, F.L.d.C., Costa, J.M.C., Batista, E.N., 2012. Application of carnauba-based wax maintains postharvest quality of 'Ortanique'tangor. Food Science and Technology 32, 261-268.

Maftoonazad, N., Ramaswamy, H., 2005. Postharvest shelf-life extension of avocados using methyl cellulose-based coating. LWT-Food Science and Technology 38, 617-624.

Magwaza, L.S., Opara, U.L., Cronje, P.J., Landahl, S., Terry, L.A., 2013. Canopy position affects rind biochemical profile of ‘Nules Clementine’mandarin fruit during postharvest storage. Postharvest Biology and Technology 86, 300-308.

Manthey, J.A., 2004. Fractionation of orange peel phenols in ultrafiltered molasses and mass balance studies of their antioxidant levels. Journal of Agricultural and Food Chemistry 52, 7586-7592.

Maqbool, M., Ali, A., Ramachandran, S., Smith, D.R., Alderson, P.G., 2010. Control of postharvest anthracnose of banana using a new edible composite coating. Crop Protection 29, 1136-1141.

McHugh, T., Senesi, E., 2000. Apple wraps: A novel method to improve the quality and extend the shelf life of fresh-cut apples. Journal of Food Science 65, 480-485.

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

Mitra, S.K., 1997. Postharvest physiology and storage of tropical and subtropical fruits. CAB international Wallingford.

Navarro-Tarazaga, M.-L., Plotto, A., Goodner, K., Baldwin, E., Perez-Gago, M.-B., 2007. Effect of edible coatings on the flavor of'Valencia'oranges, HortScience. AMER SOC Horticultural Science 113 S West ST, STE 200, Alexandria, VA 22314-2851 USA, pp. 869-869.

Navarro-Tarazaga, M.L., Del Río, M.A., Krochta, J.M., Perez-Gago, M.B., 2008. Fatty Acid Effect on Hydroxypropyl Methylcellulose− Beeswax Edible Film Properties and Postharvest Quality of Coated 'Ortanique'Mandarins. Journal of Agricultural and Food Chemistry 56, 10689-10696.

Navarro, D., Díaz-Mula, H.M., Guillén, F., Zapata, P.J., Castillo, S., Serrano, M., Valero, D., Martínez-Romero, D., 2011. Reduction of nectarine decay caused by Rhizopus stolonifer, Botrytis cinerea and Penicillium digitatum with Aloe vera gel alone or with the addition of thymol. International Journal of Food Microbiology 151, 241-246.

Olivas, G., Barbosa-Cánovas, G., 2005. Edible coatings for fresh-cut fruits. Critical Reviews in Food Science and Nutrition 45, 657-670.

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

Oussalah, M., Caillet, S., Salmiéri, S., Saucier, L., Lacroix, M., 2004. Antimicrobial and antioxidant effects of milk protein-based film containing essential oils for the preservation of whole beef muscle. Journal of Agricultural and Food Chemistry 52, 5598-5605.

Palou, L., Usall, J., Smilanick, J.L., Aguilar, M.J., Vinas, I., 2002. Evaluation of food additives and low-toxicity compounds as alternative chemicals for the control of Penicillium digitatum and Penicillium italicum on citrus fruit. Pest management science 58, 459-466.

Palou, L., Valencia-Chamorro, S.A., Pérez-Gago, M.B., 2015. Antifungal edible coatings for fresh citrus fruit: A review. Coatings 5, 962-986.

Panebianco, S., Vitale, A., Platania, C., Restuccia, C., Polizzi, G., Cirvilleri, G., 2014. Postharvest efficacy of resistance inducers for the control of green mold on important Sicilian citrus varieties. Journal of Plant Diseases and Protection 121, 177-183.

Pavlath, A.E., Orts, W., 2009. Edible films and coatings: why, what, and how?, Edible films and coatings for food applications. Springer, pp. 1-23.

Pereira, G.d.S., Machado, F.L.d.C., Costa, J.M.C.d., 2013. Quality of'Delta Valencia'orange grown in semiarid climate and stored under refrigeration after coating with wax. Food Science and Technology 33, 276-281.

Peressini, D., Bravin, B., Lapasin, R., Rizzotti, C., Sensidoni, A., 2003. Starch–methylcellulose based edible films: rheological properties of film-forming dispersions. Journal of Food Engineering 59, 25-32.

Pérez-Jiménez, R.M., 2008. Significant avocado diseases caused by fungi and oomycetes. Eur J Plant Sci Biotechnol 2, 1-24.

Perez-Gago, M., Rojas, C., DelRio, M., 2002. Effect of lipid type and amount of edible hydroxypropyl methylcellulose-lipid composite coatings used to protect postharvest quality of mandarins cv. Fortune. Journal of Food Science 67, 2903-2910.

Petracek, P.D., Dou, H., Pao, S., 1998. The influence of applied waxes on postharvest physiological behavior and pitting of grapefruit. Postharvest Biology and Technology 14, 99-106.

Ponce, A.G., Roura, S.I., del Valle, C.E., Moreira, M.R., 2008. Antimicrobial and antioxidant activities of edible coatings enriched with natural plant extracts: in vitro and in vivo studies. Postharvest Biology and Technology 49, 294-300.

Porat, R., Weiss, B., Cohen, L., Daus, A., Biton, A., 2005. Effects of polyethylene wax content and composition on taste, quality, and emission of off-flavor volatiles in ‘Mor’mandarins. Postharvest Biology and Technology 38, 262-268.

Poverenov, E., Danino, S., Horev, B., Granit, R., Vinokur, Y., Rodov, V., 2014. Layer-by-layer electrostatic deposition of edible coating on fresh cut melon model: anticipated and unexpected effects of alginate–chitosan combination. Food and Bioprocess Technology 7, 1424-1432.

Pranoto, Y., Rakshit, S., Salokhe, V., 2005. Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. LWT-Food Science and Technology 38, 859-865.

Qin, Y., Liu, D., Wu, Y., Yuan, M., Li, L., Yang, J., 2015. Effect of PLA/PCL/cinnamaldehyde antimicrobial packaging on physicochemical and microbial quality of button mushroom (Agaricus bisporus). Postharvest Biology and Technology 99, 73-79.

Quintavalla, S., Vicini, L., 2002. Antimicrobial food packaging in meat industry. Meat Science 62, 373-380.

Rappussi, M.C.C., Benato, E.A., Cia, P., Pascholati, S.F., 2011. Chitosan and fungicides on postharvest control of Guignardia citricarpa and on quality of 'Pêra Rio'oranges. Summa Phytopathologica 37, 142-144.

Rappussi, M.C.C., Pascholati, S.F., Benato, E.A., Cia, P., 2009. Chitosan reduces infection by Guignardia citricarpa in postharvest'Valencia'oranges. Brazilian Archives of Biology and Technology 52, 513-521.

Rehman, M., Singh, Z., Khurshid, T., 2018. Pre-harvest spray application of abscisic acid (S-ABA) regulates fruit colour development and quality in early maturing M7 Navel orange. Scientia Horticulturae 229, 1-9.

Rojas-Argudo, C., Del Río, M., Pérez-Gago, M., 2009. Development and optimization of locust bean gum (LBG)-based edible coatings for postharvest storage of ‘Fortune’mandarins. Postharvest Biology and Technology 52, 227-234.

Rooney, M., 1995. Overview of active food packaging, Active food packaging. Springer, pp. 1-37.

Rudnitskaya, A., Kirsanov, D., Legin, A., Beullens, K., Lammertyn, J., Nicolaï, B.M., Irudayaraj, J., 2006. Analysis of apples varieties–comparison of electronic tongue with different analytical techniques. Sensors and Actuators B: chemical 116, 23-28.

Sandhu, A.K., Gu, L., 2010. Antioxidant capacity, phenolic content, and profiling of phenolic compounds in the seeds, skin, and pulp of Vitis rotundifolia (muscadine grapes) as determined by HPLC-DAD-ESI-MS n. Journal of Agricultural and Food Chemistry 58, 4681-4692.

Sdiri, S., Bermejo, A., Aleza, P., Navarro, P., Salvador, A., 2012. Phenolic composition, organic acids, sugars, vitamin C and antioxidant activity in the juice of two new triploid late-season mandarins. Food Research International 49, 462-468.

Shah, S.W.A., Jahangir, M., Qaisar, M., Khan, S.A., Mahmood, T., Saeed, M., Farid, A., Liaquat, M., 2015. Storage stability of kinnow fruit (Citrus reticulata) as affected by CMC and guar gum-based silver nanoparticle coatings. Molecules 20, 22645-22661.

Sharma, R., Sandhooja, J., 1991. Effect of oil emulsion along with different chemicals on percent physiological loss in weight in kinnow during storage. Haryana. J. Hort 20, 216-221.

Shin, D.H., An, D.S., Koh, J.S., Lee, D.S., 1998. Wax-coating combined with grapefruit seed extract to reduce physiological quality changes and decay of Satsuma mandarin. Food Science and Biotechnology 7, 60-66.

Silva-Weiss, A., Ihl, M., Sobral, P., Gómez-Guillén, M., Bifani, V., 2013. Natural additives in bioactive edible films and coatings: functionality and applications in foods. Food Engineering Reviews 5, 200-216.

Sindhu, S., Singhrot, R., 1996. Effect of oil emulsion and chemicals on shelf life of baramasi lemon (*Citrus limon Burm*.). Haryana Journal of Horticultural Sciences 25, 67-72.

Sivakumar, D., Hewarathgamagae, N., Wijeratnam, R.W., Wijesundera, R., 2002. Effect of ammonium carbonate and sodium bicarbonate on anthracnose of papaya. Phytoparasitica 30, 486-492.

Sivakumar, D., Sultanbawa, Y., Ranasingh, N., Kumara, P., Wijesundera, R., 2005. Effect of the combined application of chitosan and carbonate salts on the incidence of anthracnose and on the quality of papaya during storage. The Journal of Horticultural Science and Biotechnology 80, 447-452.

Spadoni, A., Guidarelli, M., Sanzani, S.M., Ippolito, A., Mari, M., 2014. Influence of hot water treatment on brown rot of peach and rapid fruit response to heat stress. Postharvest Biology and Technology 94, 66-73.

Suseno, N., Savitri, E., Sapei, L., Padmawijaya, K.S., 2014. Improving shelf-life of cavendish banana using chitosan edible coating. Procedia Chemistry 9, 113-120.

Teerachaichayut, S., Ho, H.T., 2017. Non-destructive prediction of total soluble solids, titratable acidity and maturity index of limes by near infrared hyperspectral imaging. Postharvest Biology and Technology 133, 20-25.

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

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

Tharanathan, R., 2003. Biodegradable films and composite coatings: past, present and future. Trends in Food Science & Technology 14, 71-78.

Thompson, J.F., Mitchell, F.G., Kasmire, R.F., 2002. Cooling methods. Postharvest Technology of Horticultural Crops 3311, 97.

Toğrul, H., Arslan, N., 2004. Carboxymethyl cellulose from sugar beet pulp cellulose as a hydrophilic polymer in coating of mandarin. Journal of Food Engineering 62, 271-279.

Tomás-Barberán, F.A., Espín, J.C., 2001. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. Journal of the Science of Food and Agriculture 81, 853-876.

Valencia-Chamorro, S.A., Palou, L., Del Rio, M.A., Pérez-Gago, M.B., 2011. Performance of hydroxypropyl methylcellulose (HPMC)-lipid edible coatings with antifungal food additives during cold storage of 'Clemenules' mandarins. LWT-Food Science and Technology 44, 2342-2348.

Valencia-Chamorro, S.A., Pérez-Gago, M.B., del Río, M.A., Palou, L., 2010. Inhibition of postharvest penicillium molds of oranges by antifungal hydroxypropyl methylcellulose-lipid edible composite films and coatings. Environmentally friendly and safe technologies for quality of fruit and vegetables, 160-165.

Valencia-Chamorro, S.A., Pérez-Gago, M.B., del Río, M.Á., Palou, L., 2009. Effect of antifungal hydroxypropyl methylcellulose (HPMC)–lipid edible composite coatings on postharvest decay development and quality attributes of cold-stored 'Valencia'oranges. Postharvest Biology and Technology 54, 72-79.

Valencia-Chamorro, S.A., Pérez-Gago, M.B., Del Río, M.A., Palou, L., 2010. Effect of Antifungal Hydroxypropyl Methylcellulose-Lipid Edible Composite Coatings on Penicillium Decay Development and Postharvest Quality of Cold-Stored "Ortanique" Mandarins. Journal of Food Science 75, S418-S426.

Wills, R., Golding, J., 2016. Postharvest: an introduction to the physiology and handling of fruit and vegetables. UNSW press.

Xu, D., Qin, H., Ren, D., 2018. Prolonged preservation of tangerine fruits using chitosan/montmorillonite composite coating. Postharvest Biology and Technology 143, 50-57.

Xu, L., 1999. Use of ozone to improve the safety of fresh fruits and vegetables. Food technology.

Xudong, S., Hailiang, Z., Yande, L., 2009. Nondestructive assessment of quality of Nanfeng mandarin fruit by a portable near infrared spectroscopy. International Journal of Agricultural and Biological Engineering 2, 65-71.

Yao, H., Tian, S., 2005. Effects of a biocontrol agent and methyl jasmonate on postharvest diseases of peach fruit and the possible mechanisms involved. Journal of Applied Microbiology 98, 941-950.

Zeng, K., Deng, Y., Ming, J., Deng, L., 2010. Induction of disease resistance and ROS metabolism in navel oranges by chitosan. Scientia horticulturae 126, 223-228.

Zerdin, K., Rooney, M.L., Vermuë, J., 2003. The vitamin C content of orange juice packed in an oxygen scavenger material. Food chemistry 82, 387-395.

CHAPTER 3

Evaluating carboxymethylcellulose encapsulating moringa leaf extract as edible coatings for controlling rind pitting disorder in 'Marsh' grapefruit (*Citrus x paradisi* Macfad.)

3.1. Abstract

Development of rind pitting disorder (RPD) in 'Marsh' grapefruit during shipping is a major concern as it reduces the market value of the fruit. This study investigated the potential of carboxymethylcellulose (CMC) with moringa leaf extracts (MLE) as edible coatings (EC) for inhibiting the RPD incidence of grapefruit during cold storage and shelf-life. 'Marsh' grapefruit were harvested from two canopy positions (CP), including the outside canopy (OC) and inside canopy (IC). Fruit were assigned to five different treatments, namely, control, CMC (0.5%) + MLE (10%), CMC (1%) + MLE (10%), CMC 0.5%, CMC 1%. The experiment was laid in a factorial design. After treatment, fruit were kept at 3±1 °C for 9 weeks and thereafter transferred to 21±1 ^{0}C for 2 weeks. Fruit mass, citrus colour index (CCI), total soluble solids (TSS), titratable acidity (TA), rind dry matter (RDM) were measured, and RPD was also evaluated at each sampling interval. Sensory evaluation was conducted at the end of the storage. The results showed that edible coatings had a significant effect on RPD. In both canopy positions, coated fruit had less RPD compared to their uncoated counterparts. CMC 0.5%+ MLE 10% and CMC 1%+ MLE 10% had RPD incidence of 5.5% while CMC 1% had 7.6% and CMC 0.5% had 9.8% compared to control with 14.5%. Canopy position had no significant effect ($p=0.36$) on RPD. Outside canopy fruit had 12.4% disorder incidence compared to 10.5% for IC fruit. The EC and CP had significant effect ($p < 0.001$) on fruit mass loss and CCI. Coated fruit had lower mass loss than uncoated fruit, while OC fruit showed high mass loss than IC fruit with an average mass loss of 2.98 and 2.63%, respectively. Significant effect of EC, CP and storage time (ST) was observed on TA and TSS/TA. While EC*CP*ST interaction had no significant effect in TSS. Coated fruit had low TSS/TA, but high TA compared to uncoated fruit. From the obtained results, it was deducted that edible coatings improved all tested the quality attributes of citrus fruit and reduced RPD irrespective of CP.

Keywords: Edible coatings (EC), carboxymethylcellulose (CMC), moringa plant extracts (MLE), canopy position (CP), rind pitting disorder (RPD).

3.2. Introduction

Citrus is globally one of the most popular fruit crops (Rafiq et al., 2018). This is largely attributed to its high nutritive value which includes vitamin C, dietary fibre, antioxidants as well as phenolic compounds (Magwaza et al., 2017; Mditshwa et al., 2017). During the 2016/17 season, South Africa produced over 2 million tons of citrus fruit, with 75.8% of that production being exported (Edmonds, 2018). During export, fruit are subjected to cold storage to maintain postharvest quality. However, cold storage treatments have been reported to cause chilling damage on susceptible citrus species such as ‘Marsh’ grapefruit (*Citrus* x *paradisi* MacFad.) (Ezz and Awad, 2009). The fruit is also susceptible to postharvest peel pitting disorder also known as rind pitting disorder (RPD) (Alférez and Burns, 2004; Petracek et al., 1998). Postharvest RPD is a non-chilling physiological disorder that affects many citrus varieties (Alférez et al., 2005). The disorder is characterized by a collapse of the sub-epidermal rind cells without any colour change taking place at initial stages. As the disorder gets severe, oil glands are affected thereby releasing intercellular content that changes colourless lesions to bronze/brown colour due to enzymatic oxidation (Agusti et al., 2001).

The primary cause of this disorder is unknown, however, it is strongly linked to water stress (Alférez et al., 2003). Rind water stress may cause the collapse of internal flavedo and external albedo cell layers (Agusti et al., 2001; Alférez and Burns, 2004). Variation of relative humidity (RH), especially changes from low to high RH, are known to exacerbate the disorder (Alférez et al., 2003). The variation in RH changes the rind water status in fruit which ultimately changes turgor pressure of both flavedo and albedo cells thereby leading to peel damage (Alférez and Burns, 2004). In ‘Marsh’ grapefruit, the symptoms of the disorder show after 3 to 5 weeks after harvest (Ncama, 2016), which unfortunately coincides with the shipping period (Magwaza et al., 2014c; Magwaza et al., 2013c). The disorder does not affect the internal fruit quality, however, it negatively affects fruit market value and the income of the producers (Alférez et al., 2003).

While the internal quality is the determinant of flavour, consumers buy fruit based on external appearance (Chen and Opara, 2013). To reduce fruit quality loss during postharvest, the physiological basis of rind disorder prior to export must be well understood (Kader, 2002). For instance, fruit from different canopy positions differ in their biochemical compound content, therefore the susceptibility to non-chilling disorders is also different (Cronje et al., 2011a; Magwaza et al., 2014a; Magwaza et al., 2014b, c). Many strategies have been approached to reduce the disorder incidence (Henriod, 2006; Lafuente and Sala, 2002; Petracek et al., 1998; Porat et al., 2004; Yehoshua et al., 2001). For instance, Alférez and Burns (2004) reported less incidences of RPD after using gas permeable waxes and cold temperature storage. Contradicting results have also been reported, for examples, fruit waxed with less permeable shellac based coating were found to have higher RPD incidence (Petracek et al., 1998).

The inconsistent effect of waxes on RPD and overall fruit quality as well as consumer concerns have led to postharvest researchers develop a keen interest in developing edible coatings (Arnon et al., 2014). Edible coatings offer several advantages than synthetic materials (Tharanathan, 2003). For example, edible coatings are biodegradable, biocompatible, and environmentally friendly and they can provide a semipermeable barrier to gases and water vapor (Perez-Gago et al., 2005; Tharanathan, 2003). In addition, their function may be improved by the addition of ingredients such as antioxidants, antimicrobials, flavours. Therefore, fruit coated with edible coatings can be freely eaten with the coating (Ali et al., 2015) as the material used for formulating edible coatings is generally regarded as safe (GRAS) (Park et al., 1994).

To date, studies for citrus edible coatings have focused more on hydroxypropyl methylcellulose (HPMC)/beeswax/shellac composites (Contreras-Oliva et al., 2011, 2012; Navarro-Tarazaga et al., 2007; Navarro-Tarazaga et al., 2008; Valencia-Chamorro et al., 2011). Although these coatings may restrict gas exchange and result to off-flavours, they have generally been shown to retain citrus quality during postharvest storage (Contreras-Oliva et al., 2011, 2012; Navarro-Tarazaga et al., 2007). Sucrose and locust bean gum based coatings have also been tested on citrus fruit (Rojas-Argudo et al., 2009; Tao et al., 2012). Carboxymethylcellulose (CMC) is a promising polysaccharide polymer, derived from cellulose (Embuscado and Huber, 2009). CMC may be used to improve fruit appearance and extend fruit preservation since it can act as a selective barrier against respiration and moisture loss (Ali and Mahmud, 2007; Ali et al., 2011). The incorporation of CMC with moringa leaf extract (MLE) as edible coatings for enhancing and providing antifungal properties to fruits has been reported to be an efficient strategy. Plant extracts have gained attention since they are one of the several non-chemical control options.

Carboxymethylcellulose is relatively cheap, non-toxic with good solubility and low viscosity while it provides uniform coating during storage (Arnon et al., 2014; Gregorová et al., 2015). Previous studies have showed that CMC has a potential to extend storage period and to control deterioration of mango fruit by microorganisms when incorporated with moringa leaf extracts (Tesfay and Magwaza, 2017) and in papaya with essential oils (Zillo et al., 2018). Arnon et al. (2014), also reported that CMC/chitosan bilayer composite may be effectively used during citrus postharvest handling. In this respect, it is worth notice that CMC as stand-alone treatment should be evaluated in citrus postharvest storage.

Plant extracts have been shown to be effective against plant pathogens and therefore safe for consumers and the environment (Hernández-Albíter et al., 2007). Moringa (*Moringa oleifera Lam*.) is known as the multifunction tree (Ashfaq et al., 2012). Moringa leaf extracts have high antioxidant activity, high protein content and exhibit antibacterial potential against several organisms (John et al., 2013). Currently, no research has reported the use of moringa leaf extract (MLE) as an edible coating material on 'Marsh' grapefruit, one of the most important commercial citrus variety. This study was conducted to determine the efficacy of moringa leaf extract combined with CMC as a novel approach to reduce rind pitting and enhance shelf-life of 'Marsh' grapefruit. The study also attempted to identify potential physicochemical markers that are related to fruit susceptibility to rind pitting. Assessing these physicochemical markers and their correlations with the disorder constitute the major context of this research prior to understanding the mechanism that influence rind pitting disorder. In this way, the occurrence of the disorder can be predicted using physicochemical attributes as pre-symptomatic markers.

3.3. Materials and methods

3.3.1. Treatment preparation

Moringa leaves were dried by exposing them to direct sunlight for 24 hours, then pulverised with pestle and mortar to make them fine powder. Thereafter, 100g of moringa leaf was weighed in a scale and added to the 1000 mL of methanol (80 % (v/v). The mixture was then placed in magnetic stirrer for 24 hours. The extract was then filtered to a clean empty volumetric flask (1000 mL). In order to make 1% of carboxymethylcellulose (CMC), 10g of

CMC powder was weigh and added to distilled water (1000 mL) then placed in a hotplate magnetic stirrer for 12 hours for powder to dissolve thoroughly.

3.3.2. Fruit samples

A total of 300 'Marsh' grapefruit were harvested during the 2017/2018 season from a commercial orchard at Dole Bolton Citrus Estate in Nkwalini (28°75'03.719''S; 31°58'26.035''E) at Eshowe, KwaZulu-Natal Province, South Africa. Fruit were harvested based on the maturity index (total soluble solids: titratable acidity ratio) and peel colour to ensure that all fruit are in the similar condition as per canopy position. To investigate the influence of canopy position on biochemical composition of fruit rind and juice, 150 fruit were harvested from both inside and outside canopy positions. The canopy positions were referred as inside canopy and outside canopy of a fruit tree. After harvesting, fruit were transported within 24 hours at ambient temperature in ventilated sack bags to the postharvest research laboratory at University of KwaZulu-Natal (Pietermaritzburg).

