Evaluating the efficacy of formulations containing hexanal, moringa leaf extracts, and carboxy methylcellulose as postharvest treatments for fresh tomatoes (*Solanum lycopersicum* L.)

By

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Declaration

I, Sisanda, Sibusiso, Luyanda, Mthembu (student no.: 213558719), declare that this is my original work, except where acknowledged. I further declare that these results have not otherwise been submitted in any form for any degree or diploma to any university. The work does not contain any other people's information or data such as graphs, tables and pictures unless acknowledged to be found or sourced from other researchers.

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General abstract

Tomato fruit have a relatively short shelf life due to their highly perishable nature. This presents a challenge for long distance transportation of tomatoes. The use of non-refrigerated trucks (used by most commercial entities in South Africa) for transportation exacerbates the loss of fresh tomatoes. The adoption of low temperature storage units during transit is an expensive technology for farmers in developing countries. The application of postharvest treatments such as chemical treatments is used to reduce these losses. However, chemical treatments contain residues that negatively impact the environmental and human health. In addition, it causes off-flavours which negatively affects consumer acceptability and market value of tomatoes. The inefficiency of chemical treatments and the pressure experienced by producers to meet the increase in demand for fresh tomatoes without any hazardous residues by both consumers and regulatory agencies, encourages further research to investigate eco-friendly and sustainable treatments as an alternative to chemical treatments. The use of total soluble solids as a predictor of tomato quality does not provide an accurate description of internal biochemical changes. The use of accurate predictors such as sugar content and sweetness indices, provides a precise description of internal quality and estimation of shelf-life capacity. However, conventional measurements of these parameters are laborious. Thus, probing the use of a rapid and non-destructive technology (Vis/NIRS) to predict and determine sugar content and sweetness indices, in order to facilitate quality management and accurate grading of tomato produce along the supply chain. The research findings obtained in this present study, demonstrated the ability of the hexanal formulation and moringa based edible coating to optimise organoleptic quality and improve the nutritional quality of tomato fruit, harvested at different maturity stages. Vis/NIRS accurately predicted important internal quality parameters relating to the market value of tomatoes such as sugar content and sweetness indices. Results obtained by evaluating the effects of these treatments to extend shelf life and reduce losses of tomato show the potential

of adopting these treatments to serve as an alternative to the currently used treatments in the tomato industry. The successful prediction and accurate determination of sugar content and sweetness indices using Vis/NIRS, has the potential to enable rapid and precise grading of tomato produce.

Publication and conference presentation

Part of chapter 3 of this thesis has been submitted for publication. The manuscript has been reviewed and accepted for publication in the Acta Horticulturae journal. The findings of this manuscript have been presented at the 2nd International Symposium on Moringa 2019 (ISM2019).

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Nomenclature

1-MCP	1-Methylcyclopropene
ACC	1-aminocyclopropane-1-carboxylic acid
ACS	ACC synthase
APX	Ascorbate peroxidase
BHT	Butylated hydroxytoluene
C_2H_4	Ethylene
CAT	Catalase
CMC	Carboxy methylcellulose
CO_2	Carbon dioxide
CRTISO	Carotenoid isomerase
CV%	Coefficient of variation
CV.	Cultivar
DHA	Dehydroascorbic acid
DMAPP	Isomer dimethylallyl diphosphate
DPPH	1,1-diphenyl-2-picrylhydrazyl
DXS	1-deoxy-D-xylulose 5-phosphate synthase
EFF	Enhanced freshness formulation
ETR4	Ethylene receptors 4
FDA	Food and Drug Administration
FRAP	Ferric Reducing Antioxidant Power
GAE	Gallic acid equivalent
GGPP	Geranylgeranyl pyrophosphate
GGPPS	Geranylgeranyl pyrophosphate synthase

GR	Glutathione reductase
GRAS	Generally Regarded as Safe
GSH	Reduced glutathione
GSSG	Oxidized glutathione
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
IPP	Isopentenyl pyrophosphate
LCYB	Lycopene β-cyclase
LOX	Lipoxygenase
MADS-RIN	MADS box transcription factor
MDA	Malondialdehyde
MEP	2-C-methyl-D-erythriol 4-phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
NaOH	Sodium hydroxide
NFPM	National fresh produce market
O_2	Oxygen
O_2^-	Superoxide
PA	Phosphatidic acid
PAL	Phenylalanine ammonia-lyase
PDI	Percentage disease index
PDS	Phytoene desaturase
PG	Polygalacturonase
PLD	Phospholipase D

PLS	Partial least square
PLW	Physiological loss in weight
PME	Pectin methylesterase
POX	Guaiacol peroxidase
PSY	Phytoene synthase
RE	Rutin equivalent
RH	Relative humidity
RID	Refractive-index detector
RMSEC	Root mean square error of calibration
RMSEP	Root mean square error of prediction
ROS	Reactive oxygen species
RPD	Residual predictive deviation
SAM	S-adenosyl methionine
SD	Standard deviation
SE	Standard error
SEC	Standard error of calibration
SOD	Superoxide dismutase
TA	Titratable acidity
TCI	Tomato Colour Index
TPTZ	2,4,6-tris (1-pyridyl)-5-triazine
TSI	Total sweetness index
TSS	Total soluble solids
Vis/NIRS	Visible to near infrared spectroscopy
ZDS	ζ-carotene desaturase

Chapter 1: General introduction

1.1. Introduction

Tomatoes are rich in health-related compounds, these beneficial effects are believed to be due to the action of antioxidant compounds that reduce oxidative damage in the human body (Odriozola-Serrano et al., 2008), as they are good sources of polyphenol compounds (phenolics and flavonoids), β-Carotene, lycopene and ascorbic acid (Frusciante et al., 2007). Lycopene is a powerful natural antioxidant which is an efficacious free radical scavenger (Sascio et al., 1989) which may provide cardioprotective benefits (Ito et al., 2006). As antioxidants, polyphenols may protect cell constituents against oxidative damage, thus limiting the risk of various degenerative diseases associated with oxidative stress (Manach et al., 2005).

Tomatoes are the second most important vegetable crop in South Africa, after potatoes. In 2017, FAOSTAT (2019) reported a tomato harvest of 608 306 tonnes, valued at R6221 per ton, which is the highest production and average price reported since 2008 (DAFF, 2018). Tomato is a highly perishable fruit, thus having a relatively short shelf life (Babatola et al., 2008), which has resulted in limited long-distance trading of South African tomatoes. However, minimal quantities - 16 676 tons (DAFF, 2017) valued at $9.7 million (Workman, 2018), have been reported to be exported to Southern African countries and the majority of tomatoes produced are consumed by the local market. The global tomato trade is similar to that of South Africa, with the top tomato exporters such as Netherlands, Mexico, Spain, Morocco, and Canada exporting to neighbouring countries, with minimal exports to distant destinations (Collen, 2013; European Commission, 2012).

The value of tomatoes increases more significantly than potatoes or citrus along the value chain. Tomatoes are valued at R14 000 per ton at the consumption stage from R3 500 per ton at the production stage, potatoes are valued at R8 200 per ton at the consumption stage from

R2 100 per ton at the production stage and citrus is valued at R5 916 per ton at the consumption stage from R3 708 per ton at the production stage (Nahman and de Lange, 2013). Therefore, it is of great importance to optimize the postharvest handling of tomatoes in the industry.

In South Africa, 83 – 87 % of tomatoes are sold through the national fresh produce markets (NFPMs), with a limited quantity delivered directly to supermarket chains. In developing countries, 10 – 20 % of postharvest losses of fresh produce is incurred during long-distance transportation (Sugri et al., 2013). Tomato fruit have a high perishable nature and a short postharvest life, as a result, quality of fresh tomatoes declines when transported over long distances (Roy et al., 2008). The use of non-refrigerated vehicles for transport exacerbates the loss in quality. In South Africa and other developing countries, non-refrigerated vehicles are used by most commercial and emerging farmers to transport tomatoes to NFPMs (Sarma, 2018; Sibomana et al., 2016). This results in poor storage conditions during transit because the temperature in these trucks is not regulated (Sibomana et al., 2017).

In summer, conditions are warmer than the recommended storage temperature, thus promoting the ripening of tomatoes, reducing marketability of the fruit by the activation of enzymes. The activation of these enzymes results in off-flavours and fruit discolouration (Workneh and Osthoff, 2010), as well as increasing pectin and polygalacturonase activities which reduce firmness (Yoshida et al., 1984). The enzymes responsible for off-flavours include lipolytic acyl hydrolase, lipoxygenase, peroxidase, catalase and protease. Whereas the enzyme responsible for fruit discolouration is polyphenol oxidase (Wiley, 1994).

Low-temperature storage is important in retaining the quality of tomato produce during transit. However, this technology is expensive for emerging farmers, small scale farmers and a few commercial farmers in developing countries, which leads to a continuation of postharvest losses of fresh produce (Minten and Kyle, 1995). The current methods used to deal with this

problem is the application of postharvest treatments. The common treatments used to treat tomatoes (including South African tomatoes) are chemical treatments such as chlorinated water (Bartz et al., 2013; Sibomana et al., 2017; Workneh and Osthoff, 2010).

The use of chlorinated water causes off-flavours (Hassenberg et al., 2008) and poses threat to environmental and human health (Huang et al., 2008). Alternative postharvest treatments that have been proposed are hot water treatment and application of 1-Methylcyclopropene (1-MCP). The limitations of using hot water treatment are the high-energy costs, added labour (Mahajan et al., 2014) and the rapid loss of marketability (up to 80%) during storage at ambient temperature (Tolesa and Workneh, 2017).

1-MCP is used in other countries to extend the shelf life of climacteric fruit. Although this treatment is the common method used for fresh produce preservation, it has been reported to have negative effects on the quality of tomatoes (Cheema et al., 2014). For instance, the partial colour development due to the incomplete suppression of the colour biosynthesis (Tiwari and Paliyath, 2011), which leads to blotchy ripening in tomato, has been previously reported (Cliff et al., 2009; Lurie and Paliyath, 2009; Tiwari and Paliyath, 2011).

1-MCP treatment favours the accumulation of organic acids (Guillén et al., 2006; Opiyo and Ying, 2005; Tiwari and Paliyath, 2011) and physiological disorders such as external CO_2 injury (DeEll et al., 2003; Tiwari and Paliyath, 2011). Tiwari and Paliyath, (2011) demonstrated that 1-MCP favours the accumulation of organic acids by downregulating a gene for phosphoenolpyruvate carboxykinase (an enzyme involved in converting oxaloacetate to phosphoenolpyruvate, which results in a decrease in organic acids and increase in sugars through gluconeogenesis during ripening of fruit) and enhancing phosphoenolpyruvate carboxylase transcripts (an enzyme involved in converting phosphoenol to oxaloacetate). This

ultimately affects the total soluble solids to titratable acidity (TSS:TA) ratio of tomatoes. Hence, flavour biosynthesis is reduced by 1-MCP treatment

For fresh tomatoes, texture, flavour and colour are important quality attributes, which directly relate to their marketing value (Liu et al., 2009). The characteristic tomato flavour is formed by the action of volatile aroma compounds and non-volatile constituents. Dissatisfaction of consumers with an absence of characteristic fresh tomato flavour is the main marketing complaint (Yilmaz, 2001). The physiological effects caused by chlorinated water, 1-MCP and hot water treatment such as loss of flavour, inferior colour development and reduced marketability have a negative effect on the organoleptic quality of tomato fruit.