3.3.3. Postharvest treatments and storage

Edible coating treatments were prepared before fruit were harvested. 1% of Carboxymethylcellulose (CMC) was prepared by weighing 10g of CMC and mixed with 1000 mL of warm water then stirred for 24 hours. 10% of moringa leaf extracts (MLE) was prepared by weighing 100g of moringa leaves and mixed with 1000 mL of methanol (80%) then stirred for 24 hours, thereafter, the extract was transferred to a clean bottle (1L). Harvested 'Marsh' grapefruit were assigned to five (5) different treatments, namely, control (untreated), T_1 CMC (0.5%) + MLE (10%), T_2 CMC (1%) + MLE (10%), T_3 CMC 0.5% and T_4 CMC 1%. Each treatment comprised of 4 fruit per canopy position (4x2x5) that is 40 fruit for each sampling day from week 0 to week 11. Treatments were organised in a factorial design with edible coatings and canopy position as main factors. Coatings were applied in fruit with glycerol added as a plasticiser, and then left to dry for 5 hours on a laboratory bench at room temperature. Thereafter, fruit were subjected to cold storage which ranged from 3 ± 0.5 °C and 90-95% relative humidity (RH), for nine weeks simulating a maximum period of shipment. Thereafter, fruit were taken to room temperature (22 ± 2 °C) for two weeks to simulate shelf life, which would happen after reaching a designated export market.

3.3.4. Disorder rating

Since predictions for fruit susceptibility to rind pitting disorders should be done before shipping, parameters sampling was done immediately after harvesting. A visual rating scale was used to estimate the extent of non-chilling peel-pitting development. Peel pitted 'Marsh' grapefruit showed collapsed areas of the flavedo and part of the albedo. Those areas of the flavedo become dark brown with time. A rating scale from 0 (no pit), 1 (minor pitting), 2 (moderate pitting) to 3 (severe pitting) based on surface damage and intensity of browning was used (see Table 3.1). The average rind pitting index calculated using Eq. (1), previously reported by Lafuente and Sala (2002).

Table 3. 1: The scale used to label rind pitting disorder in 'Marsh' grapefruit.

Hedonic scale	**Sensory**
1-3	Non-acceptable
4-6	Acceptable
7-9	Excellent

$$\text{Rind pitting index} = \frac{\text{rindstaining scale (0–3)} \times \text{number of fruit in each class}}{total\ number\ of\ fruit} \qquad (1)$$

3.3.5. Fruit mass loss

Fruit mass was measured from the constant fruit throughout the experiment at two week intervals using a calibrated weighing scale (RADWAG Wagi Electronic Inc., Poland). The results were expressed as the percentage of mass loss relative to the initial mass according to Eq. (2).

$$\text{Mass loss \%} = \frac{\text{Initial weight} - \text{recorded weight}}{\text{Initial weight}} \text{x } 100 \qquad (2)$$

3.3.6. Fruit colour

Forty fruit were randomly selected from each treatment for colour determination. Same fruit were also used throughout the study for assessing changes in fruit colour. The fruit colour was measured from three marked, randomly selected spots on the equatorial position of a fruit using portable colorimeter (Chroma Meter, Konica Minolta Sensing, INC., Japan), which was calibrated by scanning a 100% white reference brick prior fruit scanning and periodically at 30 min intervals. L* represents the lightness of the fruit colour (0–100, black to white), while a* indicates the redness (+a*) or greenness (-a*), and b* indicates the yellow (+b*) or blue (-b*) colour of fruit skin (Rehman et al., 2018). The fruit colour was expressed as a citrus colour index (CCI) according to Eq. (3) (Pathare et al., 2013; Vidal et al., 2013).

$$\mathrm{CCI} = \frac{1000 * \mathrm{a}}{\mathrm{L} * \mathrm{b}} \qquad (3)$$

3.3.7. Total soluble solids (TSS), titratable acidity (TA)

During sampling, each fruit was cut in half and squeezed to collect juice for total soluble solids (TSS) and titratable acidity (TA) analysis. The collected juice was tested for TSS using a digital refractometer (RFM340+ BS®, Bellingham and Stanley Ltd, Basingstoke, Hants, UK). The remaining juice was snap frozen in 120 mL plastic specimen jars and stored in a freezer for further analysis for TA. Titratable acidity was analysed using Compact Titrator G20S (Mettler-Toledo GmbH, Greifensee, Switzerland) by mixing 10 mL juice with 40 mL distilled water and titrating with 0.1M sodium hydroxide (NaOH) to the end point (pH of 8.2). The titre was recorded and expressed as citric acid using Eq. (4) (Ncama, 2016).

$$TA\ (\%\ citric\ acid) = \frac{0.0064\ \mathrm{X\ titre\ (NaOH)\ ml\ X}\ 100}{10\ \mathrm{mL\ juice}} \qquad (4)$$

The ratio of TSS to TA, also known as citrus maturity index was calculated using Eq. (5).

$$\text{TSS to TA ratio (maturity index)} = \frac{\mathrm{TSS}}{\mathrm{TA}} \qquad (5)$$

3.3.8. Rind dry matter

To measure rind dry matter, the fresh rind sample was weighed to obtain fresh mass, after it was snap frozen in -20 °C, samples were then freeze dried for three days using Virtis Benchop freeze drier system (ES Model, SP Industries Inc., Warmister, USA) at 0.013-0.026 kPa and -40 °C. The freeze drier machine works by freezing the material, then reduce the pressure and adding heat to allow the frozen water in the material to sublimate. After the samples were dried, they were again weighed. Rind dry matter (RDM) was calculated using Eq. (6) (Ncama, 2016).

$$Dry\ matter\ (\%) = \frac{\text{dried mass}}{\text{initial fresh mass}} \text{ x } 100 \tag{6}$$

3.3.9. Sensory evaluation

For sensory analysis, fruit were hand-peeled, and separated segments were cut into halves and placed in different plates. Each treatment comprised of multiple cut segments from eight different fruit, for evaluation by 20 untrained panellists. Sensory preference was evaluated according to a 9-point hedonic scale ranging from “very strong dislike” to “very strong like” where 1-3 represent a range of non-acceptable quality, 4-6 is an acceptable quality range and 7-9 represent a range of excellent quality (Obenland et al., 2011). Quality attributes evaluated include evaluation of citrus colour, taste, gloss, odour, and overall acceptance. Fruit segments were presented to panellists in trays and served at room temperature. Water was provided for rinsing the pallates between samples.

3.3.10. Data analysis

Statistical analyses were performed using GenStat® 18th Edition (VSN International, Hemel Hempstead, UK). Data were subjected to analysis of variance (ANOVA) with coatings and canopy position as main factors. The least significant difference (LSD) at 5% level was considered significant. Data was also subjected to multivariate statistical analyses for principal component analysis (PCA) using SPSS V25 (SPSS Inc, Chicago, IL, USA, 2017). To measure the degree of linear relationship among fruit physicochemical parameters and the disorder, data was subjected to Pearson correlation analysis.

3.4. Results and discussion

3.4.1 Fruit mass loss

There was no significant interaction effect among canopy position, edible coatings and storage time (CP*EC*ST) ($p = 0.592$) on mass loss. Yet, highly significant interactions were observed between edible coatings and canopy position (EC*CP) ($p < 0.001$), as well as edible coatings and storage time (EC*ST) (p < 0.001). Individual factors such as CP, EC and ST had a highly significant effect (p < 0.001) on fruit mass loss during postharvest period. Edible coatings provides barrier between inner and outer environment of the fruit which significantly decreased respiration and transpiration rate (Abbas et al., 2008). Fruit mass loss is strongly linked to water loss during storage and postharvest handling (Parra et al., 2014). However, changes in fruit water status during postharvest handling has been reported to elevate the development of rind pitting disorder in ‘Navel’ oranges (Alferez et al., 2005) and ‘Marsh’ grapefruit (Alférez and Burns, 2004). Development of rind pitting had a linear relationship with water loss (Alferez et al., 2005). Alférez and Burns (2004) also reported a significant positive relationship between cumulative weight loss and variation in water status in the rind with an increase in postharvest pitting index. The loss of water in ‘Marsh’ grapefruit and ‘Villafranca’ lemons was reported to be associated with rind pitting disorder development (Cohen et al., 1994) and with the symptoms of peel pitting of ‘Fortune’ mandarins (Vercher et al., 1994).

The results showed that, compared to treated fruit, uncoated fruit had the highest mass loss in both canopies during the entire storage time (Figure 3.1). There was a noticeable high mass loss during shelf life week 10 (W10) as compared to earlier sampling dates. This increment could be attributed to an increased in temperature and decrease in relative humidity causing higher respiration rate. Also, the reduced weight loss in coated fruit could be linked to the fact that CMC provide semipermeable barrier against O_2, CO_2, moisture and solute movement, thereby reducing respiration and water loss (Baldwin et al., 1999).

High significant effect of canopy position on mass loss may be attributed by the fact that IC fruit size is relatively larger than OC fruit size. Sastry et al. (1977) suggested that surface to volume ratio is better indicator of water loss rate. In this study, OC fruit had high surface to volume ratio therefore would mean greater diffusional area per volume of water saturated space. Fruit harvested from inside canopy appeared to have lower average weight loss than outside canopy fruit (IC: 2.63 vs OC: 2.98 %). Contradicting results have previously been reported by Ehlers (2016) where canopy position showed no significant effect on rind pitting disorder in ‘Turkey’ Valencia. Notably, Ehlers (2016) also reported a higher pitting index in

'Benny' Valencia harvested from the IC position during the first season (2013/14) while the OC position had the highest pitting index during second season (2014/15). Although this study was conducted on only one citrus cultivar, it could be argued based on literature and findings of this study that the effect of canopy position is cultivar-specific.

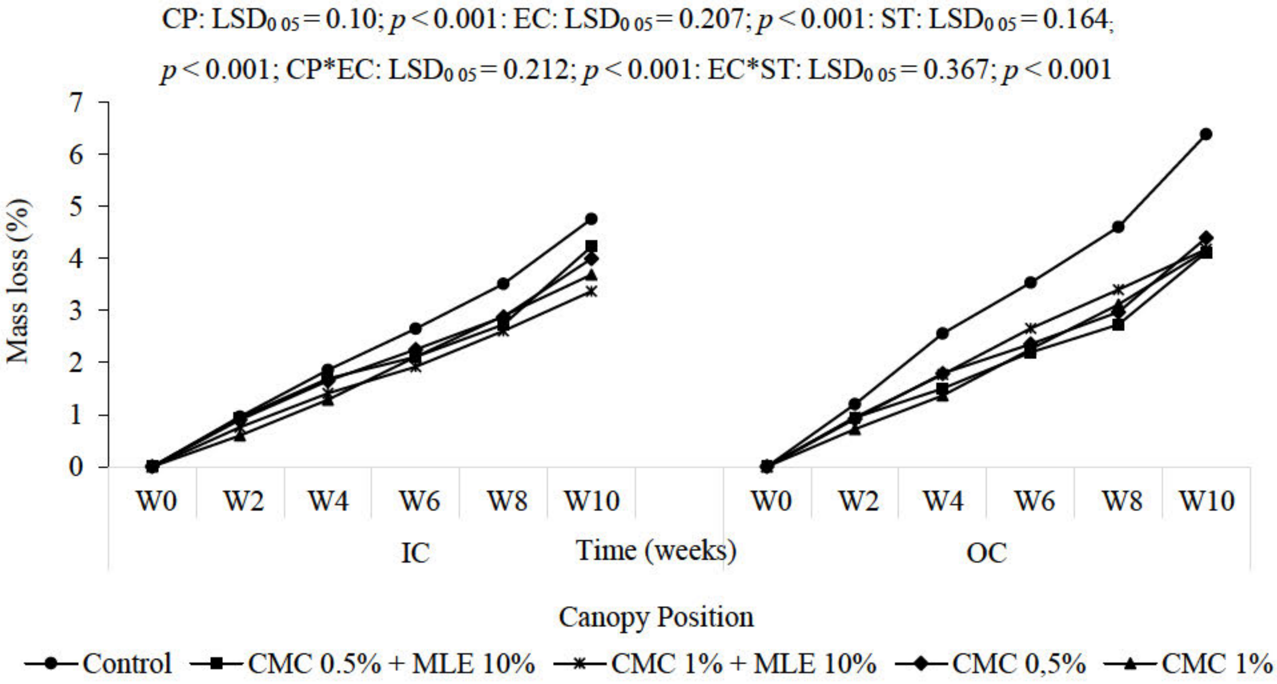


Figure 3. 1: Effect of edible coatings CMC 0.5% + MLE 10%, CMC 1% + MLE 10%, CMC 0.5% and CMC 1% on weight loss of 'Marsh' grapefruit with respect to canopy positions (IC and OC) during cold storage (3±1 ° C) for 9 weeks and (21±1 °C) at shelf life. IC: inside canopy; OC: outside canopy; CMC: carboxymethylcellulose; MLE: moringa leaf extracts; LSD: least significant difference; CP: canopy position; EC: edible coatings; ST: storage time.

3.4.2. Fruit TSS, TA and TSS/TA

There was no significant interaction among canopy position, edible coatings and storage time (CP*EC*ST) (p = 0.901) on TSS (Figure 3.2). The significant difference was only observed in CP and ST (p < 0.001), individually. Total soluble solids for OC fruit was higher (9.33%) than that of IC fruit (8.99%). A study by Ncama (2016) clearly showed that TSS and TA in relative to canopy position affect rind pitting disorder susceptibility. The study reported that OC fruit had higher TSS and lower TA and that was related to higher RPD when compared to IC fruit where fruit were harvested from different growing regions. Since TSS, TA and their ratio are fruit maturity indices, Ehlers (2016) conducted a study based on ‘Turkey’ and ‘Benny’ Valencia on two seasons and reported that fruit maturity significantly influenced peel pitting index. Although, edible coatings had no significant difference on TSS, the final TSS at shelf life was observed to be higher in control (10.82%) compared to CMC 0.5% + MLE 10%, CMC 1% + MLE 10%, CMC 0.5% and CMC 1% with 10.04%, 10.27%, 9.93% and 10.65%, respectively. These findings were comparable to Xu et al. (2018), who also reported that chitosan and montmorillonite coated tangerine fruit had low TSS in the later stage of cold storage. Contrary, Shah et al. (2015) reported that Kinnow fruit (*Citrus reticulata*) coated with CMC and Guar Gum-Based Silver Nanoparticle had an increase in TSS although the effect was not significant. This might be due to barrier provided by coatings against respiration and evaporation since TSS in citrus has been related to an increased rate of respiration and evaporation from the surface of the fruit (Thakur et al., 2002).

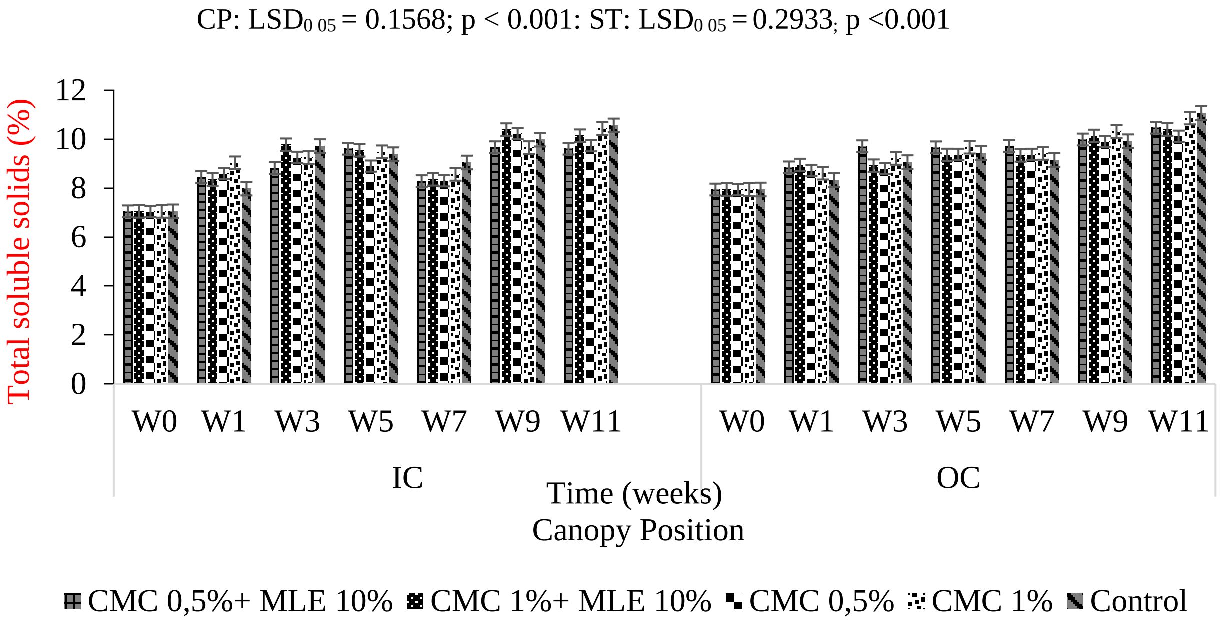


Figure 3. 2: Effect of edible coatings CMC 0.5% + MLE 10%, CMC 1% + MLE 10%, CMC 0.5% and CMC 1% on total soluble solids of ‘Marsh’ grapefruit in different canopy positions during cold storage (3±1 ºC) for 9 weeks and shelf-life (21±1 ºC) for 2 weeks. CMC: carboxymethylcellulose; MLE: moringa leaf extracts; LSD: least significant difference; CP: canopy position; EC: edible coatings; * stands for an interaction between factors.

There was a highly significant (*p* < 0.001) interaction of canopy position, edible coatings and storage time on TA (Figure 3.3). OC fruit appeared to have higher TA than IC fruit

(OC=1.24% vs IC=1.22%) during cold storage. At the end of storage (week 11), compared to uncoated fruit which had the TA of 0.92%, fruit coated with CMC 0.5% + MLE 10%, CMC 1% + MLE 10%, CMC 0.5% and CMC 1% had higher TA of 0.97%, 0.98%, 0.96%, 0.96%, respectively. Lower TA concentrations are strongly correlated with high RPD susceptibility and incidence (Ncama, 2016). Based on these results, it could be reasoned that edible coatings reduced the consumption of organic acids by slowing down the fruit respiration rate. These results are comparable to those of Cancolon and Xu (2002) who indicated that the reduced loss of TA in coated fruit was probably due to the slow rate of respiration and metabolic processes converting citric acid into sugars.

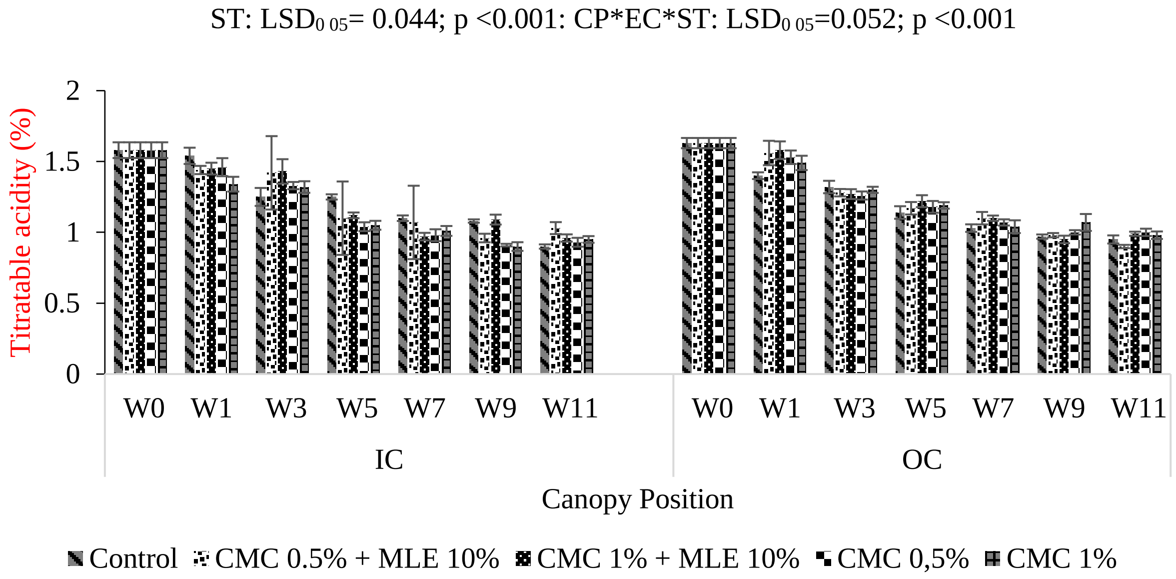

ST: LSD$_{0.05}$= 0.044; p <0.001: CP*EC*ST: LSD$_{0.05}$=0.052; p <0.001

Figure 3. 3: Effect of edible coatings CMC 0.5% + MLE 10%, CMC 1% + MLE 10%, CMC 0.5% and CMC 1% on titratable acidity of 'Marsh' grapefruit in different canopy positions during cold storage (3±1 ºC) for 9 weeks and shelf-life (21±1 ºC) for 2 weeks. CMC: carboxymethylcellulose; MLE: moringa leaf extracts; LSD: least significant difference; CP: canopy position; EC: edible coatings; * stands for an interaction between factors.

The interaction of edible coatings, canopy position and storage time had a significant effect (*p* = 0.049) on TSS/TA (Figure 3.4). TSS/TA generally determines fruit flavour in various horticultural crops (Mattheis and Fellman, 1999). In this study, TSS/TA slightly increased during storage. The slow increase is reportedly due to fruit decrease in acidity using acids as substrates in respiration (Tri carboxylic acids cycle) (Rana et al., 1992). Since TSS/TA is considered one of the maturity indices, Ehlers (2016) conducted a study on ‘Turkey’ and ‘Benny’ Valencia on two seasons and reported that fruit maturity significantly influenced peel pitting index.

ST: $\text{LSD}_{0.05}$= 0.3439; p<0.001: CP*EC*ST: $\text{LSD}_{0.05}$ =1.0874; p<0.05; CP*EC: $\text{LSD}_{0.05}$ = 0.411; p<0.05

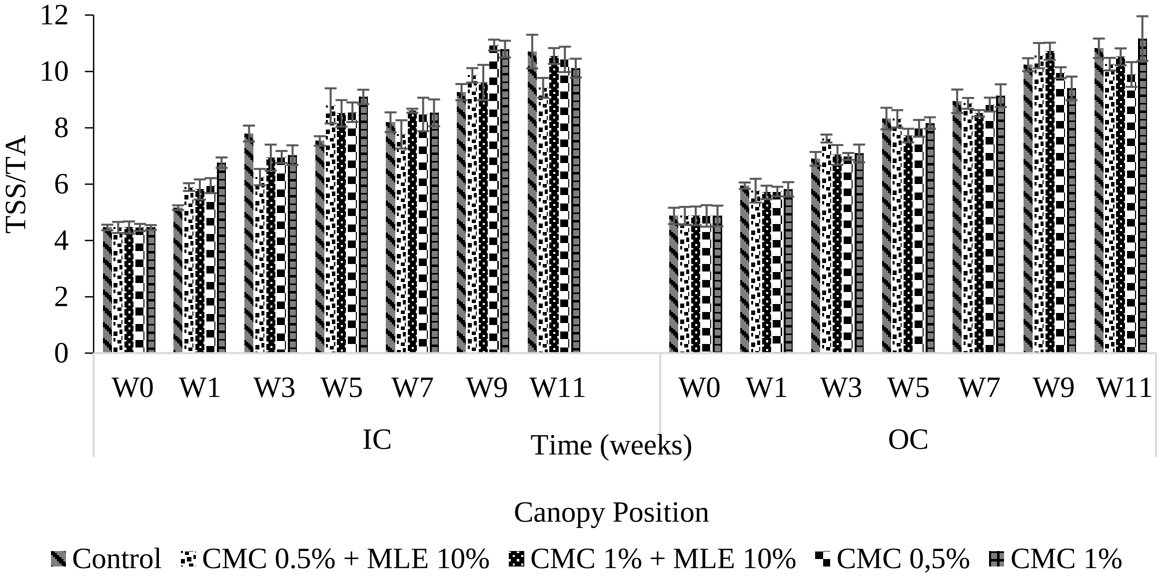


Figure 3. 4: Effect of edible coatings CMC 0.5% + MLE 10%, CMC 1% + MLE 10%, CMC 0.5% and CMC 1% on TSS/TA Marsh’ grapefruit in different canopy positions during cold storage (3±1 ºC) for 9 weeks and shelf-life (21±1 ºC) for 2 weeks. CMC: carboxymethylcellulose; MLE: moringa leaf extracts; LSD: least significant difference; CP: canopy position; EC: edible coatings; * stands for an interaction between factors.