Edible coatings are a safe way of reducing postharvest losses of fruit because they consist of organic biocide activities and antimicrobial compounds (Petersen et al., 1999) and are required by fruit that deteriorate quickly such as papaya, mango, strawberries, tomatoes and peaches, due to a high rate of water loss during storage (Nunes and Emond, 2007). The application of edible coatings prolongs the storage life of fruit by forming a semi-permeable membrane on the fruit surface, which alleviates rapid deterioration by the suppression of respiration, transpiration, ethylene production and disease incidence (Elsabee and Abdou, 2013). Carboxy methylcellulose (CMC) is a polysaccharide-based edible coating which facilitates in firmness retention and crispness of apples, berries, peaches, celery, lettuce, and carrots when used in a dry coating process (Mason, 1969).

Moringa oleifera Lam. is a tree that grows widely in many tropical and subtropical countries. Moringa leaf extracts (MLE) possess antimicrobial properties due to its active phytochemicals which include, β-sitosterol, stigma sterol, kaempferol and quercetin (Maiyo et al., 2016; Talreja and Goswami, 2016). The leaves of moringa have an antibacterial potential against several organisms (John et al., 2013). Moringa leaf extracts are not adhesive; therefore, they will be

incorporated with CMC which can adhere well to fruit surfaces. Due to the properties that moringa and CMC possess, their incorporation makes them an ideal postharvest treatment that can be used to prolong shelf life and optimize the quality of fresh produce (Tesfay and Magwaza, 2017; Tesfay et al., 2017).

A product called enhanced freshness formulation (a hexanal formulation) has the potential to preserve quality, reduce postharvest losses and assure longevity of fresh produce without compromising on fruit quality, environmental and human health. Hexanal is naturally produced during lipid peroxidation mediated by lipoxygenase and the hydroperoxide lyases. Hexanal is also a component of GRAS (Generally Regarded as Safe) status (Paliyath and Subramanian, 2008). Fresh produce that has been treated with enhanced freshness formulation include oriental sweet melons (Qi et al., 2011), mango (Jincy et al., 2017), tomato (Cheema et al., 2014) and sweet bell peppers (Cheema et al., 2018).

Phospholipase D (PLD) is the key enzyme involved in the initiation of membrane deterioration, leading to a loss in the firmness of tomato and reduces the shelf life (Paliyath and Droillard, 1992). Enhanced freshness formulation (EFF) can inhibit PLD activity, resulting in enhanced membrane stability, thus increasing the longevity of horticultural crops (Paliyath et al., 1999; Tiwari and Paliyath, 2011). EFF does not reduce the colour or flavour development of tomatoes during storage but enhances firmness and certain flavours such as terpenes and alcohols, providing better aroma for tomatoes. Thus, making EFF more efficient than the current treatments used to preserve the storage life of tomatoes. This is of importance because, firmness and colour are features that are valued by consumers (Gómez et al., 2006).

Losses of tomatoes depend on the ripening stage at harvest (Brackmann et al., 2007; Goojing et al., 1999; Oliveira et al., 2016; Opara et al., 2011). Consumer preferences and marketing strategies determine when tomatoes should be harvested (Ozgen et al., 2012). For long-distance

transportation, the fruit are harvested at the green mature to breaker stages while fruit sold locally and for immediate consumption are harvested at the fully ripe stage.

In previous literature, CMC and EFF treatment have been applied to tomato fruit to optimize the quality of the fruit during postharvest storage. However, the studies conducted on the use of CMC and EFF have focused on applying these treatments at the mature green stage. Thus, the objective of this study is to evaluate the response of the fruit to the proposed treatments at different maturity stages so producers will know how the fruit will perform when harvested at a certain maturity stage.

Postharvest life of tomatoes is considered low as current methods do not provide efficient means of maintaining quality. This research project will aim at reducing the postharvest losses of tomato, in order to increase market participation. The potential impact of this project may improve the welfare of tomato producers, increase food availability and contribute positively to food security in a manner that does not compromise the quality of the produce and is safe for human consumption and the environment.

1.2. Research aim

To reduce postharvest losses of tomato at the pre-consumer level by retaining quality and shelf life using natural postharvest treatments.

1.3. Research objectives

- Evaluate the effect of enhanced freshness formulation and the moringa based edible coating on the physicochemical attributes of fresh tomatoes harvested at three maturity stages.
- Assess the efficacy of enhanced freshness formulation and the moringa based edible coating on bioactive compounds and the antioxidant activity of fresh tomatoes.

- Utilize Vis/NIRS technology for rapid determination of changes of internal quality and sugar content of tomato during storage in order to facilitate quality management during the supply chain.

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Chapter 2: Literature Review

Effect of hexanal-based technologies on physiological and biochemical attributes of horticultural produce

2.1. Abstract

Fruit ripening leads to the development of desirable organoleptic properties and nutritional qualities. Over-ripening reduces marketability and nutritional composition of fresh produce. Fruit softening, postharvest pathogens, high ethylene production, respiration and transpiration rates, contribute to over-ripening of fresh produce. Fresh produce markets incur a financial loss due to postharvest pathogens, which contribute to over-ripening of fresh produce. Fungicides are used to eradicate this problem, but they pose a harmful threat to human health and the environment. In addition, chlorinated water has been reported to cause off-flavours of fruit. The adoption of an environmentally safe postharvest treatment, with the capacity to preserve fresh produce without causing adverse effects is essential. Agricultural research conducted on the use of hexanal formulations as postharvest treatments, has yielded promising results in this regard. Hexanal treatment has been successful in optimising shelf life and preserving nutritional quality of fresh produce, without causing negative effects on the produce and it is noteworthy to highlight that hexanal is a GRAS (generally regarded as safe) compound, approved by the United States Food and Drug Administration (FDA). Thus, these research findings suggest that hexanal may be an alternative to the current postharvest treatments. This review gives an insight on the mechanism of hexanal in relation to the preservation of fresh produce, as well as the potential benefits hexanal treatment may provide to the agricultural industry upon commercialization.

Keywords: *Postharvest, Ripening, Fruit quality, Enzymes, Ethylene, Phenolics*

2.2. Introduction

The ripening of fruit is a process involving the changes in physiological, biochemical and organoleptic properties, resulting in the development of aroma, colour, flavour, nutritional quality and texture (Paliyath and Padmanabhan, 2018). During ripening, the membrane structure is altered due to an increase in sterol levels and catabolism of phospholipids and membrane proteins. This leads to a loss of cellular compartmentalization followed by tissue structure (Paliyath and Droillard, 1992). Other dynamics of ripening include the breakdown of carbohydrates, leading to an increase in sugars, a decrease in organic acids and a production of colour and volatile aroma components (Paliyath et al., 2012).

Factors such as postharvest pathogens, increased respiration, transpiration, ethylene production and loss of membrane integrity, contribute to accelerated ripening and senescence of horticultural produce. It has been reported that membrane deterioration is initiated by phospholipase D (PLD; EC 3.1.4.4), a membrane lipid degrading enzyme (Paliyath and Subramanian, 2008). PLD binds to the membrane, initiating a cascade of catabolic reactions leading to the generation of several neutral lipids, the accumulation of which results in the destabilization of the membrane (Paliyath and Droillard, 1992).

Hexanal, a GRAS (generally regarded as safe) compound (Paliyath and Subramanian, 2008), is a natural volatile C6 aldehyde derived from the catabolism of unsaturated fatty acids that are released during membrane phospholipid degradation. Linolenic acid undergoes lipid peroxidation catalysed by lipoxygenase, and further degradation by hydroperoxide lyase results in the formation of 3Z-hexenal. The action of alkenal oxidoreductase on hexenal results in the formation of hexanal (Schwab et al., 2008). Hexanal biosynthesis is stimulated after wounding or during fruit ripening. In addition to hexanal's GRAS status, hexanal has been extensively tested and approved by the United States Food and Drug Administration (FDA).

The current postharvest treatments used are chlorine, hot water treatment and 1-MCP. The limitations of using hot water treatment are the high-energy costs, added labour (Mahajan et al., 2014) and the rapid loss of marketability (up to 80 %) during storage at ambient temperature (Tolesa and Workneh, 2017). The use of chlorinated water and 1-MCP causes off flavours and physiological disorders in fruit, such as blotchy ripening and external CO_2 injury. The physiological effects caused by chlorinated water, 1-MCP and hot water treatment such as loss of flavour, inferior colour development and reduced marketability have a negative effect on the organoleptic quality of fruit. Studies (discussed below) have demonstrated the potential of hexanal to preserve quality, reduce postharvest losses and assure longevity of fresh produce without compromising fruit quality, environmental and human health.

Hexanal and hexanal formulations known as enhanced freshness formulation (EFF) can inhibit PLD activity, resulting in enhanced membrane stability thus increasing longevity of horticultural crops. This has been proven by previous studies conducted by (Paliyath et al., 1999) and (Tiwari and Paliyath, 2011). In addition to suppressing the PLD enzyme, studies (summarised in Table 2.10 and 2.11) conducted on hexanal technology have demonstrated that hexanal has the capacity to extend shelf life and improve fruit quality by enhancing the activity of enzymes involved in scavenging reactive oxygen species (ROS), suppressing cell membrane related enzymes and delaying the ripening processes of fresh produce. The development of hexanal based technologies to inhibit PLD activity, improve shelf life and reduce postharvest losses of horticultural produce was patented by Paliyath et al. (1999) (US Patent # 6,514,914; 7,198,811).

S-adenosyl methionine (SAM) is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase (ACS). ACC oxidase uses ACC as a substrate to catalyse ethylene production. Hexanal being a potent inhibitor of PLD, which reduces membrane deterioration, also has a direct effect on gene expression. Hexanal application resulted in the

downregulation of the expression of the ACC synthase (ACS) gene, as well as the transcription factors active in regulation of ripening (Tiwari and Paliyath, 2011). Hexanal formulations can be applied at the preharvest stage by spray treatment on the fruit and at the postharvest stage by dip or vapour treatment.

The aim of this review is to provide an overview of the effect hexanal technologies have on physiological and biochemical attributes of fresh produce, the response of the produce towards the treatment as well as to gain an understanding on the mode of action of hexanal formulation in relation to improving fruit quality and shelf life.

2.3. Physiological Attributes

2.3.1. Ethylene

Ethylene is a natural gas hormone that can be found in plants and is the key regulator in the ripening of climacteric fruit (Paul et al., 2012). Ethylene is essential for proper fruit ripening and the development of desired fruit attributes such as colour, flavour, texture and nutritional qualities. Over-ripening reduces shelf life of produce by the development of physiological disorders, senescence and increased pathogen susceptibility (Delong et al., 2004; Paul et al., 2012).

Ethylene is synthesized from the amino acid methionine which gets converted to S-adenosyl methionine (SAM) via the enzyme methionine S-adenosyl transferase (Arc et al., (2013). S-adenosyl methionine is converted to the four-carbon compound 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase (ACS). However, ACC can undergo two fates, namely, it can either be converted to malonyl ACC (an in-active end product) (Kader, 1992) or be converted to ethylene by the enzyme ACC oxidase which is known as an ethylene forming enzyme (Adams and Yang, 1979).

Fruit can be classified based on the nature of their ripening process. Non-climacteric fruit are characterized by the lack of ethylene associated respiratory peak. Hence, non-climacteric fruit will not ripen if detached from the tree while unripe. Examples of non-climacteric fruit include strawberry, citrus and grape. Climacteric fruit differ from non-climacteric fruit as they experience a great upsurge of ethylene production as well as a rise in respiration (Jobling, 2000). This gives climacteric fruit the ability to ripen when harvested at their green mature stage. Examples of climacteric fruit include tomato, papaya and banana.

Controlling ethylene action at appropriate maturity levels can effectively enhance the shelf life of horticultural produce. Ethylene action can be minimized by inhibiting ethylene biosynthesis and perception of ethylene by the receptor. The formation of ACC is the rate-limiting step in ethylene production catalysed by ACS (Fluhr and Mattoo, 1996). Hexanal causes a moderate reduction in ethylene production by the downregulation of only a single ortholog of ACS (Tiwari and Paliyath, 2011).

Previous studies have demonstrated the efficacy of hexanal and hexanal formulations; enhanced freshness formulation (EFF) to reduce ethylene production and delay the ethylene peak of various fruit when applied at the preharvest and postharvest stage. EFF applied at the preharvest stage on mango (Anusuya et al., 2016) and banana (Yumbya et al., 2018), exhibited a significant reduction in ethylene production in comparison to untreated fruit. Hexanal applied at the postharvest stage on mango (Jincy et al., 2017), oriental sweet melons (Qi et al., 2011) and banana (Yumbya et al., 2018) exhibited a reduction in ethylene production compared to the untreated fruit. Qi et al. (2011) and Yumbya et al. (2018) further demonstrated that EFF applied at the pre and postharvest stage reduced ethylene production as well as delayed the ethylene peak. A study conducted by Tiwari and Paliyath (2011) as well as Pak Dek et al. (2018) on tomato fruit, demonstrated that EFF and hexanal treated tomato fruit respectively had greater ethylene production than untreated fruit. Tiwari and Paliyath (2011) showed that

untreated fruit reached their ethylene peak on day 10 of storage followed by a decline on day 20, whereas the treated fruit reached their ethylene peak on day 20 of storage before declining, although ethylene production of EFF and untreated fruit was not significant. As for the study conducted Pak Dek et al. (2018), the ethylene production of hexanal treated fruit was significantly higher than that of untreated fruit with increased storage time.

Tomato fruit were harvested at the mature green stage for both studies, however, different cultivars namely, 'Rapsodie' and 'De Ruiter' were used, respectively. The tomato fruit from the study conducted by Tiwari and Paliyath (2011) were stored at 15 °C for two weeks, then brought to room temperature and stored for another week. Tomatoes from the study conducted by Pak Dek et al. (2018) were stored at ambient temperature for ten days. Regardless of the different storage conditions of these two studies, the tomato fruit behaved in a similar manner in terms of ethylene production. The cultivars and storage conditions of the tomato fruit were different (but harvested at the same maturity stage), hexanal treatment did not reduce the ethylene production as it did on other fruit mentioned above.

Pak Dek et al. (2018) further conducted investigations of hexanal treatment on the ethylene signal transduction of tomato fruit. Their findings may explain the different outcomes obtained for tomato fruit, in relation to other fruit in studies mentioned above. Pak Dek et al. (2018) reported that the expression levels of several genes involved in ethylene signal transduction, namely: ethylene receptors 4 (ETR4) and MADS box transcription factor (MADS-RIN), were upregulated by hexanal treatment. It may be hypothesized that greater ethylene production may be due to the upregulation of these genes.

The studies mentioned above illustrated that hexanal based treatments reduced the production of ethylene more effectively when applied at the pre-harvest stage rather than the postharvest stage, as results obtained by these authors showed that hexanal treatments at the postharvest

stage showed a slight reduction in ethylene production, whereas treatments applied at the pre-harvest stage showed a greater reduction in the ethylene production during storage. However, the reduction in ethylene production is dependent on the type of fruit applied with the hexanal formulation, as fruit respond differently to the treatment in terms of ethylene production.

Table 2.1: Studies conducted on fruit ethylene production in response to hexanal treatment

Fruit/Vegetable	Cultivar	Treatment	Application	Storage Condition	Findings	Author
Mango	Alphonso and Banganapalli	EFF	Preharvest	14 ± 1 °C, 85 ± 5% RH and 28 ± 2 °C	Reduced C_2H_4 production	Anusuya et al. (2016)
Banana	Grand Nain	EFF	Preharvest	25 ± 1 °C, 60 ± 5% RH	Reduced C_2H_4 production	Yumbya et al. (2018)
Mango	Neelum	Hexanal	Postharvest	25 ± 0.8 °C, 60 ± 10% RH	Reduced C_2H_4 production	Jincy et al. (2017)
Oriental sweet melons	Jinheng No. 2	EFF	Postharvest	10 °C, 85% RH	Reduced C_2H_4 production	Qi et al. (2011)
Banana	Grand Nain	EFF	Postharvest	25 ± 1 °C, 60 ± 5% RH	Reduced C_2H_4 production	Yumbya et al. (2018)
Tomato	Rapsodie	EFF	Postharvest	15 °C and room temperature	Higher C_2H_4 production	Tiwari and Paliyath (2011)
Tomato	De Ruiter	Hexanal	Postharvest	Room temperature	Higher C_2H_4 production	Pak Dek et al. (2018)

EFF - Enhanced freshness formulation; RH – Relative humidity.

2.3.2. Respiration

Respiration is the process whereby the energy derived from oxidative catabolism of stored organic material such as carbohydrates, fats and proteins (Wilson et al., 1999) is used to keep the fruit alive and support developmental changes. Respiration occurs in the mitochondria of living cells and maintains synthetic reactions that occur after harvest by mediating the release of carbon skeletons and energy (Kays, 1991). Increased respiration means loss of stored food reserves leading to speeding up of the senescence processes. This leads to reduced food value for the consumer, loss of flavour, especially sweetness and loss of saleable dry weight and rapid deterioration (Kader, 1992; Wilson et al., 1999). In addition, increased respiration causes temperature to rise up and the growth rate of pathogens are accelerated (Kays, 1991).

The efficacy of hexanal treatments to lower the respiration rate of fruit has been proven by studies summarised in Table 2.2. EFF significantly reduced the rate of respiration of banana (Yumbya et al., 2018) and mango fruit (Anusuya et al., 2016) when applied at the preharvest stage. Hexanal treatment at the postharvest stage significantly reduced the respiration of strawberry fruit even though it is classified as a non-climacteric fruit (Yuan et al., 2009). However, hexanal applied at the postharvest stage, increased the respiration rate of tomato (Pak Dek et al., 2018) over a storage period of ten days (at room temperature), however treated fruit were still green in colour, unlike the untreated fruit, which were red in colour on the tenth day of storage.

Tiwari and Paliyath, (2011) obtained similar results for EFF treated tomatoes at the postharvest stage with treated fruit exhibiting a greater respiration rate, however, treated fruit remained firmer than the control throughout the storage period. Results of a study conducted by Cheema et al. (2018), where EFF was applied at the postharvest stage on sweet bell pepper displayed similar results to studies mentioned above. Even though the sweet bell peppers exhibited a

greater CO_2 production throughout the storage period (12 °C), treated fruit had a significantly lower loss in firmness and mass.

Hexanal formulations applied at the preharvest stage reduce the CO_2 production, whereas the respiration rate seems to be increased when the treatment is applied at the postharvest stage. Although the loss in quality and ripening is delayed, hexanal formulations should be applied at the preharvest stage of tomato and sweet bell pepper, in order to find out whether or not pre or postharvest application of hexanal, has an effect on gaseous exchange. In addition, Yumbya et al. (2018) demonstrated that regardless of the stage of application (pre or postharvest stage), EFF delayed the respiration peak, which may explain how fruit quality is maintained even though higher CO_2 production is exhibited by fruit treated at the postharvest stage.

A reduction in PLD activity by hexanal treatment has the potential of reducing the catabolism of free fatty acids liberated during the membrane phospholipid catabolism. However, this may be compensated by channelling more carbon intermediates into the respiratory cycle, resulting in increased respiration due to the lowered demand for substrates to replace membrane phospholipids that are broken down by PLD action. This may explain the reason why fruit treated with hexanal at the postharvest stage experienced an increase in respiration rate.

Table 2.2: Studies conducted on fruit carbon dioxide production in response to hexanal treatment

Fruit/Vegetable	Cultivar	Treatment	Application	Storage Condition	Findings	Author
Mango	Alphonso and Banganapalli	EFF	Preharvest	14 ± 1 °C, 85 ± 5% RH and 28 ± 2 °C	Reduced CO_2 production	Anusuya et al. (2016)
Banana	Grand Nain	EFF	Preharvest	25 ± 1 ºC, 60 ± 5% RH	Reduced CO_2 production	Yumbya et al. (2018)
Strawberry	Darselect	Hexanal	Postharvest	4°C	Reduced CO_2 production	Yuan et al. (2009)
Tomato	Rapsodie	EFF	Postharvest	15 °C and room temperature	Increased CO_2 production	Tiwari and Paliyath (2011)
Tomato	De Ruiter	Hexanal	Postharvest	Room temperature	Increased CO_2 production	Pak Dek et al. (2018)
Sweet bell pepper	Felicitas	EFF	Postharvest	12 °C, 90 – 95% RH	Increased CO_2 production	Cheema et al. (2018)
Banana	Grand Nain	EFF	Postharvest	25 ± 1 ºC, 60 ± 5% RH	Reduced CO_2 production	Yumbya et al. (2018)

EFF - Enhanced freshness formulation; RH – Relative humidity.

2.3.3. Mass loss

Physiological loss in weight (PLW) is one of the prime parameters indicating the ability of fruit to retain their freshness during storage. Water loss is one of the major factors that contribute to fruit deterioration and loss of marketability. Since the loss of water is irreplaceable once the fruit has been harvested, transpiration represents an economic loss in the supermarket industry, as it results in a loss of saleable mass (Chakraverty et al., 2003). Fruit that have lost 5 – 8 % of their initial mass begin to show signs of mass loss and become shrivelled thus losing their marketability (Wilson et al., 1999; Mahajan et al., 2009).

Fruit surface acts as a protective barrier preventing water loss, dehydration and leakage of solutes. Hexanal applications have been reported to reduce transpiration, by facilitating the preservation of cell structures and membrane integrity which leads to the reduction of water loss and mass loss of treated fruit (Tiwari and Paliyath 2011; Paliyath and Subramanian 2008). The efficacy of hexanal treatments to reduce the mass loss of fruit has been proven by studies summarised in Table 2.3.

EFF treatment applied at the preharvest stage, significantly reduced the mass loss of guava fruit (Gill et al., 2015), sweet oranges (Mwatawala et al., 2018), blueberry fruit (Padmanabhan et al., 2018) and mango fruit (Anusuya et al., 2016). Hexanal and EFF treatment applied at the postharvest stage significantly reduced mass loss of mango (Jincy et al., 2017), strawberry (Kayal et al., 2017), oriental sweet melons (Qi et al., 2011) and sweet bell pepper (Cheema et al., 2018).

Anusuya et al. (2016) demonstrated that the efficacy of hexanal at reducing mass loss of mango fruit might be cultivar-dependant as the ‘Alphonso’ cultivar retained mass more effectively than the ‘Banganapalli’ cultivar, when stored at ambient conditions. However, when stored at cold storage (14 ± 1 °C, 85 ± 5 % RH) both cultivars experienced a mass loss below 3 % for

both treated and untreated fruit after 21 days. This has also been shown by Jincy et al. (2017) for mango fruit stored at 25 ± 0.8 ℃ and 60 ± 10 % RH, for the 'Neelum' mango cultivar, whereby hexanal treatment significantly ($p < 0.01$) reduced the mass loss of mango fruit at ambient storage. The study conducted by Padmanabhan et al. (2018) showed similar results to that of Anusuya et al. (2016), with blueberry fruit stored at 21 ℃ exhibiting a mass loss of 10 - 12 % within a week of postharvest storage and fruit stored at 4 ℃ exhibiting a mass loss of 4 – 6 % after 21 days of storage.

Studies have demonstrated that hexanal formulations are more effective at reducing mass loss at cold storage (Anusuya et al., 2016; Padmanabhan et al., 2018). The results obtained by these authors contradict results obtained by Jincy et al. (2017) who reported that hexanal was effective at reducing mass loss at ambient temperature. However, it is important to highlight that hexanal was not effective at reducing mass loss at ambient temperature when applied at the preharvest stage and was effective at reducing mass loss at ambient temperature when applied at the postharvest stage.