3.4.3. Fruit colour

Interaction of edible coatings, canopy position and storage time (EC*CP*ST) showed no significant effect on citrus colour index (CCI) (*p* = 0.901) (Figure 3.5). Whilst edible coatings, canopy position and storage time as single factors showed high significant effect (*p* <0.001) on CCI. All coated fruit had low CCI compared to uncoated sample fruit. This suggest that edible coatings were able to delay fruit colour change and prolong the shelf-life. The grand mean of CCI for OC fruit was higher than that of IC fruit (OC: -1.11 vs IC: -1.51). Findings similar with these have previously been reported (Cronje et al., 2011b; Khalid et al., 2012; Magwaza et al., 2014c) where OC fruit had higher CCI than fruit from IC. This is probably due to the fact that fruit harvested inside the canopy received a reduced light intensity during fruit growth and development. Hence, low photosynthetic rate led to delayed colour change in IC fruit.

CP: $\text{LSD}_{0.05} = 0.059$; $p<0.001$: EC: $\text{LSD}_{0.05} = 0.093$ $p<0.001$; ST: $\text{LSD}_{0.05}=0.102$; $p<0.001$

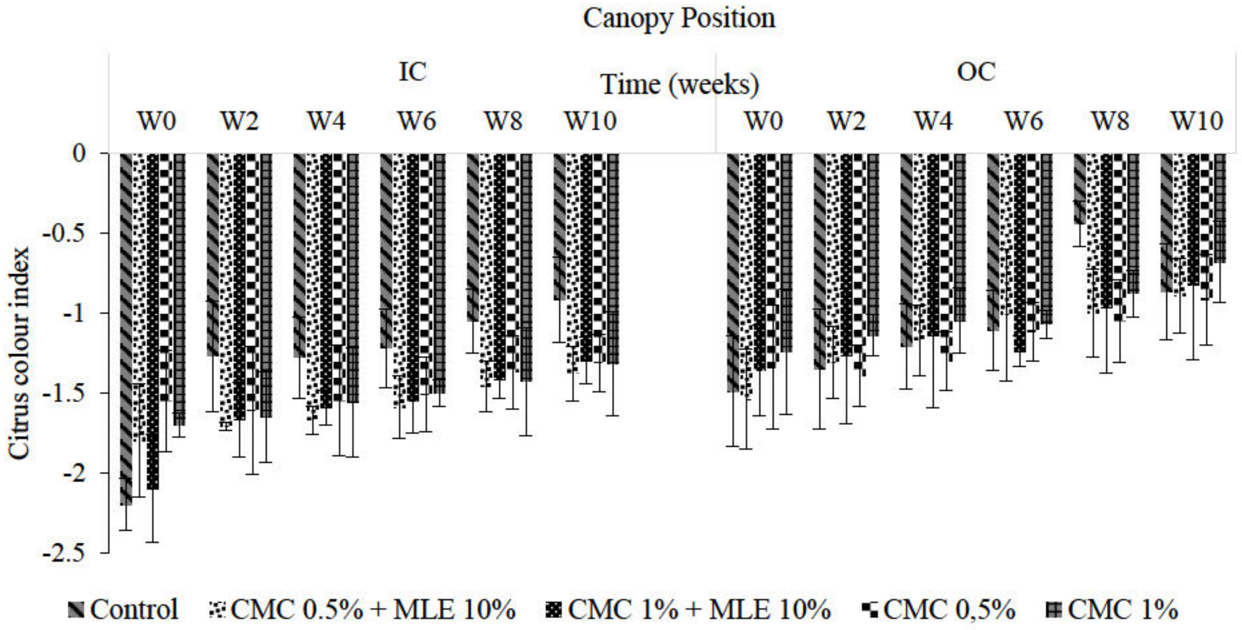


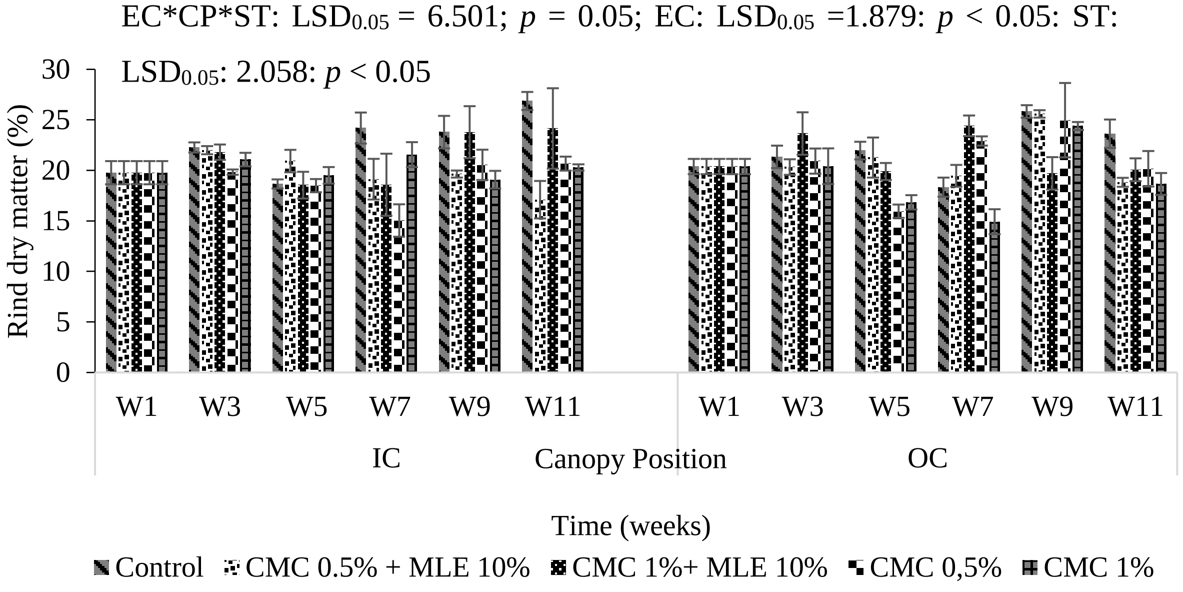
Figure 3. 5: Effect of edible coatings CMC 0.5% + MLE 10%, CMC 1% + MLE 10%, CMC 0.5% and CMC 1% on citrus colour index (CCI) of 'Marsh' grapefruit during cold storage (3±1 ℃) for 9 weeks and shelf-life (21±1 ℃) for 2 weeks. CMC: carboxymethylcellulose; MLE: moringa leaf extracts; LSD: least significant difference; CP: canopy position; EC: edible coatings; * stands for an interaction between factors.

The colour of the fruit surface is the first quality constraint valued by the consumer and it is critical in the consumer acceptance of any product (Zheng et al., 2016). A significant correlation between peel colour score and fruit maturity was also reported by Medlicott et al. (1992). However, fruit maturity was reported as a critical factor that influence the development of citrus postharvest rind disorders (Wild, 1991). Low CCI, as shown by a slightly green colour observed in IC fruit, is an indication of reduced expression of carotenoids during colour development (Cronje et al., 2013; Khalid et al., 2012). Yellower fruit were reported to be susceptible to rind pitting disorder (Duarte and Guardiola, 1993). Fruit susceptibility to rind pitting is mostly determined at the time when fruit change colour during pigmentation until harvest (Assimakopoulou et al., 2009).

3.4.4. Rind dry matter

The interaction between edible coatings, canopy position and storage time had a significant effect ($p = 0.05$) on rind dry matter (RDM) (Figure 3.6). At the end of the storage, uncoated samples had slightly higher rind dry matter, which was 23.97 compared to coated samples with 20.02 for CMC 0.5% + MLE 10%, 20.30 for CMC 1% + MLE 10%, 20.27 for CMC 0.5% and 20.12 for CMC 1%, all in percentage.

This suggests that edible coatings reduced water loss in rind during cold storage. OC fruit were recorded to have slightly higher rind dry matter percentage (21.15%) compared to IC fruit (20.44%). These findings correspond with those reported by (Ncama, 2016), who also reported a high RDM on 'Marsh' grapefruit harvested at OC compared to their IC counterparts. It is hypothesized that fruit from IC were exposed to low temperature and humid environment since they do not receive enough sunlight due to shading by leaves and other outside fruit, this limit IC fruit from losing significant moisture through the process of evapotranspiration to toughen their skin during fruit development. Hence, IC fruit had thick but soft rinds with weak structure which easily got damaged under cold conditions.



EC*CP*ST: $LSD_{0.05} = 6.501$; $p = 0.05$; EC: $LSD_{0.05} = 1.879$: $p < 0.05$: ST: $LSD_{0.05}$: 2.058: $p < 0.05$

Figure 3. 6: Effect of edible coatings CMC 0.5% + MLE 10%, CMC 1% + MLE 10%, CMC 0.5% and CMC 1% on rind dry matter % of 'Marsh' grapefruit during cold storage (3±1 °C) for 9 weeks and shelf-life (21±1 °C) for 2 weeks. CMC: carboxymethylcellulose; MLE:

moringa leaf extracts; LSD: least significant difference; EC: edible coatings, CP: canopy position, * stands for an interaction between factors.

3.4.5. Rind pitting disorder response to edible coatings application

Interaction of edible coatings, canopy position and storage time (EC*CP*ST) showed no significant effect on rind pitting disorder (RPD) ($p > 0.05$) (Figure 3.7). Though, edible coatings had a highly significant effect ($p < 0.001$) on RPD. While canopy position had no significant effect on RPD ($p = 0.36$), the disorder appeared to be higher on OC fruit compared to IC fruit (OC = 12.4 vs IC = 10.5). Similar results have previously been reported in other fruit in relationship to postharvest physiological disorders. For instance, Almela et al. (1992) who conducted a study in Spain, reported that the incidence of rind spots in 'Fortune' mandarins was higher in sun-exposed fruit than in non-exposed fruit. Similarly, rind breakdown in 'Navel' oranges (Agusti et al., 2001) and 'Encore' mandarin (Vitor et al., 2001) has been reported to dominate in sun-exposed fruit. This occurrence can be attributed to the fact that OC fruit is exposed to high temperature and irradiation which may induce localized flavedo dehydration, plasmolysis, and cell collapse, thereby resulting in rind pitting (Medeira et al., 1999).

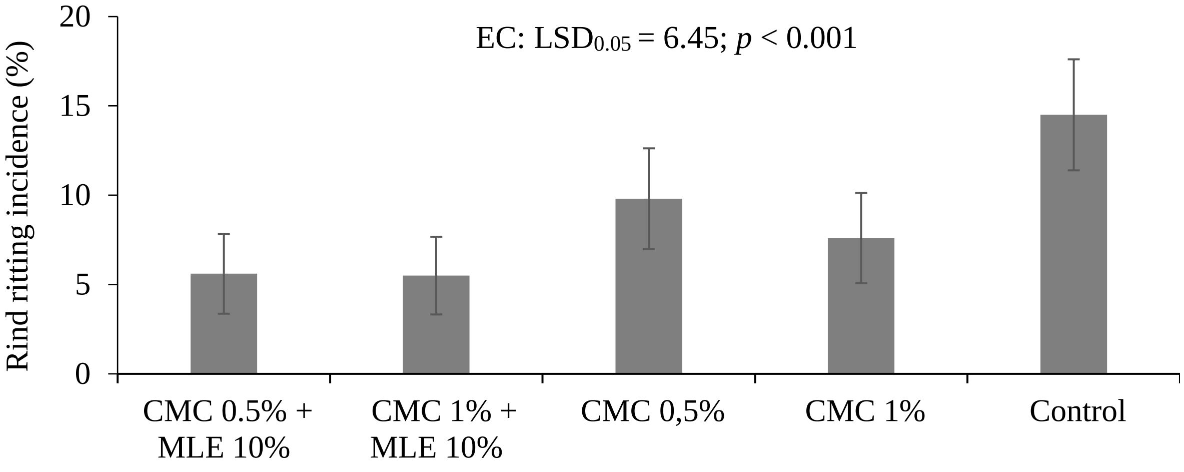


Figure 3. 7: Effect of edible coatings CMC 0.5% + MLE 10%, CMC 1% + MLE 10%, CMC 0.5% and CMC 1% on postharvest rind pitting disorder of 'Marsh' grapefruit during cold storage (3±1 ºC) for 9 weeks and shelf-life (21±1 ºC) for 2 weeks. CMC:

carboxymethylcellulose; MLE: moringa leaf extracts; LSD: least significant difference; EC: edible coatings.

As mentioned above, RPD is closely linked to fruit water loss during storage and postharvest handling. Due to semipermeable barrier provided by edible coatings which minimized water loss, coated fruit experienced low disorder incidence compared to uncoated fruit. In this study, coated fruit had low citrus colour index which was linked to low RPD incidence compared to fruit with high citrus colour index (control fruit).

The disorder incidence was rated as described in equation (1). Figure 3.8 shows the RPD incidence during storage.

Figure 3. 8: Symptoms of rind pitting in 'Marsh' grapefruit from A (little pit), B (moderate pit) and C (severe pit) during sampling.

3.4.6. The correlation of grapefruit physicochemical parameters to rind disorders

The Pearson correlations of physicochemical parameters and rind disorder were tested after normalization of data to equalize every parameter and disorder values contribution (Table 3.2). The correlation of most of these parameters to rind pitting disorder were very weak. Weak correlation makes it difficult to spot the exact factors contributing to the disorders. Fruit luminosity, titratable acidity and rind dry matter showed a negative correlation to rind pitting with correlation values (r) of -0.11, -0.40 and -0.38, respectively. This simply mean that fruit with dimmer colour, sweeter in taste and lower rind moisture had higher RPD incidence. Similar findings were reported by Ncama (2016) on 'Marsh' grapefruit stored at 5 °C for 7 weeks. Low rind moisture content have been reported to correlate with rind disorders in previous studies (Alférez and Burns, 2004; Cohen et al., 1994; Magwaza et al., 2014a). Although most parameters had weak correlation to RPD, fruit mass loss had highest positive correlation (r = 0.56) followed by CCI (r = 0.33), b* (r = 0.32), TSS (r = 0.32). As discussed above and supported by literature, the change in water status especially loss of water in fruit is a critical postharvest factor determining citrus fruit susceptibility to RPD (Alferez et al., 2010). Since fruit maturity plays a significant role in citrus rind disorders development (Wild, 1991) and TSS is one of the maturity indices of citrus, this suggest that fruit with high TSS% are more prone to the disorder. Moreover, CCI being correlated to RPD is supported by (Duarte and Guardiola, 1993) who stated that green fruit are less susceptible to rind pitting disorder. The relationship between 'Marsh' grapefruit physicochemical parameters and the disorder was satisfied by finding whether the correlation was positive or negative.

The noticeable trend on the correlation between grapefruit physicochemical parameters was that there is higher correlation of fruit colour indices and they all positively correlated with mass loss with r = 0.50, 0.70 and 0.57 for a*, b* and CCI, respectively. It was also found that mass loss had high positive correlation to TSS (r = 0.58). This means that as time progresses, not only does the fruit lose water, but it also get sweeter.

Table 3. 2: The correlation between physicochemical parameters and their correlation to rind pitting disorder of 'Marsh' grapefruit stored at 3±1°C for 9 weeks and then at 21±1 °C for 2 weeks.

	Mass loss	**RDM**	**TSS**	**TA**	**L***	**a***	**b***	**CCI**	**RPD**
Mass loss	1								
RDM	-0.15	1							
TSS	**0.58**	0.02	1						
TA	**-0.75**	0.08	-0.45	1					
L*	-0.11	0.15	-0.06	-0.06	1				
a*	**0.50**	-0.07	0.39	-0.35	-0.38	1			
b*	**0.70**	0.05	**0.55**	**-0.68**	-0.10	**0.54**	1		
CCI	**0.57**	-0.04	0.44	-0.44	-0.29	**0.98**	**0.65**	1	
RPD	**0.56**	-0.38	0.32	-0.40	-0.11	0.31	0.32	0.33	1

Parameters with values >/= 0.5 considered show high correlation to each other and to disorder. RDM- rind dry matter, TSS- total soluble solids, TA- titratable acidity, L- Luminosity, a*- fruit greenness, b*- fruit yellowness, CCI- citrus colour index and RPD- rind pitting disorder.

3.4.7. The use of principal component analysis models to separate fruit based on canopy position and PCA-based correlation of fruit physicochemical parameters to disorders

Principal component analysis (PCA) is a standard tool in modern data analysis, a simple, non-parametric method for extracting relevant information from confusing data sets (Shlens, 2014). In this study, PCA was performed to reveal the correlation of physicochemical parameters to physiological rind pitting disorder separated with canopy position. Table 3.2 shows the correlation of physicochemical parameters and the disorder in PC-1, PC-2 and PC-3. Rind dry matter, citrus colour index, TSS and TA content varied with canopy position and can be used as main quality attributes between canopy positions. The variation of parameters between canopies can be traced back during fruit growth and development.

In the first biplot, principal component contributed to a total of 87.3%, with 63.9% allocated to PC-1 and 23.9% allocated to PC-2 (Figure 3.9). While in second bi-plot, principal component accounted for 80.23% of total variability of fruit, with 62.19% accounted for PC-1 and 18.04% accounted for PC-2 of variation during mapping (Figure 3.10). A positive correlation was based on parameters proximity to fruit origin. Smaller angles between vectors facing the same direction means that those parameters and/or disorder are positive correlated. Also, parameters on the same quadrant with a disorder was considered positively correlated with the disorder and contributed significantly to the difference in fruit susceptibility, and fruit distinction according to edible coating treatments during mapping.

Table 3. 3: Principal component analysis (PCA) showing eigenvectors, eigenvalues and percent variance explained by three principal components (PCs) on physicochemical quality attributes of ‘Marsh’ grapefruit in response to canopy position.

	Outside canopy			Inside canopy		
Coating	PC1	PC2	PC3	PC1	PC2	PC3
CCI	0.04	**0.75**	**0.50**	**0.49**	0.03	0.34
Mass loss	**0.50**	-0.06	-0.17	**0.50**	0.04	-0.08
RPD	0.46	0.14	0.41	0.46	-0.06	0.12
Rind dry matter	**-0.51**	0.00	0.17	-0.47	0.35	-0.03
TA	-0.48	-0.14	0.37	0.24	0.18	**-0.91**
TSS	-0.22	**0.62**	**-0.63**	0.12	**0.92**	0.19
Explained variance (Eigenvalue)	3.80	1.44	0.64	3.73	1.08	0.90
% of variance	63.39	23.91	10.64	62.19	18.04	14.92
Cumulative variance (%)	63.39	87.30	97.94	62.19	80.23	95.15

Loadings with values ≥ 0.5 considered show relative high correlation. PC-1, PC-2 and PC-3 refer to the three principal components.

The PCA biplot is presented in two different set, as outside canopy position (Figure 3.9) and inside canopy position (Figure 10). In outside canopy, uncoated fruit (control) had a higher rind pitting incidence which was aligned with a positive contribution of fruit mass loss, TSS and CCI parameters, this was observed from the fact that vectors were faced in the same direction (Figure 3.9). Although, TSS and CCI had a weaker correlation because of larger angles among parameters and the disorder. However, mass loss had high positive correlation because the distance between the loadings was smaller compared to other parameters. Positive correlation of RPD and TSS was also observed in ‘Marsh’ grapefruit (Ezz and Awad (2009); Ncama (2016). Whereas in inside canopy, uncoated fruit (control) again had a higher rind pitting incidence which was aligned with a positive contribution of fruit mass loss and CCI. Coated fruit was observed to be negatively correlated with RPD in both canopy position, as shown in Figure 3.9 and 3.10.

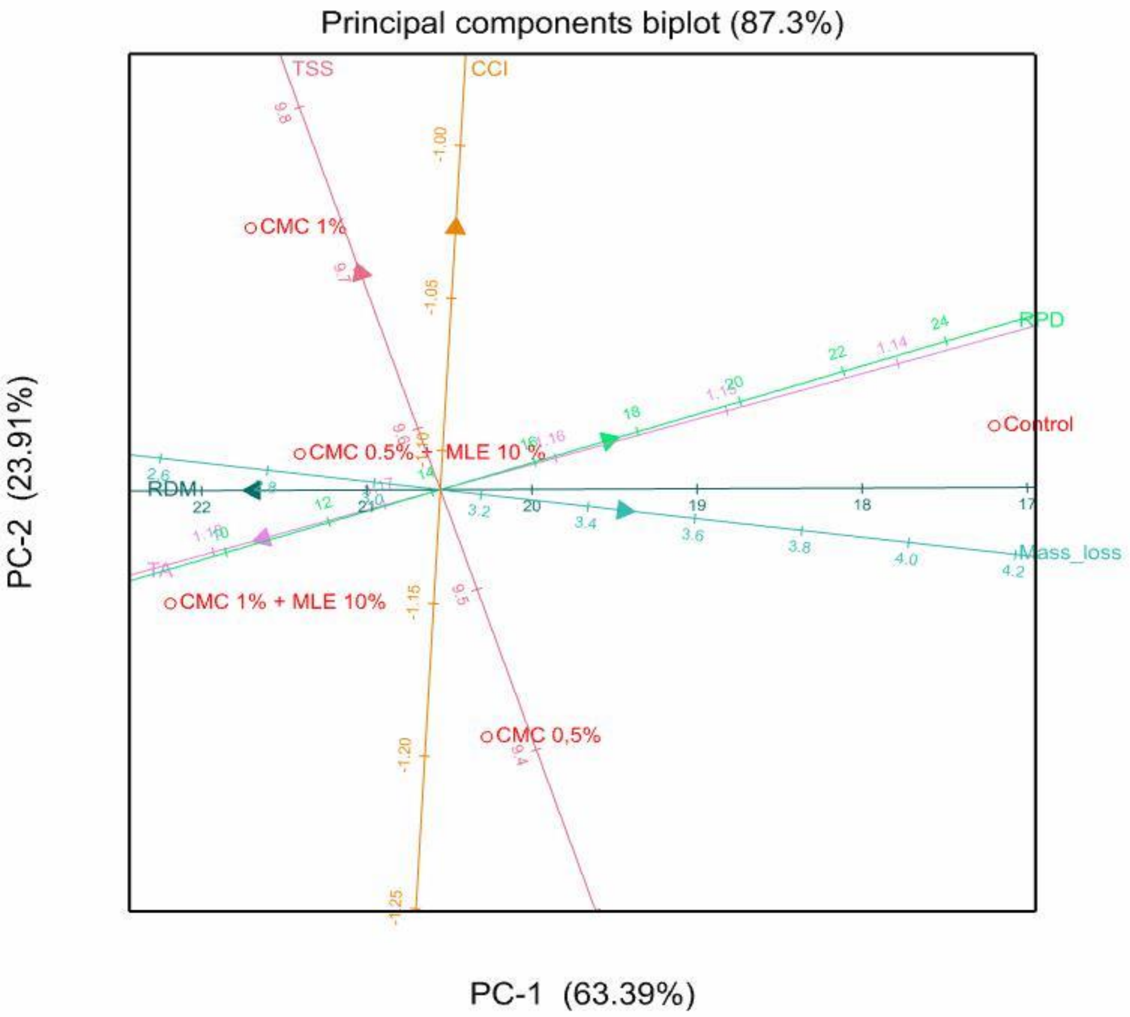


Figure 3. 9: The principal component analysis-based correlation of physicochemical parameters and edible coatings to fruit susceptibility to rind pitting disorder. The above figure is based on fruit from outside canopy position. RPD: rind pitting disorder, TSS: total soluble solutes, TA: titratable acidity, CCI: citrus colour index, CMC: carboxymethylcellulose, MLE: moringa leaf extracts.