Table 2.3: Studies conducted on fruit mass loss in response to hexanal treatment

Fruit/Vegetable	Cultivar	Treatment	Application	Storage Condition	Findings	Author
Guava	Allahabad Safeda	EFF	Preharvest	6 – 8 °C, 90 – 95% RH	Reduced mass loss	Gill et al. (2015)
Sweet orange	Valencia and Jaffa	EFF	Preharvest	Storage conditions not specified by the authors.	Reduced mass loss	Mwatawala et al. (2018)
Blueberry	Elliott	EFF	Preharvest	4 and 21 °C	Reduced mass loss	Padmanabhan et al. (2018)
Mango	Alphonso and Banganapalli	EFF	Preharvest	14 ± 1 °C, 85 ± 5% RH and 28 ± 2 °C	Reduced mass loss	Anusuya et al. (2016)
Mango	Neelum	Hexanal	Postharvest	25 ± 0.8 °C, 60 ± 10% RH	Reduced mass loss	Jincy et al. 2017
Strawberry	Jewel and Wendy	EFF	Preharvest	4 °C	Reduced mass loss	Kayal et al. (2017)
Oriental sweet melons	Jinheng No. 2	EFF	Postharvest	10 °C, 85% RH	Reduced mass loss	Qi et al. (2011)
Sweet bell pepper	Felicitas	EFF	Postharvest	12 °C, 90 – 95 % RH	Reduced mass loss	Cheema et al. (2018)

EFF - Enhanced freshness formulation; RH – Relative humidity.

2.3.4. Firmness

Biochemical and genetic studies of fruit softening indicated that cell wall biosynthesis and degradation is the result of coordinated expression of several families of genes encoding cell wall metabolism proteins. Which include expansins, pectin methylesterases, polygalacturonases, pectate lyases and phospholipase D (PLD) (Benítez-Burraco et al., 2003; Redondo-Nevado et al., 2001; Salentijn et al., 2003; Trainotti et al., 2001; Tiwari and Paliyath, 2011). PLD is a phospholipid degrading enzyme which catalyses the hydrolysis of membrane phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidyl-glycerol) to yield phosphatidic acid and is the key enzyme involved in the initiation of membrane deterioration.

Pectin substances are responsible for the cohesiveness of the fruit and the main components of the middle lamella and structural elements in the primary cell wall. Middle lamella dissolution reduces cell to cell adhesion and disassembly of the cell wall (Brummell and Harpster, 2001; Brummell, 2006). Hexanal is a potent inhibitor of PLD activity (Paliyath et al., 1999). Hexanal and EFF treatment down regulates the transcript levels of polygalacturonase (involved in pectin degradation) (Tiwari and Paliyath, 2011). Suppression of these enzymes in fruit tissues results in enhanced retention of fruit firmness, fruit quality and extension of shelf life (Brummell et al., 1999; Meli et al., 2010).

Previous studies have demonstrated the efficacy of hexanal formulations at retaining firmness of horticultural commodities, summarised in Table 2.4. Preharvest spraying of aqueous formulations of hexanal and EFF increased the firmness of tomato fruit with time on the vine. The results depicted a significant difference in firmness between treated and untreated fruit, with treated fruit having higher firmness values than untreated fruit (Cheema et al., 2014). Preharvest application of EFF reduced loss in firmness of guava (Gill et al., 2015), strawberry

(El Kayal et al., 2017), blueberry (Padmanabhan et al., 2018) and nectarine fruit (Kumar et al., 2018), with a significant difference between untreated and treated fruit.

Studies conducted on the postharvest application of EFF treatment exhibited a similar trend in relation to fruit firmness loss in comparison to studies based on the preharvest application of hexanal. The application of hexanal at the postharvest stage on sweet cherry (Sharma et al., 2010), oriental sweet melons (Qi et al., 2011), tomato (Cheema et al., 2014) and sweet bell pepper (Cheema et al., 2018), resulted in a significantly higher retention of fruit firmness in comparison to the untreated fruit. In addition to firmness retention during storage, hexanal formulations applied at the preharvest stage, resulted in firmer fruit compared to untreated fruit at harvest.

El Kayal et al. (2017) showed that firmness of preharvest sprayed strawberry fruit was approximately 21 – 30 % higher, relative to the untreated fruit. Similar results were reported by Gill et al. (2015) where hexanal treated guava fruit exhibited 16 % higher firmness in comparison to the control. A study conducted by Cheema et al. (2014) showed that hexanal and EFF treated tomato fruit respectively exhibited a 32 % and 49 % higher firmness than the control fruit. Padmanabhan et al. (2018), as well as Kumar et al. (2018), illustrated that blueberry and nectarine fruit had 9 % and 8.11 % higher firmness values at harvest respectively, when EFF is applied at the preharvest stage.

Padmanabhan et al. (2018) and Pak Dek et al. (2018) who worked on blueberry and tomato respectively, showed that hexanal treatments were not effective at firmness retention of fruit stored at ambient temperature, regardless of the stage of application (pre or postharvest application). However, a single study conducted by Yumbya et al. (2018), contradicts the findings of the authors mentioned above. Yumbya et al. (2018) reported that banana fruit treated with hexanal formulation at the pre and the postharvest stage was effective at firmness

retention, with significantly higher firmness values compared to the control for banana fruit stored at 25 ± 1 °C and 60 ± 5 % RH. The author further showed that hexanal was more effective at firmness retention when applied at the postharvest stage rather than the preharvest stage.

The ideal storage condition for blueberry fruit is around 0 – 8 °C at 90 – 95 % relative humidity. Blueberry fruit should be cooled immediately after harvest to reduce loss of firmness. The ambient storage temperature may be the reason behind the hexanal treatments inefficiency to retain firmness of blueberry fruit.

Table 2.4: Studies conducted on fruit firmness in response to hexanal treatment

Fruit/Vegetable	Cultivar	Treatment	Application	Storage Condition	Findings	Author
Guava	Allahabad Safeda	EFF	Preharvest	6 – 8 °C, 90 – 95% RH	Retained firmness	Gill et al. (2015)
Strawberry	Jewel and Wendy	EFF	Preharvest	4 °C	Retained firmness	Kayal et al. (2017)
Blueberry	Elliott	EFF	Preharvest	4 and 21 °C	Greater firmness at harvest	Padmanabhan et al. (2018)
Nectarine	Fantasia	EFF	Preharvest	2 ± 1 °C	Greater firmness at harvest	Kumar et al. (2018)
Sweet cherry	Bing	Hexanal	Postharvest	4 °C, 90 – 95% RH	Retained firmness	Sharma et al. (2010)
Oriental sweet melons	Jinheng No. 2	EFF	Postharvest	10 °C, 85% RH	Retained firmness	Qi et al. (2011)
Tomato	Prunus	Hexanal and EFF	Postharvest	15 °C	Retained firmness	Cheema et al. (2014)
Sweet bell pepper	Felicitas	EFF	Postharvest	12 °C, 90 – 95% RH	Retained firmness	Cheema et al. (2018)
Banana	Grand Nain	EFF	Preharvest	25 ± 1 ºC, 60 ± 5% RH	Retained firmness	Yumbya et al. (2018)
Tomato	De Ruiter	Hexanal	Postharvest	Room temperature	No effect on firmness	Pak Dek et al. (2018)

EFF - Enhanced freshness formulation; RH – Relative humidity.

2.3.5. Fruit colour

Changes in colour intensity are key indicators of maturity and quality of fresh fruit and vegetables. The development of red colour in tomatoes is considered as an index of maturity (Lopez Camelo and Gomez, 2004). In mango fruit, the key indicator of ripening in many cultivars is the loss of green colour. The development of the optimum skin colour is very important for fruit quality since it is an important factor by which most consumers define mango quality.

Sweet cherry fruit transition from red to purple during ripening and senescence. Sharma et al. (2010) observed a bright red colour of sweet cherries in trees sprayed with EFF in comparison to a purple-red colour observed in the untreated fruit. These observations illustrate the efficacy of hexanal formulation in delaying the ripening process. Treated cherries had a significantly higher chroma intensity (an indicator of redness), brightness (L) value and hue angle than control fruit at harvest. During postharvest storage, chroma intensity was maintained at a slightly but significantly higher level in EFF treated fruit. Hexanal and antioxidant compounds such as ascorbic acid in the formulation may have facilitated in fruit colour retention (Paliyath and Murr, 2007).

In many mango cultivars, the skin colour changes from dark green to olive green, sometimes reddish, orange-yellow or yellowish hues appear from the base colour, depending on the cultivar. Anusuya et al. (2016) observed that EFF applied at the preharvest stage retained green colour during storage, as depicted by the hue values which were significantly lower than that of untreated fruit.

In most tomato cultivars, peel colour changes from green to red during ripening. Cheema et al. (2014) observed an improved red colour in EFF treated tomatoes in comparison to control fruit at harvest. This is illustrated by the decrease in hue angle and increase of red colour intensity

and unsprayed fruit recorded higher hue values than sprayed fruit. The red colour intensity observed in this experiment as a result of EFF treatment was slightly higher than hexanal alone, suggesting that EFF may be more effective in delaying ripening. It is interesting to note that the yellow colour component that predominantly indicates the level of carotenoids (Itle and Kabelka, 2009), remained nearly constant, irrespective of treatments, suggesting that the carotenoid biosynthetic pathway was unaffected by the treatments.

The efficacy of postharvest application of hexanal and EFF on delaying ripening and senescence has been reported. Tomatoes dipped in EFF and hexanal showed higher L values, hue angle, and reduced red colour intensity than control fruit during storage, suggesting a delay in ripening (Cheema et al., 2014). However, the initial delay in red colour development does not inhibit the full colour development potential of tomatoes. This has been demonstrated by Pak Dek et al. (2018) and Tiwari and Paliyath, (2011), where tomatoes treated with hexanal and EFF (applied at the postharvest stage) developed red colour similar to that of the control during the storage period.

Lycopene is converted to β-carotene by the enzyme sesquiterpene cyclase during fruit development. Once ripening is activated, sesquiterpene cyclase levels are reduced which leads to the accumulation of lycopene and red colour (Ronen et al., 1999; Srivastava and Handa, 2005). Therefore, reduced red colour intensity in hexanal formulation treated tomatoes is a clear indication that ripening processes are delayed without causing any changes in the expression of these genes and as a result, hexanal treated tomatoes developed a full red colour. Hexanal vapour treatment delayed the ripening of bell peppers by reducing the development of red colour during storage, with respect to untreated fruit (Cheema et al., 2018). The delay in colour development was greater at the highest concentration. Treated peppers retained the green colour as evidenced by negative a* values, as compared to untreated peppers that showed an increase in a* which were positive at the end of the storage period.

Table 2.5: Studies conducted on fruit colour in response to hexanal treatment

Fruit/Vegetable	Cultivar	Treatment	Application	Storage Condition	Findings	Author
Sweet cherry	Bing	EFF	Preharvest	4 °C, 90 – 95%	Fruit colour retention	Sharma et al. (2010)
Mango	Alphonso and Banganapalli	EFF	Preharvest	14 ± 1 °C, 85 ± 5% RH and 28 ± 2 °C	Fruit colour retention	Anusuya et al. (2016)
Tomato	Prunus	Hexanal and EFF	Postharvest	12 °C and room temperature	Fruit colour retention	Cheema et al. (2014)
Tomato	De Ruiter	Hexanal	Postharvest	Room temperature	Fruit colour retention	Pak Dek et al. (2018)
Tomato	Rapsodie	EFF	Postharvest	15 °C and room temperature	Fruit colour retention	Tiwari and Paliyath (2011)
Sweet bell pepper	Felicitas	EFF	Postharvest	12 °C, 90 – 95% RH	Fruit colour retention	Cheema et al. (2018)

EFF - Enhanced freshness formulation; RH – Relative humidity.