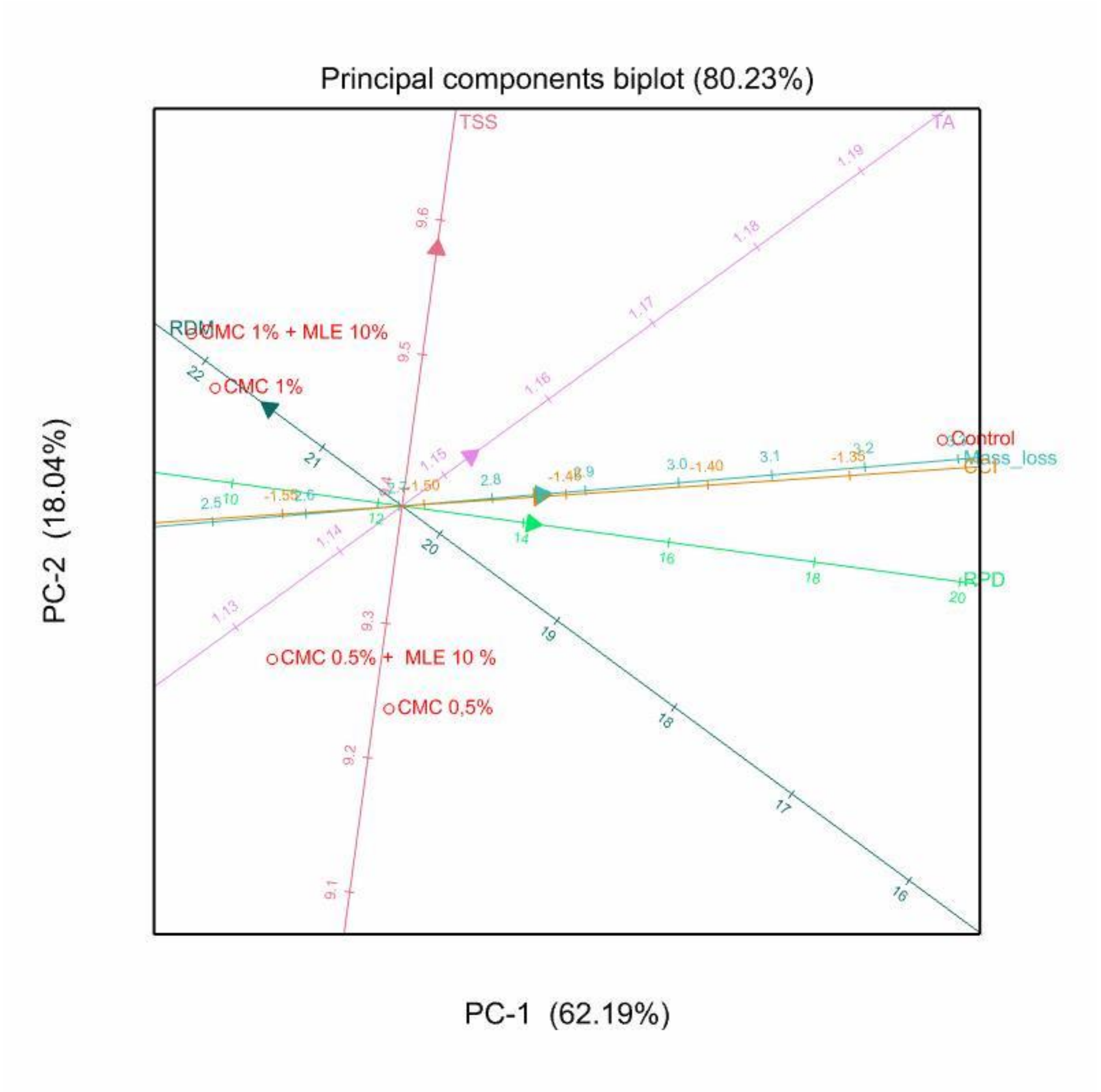


Figure 3. 10: The principal component analysis-based correlation of physicochemical parameters and edible coatings to fruit susceptibility to rind pitting disorder. The above figure is based on fruit from inside canopy position. RPD: rind pitting disorder, TSS: total soluble solutes, TA: titratable acidity, CCI: citrus colour index, CMC: carboxymethylcellulose, MLE: moringa leaf extracts.

3.4.8. Sensory quality

Sensory quality evaluation is one of the most significant determinants of quality. Instruments may be used to measure fruit quality; however, consumers contribute a lot in judging a produce by using their own senses (Ladaniya, 2008). Therefore, fruit marketability is attributed by qualities such as taste, aroma, colour, gloss etc. Taste, aroma and colour are significant fruit quality factors that determine consumer preference. Rind pitting disorder is characterized by sunken areas or 'pit' on the flavedo followed by browning and dryness of affected areas (Alférez and Burns, 2004). This means that consumers would not be satisfied by quality of affected fruit. In this study, 20 untrained panellists gave the pitted fruit low sensory scores.

Sensorial attributes (colour, taste, gloss, odour) were all scored high in coated fruit than uncoated fruit (Figure 11). These evaluations suggest that edible coatings do improve quality of citrus fruit after storage period, irrespective of canopy position.

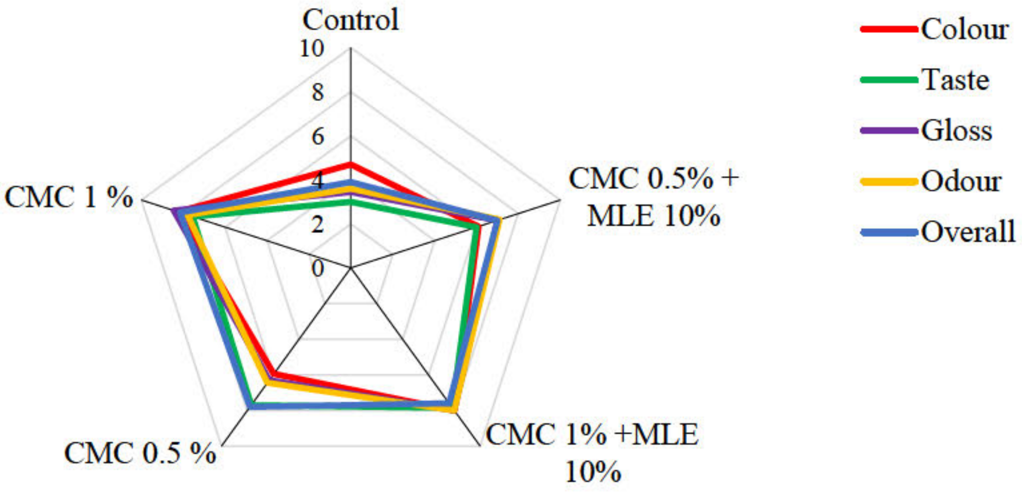


Figure 3. 11: Sensory quality evaluation of 'Marsh' grapefruit after 9 weeks of cold storage (3±1 ºC), and 2 weeks of postharvest shelf life (21 ± 1 °C). Data is represented by means. CMC: carboxymethylcellulose; MLE: moringa leaf extracts.

3.5. Conclusion

This study assessed the effect of moringa leaf extracts in carboxymethylcellulose edible coatings on rind pitting disorder and physicochemical quality of 'Marsh' grapefruit. Overall, this study demonstrated that ‘Marsh’ grapefruit coated with CMC 1% and a combination of CMC 1% and MLE 10% had lower weight loss, maintained TSS:TA ratio and CCI, lower rind dry mass. Fruit mass loss, CCI, b* and TSS were found to have a positive correlation to RPD. While, L*, TA and rind dry matter showed a negative correlation to RPD. Although the correlations of most physicochemical parameters to disorder were weak, it was significant that the relationship of each parameter and the disorder is known, either negative or positive. Fruit coated with edible coatings had lower RPD incidence compared to uncoated fruit. PCA-based discrimination of fruit from different canopy position in response to edible coatings was successful. This can enable the prediction of RPD-linked physicochemical parameters which can be used for estimating the likelihood disorder development during storage and the postharvest handling chain. Results signified that OC fruit were more susceptible RPD than IC fruit. This means that citrus industries can consider exporting more IC fruit than OC fruit, OC fruit can be sold to local markets. This study also concluded that physicochemical parameters such as TSS, RDM, CCI and mass loss can be useful markers for predicting susceptibility of ‘Marsh’ grapefruit to rind pitting disorder. The results also showed that treated fruit were more palatable to consumers than control fruit since the overall acceptability was higher in all treated fruit. Furthermore, edible coated fruit showed an improved quality and a prolonged shelf life.

3.6. References

Abbas, H., Abassi, N.A., Yasin, T., Maqbool, M., Ahmad, T., 2008. Influence of irradiated chitosan coating on postharvest quality of Kinnow (*Citrus reticulata Blanco*). Asian J. Chem 20, 6217.

Agusti, M., Almela, V., Juan, M., Alferez, F., Tadeo, F., Zacarias, L., 2001. Histological and physiological characterization of rind breakdown of 'Navelate'sweet orange. Annals of Botany 88, 415-422.

Alférez, F., Agusti, M., Zacarıas, L., 2003. Postharvest rind staining in Navel oranges is aggravated by changes in storage relative humidity: effect on respiration, ethylene production and water potential. Postharvest Biology and Technology 28, 143-152.

Alferez, F., Alquezar, B., Burns, J.K., Zacarias, L., 2010. Variation in water, osmotic and turgor potential in peel of 'Marsh'grapefruit during development of postharvest peel pitting. Postharvest Biology and Technology 56, 44-49.

Alférez, F., Burns, J.K., 2004. Postharvest peel pitting at non-chilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage. Postharvest Biology and Technology 32, 79-87.

Alférez, F., Sala, J.M., Sanchez-Ballesta, M.T., Mulas, M., Lafuente, M.T., Zacarias, L., 2005. A comparative study of the postharvest performance of an ABA-deficient mutant of oranges: I. Physiological and quality aspects. Postharvest Biology and Technology 37, 222-231.

Alferez, F., Zacarias, L., Burns, J.K., 2005. Low relative humidity at harvest and before storage at high humidity influence the severity of postharvest peel pitting in citrus. Journal of the American Society for Horticultural Science 130, 225-231.

Ali, A., Mahmud, T., 2007. The potential use of locally prepared chitosan to control in vitro growth of Colletotrichum gloeosporioides isolated from papaya fruits, Europe-Asia Symposium on Quality Management in Postharvest Systems-Eurasia 2007 804, pp. 177-182.

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

Ali, M.A., Zulfiqar, A., Arif, A.M., Khan, A.-R., Iqbal, Z., Khan, M.A., 2015. Effect of natural and synthetic fruit coatings on the postharvest quality of kinnow mandarins. Agricultural Engineering International: CIGR Journal 17.

Almela, V., Agustí, M., Pons, J., 1992. Rind spots in Fortune mandarin. Origin and control. Physiologia Plantarum 85, A60.

Arnon, H., Zaitsev, Y., Porat, R., Poverenov, E., 2014. Effects of carboxymethyl cellulose and chitosan bilayer edible coating on postharvest quality of citrus fruit. Postharvest Biology and Technology 87, 21-26.

Ashfaq, M., Basra, S.M., Ashfaq, U., 2012. Moringa: A miracle plant for agro-forestry. Journal of agriculture and social sciences 8.

Assimakopoulou, A., Tsougrianis, C., Elena, K., Fasseas, C., Karabourniotis, G., 2009. Pre-harvest rind-spotting in 'Clementine'mandarin. Journal of plant nutrition 32, 1486-1497.

Baldwin, E., Burns, J., Kazokas, W., Brecht, J., Hagenmaier, R., Bender, R., Pesis, E., 1999. Effect of two edible coatings with different permeability characteristics on mango (*Mangifera indica L.)* ripening during storage. Postharvest Biology and Technology 17, 215-226.

Cancolon, P., Xu, Y., 2002. Changes in organic acid composition of citrus juices during the 2000–2001 season, Proc. Fla. State Hort. Soc, pp. 37-41.

Chen, L., Opara, U.L., 2013. Texture measurement approaches in fresh and processed foods—A review. Food Research International 51, 823-835.

Cohen, E., Shapiro, B., Shalom, Y., Klein, J., 1994. Water loss: a nondestructive indicator of enhanced cell membrane permeability of chilling-injured citrus fruit. Journal of The American Society For Horticultural Science 119, 983-986.

Contreras-Oliva, A., Rojas-Argudo, C., Pérez-Gago, M.B., 2011. Effect of solid content and composition of hydroxypropyl methylcellulose–lipid edible coatings on physicochemical, sensory and nutritional quality of ‘Valencia’oranges. International Journal of Food Science and Technology 46, 2437-2445.

Contreras-Oliva, A., Rojas-Argudo, C., Pérez-Gago, M.B., 2012. Effect of solid content and composition of hydroxypropyl methylcellulose–lipid edible coatings on physico-chemical and nutritional quality of ‘Oronules’ mandarins. Journal of the Science of Food and Agriculture 92, 794-802.

Cronje, P.J., Barry, G.H., Huysamer, M., 2011a. Fruiting position during development of ‘Nules Clementine’mandarin affects the concentration of K, Mg and Ca in the flavedo. Scientia horticulturae 130, 829-837.

Cronje, P.J., Barry, G.H., Huysamer, M., 2011b. Postharvest rind breakdown of ‘Nules Clementine’mandarin is influenced by ethylene application, storage temperature and storage duration. Postharvest Biology and Technology 60, 192-201.

Cronje, P.J., Barry, G.H., Huysamer, M., 2013. Canopy position affects pigment expression and accumulation of flavedo carbohydrates of ‘Nules Clementine’mandarin fruit, thereby affecting rind condition. Journal of the American Society for Horticultural Science 138, 217-224.

Duarte, A., Guardiola, J., 1993. Factors affecting rind pitting in the mandarin hybrids" fortune" and" nova". The influence of exogenous growth regulators, International Symposium on Quality of Fruit and Vegetables: Influence of Pre-and Post-Harvest Factors and Technology 379, pp. 59-66.

Edmonds, J., 2018. Key industry statistics for citrus growers 2018. Citrus Growers' Association of Southern Africa, 1-48.

Ehlers, J.L., 2016. Post-harvest rind pitting studies on ‘Valencia’orange. Stellenbosch: Stellenbosch University.

Embuscado, M.E., Huber, K.C., 2009. Edible films and coatings for food applications. Springer.

Ezz, T.M., Awad, R.M., 2009. Relationship between mineral composition of the flavedo tissue of “Marsh” grapefruit and chilling injury during low temperature storage. Research Journal of Agriculture and Biological Sciences 5, 892-898.

Gregorová, A., Saha, N., Kitano, T., Sáha, P., 2015. Hydrothermal effect and mechanical stress properties of carboxymethylcellulose based hydrogel food packaging. Carbohydrate Polymers 117, 559-568.

Henriod, R., 2006. Postharvest characteristics of navel oranges following high humidity and low temperature storage and transport. Postharvest Biology And Technology 42, 57-64.

Hernández-Albíter, R.C., Barrera-Necha, L.L., Bautista-Baños, S., Bravo-Luna, L., 2007. Antifungal Potential of Crude Plant Extracts on Conidial Germination of Two Isolates of Colletotrichum gloeosporioides (Penz.) Penz. and Sacc. Revista Mexicana de Fitopatología 25.

John, N.R., Gala, V.C., Sawant, C.S., 2013. Inhibitory effects of plant extracts on multi-species dental biofilm formation in-vitro. International Journal of Biological Sciences 4, 487-495.

Kader, A.A., 2002. Postharvest technology of horticultural crops. University of California Agriculture and Natural Resources.

Khalid, S., Malik, A.U., Saleem, B.A., Khan, A.S., Khalid, M.S., Amin, M., 2012. Tree age and canopy position affect rind quality, fruit quality and rind nutrient content of ‘Kinnow’mandarin (*Citrus nobilis Lour*× *Citrus deliciosa Tenora*). Scientia Horticulturae 135, 137-144.

Ladaniya, M., 2008. Fruit quality control, evaluation and analysis. Citrus fruit: Biology, Technology and Evaluation, 475-500.

Lafuente, M.a.T., Sala, J.M., 2002. Abscisic acid levels and the influence of ethylene, humidity and storage temperature on the incidence of postharvest rindstaning of ‘Navelina’orange (*Citrussinensis L. Osbeck*) fruit. Postharvest Biology and Technology 25, 49-57.

Magwaza, L.S., Landahl, S., Cronje, P.J., Nieuwoudt, H.H., Mouazen, A.M., Nicolaï, B.M., Terry, L.A., Opara, U.L., 2014a. The use of Vis/NIRS and chemometric analysis to predict fruit defects and postharvest behaviour of ‘Nules Clementine’mandarin fruit. Food Chemistry 163, 267-274.

Magwaza, L.S., Mditshwa, A., Tesfay, S.Z., Opara, U.L., 2017. An overview of preharvest factors affecting vitamin C content of citrus fruit. Scientia Horticulturae 216, 12-21.

Magwaza, L.S., Opara, U.L., Cronje, P.J., Landahl, S., Nieuwoudt, H.H., Mouazen, A.M., Nicolaï, B.M., Terry, L.A., 2014b. Assessment of rind quality of ‘Nules Clementine’mandarin during postharvest storage: 1. Vis/NIRS PCA models and relationship with canopy position. Scientia Horticulturae 165, 410-420.

Magwaza, L.S., Opara, U.L., Cronje, P.J., Landahl, S., Nieuwoudt, H.H., Mouazen, A.M., Nicolaï, B.M., Terry, L.A., 2014c. Assessment of rind quality of ‘Nules Clementine’mandarin fruit during postharvest storage: 2. Robust Vis/NIRS PLS models for prediction of physico-chemical attributes. Scientia Horticulturae 165, 421-432.

Magwaza, L.S., Opara, U.L., Cronjé, P.J., Landahl, S., Terry, L.A., Nicola ï, B.M., 2013. Nonchilling physiological rind disorders in citrus fruit. Horticultural Reviews Volume 41, 131-176.

Mattheis, J.P., Fellman, J.K., 1999. Preharvest factors influencing flavor of fresh fruit and vegetables. Postharvest Biology and Technology 15, 227-232.

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

Medeira, M., Maia, M., Vitor, R., 1999. The first stages of pre-harvest ‘peel pitting’development in ‘Encore’mandarin. An histological and ultrastructural study. Annals of Botany 83, 667-673.

Medlicott, A., Semple, A., Thompson, A., Blackbourne, H., Thompson, A., 1992. Measurement of colour changes in ripening bananas and mangoes by instrumental, chemical and visual assessments. Trop. Agric.(Trinidad) 69, 161-166.

Navarro-Tarazaga, M.-L., Plotto, A., Goodner, K., Baldwin, E., Perez-Gago, M.-B., 2007. Effect of edible coatings on the flavor of'Valencia'oranges, HortScience. Amer SOC

Horticultural Science 113 S West ST, STE 200, Alexandria, VA 22314-2851 USA, pp. 869-869.

Navarro-Tarazaga, M.L., Del Río, M.A., Krochta, J.M., Perez-Gago, M.B., 2008. Fatty Acid Effect on Hydroxypropyl Methylcellulose− Beeswax Edible Film Properties and Postharvest Quality of Coated ‘Ortanique’Mandarins. Journal of Agricultural and Food Chemistry 56, 10689-10696.

Ncama, K., 2016. Non-destructive prediction of ‘marsh’grapefruit (*Citrus x paradisi* MacFad) postharvest quality and physiological rind disorders using visible to near infrared spectroscopy.

Obenland, D., Collin, S., Mackey, B., Sievert, J., Arpaia, M.L., 2011. Storage temperature and time influences sensory quality of mandarins by altering soluble solids, acidity and aroma volatile composition. Postharvest Biology and Technology 59, 187-193.

Park, H., Chinnan, M., Shewfelt, R., 1994. Edible corn-zein film coatings to extend storage life of tomatoes. Journal of Food Processing and Preservation 18, 317-331.

Parra, J., Ripoll, G., Orihuel-Iranzo, B., 2014. Potassium sorbate effects on citrus weight loss and decay control. Postharvest Biology and Technology 96, 7-13.

Pathare, P.B., Opara, U.L., Al-Said, F.A.-J., 2013. Colour measurement and analysis in fresh and processed foods: a review. Food and Bioprocess Technology 6, 36-60.

Perez-Gago, M., Serra, M., Alonso, M., Mateos, M., Del Río, M., 2005. Effect of whey protein- and hydroxypropyl methylcellulose-based edible composite coatings on color change of fresh-cut apples. Postharvest Biology and Technology 36, 77-85.

Petracek, P.D., Dou, H., Pao, S., 1998. The influence of applied waxes on postharvest physiological behavior and pitting of grapefruit. Postharvest Biology and Technology 14, 99-106.

Porat, R., Weiss, B., Cohen, L., Daus, A., Aharoni, N., 2004. Reduction of postharvest rind disorders in citrus fruit by modified atmosphere packaging. Postharvest Biology and Technology 33, 35-43.

Rafiq, S., Kaul, R., Sofi, S., Bashir, N., Nazir, F., Nayik, G.A., 2018. Citrus peel as a source of functional ingredient: a review. Journal of the Saudi Society of Agricultural Sciences 17, 351-358.

Rana, G., Kartar, S., Singh, K., 1992. Studies on extending post harvest life of sweet orange fruits. Crop Res 5, 150-153.

Rehman, M., Singh, Z., Khurshid, T., 2018. Pre-harvest spray application of abscisic acid (S-ABA) regulates fruit colour development and quality in early maturing M7 Navel orange. Scientia Horticulturae 229, 1-9.

Rojas-Argudo, C., Del Río, M., Pérez-Gago, M., 2009. Development and optimization of locust bean gum (LBG)-based edible coatings for postharvest storage of 'Fortune'mandarins. Postharvest Biology and Technology 52, 227-234.

Sastry, S., BUFFINGTON, D., Baird, C., 1977. Transpiration rates of certain fruits and vegetables, Ashrae Journal-American Society of Heating Refrigerating And Air-Conditioning Engineers. Amer Soc Heat Refrig Air-Conditioning Eng Inc 1791 Tullie Circle Ne, Atlanta, pp. 36-36.

Shah, S., Jahangir, M., Qaisar, M., Khan, S., Mahmood, T., Saeed, M., Farid, A., Liaquat, M., 2015. Storage stability of kinnow fruit (Citrus reticulata) as affected by CMC and guar gum-based silver nanoparticle coatings. Molecules 20, 22645-22661.

Shlens, J., 2014. A tutorial on principal component analysis. arXiv preprint arXiv:1404.1100.

Tao, N.G., Ao, T.T., Liu, Y.J., Huang, S.R., 2012. Effect of sucrose-based polymers on quality of Satsuma mandarin fruit (*Citrus unshiu* Marc. cv. *Miyagawa Wase*). International Journal of Food Science and Technology 47, 997-1003.