2.4. Biochemical Attributes

2.4.1. Total soluble solids and titratable acidity

The increase in total soluble solids (TSS) of fruit is due to an increase in free sugars (Cheour et al., 1990) caused by the breakdown of carbohydrates into sugars such as glucose (Kittur et al., 2001). As the fruit ripens, the starch is hydrolysed into glucose, fructose and sucrose (Ito et al., 1997). This can be explained by the increase in the activity of sucrose synthase, invertase and amylase enzyme, which hydrolyse starch to sucrose (Kumar et al., 1994). The increase in TSS content during storage might result from hydrolysis of starch into sugars. On completion of starch hydrolysis, no further increase in sugar content occurs and subsequently the TSS content declines as the sugars are metabolized during respiration (Wills et al., 1980). The decrease in titratable acidity of fruit during storage could be attributed to the use of organic acids in the respiratory process (Echeverria and Valich, 1989).

Previous studies investigating the effect of hexanal formulations on TSS and TA are presented in table 2.6. Gill et al. (2015) reported that TSS of EFF treated fruit at the preharvest stage, was higher than untreated fruit at harvest. TSS content of untreated fruit increased at a faster rate than treated fruit and declined seven days before a decrease was observed in treated fruit. Cheema et al. (2014) also reported a significantly higher soluble solid content in EFF treated fruit with respect to untreated fruit. Titratable acidity (TA) of untreated fruit was higher than treated fruit at harvest. A decline in TA was more rapid in the untreated fruit, whereas the EFF treated fruit maintained significantly higher TA. Fruit treated with EFF maintain a higher acid concentration during storage, which may be due to the reduction in respiration rate and delayed ripening.

Anusuya et al. (2016) also reported higher TSS of EFF treated mango fruit with respect to control at harvest and TA of mango fruit from sprayed trees was lower than that of untreated trees. Treated fruit retained a significantly higher TSS level throughout the storage period than

untreated fruit. There was no significant difference in the level of TA between hexanal and untreated fruit. During storage, the sugars accumulated in the pulp of fruit while starch declined with the progression of ripening. This may be due to EFF maintaining starch levels in the pulp and preserving the structural integrity of tissues in treated fruit, unlike untreated fruit which exhibit exhaustion of starch and smudging of tissues. Kayal et al. (2017) observed no significant difference in TSS and TA between hexanal and untreated fruit however, TA was higher for hexanal treated fruit than the untreated fruit.

Yuan et al. (2009) and Jincy et al. (2017) reported that strawberry and mango fruit treated at the postharvest stage respectively, had lower TSS and higher TA than untreated fruit during storage. The lower TSS content of hexanal treated strawberry fruit may reflect delayed ripening. However, there was no significant difference observed between treated and untreated strawberry fruit. In addition, hexanal treated mango fruit had increased starch granules, thus delaying the ripening process.

A study conducted by Yumbya et al. (2018) showed that the stage of application of hexanal had a significant effect on TSS and TA of banana fruit. Fruit treated with EFF at the preharvest stage had a greater and rapid increase in TSS in comparison to fruit that were dipped in EFF. However, fruit treated with EFF at the preharvest stage possessed higher levels of TA than fruit treated at the postharvest stage. In relation to the untreated fruit, hexanal treatments significantly delayed the increase of TSS and decrease in TA during storage. This may be due to the reduced activity of the enzymes involved in the hydrolysis of stored carbohydrates into soluble sugars and reduced activities of enzymes such as malate dehydrogenase, which influence the level of malic acid in banana.

Table 2.6: Studies conducted on TSS and TA in response to hexanal treatment

Fruit/Vegetable	Cultivar	Treatment	Application	Storage Condition	Findings	Author
Guava	Allahabad Safeda	EFF	Preharvest	6 – 8 °C, 90 – 95% RH	Higher TSS and TA at harvest	Gill et al. (2015)
Tomato	Prunus	Hexanal and EFF	Postharvest	15 °C	Higher TSS at harvest and TA	Cheema et al. (2014)
Mango	Alphonso and Banganapalli	EFF	Preharvest	14 ± 1 °C, 85 ± 5% RH and 28 ± 2 °C	Higher TSS at harvest and TA	Anusuya et al. (2016)
Strawberry	Jewel and Wendy	EFF	Preharvest	4 °C	No significant difference	Kayal et al. (2017)
Banana	Grand Nain	EFF	Preharvest	25 ± 1 ºC, 60 ± 5% RH	Reduced TSS and retained TA	Yumbya et al. (2018)
Banana	Grand Nain	EFF	Postharvest	26 ± 1 ºC, 60 ± 5% RH	Reduced TSS and retained TA	Yumbya et al. (2018)
Strawberry	Darselect	Hexanal	Postharvest	4 °C	Reduced TSS and retained TA	Yuan et al. (2009)
Mango	Neelum	Hexanal	Postharvest	25 ± 0.8 °C, 60 ± 10% RH	Reduced TSS and retained TA	Jincy et al. 2017

EFF - Enhanced freshness formulation; RH – Relative humidity.

2.4.2. Ascorbic acid

Ascorbic acid (vitamin C) is a low molecular weight metabolite, which functions as an antioxidant agent in plants (Fotopoulos et al., 2008), and its accumulation in fruit and vegetables is important for the human diet and health (Davey et al., 2000). Ascorbic acid of fruit and vegetables gradually declines as the storage temperature or duration increases (Adisa, 1986; Tsaniklidis et al., 2014), due to an increase in dehydroascorbic acid (DHA) (Lee and Kader, 2000).

Ascorbate oxidase is a multi-copper enzyme that catalyses the oxidation of ascorbic acid to yield dehydroascorbic acid, followed by the decomposition of dehydroascorbic acid (Mapson 1970; Shimada and Ko, 2008). Fruit and vegetables that exhibit a high enzymatic activity of ascorbate oxidase, are characterised by an increase in the residual ratio of dehydroascorbic acid and a decrease in the total ascorbic acid. In addition, it has been found that low temperatures also stimulate ascorbic acid oxidation through the activity of ascorbate peroxidase (APX) (Loannidi et al., 2009).

Previous studies demonstrated the efficacy of hexanal formulations in retaining ascorbic acid levels in various horticultural produce such as tomato (Cheema et al., 2014), guava (Gill et al., 2015), mango (Jincy et al., 2017) and banana (Yumbya et al., 2018), presented in table 2.7. The studies mentioned above showed that levels of ascorbic acid declined with storage time, however, hexanal and EFF treated fruit exhibited a slower degradation of ascorbic acid during storage. In addition, levels of ascorbic acid were higher in fruit treated with hexanal at the preharvest stage, with respect to the untreated fruit. The gradual decline in ascorbic acid in hexanal and EFF treated fruit might be due to increased biosynthesis, or decreased oxidation during storage.

EFF treated tomato fruit (at the preharvest stage) registered a significant increase in ascorbic acid content (27 % higher than untreated fruit) at harvest, whereas the untreated fruit showed a declining trend in ascorbic acid content after the second and third week of harvest (Cheema et al., 2014). The concentration of ascorbic acid in EFF treated tomato fruit after 21 days of postharvest storage (12 °C) was nearly 141 % higher than that of untreated fruit (Cheema et al., 2014).

A similar increasing trend in the concentration of ascorbic acid was also reported in EFF treated guava fruit with over 24 % higher levels after 35 days in postharvest storage compared to untreated fruit (Gill et al., 2015). The hexanal treated mango fruit registered significantly higher ascorbic acid content after 12 days in storage (Jincy et al., 2017). Hexanal treatment significantly delayed the rate of oxidation of ascorbic acid of banana fruit, irrespective of the mode of application at the pre and postharvest stage (Yumbya et al., 2018).

The literature based on hexanal treatments has shown that the hexanal formulation has the capacity to increase the synthesis of ascorbic acid during fruit development (on the tree/vine) as well as reduce the decrease of ascorbic acid during storage regardless of the stage of application. However, the efficacy of the stage of application on the retention of ascorbic acid has not been demonstrated, as no studies have compared which mode of application is more effective at retaining the concentration of ascorbic acid after harvest.

Table 2.7: Studies conducted on ascorbic acid content in response to hexanal treatment

Fruit/Vegetable	Cultivar	Treatment	Application	Storage Condition	Findings	Author
Tomato	Prunus	Hexanal and EFF	Preharvest	15 °C	Higher ascorbic acid at harvest	Cheema et al. (2014)
Tomato	Prunus	Hexanal and EFF	Postharvest	12 °C and room temperature	Retained ascorbic acid	Cheema et al. (2014)
Guava	Allahabad Safeda	EFF	Preharvest	6 – 8 °C, 90 – 95% RH	Retained ascorbic acid	Gill et al. (2015)
Banana	Grand Nain	EFF	Preharvest	25 ± 1 ºC, 60 ± 5% RH	Retained ascorbic acid	Yumbya et al. (2018)
Banana	Grand Nain	EFF	Postharvest	26 ± 1 ºC, 60 ± 5% RH	Retained ascorbic acid	Yumbya et al. (2018)
Mango	Neelum	Hexanal	Postharvest	25 ± 0.8 °C, 60 ± 10 % RH	Retained ascorbic acid	Jincy et al. (2017)

EFF - Enhanced freshness formulation; RH – Relative humidity

2.4.3. Phenolic compounds

Polyphenols are secondary metabolites/group of antioxidants in plants that protect lipids and cell constituents against oxidative damage, thus have the ability to protect human body tissues against oxidative attacks (Romanazzi et al., 2002). They possess a broad spectrum of biochemical activities such as antioxidant, antimutagenic and anticarcinogenic (Nakamura et al., 2003). The synthesis of phenolic compounds may be attributed to the activity of phenylalanine lyase (PAL), which is the key enzyme that uses phenylalanine to synthesise phenolic compounds (Ke and Salveit, 1989). The accumulation of phenolic compounds is caused by PAL activity, which is activated under oxidative stress conditions. Such stress conditions involve high levels of CO_2 and low levels of O_2 (Frusciante et al., 2007; Romanazzi et al., 2002; Odriozola-Serrano et al., 2008).

Phenolics can function as effective antioxidants by scavenging singlet oxygen and free radicals via their ability to donate hydrogen from hydroxyl groups positioned around the aromatic ring (Hertog et al., 1992; Foti et al., 1994; Hertog et al., 1995; Rice-Evans et al., 1995; Jorgensen et al., 1999). Phenolic compounds play a crucial role in enhancing the shelf life and quality of fresh fruit by delaying senescence induced by oxidative degradation (Khanizadeh et al., 2009). Therefore, preservation of antioxidant levels during the postharvest storage is imperative for the preservation of fresh produce. Previous studies investigating the effect of hexanal formulations on phenolic compounds are presented in table 2.8.

Anthocyanins are the most abundant phenolic compounds in strawberry fruit and pelargonidin-3-glucoside has been reported to be the major anthocyanin component (Aaby et al., 2012; Misran et al., 2014). Misran et al. (2015) demonstrated that EFF treatment applied to strawberry fruit at the preharvest stage resulted in enhanced levels of pelargonidin-3-glucoside, which were significantly higher than the untreated fruit. The enhanced freshness formulation used in

this particular experiment contained cinnamic acid, which may have stimulated the synthesis of anthocyanins, such as pelargonidin-3-glucoside through UDP-glucose:flavonoid-3-O-glucosyltransferase (3GT) activity in treated fruit (Misran et al., 2014). However, on strawberry fruit, this has been proven to be cultivar-dependant. The 'Jewel' cultivar exhibited an increase in pelargonidin-3-glucoside, in response to the EFF treatment. However, EFF treatment had no significant effect on the level of pelargonidin-3-glucoside of the 'Mira' cultivar, in comparison to the untreated fruit.

During the ripening of guava fruit, the total phenolic content of guava fruit tends to decline during postharvest storage (Singh and Pal, 2008). A study conducted by Gill et al. (2015), showed that the application of EFF treatment to guava fruit at the preharvest stage, resulted in a higher concentration of phenolic content in treated fruit, in comparison to the untreated fruit. In addition, treated fruit maintained their phenolic content more efficiently than the control and treated fruit had significantly higher levels of phenolics throughout the storage period.

Sharma et al. (2010) demonstrated that the total phenolic content of sweet cherries showed an increasing and decreasing trend during storage. EFF treated sweet cherries (applied at the preharvest stage) showed a significant increase in phenolic content, with respect to the untreated fruit, followed by a decline in the phenolic content. However, the concentration of phenolics in EFF treated fruit remained higher than the untreated control at the end of the storage period. In the same study, the prominent phenolic compounds were neochloro-genic acid, p-coumarylquinic acid and feruloylglucose. The neochlorogenic acid levels of untreated cherries exhibited a 42 % decrease whereas EFF treated fruit exhibited a 76 % increase during storage. The feruloyl glucose levels of untreated fruit exhibited a 46 % decrease whereas EFF treated fruit exhibited a 28 % increase during storage. The phenolic components of untreated fruit showed a decreasing trend throughout the storage period, whereas the treated fruit showed

an increase followed by a decreasing trend. However, the phenolic components of treated fruit remained higher than that of control during storage.

The efficacy of EFF treatment to maintain the level of phenolics in treated fruit may be attributed to its ability to delay ripening and senescence of fruit during postharvest storage. In relation to the accumulation of phenolic compounds as a result of PAL activity, in section 2.2 above, previous studies have shown that hexanal formulations increase the CO_2 levels of fruit, thus the higher levels of phenolic content in hexanal treated fruit may be attributed to elevated levels of carbon dioxide of the fruit.

Table 2.8: Studies conducted on phenolic compounds in response to hexanal treatment

Fruit/Vegetable	Cultivar	Treatment	Application	Storage Condition	Findings	Author
Strawberry	Jewel	EFF	Preharvest	At harvest	Enhanced Phenolic compounds	Misran et al. (2015)
Strawberry	Mira	EFF	Preharvest	At harvest	No significant difference	Misran et al. (2015)
Guava	Allahabad Safeda	EFF	Preharvest	6 – 8 °C, 90 – 95% RH	Retained phenolic compounds	Gill et al. (2015)
Sweet cherry	Bing	EFF	Preharvest	4 °C, 90 – 95% RH	Retained phenolic compounds	Sharma et al. (2010)

EFF - Enhanced freshness formulation; RH – Relative humidity.

2.4.4. Pigment development

Carotenoids are synthesized from a C5 building block, a common precursor to all isoprenoids, which is the isopentenyl pyrophosphate (IPP) molecule, and its isomer dimethylallyl diphosphate (DMAPP) (Chappell et al., 1995) via the plastidial 2-C-methyl-D-erythriol 4-phosphate (MEP) pathway in plastids (Lichtenthaler, 1999; Eisenreich et al., 2001; Schwender et al., 2001). The reaction chains of carotenoid biosynthesis occur in the plastids, and carotenogenesis is dependent on precursors produced via the MEP pathway (Milborrow and Lee, 1998).

The first step in carotenoid biosynthesis is the head to head condensation of two molecules of geranylgeranyl pyrophosphate (GGPP), catalysed by the enzyme phytoene synthase (PSY), to form phytoene, the first but uncoloured carotenoid (Dogbo et al., 1988). The tomato nuclear genome contains sequences encoding three PSY enzymes, named PSY1, PSY2 and PSY3. The PSY1 enzyme is involved in the synthesis of carotenoids in ripening fruit (Bartley and Scolnik, 1993).

Following this step, phytoene undergoes a series of sequential desaturation (dehydrogenation) and isomerization reactions that increase the number of conjugated double bonds in the initial structure catalysed by phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS), which result in the formation of pro-lycopene (a pink/red coloured carotenoid). Pro-lycopene is then isomerized by carotenoid isomerase (CRTISO) into all-trans lycopene. After this step, the pathway is divided into two branches. In one branch of the synthetic pathway, lycopene is cyclized at both ends by lycopene β-cyclase (LCYB), yielding β-carotene with two β–ionone end groups.

During ripening of tomato fruit, the transcripts of genes encoding 1-deoxy-D-xylulose 5-phosphate synthase (DXS), geranylgeranyl pyrophosphate synthase (GGPPS), phytoene

synthase (PSY), phytoene desaturase (PDS), ζ-carotene desaturase (ZDS), and carotene isomerase (CRTISO) are up-regulated and contribute to the formation of lycopene (Giuliano et al., 1993; Fraser et al., 1994; Corona et al., 1996; Pecker et al., 1996). Previous studies investigating the effect of hexanal formulations on pigment development are presented in table 2.9.

Pak Dek et al. (2018) observed enhanced transcript levels of DXS, GGPP2, PSY1 and CTRISO with respect to the control. Tiwari and Paliyath (2011) reported that EFF did not affect the expression of genes involved in the GGPP biosynthesis and trans-lycopene biosynthesis. However, in both experiments, untreated tomatoes had significantly higher lycopene (Pak Dek et al., 2018), β-carotene and lycopene content (Tiwari and Paliyath, 2011), with respect to hexanal and EFF treated tomatoes, respectively. The evidence showed the efficacy of hexanal in delaying the ripening process whilst sustaining biosynthesis of the carotenoids without causing an inhibition of the isoprenoid pathway. Thus, secondary metabolic pathways such as that of lycopene biosynthesis were not downregulated by hexanal treatment.

The red colour development in tomato fruit occurs by the inhibition of sesquiterpene cyclase by ethylene during normal ripening. By reducing sesquiterpene cyclase levels, ethylene enables the accumulation of lycopene leading to the development of red colour. Hexanal treatment did not cause any changes in the expression of the genes involved in carotenoid biosynthesis, as a result, hexanal treated tomatoes developed a red colouration with increased storage time.

Table 2.9: Studies conducted on pigment development in response to hexanal treatment

Fruit/Vegetable	Cultivar	Treatment	Application	Storage Condition	Findings	Author
Tomato	Rapsodie	EFF	Postharvest	15 °C and room temperature	Delayed pigment development	Tiwari and Paliyath, (2011)
Tomato	De Ruiter	Hexanal	Postharvest	Room temperature	Delayed pigment development	Pak Dek et al. (2018)

EFF - Enhanced freshness formulation; RH – Relative humidity.

2.5. Enzyme Activity

2.5.1. Antioxidant enzymes

An efficient antioxidant system has the ability to delay senescence and oxidative damage to cell constituents. Excessive amounts of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), hydroxyl radicals and superoxide radicals (O_2^-) are produced due to stress and senescence. High levels of ROS are detrimental to plant, fruit and vegetables as they cause peroxidation, which leads to the damage of cell membranes, enzyme inhibition, oxidative damage, strand breakage in nucleic acids and protein oxidation (Allen, 1995). Plants possess well defined enzymatic and non-enzymatic antioxidant defence systems, which protect against deleterious effects of ROS.

Antioxidant enzymes that scavenge ROS include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (POX) and glutathione reductase (GR). These enzymes have the capacity to detoxify ROS, which is lethal and induce oxidative damage to cellular components. Increased activity of antioxidant enzymes, results in higher scavenging potential of ROS. This provides improved protection of cell membranes, improved prevention of water loss from fruit and reduced damage during ripening. Ultimately, an efficient antioxidant system results in improved preservation of fruit quality.

Within the cell, SOD establishes the first line of defence by catalysing the dismutation reaction of two superoxide free radicals (O_2^-) to one molecule of O_2 and one molecule of hydrogen peroxide H_2O_2. CAT (present in the cytosol and in peroxisomes) and POX further catalyse the decomposition of H_2O_2 formed through SOD to form H_2O and O_2. APX (a cytosolic enzyme that is involved in the decomposition of H_2O_2 generated both in the chloroplasts and in the cytosol) works in tandem with SOD to scavenge H_2O_2. Glutathione reductase (GR) is an NADPH-dependent enzyme that plays an important role in cell metabolism by facilitating the

maintenance of a high ratio of GSH/GSSG by catalysing the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) (Beyer and Fridovich, 1987).

Previous studies have demonstrated the efficacy of hexanal and EFF at maintaining ROS levels in fruit during postharvest storage, by significantly increasing the activity of antioxidant enzymes, presented in table 2.10. In hexanal treated (applied at the postharvest stage) strawberry fruit, Yuan et al. (2009) observed a significantly lower superoxide radical production relative to the control. Sharma et al. (2010) demonstrated that hexanal treated sweet cherries (applied at the postharvest stage) exhibited significantly higher SOD activity, with respect to the untreated fruit by approximately 288 % after 15 days of storage. In addition, this experiment showed that fruit treated with EFF applied at the preharvest stage only, exhibited a significantly lower enzyme activity than that of fruit treated with EFF at the postharvest stage. APX and GPX activities had a similar trend to SOD but hexanal treatments had no significant effect on their activity.

Qi et al. (2011) reported that EFF treated (applied at the postharvest stage) oriental sweet melons significantly increased SOD and CAT activities, relative to the untreated fruit. A study conducted by Jincy et al. (2017) showed that mango fruit treated with hexanal had a higher SOD, CAT, APX and GPX activity than control fruit. In addition, untreated fruit showed a significantly higher production of (O_2^-) and (H_2O_2). The efficient scavenging of ROS in hexanal treated fruit resulted in a decrease of oxidative damage compared with control.

Cheema et al. (2018) reported that hexanal vapour treated bell peppers at the postharvest stage, exhibited significantly higher SOD, CAT, GR and POX enzyme activities, relative to the untreated fruit.

Table 2.10: Studies conducted on antioxidant enzymes in response to hexanal treatment

Fruit/Vegetable	Cultivar	Treatment	Application	Storage Condition	Findings	Author
Strawberry	Darselect	Hexanal	Postharvest	4 °C	Lower O_2^- production	Yuan et al. (2009)
Sweet cherry	Bing	EFF	Postharvest	4 °C, 90 – 95% RH	Enhanced antioxidant enzymes	Sharma et al. (2010)
Sweet cherry	Bing	EFF	Preharvest	5 °C, 90 – 95% RH	Enhanced antioxidant enzymes	Sharma et al. (2010)
Oriental sweet melons	Jinheng No. 2	EFF	Postharvest	10 °C, 85% RH	Enhanced antioxidant enzymes	Qi et al. (2011)
Mango	Neelum	Hexanal	Postharvest	25 ± 0.8 °C, 60 ± 10% RH	Enhanced antioxidant enzymes	Jincy et al. (2017)
Sweet bell pepper	Felicitas	EFF	Postharvest	12 °C, 90 – 95% RH	Enhanced antioxidant enzymes	Cheema et al. (2018)

EFF - Enhanced freshness formulation; RH – Relative humidity.

2.5.2. Cell membrane enzymes

During fruit ripening, loss of membrane integrity and senescence leads to a loss in fruit quality. Phospholipase-D (PLD) is a membrane lipid degrading enzyme (Paliyath and Subramanian, 2008), involved in the membrane deterioration pathway (Paliyath and Droillard, 1992). PLD initiates the phospholipid breakdown by removing the head group of phospholipids, and other enzymes that act downstream such as phosphatidate phosphatise, lipolytic acyl hydrolase and lipoxygenase (LOX), that act in tandem with PLD (Paliyath and Droillard, 1992).