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

Thakur, K., Lal Kaushal, B., Sharma, R., 2002. Effect of different post-harvest treatments and storage conditions on the fruit quality of Kinnow. Journal of Food Science and Technology 39, 609-618.

Tharanathan, R., 2003. Biodegradable films and composite coatings: Past, present and future. Trends in Food Science and Technology 14, 71-78.

Valencia-Chamorro, S.A., Palou, L., Del Rio, M.A., Pérez-Gago, M.B., 2011. Performance of hydroxypropyl methylcellulose (HPMC)-lipid edible coatings with antifungal food additives during cold storage of 'Clemenules' mandarins. LWT-Food Science and Technology 44, 2342-2348.

Vercher, R., Tadeo, F.R., Almela, V., Zaragoza, S., Primo-Millo, E., Agustí, M., 1994. Rind structure, epicuticular wax morphology and water permeability of 'Fortune'mandarin fruits affected by peel pitting. Annals of Botany 74, 619-625.

Vidal, A., Talens, P., Prats-Montalbán, J.M., Cubero, S., Albert, F., Blasco, J., 2013. In-line estimation of the standard colour index of citrus fruits using a computer vision system developed for a mobile platform. Food and Bioprocess Technology 6, 3412-3419.

Vitor, R.F., Lidon, F.C., da Graça Barreiro, M., Maia, M.I., Medeira, M.C., Guerreiro, A., 2001. Peel pitting of Encore mandarin fruits: Etiology, control and implications in fruit quality. Fruits 56, 27-35.

Wild, B., 1991. Postharvest factors governing the development of peteca rind pitting on 'Meyer' lemons. HortScience 26, 287-289.

Xu, D., Qin, H., Ren, D., 2018. Prolonged preservation of tangerine fruits using chitosan/montmorillonite composite coating. Postharvest Biology and Technology 143, 50-57.

Yehoshua, S.B., Peretz, J., Moran, R., Lavie, B., Kim, J., 2001. Reducing the incidence of superficial flavedo necrosis (noxan) of 'Shamouti'oranges (*Citrus sinensis*, Osbeck). Postharvest Biology and Technology 22, 19-27.

Zheng, H., Zhang, Q., Quan, J., Zheng, Q., Xi, W., 2016. Determination of sugars, organic acids, aroma components, and carotenoids in grapefruit pulps. Food chemistry 205, 112-121.

Zillo, R.R., da Silva, P.P.M., de Oliveira, J., da Glória, E.M., Spoto, M.H.F., 2018. Carboxymethylcellulose coating associated with essential oil can increase papaya shelf life. Scientia horticulturae 239, 70-77.

CHAPTER 4

Vis/NIRS based partial least squares models for predicting rind pitting disorder in ‘Marsh’ grapefruit (*Citrus x paradisi* Macfad.) using rind phytochemical attributes as markers in response to edible coatings

4.1. Abstract

‘Marsh’ grapefruit are prone to postharvest rind pitting (RP) disorder during storage. The primary cause and etiology of this disorder are not known nor understood. This study assessed rind phytochemical properties as pre-symptomatic bio-markers linked to the susceptibility of ‘Marsh’ grapefruit during postharvest storage in response to edible coatings. Visible to near infrared spectra (Vis/NIRS) was used to predict pre-symptomatic indicators associated with RP in ‘Marsh’ grapefruit. The effect of canopy positions (inside and outside) on the fruit susceptibility to RPD was also assessed. A total of 260 fruit were harvested and treated with different concentrations edible coatings based on moringa leaf extracts (MLE) infused into carboxymethylcellulose (CMC). Postharvest treatments were as follows: T_1 = CMC 0.5 % + MLE 10%, T_2 = CMC 1% + MLE 10%, T_3 = CMC 0.5%, T_4 = CMC 1%, Control. Treated fruit were stored at 3 °C for 9 weeks and 21 °C for 2 weeks of shelf life. A total of 40 fruit were scanned using a bench-top monochromator NIR systems equipped with a quartz halogen lamp and lead sulphide (PbS) detector. Rind parameters including chlorophyll a and b, β carotene and total carotenoids, sucrose, glucose, fructose, total antioxidant capacity, ascorbic acid, phenolics, flavonoids and antioxidant activity were analysed in laboratory as reference measurements. Principal component analysis (PCA) and Partial least square (PLS) regression models based on reference data and spectral data were developed using Unscrambler® chemometric software. The Vis/NIRS model was calibrated and validated. This study managed to develop the prediction model for RP on ‘Marsh’ grapefruit which was as follows (R^2c = 0.973; root mean square error of calibration (RMSEC) = 0.118; R^2_P = 0.966; root mean square error of prediction (RMSEP) = 0174; bias = -0.036; residual predictive deviation (RPD) = 5.23). Partial Least square models for rind phytochemical parameters were developed to associate them with RP. Fruit from outside canopy were more susceptible to RP than those from the inside canopy fruit. Exporting fruit to international markets when pitting is expected could result in higher loss than in local markets when disorder is higher than anticipated. The Vis/NIRS and models developed in this study may be used as a tool to predict fruit origin within

the canopy and susceptibility of ‘Marsh’ grapefruit to rind pitting disorder using tested rind phytochemical parameters as biomarkers.

Keywords: ‘Marsh’ grapefruit, Rind pitting, Visible to near infrared, edible coatings; rind phytochemical attributes, canopy position, PLS regression models.

4.2. Introduction

Citrus is one of the most popular fruit in the world (Nekvapil et al., 2018) with the production of 112.5 million tons in 2017 season (Edmonds, 2018). The attractiveness and high consumption of citrus fruit is highly attributed to its health promoting phytochemical compounds such as phenolics and vitamin C (Lado et al., 2016; Liu et al., 2012; Rafiq et al., 2018; Sidana et al., 2013). Citrus quality is mostly evaluated based on visible external appearance such as surface colour, size, shape and the presence or absence of skin defects is significant as it contributes on price decision making in the markets (Alquezar et al., 2010; Magwaza et al., 2014a; Xudong et al., 2009). These quality attributes are commonly evaluated by humans or by machine systems (Xudong et al., 2009). Citrus fruit are susceptible to different postharvest physiological rind disorders, which are manifested during storage period (Alquezar et al., 2010; Kader, 2002; Magwaza et al., 2013a; Magwaza et al., 2012c). In the worst season, fruit affected by disorders can reach up to 60% of total production (Agusti et al., 2001). Although most of these physiological disorders affect rind, not the edible internal portion of a fruit, affected fruit have reduced market value since consumers use the external appearance as their primary quality indicator (Alquezar et al., 2010).

Citrus fruit such as ‘Marsh’ grapefruit are likely to develop postharvest rind pitting (RP) disorder during storage at chilling temperature (Alférez and Burns, 2004), while in ‘Fortune’ mandarin, peel pitting often appears before harvesting (Almela et al., 1992). Recent evidence in the literature has showed that peel water status plays a major role in RP development (Cronjé et al., 2017). Fruit with thick albedo such as ‘Marsh’ grapefruit experience early RP after fruit being transferred from low relative humidity (RH) to high RH. These changes have a linear relationship with water loss (Cronjé et al., 2017).

Rind pitting disorder is characterized by sub epidermal rind cells collapse with no discoloration taking place during the early stages of development. As the disorder persists, it affects oil glands and eventually releases intercellular content that changes colourless lesions to dark brown due to enzymatic oxidation (Agusti et al., 2001). The mechanism leading to the occurrence of this disorder and how is it related to other rind physiological disorders is not known (Agustí et al., 2002). As a result, developing a cost-effective approach for inhibiting RP and minimising postharvest losses associated with this disorder continues to be a challenge (Magwaza, 2013).

Several studies have been conducted to identify the occurrence and susceptibility citrus fruit RP. For instance, Alférez and Burns (2004) reported that commercial wax promote rind pitting in 'Marsh' grapefruit during storage. Cohen et al. (1994) also suggested that low storage temperatures might be involved in development of rind pitting. While, postharvest fruit susceptibility to this disorder is also related to ripening stage (Purvis et al., 1979; Vercher et al., 1994), as well as the position of the fruit within the tree canopy (Almela et al., 1992; El-Otmani et al., 1987), as well as each variety characteristic (Chalutz et al., 1985).

Canopy position may influence the biochemical properties of the rind and could play a significant role in fruit susceptibility to rind disorders (Cronje et al., 2011). This is largely attributed to different light intensity radiations within the canopy and their enormous influence on rind concentration of carbohydrate and rind condition (Holland et al., 2002). Since these quality attributes can be used as indicators of the presence or absence of rind disorders, non-destructive techniques are necessary to use as they will enable the assessment of the entire fruit quality rapidly and also monitor changes during postharvest storage period (Giovanelli et al., 2014). Among other non-destructive techniques such as hyperspectral imaging and multispectral (Blasco et al., 2009; Magwaza et al., 2012), magnetic resonance imaging (MRI) (Lammertyn et al., 2003), X-ray-computed tomography (CT) (Verboven et al., 2008) and optical coherence tomography (OCT) (Meglinski et al., 2010), Near infra-red spectroscopy (NIRS) is certainly the most advanced in instrumentation, applications, accessories, and chemometric software packages (Nicolai et al., 2007).

The non-destructive methods are preferred over destructive because they are simple, fast and it allows a great number of samples to be measured individually, they also reduce waste and allow repeated measures on the same item over time (Nicolai et al., 2007). Visible to near infrared spectroscopy (Vis/NIRS) is the most used for evaluation of a wide range of postharvest quality assessments of fruit and vegetables (Wedding et al., 2013). A review by Magwaza et al. (2012b) evaluated the feasibility of Vis/NIRS to observe external and internal properties in relation to citrus fruit quality. Studies that are based on monitoring and predicting fruit rind physiological disorders using NIRS are scarce. A study by Gómez-Sanchis et al. (2014); and Zheng et al. (2010) managed to detect oleocellosis and decay in citrus fruit and it was suggested that NIRS method is capable of predicting the susceptibility of citrus related rind physiological disorders development.

The knowledge of biochemical changes that occur in citrus fruit that could be used as indicators to fruit susceptibility to disorders is limited, a review by Magwaza et al. (2013b) highlighted. It is, therefore, becoming a challenge to identify possible rind phytochemical markers in fruit that are related to rind pitting disorder susceptibility. Therefore, this study assessed rind phytochemical markers and their correlation to the development of rind pitting disorder. An attempt has also been made to understand the mechanisms associated with rind pitting disorder which may influence the disorder in response to edible coatings. Vis/NIRS was used to predict pre-symptomatic indicators associated with rind pitting disorder in 'Marsh' grapefruit.

4.3. Materials and methods

4.3.1. Fruit samples

The study was conducted during the 2017/18 growing season. A total of 260 'Marsh' grapefruit were harvested at commercial maturity from Dole Farm located at Enkwalini (28°75'03.719''S; 31°58'26.035''E), KwaZulu-Natal Province, South Africa. To investigate the influence of canopy position on rind biochemical composition, an equal amount of fruit were harvested from inside and outside canopy positions (130 from each). The canopy positions were referred as inside canopy, and outside canopy of a fruit tree (Cronje et al., 2011). After harvesting, fruit were immediately transported at ambient temperature in ventilated sack bags to the Postharvest Research Laboratory of the University of KwaZulu-Natal, where the fruit were washed, sorted and assigned to different postharvest treatments.

4.3.2. Postharvest treatments and storage

Upon arrival at the Postharvest Research Laboratory, harvested fruit were assigned to five (5) different postharvest treatments based on different concentrations of edible coatings based on moringa leaf extracts (MLE) infused into carboxymethylcellulose (CMC). Postharvest treatments were as follows: T_1 = CMC 0.5 % + MLE 10%, T_2 = CMC 1% + MLE 10%, T_3 = CMC 0.5%, T_4 = CMC 1% and control. Each treatment was comprised of four fruit per canopy position for each sample day from week 0 to week 11. Treatments were organised in a factorial design with edible coatings and canopy position as main factors. Coatings were applied, and fruit left to dry on a laboratory bench top. Thereafter, fruit were stored in a cold room with delivery air temperature at 3 °C and relative humidity (RH) set at 90%. Fruit were stored for nine weeks corresponding with maximum period of shipment. Thereafter, fruit were taken back to room temperature to simulate shelf life for two weeks, which would happen after reaching designated market.

4.3.3. Disorder rating

Rind pitting incidence evaluation of the fruit was done using visual rating scale. Fruit were rated from 0 (no pit) to 3 (severe pitting). Rind pitting index was calculated using Eq. (1) previously reported by Lafuente and Sala (2002).

$$\text{Rind pitting index} = \frac{\text{rindstaining scale (0–3)}\times\text{number of fruit in each class}}{total\ number\ of\ fruit} \qquad (1)$$

4.3.4. Visible to near infrared spectroscopy (Vis/NIRS) spectra collection

Visible to near infrared spectroscopy (Vis/NIRS) spectral data was acquired in reflectance mode using a laboratory bench-top monochromator NIR Systems Model XDS spectrometer (FOSS NIR Systems, Inc.; Maryland, USA) equipped with a quartz halogen lamp and lead sulfide (PbS) detector. The system was calibrated by scanning a 100% white reference tile to provide background reference prior fruit scanning, and periodically at 30 min intervals of scanning fruit, to reduce baseline shift of spectral data (Magwaza et al., 2014a; Magwaza et al., 2014c). The spectra were acquired with a circular sample cup with a quartz window. Samples were scanned by placing them on a sample cup gentle in an enclosed box, which was designed to prevent light leakage. Reflectance spectra were obtained at 2 nm intervals from fully visible to the near infrared spectrum of 400 to 2500 nm wavelength range from two opposite sides along equatorial region of the fruit and recorded as log 1/reflectance (log 1/R). Each spectrum was the average of 32 scans recorded using Vision software (Vision TM, version 3.5.0.0, Tidestone Technologies Inc., KS, USA).

4.3.5. Sample preparation

For further analysis of rind phytochemical parameters, fresh rind samples were freeze dried for three days using Virtis Benchop freeze drier system (ES Model, SP Industries Inc., Warmister, USA) at 0.013-0.026 kPa and -40 ºC. The freeze drier machine work by freezing the material, then reduce the pressure and adding heat to allow the frozen water in the material to sublimate. After the samples had dried, rind phytochemical parameters were then analysed using freeze dried samples.

4.3.6. Determination of total rind carotenoids and chlorophylls

Total carotenoid content was determined according to Lichtenthaler (1987) with slight modifications. A freeze-dried sample of 0.5 g was weighed into a test tubes followed by the addition of 8 mL of 80% (v/v) methanol. Samples were then allowed to stand for 10 min on ice covered with aluminium foil. Thereafter, samples were centrifuged for 10 min using GenVac® (SP Scientific, Genvac LTD., Suffolk, UK). The absorbance values of the supernatants were read using UV-1800 Spectrophotometer (Shimadzu Scientific Instruments INC., Columbia, USA) at the wavelengths required for calculations of the pigments. Chlorophyll a (Chl_a), chlorophyll b (Chl_b) and total carotenoids (C_{x+c}) were respectively calculated using Eqs. 2, 3, and 4 according to Lichtenthaler (1987). The β-carotene concentration was calculated using Eq. 5 according to (Luterotti and Kljak, 2010).

$$Chl_a = 12.25\ A_{663.2} - 2.79\ A_{646.8} \quad (2)$$

$$Chl_b = 21.50\ A_{646.8} - 5.10\ A_{663\ 2} \quad (3)$$

$$C_{x+c} = (1000\ A_{470} - 1.82\ Chl_a - 85.02\ Chl_b) / 198 \quad (4)$$

$$\beta \text{ carotene} = 0.216\ A_{663.2} – 1.22\ A_{645.0} – 0.304\ A_{505.0} + 0.452\ A_{453.0} \quad (5)$$

Where, A is absorbance of a sample at subscript wavelength, *e.g.* $A_{663.2}$ is sample absorbance at 663.2 nm.

4.3.7. Extraction and quantification of rind soluble sugars

The extraction and quantification of rind soluble sugars was carried out according to (Olarewaju et al., 2017). Briefly, rind soluble sugars were extracted from 0.5 g of dried rind powder using 80% (v/v) aqueous methanol (5 mL). The mixture was then placed for 60 min in a water bath at 80 °C then the mixture was stored in a refrigerator at 4 °C overnight to facilitate the release of soluble sugars. 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 vials and ready for injection using high performance liquid chromatography (HPLC). Concentrations of sugars (glucose, sucrose and fructose) were analysed using an isocratic HPLC system equipped with a refractive index detector, method by (Liu et al., 1999). Sample extracts were injected into a Rezex RCM monosaccharide Ca+ (8%) column of 7.8 mm diameter x 300 mm (Phenomenex, Torrance, CA, USA) with a Carbo-Ca2 + guard column of 3 mm x 4 mm x (Phenomenex). The column temperature was set at 85 °C using a thermo-stated column compartment (G1316A, Agilent). The mobile phase was ultra-pure water at a flow rate of 0.6 mL/min. The presence and concentration of the selected non-structural carbohydrates was calculated by comparing peak area of samples against peak area of known standard concentrations.

4.3.8. Extraction and quantification of rind phenolic compounds

Total phenolic compound extraction and quantification were carried out using a method described by Lamien-Meda et al. (2008) with slight modifications. Briefly, a dried rind sample powder (0.5 g) was extracted with 80% (v/v) methanol (5 mL). The mixture covered with aluminium foil was kept for 10 mins at room temperature. The mixture was then centrifuged 10 min using GenVac® centrifuge (SP Scientific, Genvac LTD., Suffolk, UK) to obtain the clear extract.

Total phenolic compounds contents were determined by Folin-Ciocalteau method (Lamien-Meda et al., 2008). Each extract (100 µL) was mixed with 2N Folin-Ciocalteau reagent (125 µL) and allowed to stand for 3 min. Thereafter, 1250 µL of sodium carbonate (7.5% v/v) was added and samples were incubated for 1 h in dark room temperature. The absorbance was measured at 760 nm using a UV-1800 Spectrophotometer (Shimadzu Scientific Instruments INC., Columbia, USA) against methanol as blank. A standard calibration curve was plotted using gallic acid (0-120 µg/mL; $R^2 = 0.997$) and the total phenolic compounds were converted to mg GAE/g on dry matter basis.

4.3.9. Determination of rind flavonoid concentrations

Total flavonoid compound extraction and quantification was carried out using a method described by Zhishen et al. (1999) with slight modifications. Briefly, a dried rind sample powder (0.5 g) was extracted with 80% (v/v) methanol (5 mL). The mixture covered with aluminium foil was kept for 10 min at room temperature. The mixture was then centrifuged 10 min using GenVac® centrifuge (SP Scientific, Genvac LTD., Suffolk, UK) to obtain clear extract.

Total flavonoid compound contents were determined according to Zhishen et al. (1999) with modifications. Briefly, extract (1 mL) was mixed with 0.3 mL of 5% (m/v) sodium nitrite ($NaNO_2$) and kept for 5 minutes and 0.3 mL of 10% (m/v) aluminium chloride ($AlCl_3$) was added and kept for 6 minutes and the 3mL of 1M of sodium hydroxide (NaOH) was added to the mixture and incubated for 6 minutes in dark room temperature. After incubating, flavonoids were measured at 510 nm using UV-1800 Spectrophotometer (Shimadzu Scientific Instruments INC., Columbia, USA) against methanol as blank. Quantification was done on the basis of the standard curve of quercetin (0-1000 µg/mL; $R^2 = 0.998$), with the results expressed as mg quercetin equivalents (QTE)/g on dry matter basis.

4.3.10. Rind ascorbic acid content

Ascorbic acid content of the freeze-dried samples was analysed spectrophotometrically using the method of Chang et al. (2006). In summary, 0.5 g of a freeze-dried sample was extracted in 10 mL metaphosphoric acid (3% v/v) for 1 hour. The extract was then be centrifuged using GenVac® concentrator (SP Scientific, Genvac LTD., Suffolk, UK) for 15 minutes. One ml of the supernatant was subsequently added to 3 mL 0.05 mM DPIP and mixed for 5 seconds, and the absorbance of this solution measured at 515 nm using UV-1800 Spectrophotometer (Shimadzu Scientific Instruments INC., Columbia, USA) against a blank made by one mL 3% metaphosphoric acid (v/v) added to 9 mL 0.05 mM DIP and mixed for 15 sec. The ascorbic acid content was calculated from standard curve (0-500µg/mL; $R^2 = 0.997$) and converted to mg/g on dry matter basis.

4.3.11. Rind antioxidant activity determination

Extraction was carried out according to Aliyu et al. (2013) with slight modifications. A freeze-dried sample of 1 g was extracted using 25 mL of distilled water. The mixture was allowed to stand at room temperature for 1 h in the dark, with agitations at every 15 min intervals. The mixture was centrifuged for 20 min using GenVac® centrifuge (SP Scientific, Genvac LTD., Suffolk, UK) obtain clean extract before analysis. 0.1 mM solution of 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) scavenging assay in methanol was prepared. An aliquot (40 µL) was extracted by 3 mL of methanolic DPPH solution. The samples were allowed to react for 30 minutes. The sample was thereafter measured in 515 nm using Ultraspec UV-1800 Spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, USA) under dim light.

Radical-scavenging activities were calculated by the percentage of DPPH that were scavenged using Eq. 6

$$\text{Radical-scavenging activities (\%)} = \frac{\text{Sample absorbance} - \text{control absorbance}}{\text{control absorbance}} \text{ x } 100 \qquad (6)$$

Where control absorbance referred to methanolic DPPH only while sample absorbance referred to a mixture containing DPPH.

4.3.12. Rind total antioxidant capacity determination

Total antioxidant capacity of dried sample was evaluated by the phosphor-molybdenum method (Aliyu et al., 2013). Briefly, 80% v/v methanol (5 mL) was used for extracting the rind powder sample (0.5 g). The aqueous extract was obtained by centrifuging liquid mixture using GenVac® centrifuge (SP Scientific, Genvac LTD., Suffolk, UK) before analysis. The methanolic extracts (0.3 mL) were combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 85 °C for 90 min. The solution was allowed to cool to room temperature before the absorbance was measured at 695 nm using a UV-1800 Spectrophotometer (Shimadzu Scientific Instruments INC., Columbia, USA), against pure methanol which was used as blank. The standard curve was done based on throlox (0-120µg/mL; $R^2 = 0.989$).

4.3.13. Spectra analysis

The reflectance spectra in Vision format (Vision ^{TM}, version 3.5.0.0, Tidestone Technologies Inc., KS, USA) were transformed to MS excel format compatible with The Unscrambler® chemometric software (Version 10.3, Camo Software, AS., Norway). The spectral range of 400-2500 nm was used to develop models. The different spectral range were tested and the range that showed best results on reflecting fruit parameters was selected to create the final model. Lab measured data (reference data) and spectral data were used to develop partial least squares (PLS) regression models. PLS is commonly used in quantitative spectroscopy to link spectroscopic data (X) with related physicochemical or biochemical data (Y) (Sáiz-Abajo et al., 2005). Several pre-processing methods were applied to the spectra to convert spectral and reference data to a standardized investigative state during chemometric analyses. Pre-processing data was done to correct light scattering and reduce the changes of light path length (Magwaza et al., 2012c). Spectral pre-processing techniques are used to remove unwanted data which cannot be picked up properly by the regression techniques (Nicolai et al., 2007). Pre-processing methods such as multiplicative scattering correction (MSC), Savitzky–Golay first and second derivatives etc. Multiplicative scattering correction is the most used normalisation technique (Næs et al., 2004), it used to compensate for baseline shift and multiplicative effect in the spectral data which are typically induced by physical effects. Savitzky–Golay first and second derivatives belongs to the transformation technique often used to remove baseline shifts and superposed peaks, second derivatives being the one that is used most because they can correct additive and multiplicative like MSC (Næs et al., 2004). Therefore, these two techniques cannot be done on the same dataset. These pre-processing were performed using Unscrambler® chemometric software (Version 10.3, Camo Software, AS., Norway). These methods were tested individually and with the combination with each other. Among several pre-processing methods, best performing models for all measured rind attributes during cross validation were chosen to develop a final model (Table 4.2). To improve the performance of the model and discriminate the outliers, hoteling T^2 outlier detection technique was used in cross validation (Magwaza et al., 2014b).