The downstream enzymes cannot directly cause the catabolism of phospholipids. Therefore, the entire catabolic pathway can be slowed down by inhibiting PLD activity. Hexanal is capable of inhibiting PLD by blocking the hydrolysis at the active site of PLD, thus facilitating the preservation of fruit quality by reducing membrane deterioration and delaying senescence (Tiwari and Paliyath, 2011).

Lipoxygenases are a family of non-heme iron-containing enzymes which catalyse the deoxygenation of polyunsaturated fatty acids in lipids. LOX activity is involved in membrane deterioration, ripening and senescence. Suppressing the expression of LOX activity results in improved maintenance of membrane integrity and delays the fruit ripening process (Zheng et al., 2007).

Malondialdehyde (MDA) is a highly reactive compound that occurs naturally and is a marker for oxidative stress. MDA results from lipid peroxidation of polyunsaturated fatty acids. The degree of lipid peroxidation can be estimated by the amount of MDA in tissue cells. ROS degrade polyunsaturated lipids, forming MDA. The production of this aldehyde is used to measure the level of oxidative stress in an organism.

Pectin methylesterase (PME), is an enzyme that is involved in the hydrolysis of methyl ester groups in pectin chains, leading to the formation of carboxylate groups (Jayani et al., 2005).

PME removes the methyl group of galacturonic acid polymers of pectin. PME activity causes the de-esterification of the pectin chain, this increases the susceptibility of the pectin chain to polygalacturonase mediated degradation (Carpita and Gibeaut, 1993). This results in a rapid loss of cell wall structure. Thus, PME activity has an influence on postharvest shelf life and quality of horticultural produce.

The efficacy of hexanal treatments to suppress the expression/activity of PLD by blocking the hydrolysis at the active site of PLD, reducing the amount of MDA produced by suppressing the production of ROS, reducing the LOX activity by preserving the membrane cells, decreasing PME and polygalacturonase (PG) transcripts (reduces breakdown of pectin) results in improved quality, firmness and optimises the postharvest shelf life of horticultural produce.

Previous studies have demonstrated the effect of hexanal treatment on cell membrane enzymes, presented in table 2.11. Yuan et al. (2009) reported an increase in PLD activity of strawberry fruit during storage, with hexanal treated (applied at the postharvest stage) fruit exhibiting a higher activity of PLD. However, hexanal treatment reduced the LOX activity of the strawberry fruit. Tiwari and Paliyath, (2011) reported that EFF treatment inhibited PLD activity and downregulated the transcript levels of PME in tomato fruit.

A decrease in pectin can be correlated with a decrease in molecular size and esterification of pectin during storage. EFF treatment significantly reduced PME activity of guava fruit relative to untreated fruit, throughout storage. In addition, the peak in PME activity was delayed by 14 days in treated fruit, with respect to the untreated fruit. A study conducted by Jincy et al. (2017), showed that hexanal dip treatment significantly reduced PLD activity and MDA production, relative to the untreated fruit. Results obtained by these authors are indicative of less oxidative damage in hexanal treated mango fruit.

Hexanal treated tomato fruit, significantly inhibited PLD activity throughout the storage period, with respect to the untreated fruit. These results suggest that the ripening of tomato was delayed as a result of less generation of phosphatidic acid (PA) in hexanal treated fruit. Anusuya et al. (2016), demonstrated that EFF applied at the preharvest stage, reduced PME and PG activity for two mango cultivars at cold and ambient storage temperature.

Table 2.11: Studies conducted on cell membrane enzymes in response to hexanal treatment

Fruit/Vegetable	Cultivar	Treatment	Application	Storage Condition	Findings	Author
Strawberry	Darselect	Hexanal	Postharvest	4 °C	Reduced LOX activity	Yuan et al. (2009)
Tomato	Rapsodie	EFF	Postharvest	15 °C and room temperature	Inhibited PLD activity	Tiwari and Paliyath (2011)
Guava	Allahabad Safeda	EFF	Preharvest	6 – 8 °C, 90 – 95% RH	Reduced PME activity	Gill et al. (2015)
Mango	Alphonso and Banganapalli	EFF	Preharvest	14 ± 1 °C, 85 ± 5% RH and 28 ± 2 °C	Reduced PME and PG activity	Anusuya et al. (2016)
Mango	Neelum	Hexanal	Postharvest	25 ± 0.8 °C, 60 ± 10% RH	Reduced PLD activity	Jincy et al. (2017)

EFF - Enhanced freshness formulation; RH – Relative humidity.

2.6. Commercialization

Fungicides are used to preserve fruit in order to control postharvest decay and optimise fruit shelf life (Palou et al., 2015). The common synthetic fungicides used to control fruit decay during the supply chain are prochloraz and chlorinated water. Residues of this fungicide may be harmful to consumers (Johnson and Sangchote, 1993) and disposal of the fungicide solution used for dip treatment is harmful to the environment and a major problem for packhouses.

Updated regulations from many countries including South Africa, are increasingly restricting the use of agrochemicals and every day more export markets are demanding fruit with residue levels even lower than those established by official regulations (DAFF, 2010; Palou, 2015). Due to this situation, research should focus on anticipating a scenario in which the use of conventional chemical fungicides is reduced.

Chlorinated water is a common method used in South African packhouses to disinfect tomatoes. In addition to the minor threats chlorinated water causes towards human health and the environment (Boyette et al., 1994), it is not an efficient method as it causes off-flavours in some fruit (Hassenberg et al., 2008). In addition, increase in consumer awareness regarding food safety and demand for organically produced fruit (Bill et al., 2014) and several European countries forbidding its use (Pinheiro et al., 2013), there is a trend for eliminating chlorine from the disinfection process (Olmez and Kretzschmar, 2009). Hexanal treatment leaves no residue on fruit and vegetables upon application, thus posing no threat to human health and the environment.

Studies conducted on hexanal treatments have demonstrated that hexanal formulations have no adverse effects such as disorders or off-flavours on fresh produce and optimise the shelf life of fresh produce during postharvest storage. The ability of this treatment to maintain quality during storage, show it's potential of increasing marketability of fresh produce in the

postharvest industry, in a manner that does not put farmers involved in exporting fresh produce under pressure to adhere to strict regulations regarding harmful residues as hexanal leaves no residues when applied on the commodity.

Adoption of hexanal technology has been reported to improve food security and economic independence by allowing producers to wait for a better price, as the treatment has been estimated to provide farmers with an average of 15 – 25 % more income per acre. This is due to the extension of shelf life provided by the treatment. In addition, hexanal technology is an affordable and cost-effective technology that can even be adopted by smallholder farmers, as the enhanced freshness formulation uses low quantities of hexanal and the price of hexanal is estimated at $15 per lire.

2.7. Conclusion

The discovery of hexanal and development of the enhanced freshness formulation has resulted in research in the postharvest science field to enhance quality of fresh produce during shelf life. The studies conducted on this technology have been successful in optimising quality of fresh produce without causing adverse effects on the produce. Furthermore, scientific studies have been conducted to understand and show the mode of action of hexanal in maintaining quality of fresh produce. These studies involved experiments related to antioxidant enzymes, where the activity of these enzymes was enhanced by the treatment. Which resulted in reduced oxidative damage in the fruit and more efficient scavenging of reactive oxygen species.

The suppression of membrane enzymes by hexanal treatment showed that fruit were of superior quality in comparison to untreated fruit, as shown by results illustrating firmness retention during storage. The suppression of cell membrane enzymes that lead to fruit softening and deterioration, as well as enhanced activity of antioxidant enzymes, is indicative of improved quality maintenance owed to the hexanal treatment.

Hexanal may be applied at the preharvest and postharvest stage. The versatility of this treatment regarding its mode of action makes it easy to incorporate and adopt within the industry. This makes it beneficial and applicable to small scale and smallholder farmers who cannot afford packhouse facilities as it can be applied at the preharvest stage and quality can be maintained after harvest.

Even though hexanal technology has been proven to enhance the quality of fresh produce and reduce postharvest losses caused by excessive ripening, fruit softening and postharvest decay, there are a wide range of horticultural produce that experience physiological disorders that contribute to postharvest losses. Further research based on hexanal technology could be conducted in an attempt to reduce such physiological disorders during storage, in an effort to reduce postharvest losses caused by these factors, in addition to enhancement of quality.

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Chapter 3: Efficacy of moringa leaf extracts, enhanced freshness formulation and carboxy methylcellulose on optimising postharvest quality of tomato fruit (*Solanum lycopersicum* L.) harvested at different maturity stages

3.1. Abstract

Tomato fruit (*Solanum lycopersicum* L.) have a relatively short postharvest life and are prone to several problems during transportation and storage, affecting their market value. This study evaluated the efficacy of enhanced freshness formulation (EFF), and carboxyl methylcellulose (CMC) infused with moringa leaf extract (MLE) on physico-chemical attributes of tomato fruit (cv. 'Star 9037'), harvested at three maturity stages: namely mature green, breaker and turning. Treatments comprised of untreated fruit and a combination of 1 % CMC + 10 % MLE, 0.02 % (v/v) EFF and 0.02 % (v/v) EFF + 10 % MLE. Fruit were then stored for 21 days in a cold room with a delivery air temperature of 11.5 ºC and (90 % RH). Thereafter, fruit were transferred to ambient conditions at 22 ± 2 ºC for 8 days, to simulate shelf life at retail. Fruit were evaluated for physico-chemical quality attributes including mass loss, total soluble solids, colour and decay. The results showed that CMC + MLE and EFF significantly ($p < 0.001$) delayed changes in quality attributes and reduced spoilage in comparison to the untreated fruit, with a mass loss of less than 10 % at the mature green and breaker stage. The treatment effects were more pronounced on the mature green stage followed by the breaker and turning stage. The present study illustrated the efficacy of these treatments in optimising the overall quality. Thus, results obtained in this study show that these treatments may be used to preserve the quality of fresh tomato fruit during the supply chain.

Keywords: *physicochemical attributes, shelf-life, decay, edible coating, colour index, hexanal*

3.2. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops in the world and the second most important vegetable in South Africa. Tomato being a climacteric fruit, exhibits a relatively short postharvest life and high perishability during the postharvest handling chain. This is largely attributed to the high rate of moisture loss during storage (Nunes and Emond, 2007). The high perishability and moisture content of tomato, subjects the fruit to problems during transportation and storage, thus limiting its marketability. In addition, the climacteric nature of tomato and the development of fungal infections is another factor which contributes to poor quality and postharvest losses. Texture, flavour and colour are important quality attributes which relate directly to the marketing value of fresh tomatoes (Liu et al., 2009).

The maturity stage at harvest has an enormous effect on storage life and nutritional composition of tomato fruit. Consumer preferences and marketing strategies such as the distance of the market, purpose of use and production area determine when tomatoes should be harvested (Ozgen et al., 2012; Abebe et al., 2017). For long-distance transportation, the fruit are harvested at the mature green to breaker stages while fruit sold locally and for immediate consumption are harvested at the fully ripe stage. The perishable nature of tomatoes, caused by several factors such as ethylene production, respiration, transpiration, membrane deterioration and postharvest diseases, lead to excessive ripening and loss of fruit quality, resulting in high economic repercussions (Guillén et al., 2006). Therefore, effective technologies to reduce postharvest decay and maintain quality of tomatoes during storage are required (González-Aguilar et al., 2009).