Principal component analysis (PCA) was also performed using Unscrambler® chemometric software (Version 10.3, Camo Software, AS, Norway) to identify which canopy position contributed more in RP disorder development and as well as potential coating treatment that can reduce the disorder during postharvest storage and handling.

4.3.14. Evaluating model's performance

Calibration and validation sets were selected using multivariate sampling which constituted 50% of calibration and 50% of validation sets. Dataset (spectral data and reference data) was sampled according to canopy position. The developed PLS regression models were defined by the following statistical terms; highest coefficient of determination (R^2), it basically signifies the proportion of explained variance of the response variable in calibration (R^2c) or validation (R^2v) set, Eq. (7); highest residual predictive deviation (RPD) Eq. (8), an RPD between 1.5 and 2 means that the model can discriminate low from high values of the response variable; a value between 2 and 2.5 indicates that coarse quantitative predictions are possible, and a value between 2.5 and 3 or above corresponds to good and excellent prediction accuracy, respectively (Nicolai et al., 2007); lowest root mean square error of calibration (RMSEC), Eq. (9); lowest root mean square prediction (RMSEP), Eq. (10); root mean square error analytical value must be as low as possible; a lowest measure of favour in samples evaluation (bias; Eq. 11), bias analytical value must be as small as possible for a model to be stable. Selected models were used to develop calibration models for predicting 'Marsh' grapefruit biochemical parameters.

$$R^2 = 1 - \frac{\sum(Ycal - Yact)^2}{\sum(Ycal - Ymean)^2} \tag{7}$$

$$RPD = \frac{SD}{RMSEP} \tag{8}$$

$$RMSEC = \sqrt{\sum(Ycal - Yact)^2/n} \tag{9}$$

$$RMSEP = \sqrt{\sum(Ypred - Yact)^2/n} \tag{10}$$

$$Bias = \frac{1}{n}\sqrt{\sum(Ypred - Yact)^2} \tag{11}$$

Where n is the number of spectra; Y_{act} is the actual value measured by the destructive method; Y_{mean} is the average value of predicted data; Y_{pred} is the Vis/NIRS predicted value of fruit quality parameter and SD is the standard deviation of measured data values

4.4. Results and Discussion

4.4.1. Spectra

A typical reflectance spectrum (400 to 2500 nm) obtained from 'Marsh' grapefruit harvested from outside canopy (OC) and inside canopy (IC) is presented in Figure 4.1. The spectra of 'Marsh' grapefruit of both canopy positions showed similar absorbance characteristics with the exception of higher peaks for OC at 450-750 nm compared to IC (Figure 4.1). It was hypothesized that OC fruit were more yellow to orange colour than IC fruit. Orange colour is found at 590-680 nm range of the spectrum (Chittka and Waser, 1997). The absorbance continued to decrease until it reaches 620 nm for both canopies. The peak observed in 682 nm wavelength was correlated to red colour absorbing pigments, particularly green chlorophylls that gives fruit their green colour at an immature stage (Gomez et al., 2006). NIR region showed to have high absorbance (radiation penetration) for both canopy positions compared to the visible region. This was related to bright rind colour causing reflection which may lead to poor prediction model performance using region below near infrared (750 nm). However, absorbance along the wavelengths in the NIR region showed the better absorbance which was correlated with the better performance of models developed using NIR region. These findings were in agreement with studies related to poor performance of long wavelengths to penetrate citrus with thick rind (Gomez et al., 2006; Magwaza et al., 2011; Ncama et al., 2017b). NIR spectra is comprised of broad bands which arise from overlapping mainly to overtones and combination of vibrational modes involving chemical bonds such as C-H, O-H, N-H and S-H (Osborne, 2006). The peaks observed at approximately 960 and 1170 nm were related to water and carbohydrates absorptions that usually occurs at 935 and 958 nm (Mcglone and Kawano, 1998). The absorption peaks at 1450 and 1950 nm match the first and second vibrational overtones that are related with H-O-H stretching modes of water absorption bands that are usually found at 970, 1200, 1450, 1950 and 2250 nm in biological samples (Ncama et al., 2018; Olarewaju et al., 2016; Williams and Norris, 1987). Magwaza et al. (2012b) also, highlighted that strong water absorption peaks on intact fruit are normally observed at 1184 and 1457 nm.

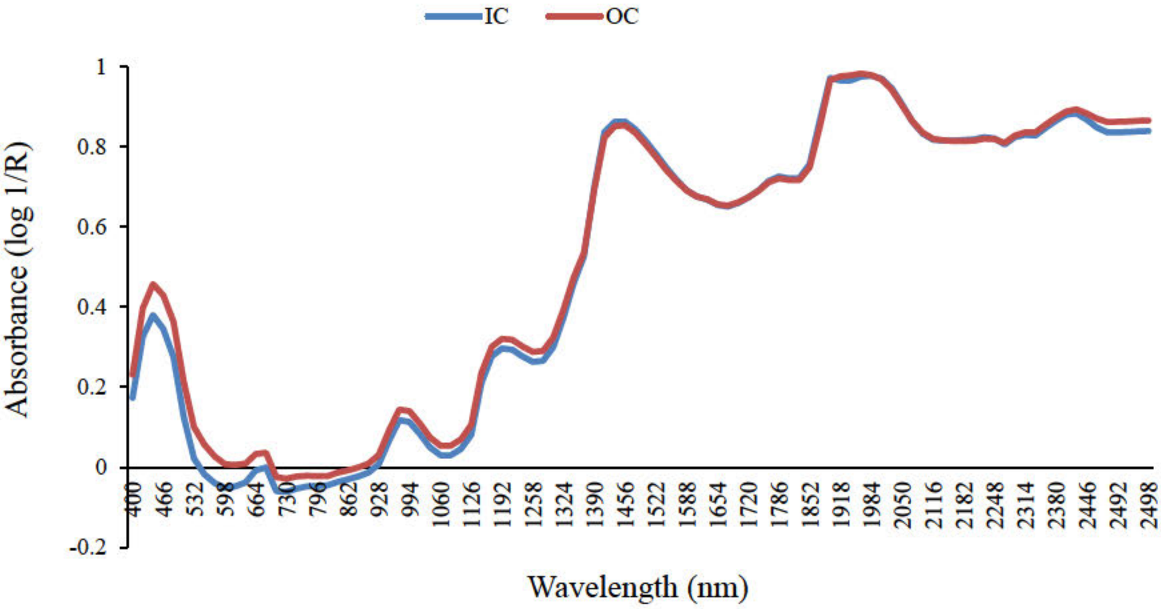


Figure 4. 1: A typical average visible to near infrared (400-2500 nm) spectrum illustrating inside canopy (IC) and outside canopy (OC) of 'Marsh' grapefruit.

4.4.2. Descriptive analysis

The descriptive statistics of reference data used to during model calibration and validation of rind parameters was analysed and displayed in Table 4.1. Rind parameters were distributed around the mean values with their respective standard deviation (SD) covering a wide range and variation, represented by the coefficient of variation as percentage (CV %). High variability of reference data is helpful in developing a reliable prediction model for visible to near infrared spectra (Vis/NIRS) (CléMent et al., 2008). The mean of all tested rind phytochemical parameters (rind ascorbic acid, rind phenolics, rind flavonoids, antioxidant activity, total antioxidant capacity, rind pigments and rind sugars) for 'Marsh' grapefruit was predicted accurately (Table 4.1). The predicted mean was allied to the values between the samples within the batch. The standard deviation for rind phytochemical parameters except total carotenoids were lower, which means that the predicted values were closer to the mean. The results were classified as good because predicted values deviated around the mean which means very low chances of predicting outlier samples. Additionally, the skewness of the data may be affected by the change in SD and results in a bias statistical parameter. The coefficient of variations of rind phytochemical parameters for 'Marsh' grapefruit were lower except for chlorophyll a (Chla) and chlorophyll b (Chlb), which was indicating that the variation within the batch sample is not wide. However, for chlorophylls the reason might be caused by the negative values obtained from the laboratory which made the variation wide within the samples. Though, it is vital to maintain CV, since it indicate the prediction if it closer to the actual values or not.

Table 4. 1: The descriptive statistics of ‘Marsh’ grapefruit rind phytochemical parameters

Parameters	**AA** (mg/g)	**Phenolics** (mg/g)	**Flavonoids** (mg/g)	**DPPH** %	**TAO** (mg/g)	**Chl a** (µg/g)	**Chl b** (µg/g)	**β caro** (µg/g)	**T.caro** (mg/g)	**Sucrose** (mg/g)	**Glucose** (mg/g)	**Fructose** (mg/g)
Max	4.09	1.42	0.97	79.29	6.46	3.96	2.37	0.35	951.84	9.87	9.60	5.91
Min	1.72	0.95	0.57	63.67	3.22	-1.84	-1.98	0.08	410.82	2.78	0.71	1.87
Mean	3.65	1.08	0.86	70.77	5.33	1.31	1.81	0.30	632.37	6.06	5.30	3.95
SD	0.22	0.05	0.04	1.78	0.27	1.02	0.80	0.03	55.62	0.63	1.28	0.42
CV%	5.95	4.61	4.74	2.51	5.12	77.94	43.91	10.77	8.80	10.43	24.13	10.54

Max: maximum; Min: minimum; SD, standard deviation; CV: coefficient of variance; AA; ascorbic acid; DPPH represent antioxidant activity; TAO: total antioxidant capacity; Chl a, chlorophyll a; Chl b, chlorophyll b; β caro.; carotene; T caro, total carotenoids.

4.3.3. PLS regression models of rind phytochemical parameters and rind pitting disorder obtained from visible to near infrared spectroscopy (Vis/NIRS)

The results of prediction models *vs* measured results for rind phytochemical quality parameters of 'Marsh' grapefruit, namely ascorbic acid, phenolics, flavonoids, antioxidant activity are presented in Figure 4.2, total antioxidant capacity, chlorophyll a, chlorophyll b, β carotene presented in Figure 4.3, while total carotenoids, sucrose, glucose and fructose were presented in Figure 4.4. The validity and prediction accuracy of models is mostly dependent on the precision of the reference data and the presence of enough variation in datasets (Magwaza et al., 2014c). As a common practice in Vis/NIRS, different pre-processing treatment techniques were tested before final calibration, in order to determine pre-treatment that will give best model. Table 4.2 summarise results obtained using different pre-processing treatments and spectral regions. The prediction of rind phytochemicals was good during calibration using inside canopy fruit, the calibration set was then validated using outside canopy fruit to produce prediction models. Best and stable models were selected and are presented in Table 4.2. The best prediction of rind pitting disorder index on 'Marsh' grapefruit was obtained as (R^2c = 0.973; RMSEC =0.118; R^2_P = 0.966; RMSEP= 0174; bias = -0.036; RPD = 5.23). The validation model for the disorder had a good fit for the developed models. The RP model is exhibited low value of bias (-0.036).

The predictive model was also characterized by high RPD higher than three (3). According to a system by Davey et al. (2009), the model was regarded as suitable for quantitative prediction, since its value was higher than 2. Various studies have suggested that is it important to confirm the reliability of the model by it RPD value even when high correlation occurs between NIR predicted and measured reference (Magwaza et al., 2012c; Williams, 2014). Davey et al. (2009) categorized RPD accurate level as unreliable (RPD < 1.5), appropriate for rough prediction (1.5 < RPD < 2.0), suitable for quantitative predictions (2.0 < RPD < 2.5), good (2.5 > RPD < 3.0) and models >3.0 are regarded as satisfactory and excellent for prediction.

PLS models were developed for predicting rind pitting using rind phytochemical quality attributes of 'Marsh' grapefruit as displayed in Table 4.2. Refer to Table 4.2, neither RMSEC nor RMSEP were greater than one (<1) among all rind phytochemical parameters except for total carotenoids. The exception of total carotenoids may be attributed by the latent value (LV) used to obtain the model is low as one, which may determine the RMSEC or RMSEP. The lower latent variables and high prediction accuracy of total carotenoids could be attributed to the significant waveband for this parameter occurring in the visible range. Previous studies based on citrus species has also reported root mean square error less than one, Lee et al., 2004 in *Citrus x limon* (L) Burm.f., Ncama et al., 2016 in 'Star Ruby" grapefruit. Table 4.2 also shows very low bias for all rind phytochemical parameters, which means PLS models can reliable. Golic and Walsh 2006 reported that in fruit, bias is mainly caused by temperature differences between calibration and validation which was not the case in this study. The validity and prediction accuracy of calibration models depends on the accuracy of reference data and enough variation existence in both calibration and validation datasets (Huishan et al., 2005).

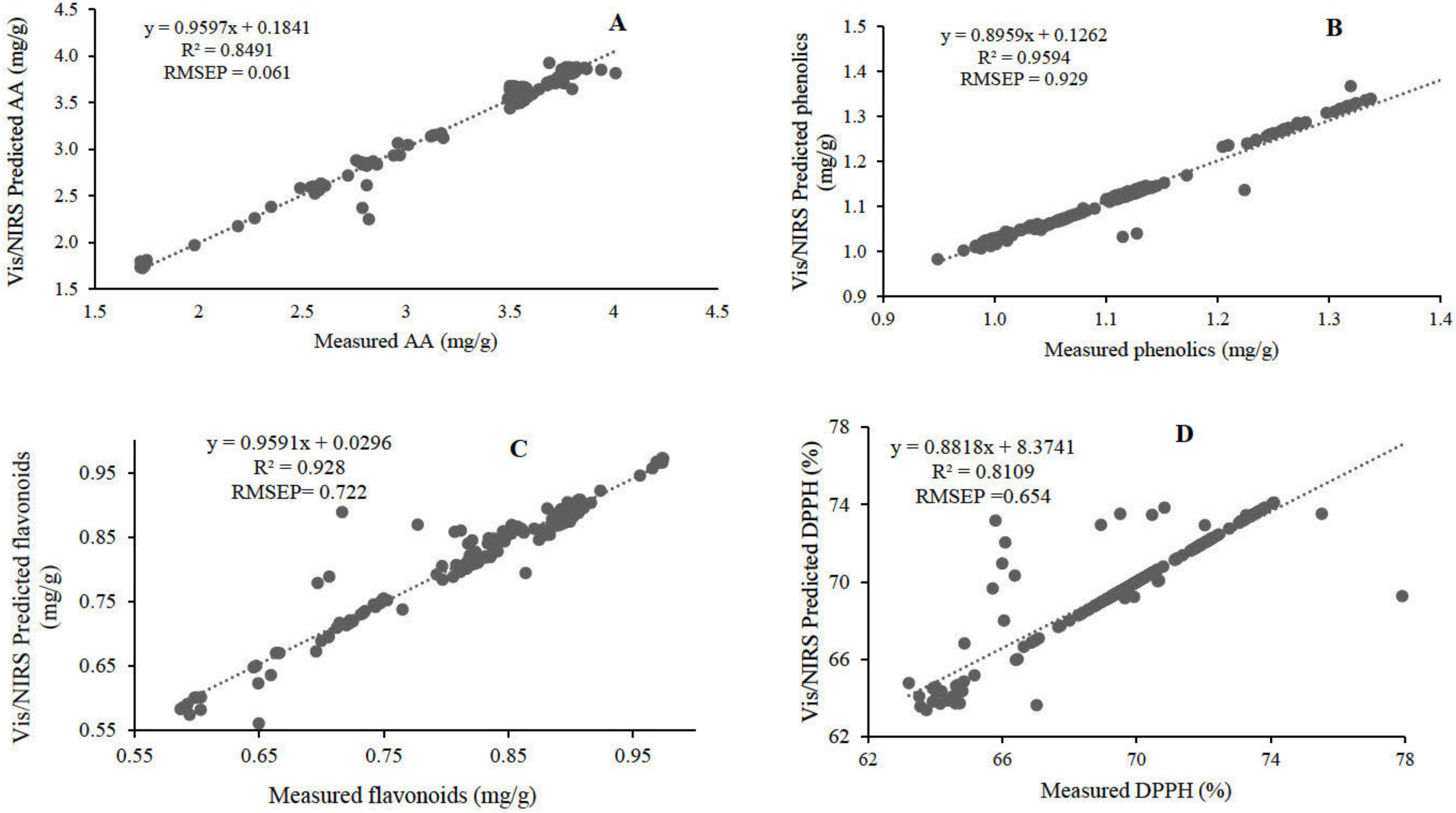


Figure 4. 2: Scatter plot of Vis/NIRS of predicted versus measured data of rind AA (A), phenolics (B), flavonoids (C), DPPH (D) RMSEP: root mean square error of prediction; Vis/NIRS: visible to near infrared spectroscopy (number of samples = 220).

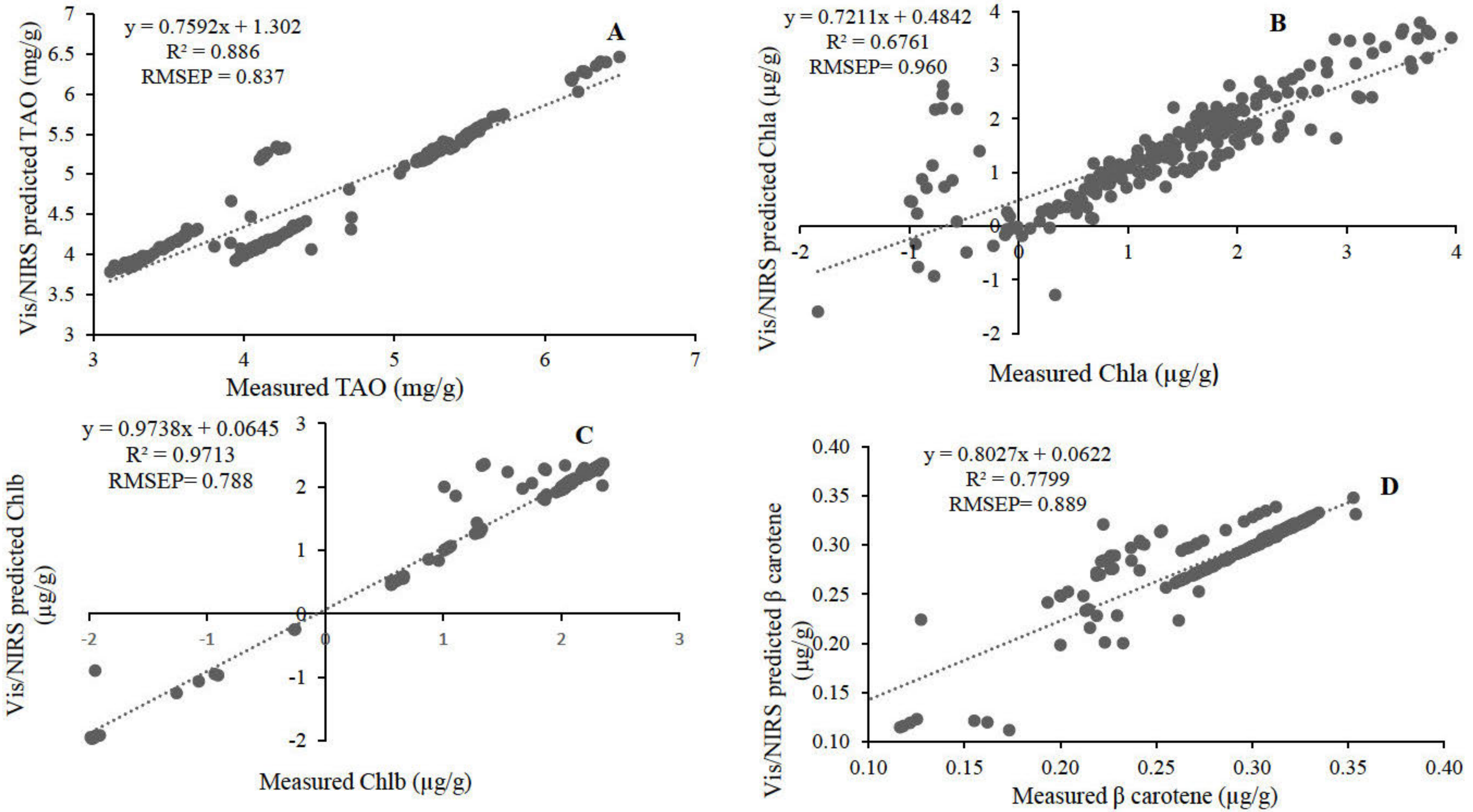


Figure 4. 3: Scatter plot of Vis/NIRS of predicted versus measured data of rind TAO (A), Chla (B), Chlb (C), β caro (D). RMSEP: root mean square error of prediction; Vis/NIRS: visible to near infrared spectroscopy (number of samples = 220).

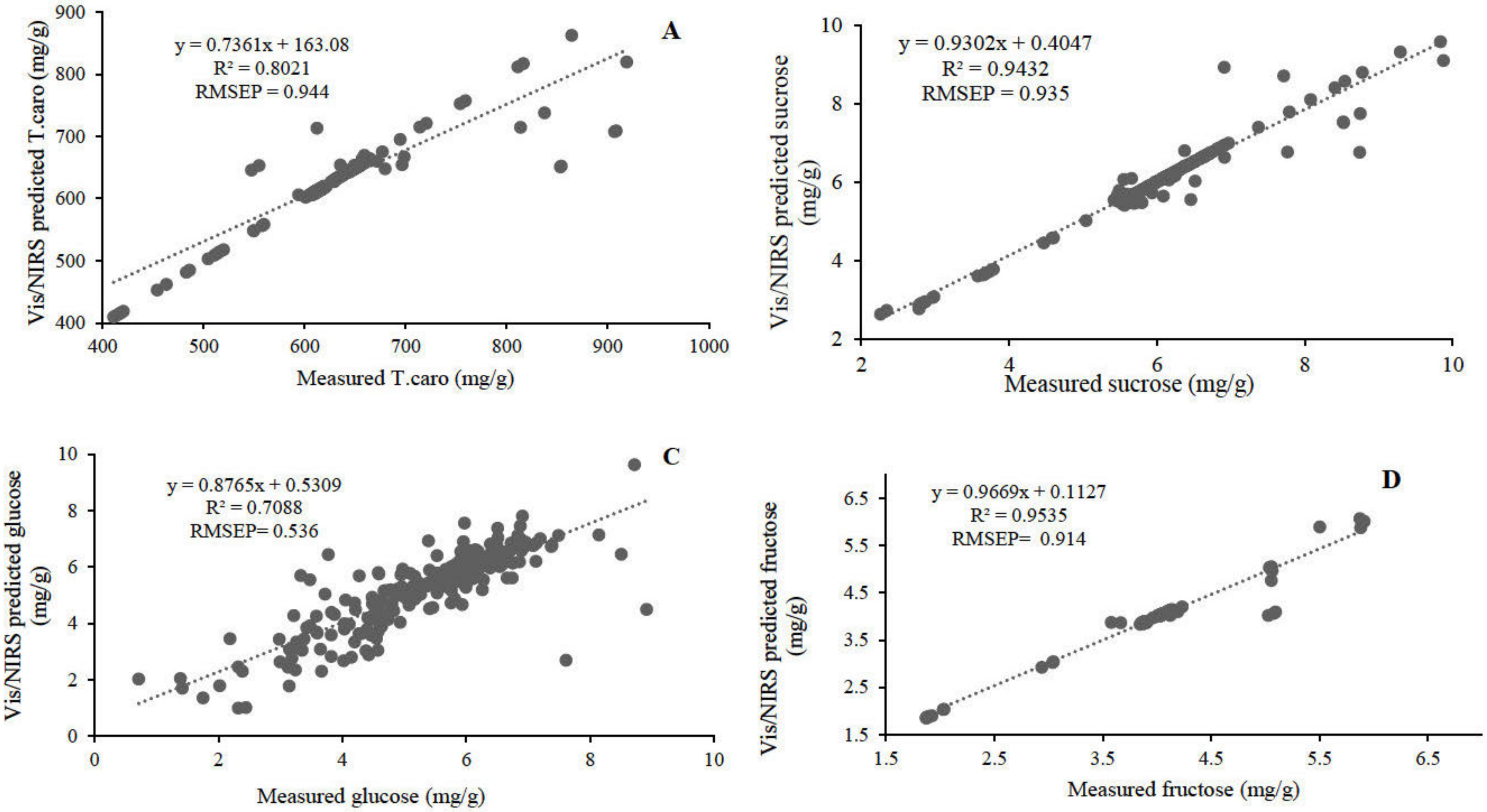


Figure 4. 4: Scatter plot of Vis/NIRS of predicted versus measured data of rind T.caro (A), sucrose (B), glucose (C), fructose (D) of 'Marsh' grapefruit. RMSEP: root mean square error of prediction; Vis/NIRS: visible to near infrared spectroscopy (number of samples = 220).

Table 4. 2: Performance statistics of the partial least squares calibration and prediction models for quantifying rind phytochemical quality and rind pitting disorder of ‘Marsh’ grapefruit.

		Calibration set			Validation set					
Parameters	LV	R^2	RMSEC	Slope	R^2	RMSEP	Slope	Bias	RPD	Region
Ascorbic acid (mg/g DW)	7	0.748	0.064	0.748	0.745	0.061	0.959	0.037	3.607	1100-2500
Phenolics (mg GAE/g DW)	1	0.673	0.028	0.673	0.545	0.046	0.461	0.008	1.087	400-2500
Flavonoids (mg QTE/g) DW	3	0.437	0.034	0.437	0.722	0.018	0.815	-0.004	2.222	1100-2500
DPPH (% DW)	2	0.742	0.991	0.742	0.889	0.543	0.086	0.086	3.278	400-2500
TAO (mg/g DW)	3	0.507	0.110	0.507	0.833	0.065	0.882	0.006	4.154	1100-2500
Chlorophyll a (µg/g DW)	7	0.944	0.243	0.944	0.690	0.676	0.758	0.105	1.509	400-2500
Chlorophyll b (µg/g DW)	2	0.689	0.093	0.689	0.788	0.072	0.791	-0.006	11.11	400-2500
B carotene (µg/g DW)	2	0.780	0.009	0.780	0.889	0.007	0.873	-0.001	4.286	400-2500
Total carotenoids (mg/g DW)	1	0.939	4.320	0.939	0.944	4.485	0.904	-0.432	12.40	400-2500
Sucrose (mg/g DW)	2	0.929	0.111	0.929	0.935	0.113	0.956	-0.013	5.575	1100-2500
Glucose (mg/g DW)	7	0.731	0.600	0.731	0.536	0.930	0.863	-0.147	1.376	1100-2500
Fructose (mg/g DW)	2	0.771	0.050	0.771	0.914	0.027	0.921	-0.005	15.56	400-2500
Rind pitting index	2	0.973	0.118	0.973	0.966	0.174	0.956	-0.036	5.230	400-2500

DPPH: antioxidant activity; TAO: total antioxidant capacity; LV: latent variable; R^2: coefficient of determination; RMSEC: root mean square error of calibration, RMSEP: root mean square error of prediction, RPD: residual predictive deviation: DW = dry weight.

4.3.4. The correlations of rind phytochemical attributes and to rind pitting disorder

Pearson correlations of rind phytochemical quality attributes and rind pitting disorder of ‘Marsh’ grapefruit was tested, and the results are displayed in Table 4.3. Phytochemical parameters and rind pitting disorder correlation was not that strong, but parameters correlated to the disorder were noticed. Rind phytochemical parameters such as ascorbic acid, chlorophyll a, chlorophyll b and fructose exhibited negative correlation to rind pitting disorder with r values of -0.04; -0.43; -0.36 and -0.26, respectively. Phenolics, flavonoids, DPPH, β carotene and total carotenoids on the other hands were weakly positive to RP with respective r values of 0.24, 0.46, 0.07, 0.25 and 0.35. Total antioxidant capacity, sucrose and glucose had relatively high positive correlation with RP with r values 0.51, 0.53 and 0.54, respectively.

The relationship of rind parameters with negative correlation to the disorder displays an inversely proportional relationship with the disorder, although the correlation is weak. Rind phytochemical parameters that are not strongly correlated with the disorder may not be used as primary bio-markers to the susceptibility of rind pitting development. Whereas, rind sucrose, glucose and total antioxidant capacity can be used as primary bio-markers to rind pitting disorder susceptibility development. The high negative correlation of chlorophylls (a and b) to carotenoids were observed to be -0.68 and -0.52, respectively. This negative correlation is not surprising since chlorophyll degrades as the fruit matures while carotenoids accumulate in citrus peel (Goldschmidt, 1988). The accumulation of carotenoids in fruit encourage senescence, however, Alferez and Zacarías (2014) reported that fruit are being more susceptible to peel pitting disorder as the peel ages. A high negative correlation was also noticed in chlorophyll a and b with rind sugars, sucrose with an r^2 value of -0.81, glucose -0.81, and fructose -0.54. This correlation was due to the fact that as citrus fruit respires during postharvest handling, chlorophyll gradually degrades while sugars continue to accumulate till senescence. Rind sucrose and glucose had a noticeable high positive correlation of (r^2 =0.83), this was understandable because sucrose is a macromolecule made up of glucose molecules, therefore an increase of glucose consequently increase the amount of sucrose present in the fruit (Bewley and Black, 1994).

Table 4. 3: The correlation between rind phytochemicals and rind pitting disorder of ‘Marsh’ grapefruit.

	AA (mg/g)	Phenolics (mg/g)	Flavonoids (mg/g)	DPPH %	TAO (mg/g)	Chla (µg/g)	Chlb (µg/g)	β caro (µg/g)	T.caro (mg/g)	Sucrose (mg/g)	Glucose (mg/g)	Fructose (mg/g)	RP
AA (mg/g)	1.00												
Phenolics (mg/g)	0.15	1.00											
Flavonoids(mg/g)	-0.01	0.37	1.00										
DPPH (%)	0.41	-0.10	-0.05	1.00									
TAO (mg/g)	0.06	-0.02	0.11	-0.18	1.00								
Chla (µg/g)	0.36	0.26	-0.20	0.45	**-0.59**	1.00							
Chlb (µg/g)	0.31	0.14	-0.09	0.36	-0.49	**0.87**	1.00						
β caro (µg/g)	-0.08	0.08	0.19	-0.14	0.13	-0.42	-0.36	1.00					
T.caro (mg/g)	-0.09	-0.19	-0.10	-0.30	0.36	**-0.68**	**-0.52**	0.21	1.00				
Sucrose (mg/g)	-0.05	-0.21	0.09	-0.44	0.42	**-0.81**	**-0.58**	0.37	**0.67**	1.00			
Glucose (mg/g)	-0.04	-0.18	0.05	-0.49	0.37	**-0.81**	**-0.55**	0.38	**0.61**	**0.83**	1.00		
Fructose (mg/g)	0.09	-0.35	0.30	-0.34	0.28	**-0.54**	**-0.50**	-0.24	-0.18	-0.28	-0.21	1.00	
RP	-0.04	0.24	0.46	0.07	**0.51**	-0.43	-0.36	0.25	0.35	**0.53**	**0.54**	-0.26	1.00

AA (ascorbic acid); DPPH: antioxidant activity; TAO: antioxidant capacity; Chla: chlorophyll a: chlorophyll b; β caro: beta carotene: T. caro: total carotenoids.

4.3.5. Principal component analysis

In order to gain a better insight on how measured rind biochemical quality parameters correlate with rind pitting (RP) disorder, data measured from the laboratory were subjected to principal component analysis (PCA). The PCA biplot (Figure 4.5) shows that the total variability was explained by the first two principal components (PCs), with PC1 account 54% and PC2 account 19% of the variation in the data. The total variability of the first two PCs is 73%.

PCA showed that sucrose, flavonoids, total antioxidant capacity were positively correlated with rind pitting (RP), therefore contributing significantly to the development of rind pitting disorder. Moreover, control samples from both canopies were correlated to RP. The direction of the vector of chla (chlorophyll a) was appeared to be opposite to that of RP, indicating a negative correlation as shown in Table 4.3. Although glucose and total carotenoids are located in the same plane as RP, the correlation was weak. This explains that glucose and carotenoids may not be considered as primary markers of fruit susceptibility to the disorder compared to sucrose, flavonoids, total antioxidant capacity which showed very strong correlations with the disorder. Yet, glucose and carotenoids can still be used as secondary rind biochemical indicators of ‘Marsh’ grapefruit sensitivity to the disorder. The response of fruit to the postharvest treatments for both canopy positions were observed and are presented in Figure 4.5.

Uncoated samples, both from outside and inside canopy appeared to experience more rind pitting than coated fruit. Among edible coated fruit, T1OC (CMC 0.5% + MLE 10%) experienced more rind pitting disorder than other treatments. T2OC (CMC 1% + MLE 10%), T3OC (CMC 0.5%) and T4OC (CMC 1%) were also in the same plane with rind pitting, meaning they were also affected by the disorder. In this study, ascorbic acid content increased for the first three weeks, thereafter it decreased until the end of the storage. The rate of decrease was lower in coated fruit compared to control fruit. This typical trend was reported in findings of Huang et al. (2008); and Shi et al. (2018) who found that application of aloe vera edible coatings combined with salicylic acid in oranges and grapefruit reduced degradation of vitamin C respectively. CMC coating probably provide a gas barrier layer that might decrease vitamin C oxidation. There is no enough evidence how CMC and MLE could lessen pitting of ‘Marsh’ grapefruit, however, by maintaining ascorbic acid therefore oxidative reactions.

Rind phenolics was almost stable for the first three weeks then slightly increased thereafter. Edible coated fruit had higher phenolics than control. These results were similar with those of Shi et al. (2018) who found that chitosan coating and salicylic acid increased the phenolic contents in grapefruit. Phenols are one of the important antioxidant constituents in fruit. The changes in phenolic content was reported to be depended on the genetics, temperature and environmental conditions during postharvest storage period (Kalt, 2005). In this study, it was hypothesized that CMC coating combined with MLE managed to increased phenylalanine ammonia-lyase (PAL) which is the key enzyme in biosynthesis of phenolics. Therefore, by elevating phenolics, fruit delay senescence process and thus reduces chances of being affected by rind pitting during storage. Therefore, CMC and MLE might ameliorate RP by increasing antioxidant systems. MLE have been reported to exhibit antioxidant activity due to abundant phenolic acids and flavonoids (Chumark et al., 2008; Verma et al., 2009).

Rind sugars (sucrose, glucose and fructose) were generally increasing during storage. Rate of increase in sucrose and glucose concentrations was much higher than that of fructose concentration. Uncoated fruit had higher rind sugars than coated fruit. Rind sugars has been linked with development of citrus rind disorders (Cronje et al., 2011). Edible coatings might have managed to maintain osmotic potential which is related to higher rind sucrose (Huang et al., 2000; Yakushiji et al., 1996). Lower osmotic potential due to high rind sucrose has been linked with development of citrus rind disorders (Cronje et al., 2011). Rind carotenoids improved during storage. De-greening of flavedo chlorophyll content declined with storage time. Coated fruit had higher chlorophyll content than uncoated fruit, in contrast, carotenoids were higher in uncoated fruit compared to coated fruit. However, it was reported that as the fruit ages, it becomes more prone to RP. Edible coatings may have delayed the accumulation of carotenoids and chlorophyll degradation.

Fruit from outside canopy were more susceptible to rind pitting than inside canopy fruit (Figure 4.5). Outside canopy had high rind sugar concentrations, possibly a stress response which was triggered by high light levels. This could encourage rind quality reduction, which establish as RP symptoms development. Chlorophyll content was higher in fruit from inside canopy compared to outside while carotenoid content was higher in outside canopy than inside. Therefore, sunlight fastened chlorophyll degradation and carotenoids accumulation.

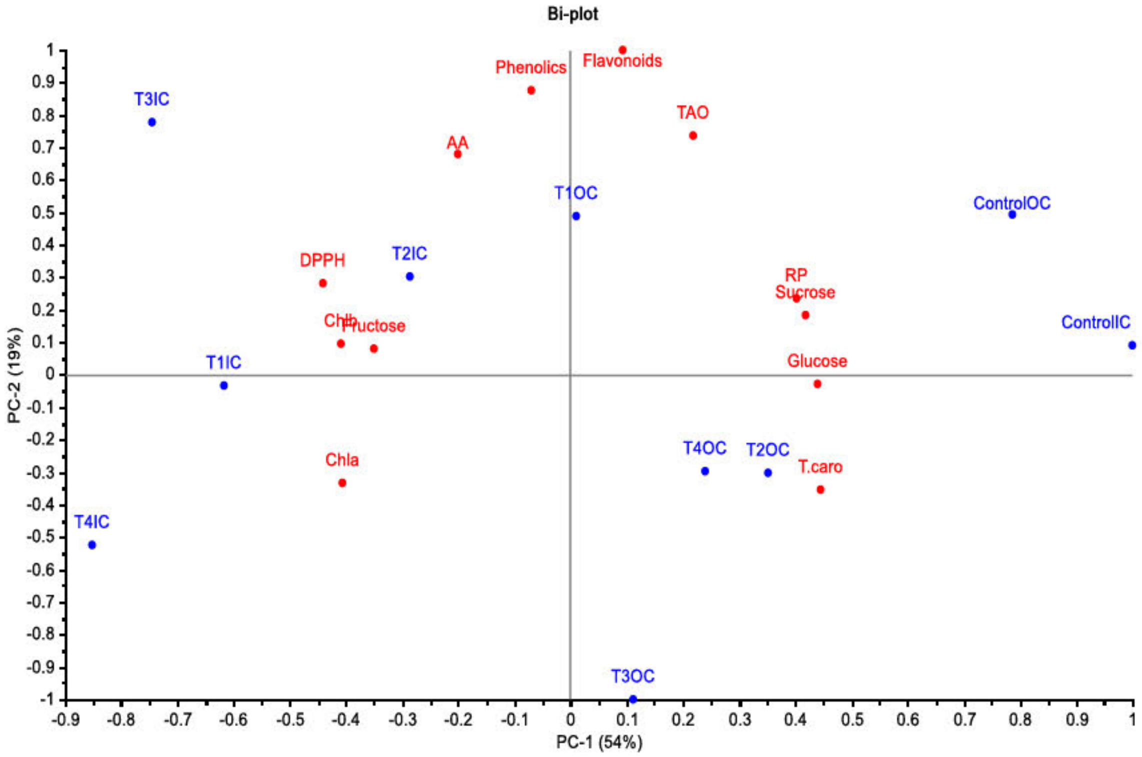


Figure 4. 5: Principal component analysis (PCA) based on the first two principal components showing a correlation between measured rind phytochemical properties and rind pitting susceptibility in 'Marsh' grapefruit. AA-ascorbic acid; Chla-chlorophyll a; Chlb-chlorophyll b; DPPH-antioxidant activity; T. caro-total carotenoids; TAO- total antioxidant capacity; RP-rind pitting: T1-CMC 0.5% + MLE 10%; T2-CMC 1% + MLE 10%; T3-CMC 0.5%; T4-CMC 1%; IC-inside canopy; OC-outside canopy.

4.4. Conclusion

The study demonstrated that the integration of Vis/NIRS and destructive data allowed the establishment of calibration and prediction equations of rind phytochemical quality and rind pitting disorder of 'Marsh' grapefruit using partial least square (PLS) multivariate analysis. Prediction model for rind pitting (RP) disorder was obtained using Vis/NIRS at full range (400-2500 nm). This study focused on rind carbohydrates (sucrose, glucose and fructose), rind pigments (chlorophyll a and b, β carotene and total carotenoids) and rind antioxidants (ascorbic acid, phenolics, flavonoids and antioxidant capacity). The data distribution in the PCA displayed clusters that allowed the distinction between fruit from different canopies and coated with different treatment concentrations. Rind sucrose, glucose, flavonoids, total antioxidant capacity and total carotenoids were associated with RP development. The rinds of fruit from outside canopy, which developed under sunlight conditions experienced higher rind pitting compared to the fruit inside canopy. Therefore, exporting fruit to international markets when pitting is expected could result in higher loss than in local markets when disorder is highly expected. Though, the information on the mechanism responsible for this rind disorder still need to be investigated. The reaction of the fruit to the edible coatings used in the experiments offers some implementable practices to minimise this disorder, however an exact concentration of coatings still requires attention. Additionally, separating fruit spectra based on canopy position can be applied by citrus farmers at postharvest handling during sorting and packing of fruit to separate fruit based on the canopy position

4.5. References

Agusti, M., Almela, V., Juan, M., Alferez, F., Tadeo, F., Zacarias, L., 2001. Histological and physiological characterization of rind breakdown of 'Navelate'sweet orange. Annals of Botany 88, 415-422.

Agustí, M., Almela, V., Juan, M., Martinez-Fuentes, A., Mesejo, C., 2002. Quality of citrus for fresh consumption as affected by water relationships, XXVI International Horticultural Congress: Citrus and Other Subtropical and Tropical Fruit Crops: Issues, Advances and 632, pp. 141-148.

Alférez, F., Burns, J.K., 2004. Postharvest peel pitting at non-chilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage. Postharvest Biology and Technology 32, 79-87.

Alferez, F., Zacarías, L., 2014. Influence of fruit maturity in the susceptibility of Navelina oranges to develop postharvest non-chilling peel pitting. Food Science and Technology International 20, 183-191.

Aliyu, A.B., Ibrahim, M.A., Musa, A.M., Musa, A.O., Kiplimo, J.J., Oyewale, A.O., 2013. Free radical scavenging and total antioxidant capacity of root extracts of Anchomanes difformis Engl.(*Araceae*). Acta Pol Pharm 70, 115-121.

Almela, V., Agustí, M., Pons, J., 1992. Rind spots in Fortune mandarin. Origin and control. Physiologia Plantarum 85, A60.

Alquezar, B., Mesejo, C., Alferez, F., Agustí, M., Zacarias, L., 2010. Morphological and ultrastructural changes in peel of 'Navelate'oranges in relation to variations in relative humidity during postharvest storage and development of peel pitting. Postharvest Biology and Technology 56, 163-170.

Bewley, J.D., Black, M., 1994. Seeds, Seeds. Springer, pp. 1-33.

Blasco, J., Aleixos, N., Gómez-Sanchis, J., Moltó, E., 2009. Recognition and classification of external skin damage in citrus fruits using multispectral data and morphological features. Biosystems Engineering 103, 137-145.

Chalutz, E., Waks, J., Schiffmann-Nadel, M., 1985. A comparison of the response of different citrus fruit cultivars to storage temperature. Scientia Horticulturae 25, 271-277.

Chang, C.-H., Lin, H.-Y., Chang, C.-Y., Liu, Y.-C., 2006. Comparisons on the antioxidant properties of fresh, freeze-dried and hot-air-dried tomatoes. Journal of Food Engineering 77, 478-485.

Chittka, L., Waser, N.M., 1997. Why red flowers are not invisible to bees. Israel Journal of Plant Sciences 45, 169-183.

Chumark, P., Khunawat, P., Sanvarinda, Y., Phornchirasilp, S., Morales, N.P., Phivthong-ngam, L., Ratanachamnong, P., Srisawat, S., Klai-upsorn, S.P., 2008. The in vitro and ex vivo antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of Moringa oleifera Lam. leaves. Journal of Ethnopharmacology 116, 439-446.

Clément, A., Dorais, M., Vernon, M., 2008. Nondestructive measurement of fresh tomato lycopene content and other physicochemical characteristics using visible− NIR spectroscopy. Journal of Agricultural and Food Chemistry 56, 9813-9818.

Cohen, E., Shapiro, B., Shalom, Y., Klein, J., 1994. Water loss: a nondestructive indicator of enhanced cell membrane permeability of chilling-injured citrus fruit. Journal of the American Society for Horticultural Science 119, 983-986.

Cronje, P.J., Barry, G.H., Huysamer, M., 2011. Fruiting position during development of 'Nules Clementine'mandarin affects the concentration of K, Mg and Ca in the flavedo. Scientia horticulturae 130, 829-837.

Cronjé, P.J., Zacarías, L., Alférez, F., 2017. Susceptibility to postharvest peel pitting in Citrus fruits as related to albedo thickness, water loss and phospholipase activity. Postharvest Biology and Technology 123, 77-82.

Davey, M.W., Saeys, W., Hof, E., Ramon, H., Swennen, R.L., Keulemans, J., 2009. Application of visible and near-infrared reflectance spectroscopy (Vis/NIRS) to determine carotenoid contents in banana (*Musa spp*.) fruit pulp. Journal of Agricultural and Food Chemistry 57, 1742-1751.

Edmonds, J., 2018. Key industry statistics for citrus growers 2018. Citrus Growers' Association of Southern Africa, 1-48.

El-Otmani, M., Arpaia, M.L., Coggins Jr, C.W., 1987. Developmental and topophysical effects on the n-alkanes of Valencia orange fruit epicuticular wax. Journal of Agricultural and Food Chemistry 35, 42-46.

Giovanelli, G., Sinelli, N., Beghi, R., Guidetti, R., Casiraghi, E., 2014. NIR spectroscopy for the optimization of postharvest apple management. Postharvest Biology and Technology 87, 13-20.

Goldschmidt, E.E., 1988. Regulatory aspects of chloro-chromoplast interconversions in senescing Citrus fruit peel. Israel Journal of Botany 37, 123-130.

Golic, M. and Walsh, K.B., 2006. Robustness of calibration models based on near infrared spectroscopy for the in-line grading of stonefruit for total soluble solids content. Analytica Chimica Acta, *555*, 286-291.

Gómez-Sanchis, J., Lorente, D., Soria-Olivas, E., Aleixos, N., Cubero, S., Blasco, J., 2014. Development of a hyperspectral computer vision system based on two liquid crystal tuneable filters for fruit inspection. Application to detect citrus fruits decay. Food and Bioprocess Technology 7, 1047-1056.

Gomez, A.H., He, Y., Pereira, A.G., 2006. Non-destructive measurement of acidity, soluble solids and firmness of Satsuma mandarin using Vis/NIR-spectroscopy techniques. Journal of Food Engineering 77, 313-319.

Holland, N., Menezes, H.C., Lafuente, M.a.T., 2002. Carbohydrates as related to the heat-induced chilling tolerance and respiratory rate of ‘Fortune’mandarin fruit harvested at different maturity stages. Postharvest Biology and Technology 25, 181-191.

Huang, R., Xia, R., Lu, Y., Hu, L., Xu, Y., 2008. Effect of pre-harvest salicylic acid spray treatment on post-harvest antioxidant in the pulp and peel of ‘Cara cara’navel orange (*Citrus sinenisis L*. Osbeck). Journal of the Science of Food and Agriculture 88, 229-236.

Huang, X.-M., Huang, H.-B., Gao, F.-F., 2000. The growth potential generated in citrus fruit under water stress and its relevant mechanisms. Scientia Horticulturae 83, 227-240.

Huishan, L., Yibin, Y., Huanyu, J., Yande, L., Xiaping, F., Wang, J., 2005. Application Fourier transform near infrared spectrometer in rapid estimation of soluble solids content of intact citrus fruits, 2005 ASAE Annual Meeting. American Society of Agricultural and Biological Engineers, p. 1.

Kader, A.A., 2002. Postharvest technology of horticultural crops. University of California Agriculture and Natural Resources.

Kalt, W., 2005. Effects of production and processing factors on major fruit and vegetable antioxidants. Journal of Food Science 70, R11-R19.

Lado, J., Cuellar, F., Rodrigo, M.J., Zacarías, L., 2016. Nutritional composition of mandarins, Nutritional Composition of Fruit Cultivars. Elsevier, pp. 419-443.

Lafuente, M.a.T., Sala, J.M., 2002. Abscisic acid levels and the influence of ethylene, humidity and storage temperature on the incidence of postharvest rindstaning of ‘Navelina’orange (*Citrus sinensis L*. Osbeck) fruit. Postharvest Biology and Technology 25, 49-57.

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

Lammertyn, J., Dresselaers, T., Van Hecke, P., Jancsók, P., Wevers, M., Nicolai, B., 2003. MRI and X-ray CT study of spatial distribution of core breakdown in ‘Conference’pears. Magnetic Resonance Imaging 21, 805-815.

Lee, K.J., Kim, G.Y., Kang, S.W., Son, J.R., Choi, D.S. and Choi, K.H., 2004. Measurement of sugar contents in citrus using near infrared transmittance. In *Key Engineering Materials* (Vol. 270, pp. 1014-1019). Trans Tech Publications Ltd.

Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes, Methods in enzymology. Elsevier, pp. 350-382.

Liu, X., Robinson, P.W., Madore, M.A., Witney, G.W., Arpaia, M.L., 1999. Hass' avocado carbohydrate fluctuations. II. Fruit growth and ripening. Journal of the American Society for Horticultural Science 124, 676-681.

Liu, Y., Heying, E., Tanumihardjo, S.A., 2012. History, global distribution, and nutritional importance of citrus fruits. Comprehensive Reviews in Food Science and Food Safety 11, 530-545.

Luterotti, S., Kljak, K., 2010. Spectrophotometric estimation of total carotenoids in cereal grain products. Acta Chimica Slovenica 57.

Magwaza, L.S., 2013. Non-destructive prediction and monitoring of postharvest quality of citrus fruit.

Magwaza, L.S., Ford, H.D., Cronje, P.J., Opara, U.L., Landahl, S., Tatam, R.P., Terry, L.A., 2013a. Application of optical coherence tomography to non-destructively characterise rind breakdown disorder of ‘Nules Clementine’mandarins. Postharvest Biology and Technology 84, 16-21.

Magwaza, L.S., Landahl, S., Cronje, P.J., Nieuwoudt, H.H., Mouazen, A.M., Nicolaï, B.M., Terry, L.A., Opara, U.L., 2014a. The use of Vis/NIRS and chemometric analysis to predict fruit defects and postharvest behaviour of ‘Nules Clementine’mandarin fruit. Food Chemistry 163, 267-274.

Magwaza, L.S., Opara, U.L., Cronje, P.J., Landahl, S., Nieuwoudt, H.H., Mouazen, A.M., Nicolaï, B.M., Terry, L.A., 2014b. Assessment of rind quality of ‘Nules Clementine’mandarin during postharvest storage: 1. Vis/NIRS PCA models and relationship with canopy position. Scientia Horticulturae 165, 410-420.

Magwaza, L.S., Opara, U.L., Cronje, P.J., Landahl, S., Nieuwoudt, H.H., Mouazen, A.M., Nicolaï, B.M., Terry, L.A., 2014c. Assessment of rind quality of ‘Nules Clementine’mandarin fruit during postharvest storage: 2. Robust Vis/NIRS PLS models for prediction of physico-chemical attributes. Scientia Horticulturae 165, 421-432.

Magwaza, L.S., Opara, U.L., Cronje, P.J., Landahl, S., Terry, L.A., 2013b. Canopy position affects rind biochemical profile of ‘Nules Clementine’mandarin fruit during postharvest storage. Postharvest Biology and Technology 86, 300-308.

Magwaza, L.S., Opara, U.L., Nieuwoudt, H., Cronje, P., 2011. Non-destructive quality assessment of ‘Valencia’orange using FT-NIR spectroscopy, Proceedings of the NIR 2011, 15th International Conference on Near-Infrared Spectroscopy.

Magwaza, L.S., Opara, U.L., Terry, L.A., Landahl, S., Cronje, P.J., Nieuwoudt, H., Mouazen, A.M., Saeys, W., Nicolaï, B.M., 20127. Prediction of ‘Nules Clementine’mandarin susceptibility to rind breakdown disorder using Vis/NIR spectroscopy. Postharvest biology and technology 74, 1-10.

McGlone, V.A., Kawano, S., 1998. Firmness, dry-matter and soluble-solids assessment of postharvest kiwifruit by NIR spectroscopy. Postharvest Biology and Technology 13, 131-141.

Meglinski, I., Buranachai, C., Terry, L., 2010. Plant photonics: application of optical coherence tomography to monitor defects and rots in onion. Laser Physics Letters 7, 307.

Næs, T., Isaksson, T., Fearn, T. and Davies-Hill, T., 2004. A user-friendly guide to multivariate calibration and classification.

Ncama, K., Opara, U.L., Tesfay, S.Z., Fawole, O.A., Magwaza, L.S., 2017. Application of Vis/NIR spectroscopy for predicting sweetness and flavour parameters of ‘Valencia’orange (Citrus sinensis) and ‘Star Ruby’grapefruit (Citrus x paradisi Macfad). Journal of Food Engineering 193, 86-94.

Ncama, K., Tesfay, S.Z., Fawole, O.A., Opara, U.L., Magwaza, L.S., 2018. Non-destructive prediction of ‘Marsh’grapefruit susceptibility to postharvest rind pitting disorder using reflectance Vis/NIR spectroscopy. Scientia Horticulturae 231, 265-271.

Nekvapil, F., Brezestean, I., Barchewitz, D., Glamuzina, B., Chiş, V., Pinzaru, S.C., 2018. Citrus fruits freshness assessment using Raman spectroscopy. Food Chemistry 242, 560-567.

Nicolai, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, K.I., Lammertyn, J., 2007. Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review. Postharvest Biology and Technology 46, 99-118.

Olarewaju, O.O., Bertling, I., Magwaza, L.S., 2016. Non-destructive evaluation of avocado fruit maturity using near infrared spectroscopy and PLS regression models. Scientia Horticulturae 199, 229-236.

Olarewaju, O.O., Magwaza, L.S., Fawole, O.A., Tesfay, S.Z., Opara, U.L., 2017. Role of canopy positions on rind biochemical concentrations and radical-scavenging activities in relation to rind breakdown of ‘Nules Clementine’mandarins stored at non-chilling temperature. Scientia Horticulturae 226, 231-240.

Osborne, B.G., 2006. Near-infrared spectroscopy in food analysis. Encyclopedia of analytical chemistry: applications, theory and instrumentation.

Purvis, A., Kawada, K., Grierson, W., 1979. Relationship between midseason resistance to chilling injury and reducing sugar level in grapefruit peel. HortScience.

Rafiq, S., Kaul, R., Sofi, S., Bashir, N., Nazir, F., Nayik, G.A., 2018. Citrus peel as a source of functional ingredient: a review. Journal of the Saudi Society of Agricultural Sciences 17, 351-358.

Sáiz-Abajo, M., Mevik, B.-H., Segtnan, V., Næs, T., 2005. Ensemble methods and data augmentation by noise addition applied to the analysis of spectroscopic data. Analytica Chimica Acta 533, 147-159.

Shi, Z., Wang, F., Lu, Y., Deng, J., 2018. Combination of chitosan and salicylic acid to control postharvest green mold caused by Penicillium digitatum in grapefruit fruit. Scientia Horticulturae 233, 54-60.

Sidana, J., Saini, V., Dahiya, S., Nain, P., Bala, S., 2013. A Review on Citrus-“The Boon of Nature”. International Journal of Pharmacological Sciences. Rev. Res 18, 20-27.

Verboven, P., Kerckhofs, G., Mebatsion, H.K., Ho, Q.T., Temst, K., Wevers, M., Cloetens, P., Nicolaï, B.M., 2008. Three-dimensional gas exchange pathways in pome fruit characterized by synchrotron X-ray computed tomography. Plant Physiology 147, 518-527.

Vercher, R., Tadeo, F.R., Almela, V., Zaragoza, S., Primo-Millo, E., Agustí, M., 1994. Rind structure, epicuticular wax morphology and water permeability of 'Fortune'mandarin fruits affected by peel pitting. Annals of Botany 74, 619-625.

Verma, A.R., Vijayakumar, M., Mathela, C.S., Rao, C.V., 2009. In vitro and in vivo antioxidant properties of different fractions of Moringa oleifera leaves. Food and Chemical Toxicology 47, 2196-2201.

Wedding, B., Wright, C., Grauf, S., White, R., Tilse, B., Gadek, P., 2013. Effects of seasonal variability on FT-NIR prediction of dry matter content for whole Hass avocado fruit. Postharvest Biology and Technology 75, 9-16.

Williams, P., 2014. The RPD statistic: a tutorial note. NIR news 25, 22-26.

Williams, P., Norris, K., 1987. Near-infrared technology in the agricultural and food industries. American Association of Cereal Chemists, Inc.

Xudong, S., Hailiang, Z., Yande, L., 2009. Nondestructive assessment of quality of Nanfeng mandarin fruit by a portable near infrared spectroscopy. International Journal of Agricultural and Biological Engineering 2, 65-71.

Yakushiji, H., Nonami, H., Fukuyama, T., Ono, S., Takagi, N., Hashimoto, Y., 1996. Sugar accumulation enhanced by osmoregulation in Satsuma mandarin fruit. Journal of the American Society for Horticultural Science 121, 466-472.

Zheng, Y., He, S., Yi, S., Zhou, Z., Mao, S., Zhao, X., Deng, L., 2010. Predicting oleocellosis sensitivity in citrus using VNIR reflectance spectroscopy. Scientia Horticulturae 125, 401-405.

Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry 64, 555-559.

CHAPTER 5

General discussion

The study reviewed literature on the efficacy of edible coatings to maintain fruit quality and prolong shelf-life of fresh horticultural produce, particularly citrus fruit. Environmentally friendly coatings have received attention in postharvest handling, due to the fact that they are biodegradable and non-toxic to humans (Tharanathan, 2003; Valencia-Chamorro et al., 2011). The review highlighted important edible coatings that are highly effective for enhancing fruit quality and extend shelf life of citrus fruit such as chitosan and their derivatives, montmorillonite, carboxymethylcellulose (CMC), aloe vera and gum arabic. These edible coatings were reported to retain physicochemical properties such as firmness and total soluble solids (TSS), delay colour change and maintain phytochemicals such as ascorbic acid, phenolics and antioxidants while reducing weight loss in citrus fruit. Other coatings, particularly the chitosan based coatings, were reported to be highly effective in inhibiting fruit diseases such as green, grey and blue molds.

Studies have shown that edible coatings maintain ascorbic acid by providing gas barrier layer that decreases ascorbic acid oxidation thereby decreasing oxidative reaction, this increases the ability of fruit to minimize detrimental effects of free radicals (Huang et al., 2008; Rasouli et al., 2019; Sayyari et al., 2009; Shi et al., 2018). Phenolic compounds are also one of the important antioxidant properties in citrus. Generally, phenolic compounds decreases with storage periods due to the fact that they are produced in response to exterior environment which regulate physiological function and metabolic reaction on fruit against stress (Brandt and Mølgaard, 2001).

The review highlighted some aspects that should be considered for future research. For instance, in order to gain and retain consumer trust, food scientists should use the known and commonly used plants for producing edible coatings (Adetunji et al., 2012; Tesfay and Magwaza, 2017). Plant extracts are promisingly effective for controlling pathogens and are generally considered to be safe. Currently, there is a little information on the use of moringa plant extracts to ameliorate postharvest quality and prolonging fruit shelf-life of citrus.

The study conducted on this dissertation zoomed into ‘Marsh’ grapefruit (*Citrus paradisi* MacFad) which are reportedly susceptible to physiological disorders such as rind pitting

(Alférez and Burns, 2004). Rind pitting disorder has been the main concern lately, simple because it does not manifest during harvest or fruit grading in the packhouse where it can be noticed early but the symptoms start developing during postharvest storage at about 3-5 weeks. This has become a challenge for the South African citrus industry since most (75%) of the production is exported to other continents. This may result to consumer complaints and subsequently financial losses. Therefore, the first objective of this study was to determine the efficacy of moringa leaf extract combined with CMC as a novel approach for reducing rind pitting and extending the shelf-life of 'Marsh' grapefruit. An attempt was also made to identify potential physicochemical markers that are related to fruit susceptibility to rind pitting disorders. Physicochemical markers and their correlations with the disorder was assessed and it constituted the major context of this research prior to understanding the mechanism that influence rind pitting disorder. This objective included the effect of microclimate characterized by canopy position in a tree, since it has been reported to influence the physicochemical attributes and susceptibility of citrus postharvest physiological disorders (Magwaza et al., 2012).

In this first objective, fruit harvested from outside canopy experienced more rind pitting disorder as compared to fruit harvest from inside canopy. Similar results were reported in 'Nova' mandarin, where fruit exposed to sunlight developed pre-and postharvest rind pitting in 14 to 28 days yet shaded fruit did not develop the disorder (Duarte and Guardiola, 1993). It was hypothesized that fruit exposed to direct sunlight loses more water than shaded fruit. The study further demonstrated the contribution of water loss in rind pitting disorder development, since fruit with high mass loss were reported to experience high rind pitting incidence compared to their counterparts. It also showed that water loss is related to field environment to which the fruit are exposed. Rind stress that occurs in cellular zone between flavedo and albedo is mainly caused by water loss, it is believed to cause cellular collapse which manifests in visible pitting lesion on rind of the fruit (Alferez et al., 2010). Rind dry matter (RDM) was reported to play a significant role in fruit susceptibility to physiological rind pitting disorder in 'Marsh' grapefruit. Coated fruit had lower RDM compared to uncoated fruit and were less susceptible to the disorder. These findings suggested that RDM could be used as a pre-symptomatic indicator of fruit susceptibility to rind pitting. The maturity of citrus fruit such as grapefruit is measured primarily based on total soluble solids (TSS), titratable acidity (TA) and the ratio between them (Kader, 2002). Total soluble solids in citrus generally increases as

TA decreases during earlier stages of maturation. These changes take place more slowly in grapefruit (Chace and Church, 1924). In this study, significant differences were not observed on TSS, however, uncoated fruit has slightly higher TSS compared to coated fruit. Fruit with higher TSS were found to be more affected by rind pitting, high TSS is a well-known indicator of fruit maturity. However, Alferez and Zacarías (2014) reported that pitting is influenced by maturation stage, it becomes more susceptible to the disorder as it ages. Studies by Ezz and Awad (2009); and Ncama (2016) also found a positive correlation between TSS and the disorder. Citrus rind colour was measured over time to determine if it contributes to development of rind pitting. Rind colour was expressed as citrus colour index (CCI) which was higher in uncoated fruit compared to coated fruit. It was suggested that edible coatings were able to delay colour change. Inside canopy fruit had low CCI as compared to outside canopy fruit and therefore less susceptible to rind pitting. It was then suggested that by identifying parameters that correlate with the disorder, the occurrence of the disorder can be predicted using physicochemical attributes as pre-symptomatic markers.

The second objective of this study explored the use of visible to near infrared spectroscopy (Vis/NIRS) models for predicting postharvest rind pitting disorder in ‘Marsh’ grapefruit at postharvest storage. The study managed to identify the pre-symptomatic phytochemical markers that can be used to predict susceptibility of ‘Marsh’ grapefruit to rind pitting. Since rind pitting was reported to be influenced by microclimate, in this objective, canopy position was also taken to considerations. Partial least square (PLS) regression were also able to predict rind phytochemical parameters such as citrus rind pigments, rind sugars and antioxidants and their constituents. These models were obtained with different latent variables (LV) at different spectra regions. The validity and prediction accuracy of calibration models depends on the accuracy of reference data and enough variation existence in both calibration and validation datasets (Huishan et al., 2005). The principal components analysis (PCA) based correlation and clustering was applied. Principal components analysis was used to make data easy to explore, visualise and explain. Principal components analysis enable us to identify weak/strong and negative/positive correlation of rind phytochemical parameters to rind pitting disorder. Rind phytochemical parameters such as rind sucrose, glucose, flavonoids and antioxidant capacity appeared to have relatively high correlation to rind pitting. While, rind chlorophyll and ascorbic acid had negative correlation to rind pitting. It was suggested that these parameters could be used as biomarkers linked to the susceptibility of rind pitting. However, rind carotenoids were observed as secondary biomarkers to rind pitting occurrence, since their correlation was positively low. It was considered useful to correlate rind phytochemical parameters with the rind disorder because of their proximity to the actual site of the disorders' action.

Edible coatings were applied to improve quality of 'Marsh' grapefruit and prolong shelf life. This study reported that treated fruit experienced lower rind pitting compared to untreated fruit. As abovementioned, this is due to the ability of coatings to maintain antioxidant components such as ascorbic acid and phenolic compounds. Coatings were hypothesized to up-regulate phenylalanine ammonia-lyase (PAL), a key enzyme in biosynthesis of phenolics to increase phenolic compounds in fruit. This may result in high antioxidant pool in fruit rind which then fight against stress imposed to rind. Noticeable, rind sugars (sucrose, glucose and fructose) were higher in untreated fruit compared to treated fruit, however, rind sugars have been linked with development of citrus rind disorders (Cronje et al., 2011). Additionally, treated fruit had low rind carotenoids, high chlorophyll content compared to untreated fruit. Therefore, coatings managed to delay the synthesis of carotenoids which resulted in fruit being less susceptible to the disorder.

General conclusion

Carboxymethylcellulose (CMC) edible coating and moringa leaf extracts (MLE) effectively reduced the incidence of rind pitting. In this study, the combination of CMC and MLE were more effective against rind pitting disorder than CMC as a standalone. This may be due to the fact that moringa leaves are rich in antioxidants, which may be released to the fruit and play their role of defence against stress after applying MLE. It is recommended that the exact concentration of edible coatings must be investigated so that producers and exporters would benefit in order to manage supply of fresh fruit to markets. Also, aligning fruit spectra according to canopy position can be applied at postharvest practice during sorting and packing of fruit to separate fruit based on the canopy position. For instance, since fruit from outside canopy (OC) experienced higher disorder development than inside canopy fruit (IC), canopy separation would enable citrus farm managers to apply recommended treatments. The effect of environmental conditions on rind condition especially oil glands that are associated with rind pitting occurrence should also receive attention for future research. To reduce potential financial losses due to postharvest physiological rind pitting, the primary cause of the disorder must be investigated. This could be done for each orchard and cultivar. The knowledge of orchard history and environmental conditions could be necessary to consider during investigation. Additionally, it would make financial sense to send fruit with higher chances of developing disorders to local markets. Exporting fruit with high chances of rind pitting development will compromise South Africa’s market share in foreign market, since buyers will search for other citrus suppliers if they receive fruit with pitting regularly. Another alternative strategy would be to process the fruit to other products such as juices and dried fruits which have a longer lifespan than the optimum shelf life of fresh fruit.

5.2. References

Adetunji, C., Fawole, O., Arowora, K., Nwaubani, S., Ajayi, E., Oloke, J., Majolagbe, O., Ogundele, B., Aina, J., Adetunji, J., 2012. Quality and safety of Citrus Sinensis coated with Hydroxypropylmethylcellulose edible coatings containing Moringa oleifera extract stored at ambient temperature. Global Journal of Science Frontier Research, 2249-4626.

Alferez, F., Alquezar, B., Burns, J.K., Zacarias, L., 2010. Variation in water, osmotic and turgor potential in peel of ‘Marsh’grapefruit during development of postharvest peel pitting. Postharvest Biology and Technology 56, 44-49.

Alférez, F., Burns, J.K., 2004. Postharvest peel pitting at non-chilling temperatures in grapefruit is promoted by changes from low to high relative humidity during storage. Postharvest Biology and Technology 32, 79-87.

Alferez, F., Zacarías, L., 2014. Influence of fruit maturity in the susceptibility of Navelina oranges to develop postharvest non-chilling peel pitting. Food Science and Technology International 20, 183-191.

Brandt, K., Mølgaard, J.P., 2001. Organic agriculture: does it enhance or reduce the nutritional value of plant foods? Journal of the Science of Food and Agriculture 81, 924-931.

Chace, E., Church, C., 1924. Composition of Marsh seedless grapefruit grown in California and Arizona. Calif. Citrograph 9, 122-134.

Cronje, P.J., Barry, G.H., Huysamer, M., 2011. Fruiting position during development of ‘Nules Clementine’mandarin affects the concentration of K, Mg and Ca in the flavedo. Scientia Horticulturae 130, 829-837.

Duarte, A., Guardiola, J., 1993. Factors affecting rind pitting in the mandarin hybrids" fortune" and" nova". The influence of exogenous growth regulators, International Symposium on Quality of Fruit and Vegetables: Influence of Pre-and Post-Harvest Factors and Technology 379, pp. 59-66.

Ezz, T.M., Awad, R.M., 2009. Relationship between mineral composition of the flavedo tissue of “Marsh” grapefruit and chilling injury during low temperature storage. Research Journal of Agriculture and Biological Sciences 5, 892-898.

Huang, R., Xia, R., Lu, Y., Hu, L., Xu, Y., 2008. Effect of pre-harvest salicylic acid spray treatment on post-harvest antioxidant in the pulp and peel of ‘Cara cara’navel orange (*Citrus sinenisis L*. Osbeck). Journal of the Science of Food and Agriculture 88, 229-236.

Huishan, L., Yibin, Y., Huanyu, J., Yande, L., Xiaping, F., Wang, J., 2005. Application Fourier transform near infrared spectrometer in rapid estimation of soluble solids content of intact citrus fruits, 2005 ASAE Annual Meeting. American Society of Agricultural and Biological Engineers, p. 1.

Kader, A.A., 2002. Postharvest technology of horticultural crops. University of California Agriculture and Natural Resources.

Magwaza, L., Opara, U., Cronje, P., Nieuwoudt, H., Landahl, S., Terry, L., 2012. Quantifying the effects of fruit position in the canopy on physical and biochemical properties and predicting susceptibility to rind breakdown disorder of 'NULES clementine'mandarin (*Citrus reticulate* Blanco) using Vis/NIR spectroscopy, II All Africa Horticulture Congress 1007, pp. 83-91.

Ncama, K., 2016. Non-destructive prediction of ‘marsh’grapefruit (*Citrus x paradisi* MacFad) postharvest quality and physiological rind disorders using visible to near infrared spectroscopy.

Rasouli, M., Saba, M.K., Ramezanian, A., 2019. Inhibitory effect of salicylic acid and Aloe vera gel edible coating on microbial load and chilling injury of orange fruit. Scientia Horticulturae 247, 27-34.

Sayyari, M., Babalar, M., Kalantari, S., Serrano, M., Valero, D., 2009. Effect of salicylic acid treatment on reducing chilling injury in stored pomegranates. Postharvest Biology and Technology 53, 152-154.

Shi, Z., Wang, F., Lu, Y., Deng, J., 2018. Combination of chitosan and salicylic acid to control postharvest green mold caused by Penicillium digitatum in grapefruit fruit. Scientia Horticulturae 233, 54-60.

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

Tharanathan, R., 2003. Biodegradable films and composite coatings: past, present and future. Trends in Food Science & Technology 14, 71-78.

Valencia-Chamorro, S.A., Palou, L., Del Rio, M.A., Pérez-Gago, M.B., 2011. Performance of hydroxypropyl methylcellulose (HPMC)-lipid edible coatings with antifungal food additives during cold storage of 'Clemenules' mandarins. LWT-Food Science and Technology 44, 2342-2348.