Phospholipase D (PLD) is the key enzyme involved in the initiation of membrane deterioration, leading to loss in firmness of tomato and reduces the shelf life. PLD binds to the membrane, initiating a cascade of catabolic reactions leading to the generation of several neutral lipids, the

accumulation of which results in the destabilization of the membrane and senescence (Paliyath and Droillard, 1992). Hexanal is a potent inhibitor of the PLD enzyme and has been proven to inhibit PLD activity (Paliyath et al., 1999; Tiwari and Paliyath, 2011). Hexanal which is a component of GRAS (generally regarded as safe) status (Paliyath and Subramanian, 2008), is a naturally occurring volatile C6 aldehyde, produced during lipid peroxidation mediated by lipoxygenase and the hydroperoxide lyases.

In addition to inhibiting PLD activity, hexanal has the capacity to effectively inhibit the ethylene signal transduction pathway (Paliyath et al., 2003; Jakubowicz et al., 2010) and possesses antifungal properties that reduce postharvest decay of fruit (Song et al., 2007; Utto et al., 2008). Thus, hexanal and hexanal formulations (comprising of hexanal and antioxidants such as α-tocopherol and ascorbic acid) known as enhanced freshness formulation (EFF), have the ability to optimise shelf life and preserve quality of fresh produce without causing adverse effects on the produce (Cheema et al., 2014).

Edible coatings are capable of enhancing fruit quality by reducing moisture loss and delaying the ripening process (Arvanitoyannis and Gorris, 1999). Edible coatings function as a semi-permeable barrier against O_2, CO_2, moisture and solute movement, thus reducing respiration, transpiration rate and oxidation reactions. Carboxy methylcellulose (CMC) is an excellent barrier for oxygen and provides strength and structural integrity (Banker, 1966) and facilitates in the retention of mass, firmness and the overall postharvest quality (Mason, 1969). *Moringa oleifera* Lam. is a widely grown tree in many tropical and subtropical countries. Moringa leaf extracts (MLE) possess antimicrobial properties against several microorganisms (John et al., 2013). The antimicrobial properties of MLE are mostly due to its active phytochemicals such as β-sitosterol, stigma sterol, kaempferol and quercetin (Maiyo et al., 2016; Talreja and Goswami, 2016; Valdez-Solana et al., 2015). Since MLE cannot adhere to the fruit surface, the incorporation of MLE with CMC makes them an ideal postharvest treatment for prolonging

shelf life and optimizing the quality of fresh produce (Tesfay and Magwaza, 2017; Tesfay et al., 2017).

This study evaluated the efficacy of enhanced freshness formulation, and carboxy methylcellulose infused with moringa leaf extracts on the maintenance of physicochemical attributes of fresh tomatoes harvested at three maturity stages.

3.3. Materials and methods

3.3.1. Fruit source

Tomato fruit (*Solanum lycopersicum* L.) cv. 'Star 9037' were harvested from TRISBN Sibani farming (Pty) Ltd, a commercial tomato farm located at Henley Dam (Latitude: 29°37′03.8″S, Longitude: 30°15′10.5″E), Pietermaritzburg, KwaZulu-Natal Province, South Africa. Fruit were harvested at three maturity stages, namely, mature green, breaker and turning. Harvested fruit were immediately transported in a ventilated vehicle to the postharvest laboratory of the University of KwaZulu-Natal, where postharvest treatments were applied, and storage experiments conducted.

3.3.2. Preparation of treatment formulations

3.3.2.1. Enhanced freshness formulation

Tomato fruit were dipped in an aqueous solution containing 0.02 % (v/v) EFF for 2.5 min, containing 2 mM hexanal. The basic ingredients of the stock formulation include 1 % (v/v) hexanal, 1 % (v/v) geraniol, 1 % (w/v) α-tocopherol, 1 % (w/v) ascorbic acid, 0.1 % (w/v) cinnamic acid, 10 % (v/v) Tween 80 dissolved in ethanol (10 % v/v). The stock solution was mixed in water (1 L to 50 L final, with hexanal concentration at 0.02 % v/v).

3.3.2.2. Carboxy methylcellulose

CMC coating was prepared by solubilising 1 g of CMC powder in 100 mL of distilled water at 75 °C under magnetic stirring for 15 min.

3.3.2.3. Plant tissue extraction

Thirty grams of moringa plant tissues (leaf extracts) were extracted with 300 mL of methanol for 24 h with constant agitation at 4 °C. Extracts were concentrated in a rotary evaporator and 20 mL of distilled water was added. Finally, crude extract was subjected to sequential liquid - liquid extraction with hexane, chloroform and finally ethyl acetate.

3.3.3. Postharvest treatments and storage

Tomato fruit were subjected to four postharvest treatments, namely, control (untreated) and 1 % CMC + 10 % MLE, 0.02 % (v/v) EFF and 0.02 % (v/v) EFF + 10 % MLE. Fruit were thereafter stored for 21 days in a cold room with temperature set at 11.5 °C and relative humidity at 90 %. Thereafter, stored fruit were transferred to ambient conditions at 22 (± 2) °C for 8 days, to simulate shelf life at retail conditions.

3.3.4. Measurement of quality attributes

3.3.4.1. Mass Loss

At regular interval of 7 days, mass of stored fruit was measured using a calibrated Mettler Toledo digital scale (± 0.01 g). Mass loss was calculated using Eq. 1:

$$\% \text{ Mass loss (ML)} = (IM - FM) / IM \times 100. \quad (1)$$

Where, ML is mass loss (%), IM, the initial mass of fruit (g) and FM, the final mass of fruit (g).

3.3.4.2. Fruit firmness

Fruit firmness was determined using a hand-held firmness tester (Bareiss, Germany). Two readings, on a scale of 100 (hard, unripe) to < 60 (ready to eat), were taken at the equatorial region of the fruit on opposite sides (Standard ISO 7619, International Organization for Standardization).

3.3.4.3. Fruit peel colour measurement

The colour of tomato fruit was assessed using a Konica Minolta Chroma meter (Model CR-400, Konica Minolta Sensing, Inc. Osaka, Japan), calibrated with a white standard tile (Y = 87.0, X = .3146, y = .3215). The brightness (L*), the green to red component (a*) and the blue to yellow value (b*) were measured and the results were expressed as the Tomato Colour Index (TCI) calculated using Eq. 2:

$(TCI = 2000a/L\ (a^2 + b^2)^{1/2})$ (Hobson et al., 1983). (2)

3.3.4.4. Total Soluble Solids (TSS) and Titratable acidity (TA)

TSS of tomato juice was determined using a desktop Refractometer (Bellingham + Stanley Ltd, Model: RFM340+, UK). Titratable acidity was determined by titrating 8 mL of tomato juice with 0.1 M NaOH to a pH value of 8.1, using a Mettler Toledo Potentiometric compact titrator (Model G20S, Greifensee, Switzerland). Titratable acidity was expressed as the percentage of citric acid equivalent on a fresh weight basis using Eq. 3:

$$\text{TA (\% citric acid)} = (0.0064 \text{ x titre (NaOH) mL})/(8 \text{ mL juice})\ x\ 100 \quad (3)$$

The ratio of TSS to TA, also known as maturity index, was calculated using Eq. 4:

$$\text{TSS to TA ratio (maturity index)} = \text{TSS/TA} \quad (4)$$

3.3.4.5. Percentage disease index (PDI)

Disease incidence was measured by assessing the number of fruit infected during the storage period. Generally, PDI was performed to examine the ability of postharvest treatment to prevent or reduce disease incidence. PDI was calculated using Eq. 5: PDI = (Number of infected fruit)/(Total number of fruits assessed) × 100. (5)

3.3.5. Statistical analysis

The data collected were subjected to the analysis of variance (ANOVA) using statistical software (GenStat 18th Edition, VSN International, Hemel Hempstead, United Kingdom). Fischer's least significant differences were calculated and used to separate means at 5 % significance level.

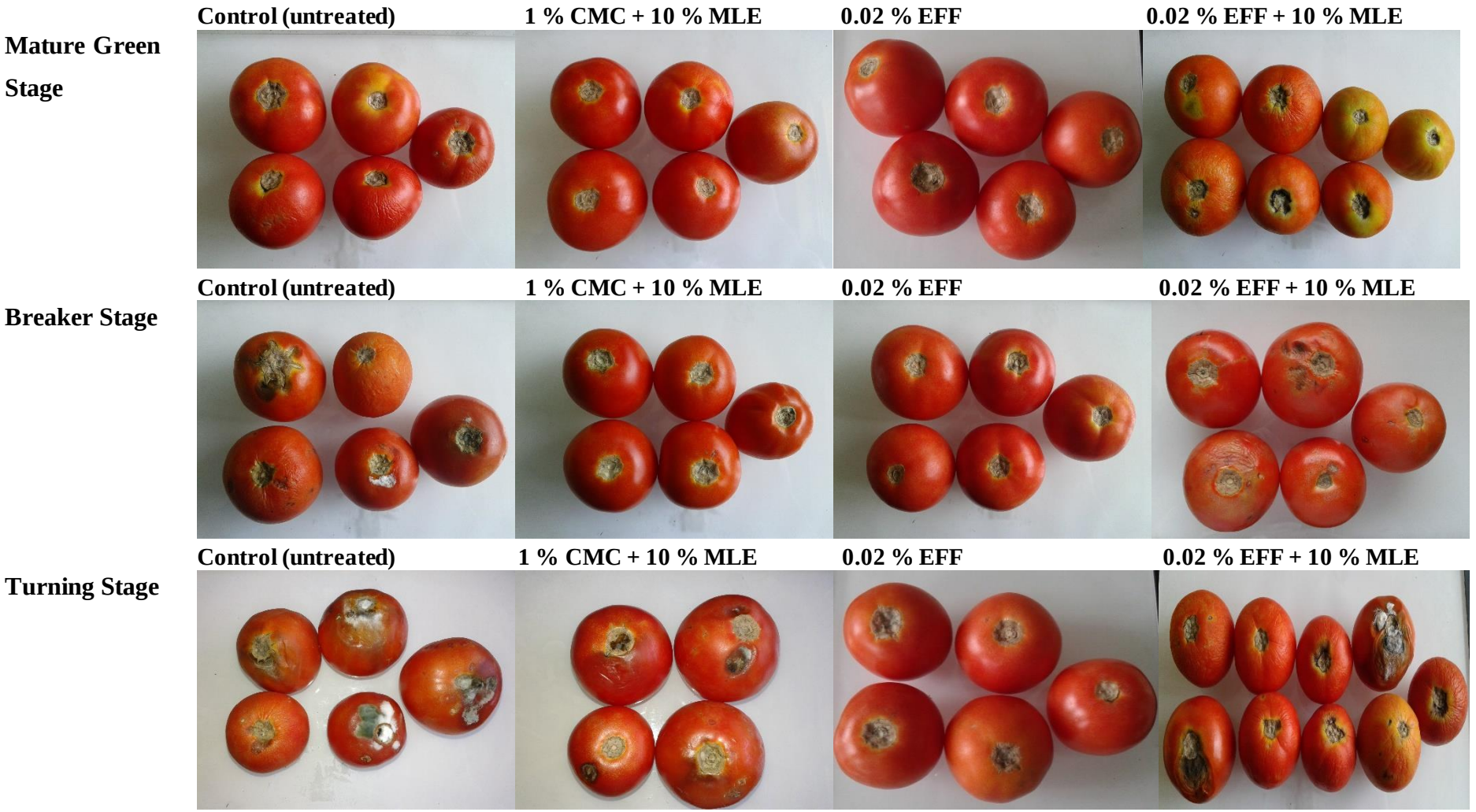

Fig 3.1. Samples of tomato fruit photographed on day 29 of storage for control, CMC + MLE and EFF treated fruit at three maturity stages. As well as day 21 of storage for EFF + MLE treated fruit at three maturity stages. Fruit were stored for 21 days at 11.5 °C and 90 % relative humidity. Thereafter, stored fruit were transferred to ambient conditions at 22 (± 2) °C for 8 days. CMC – Carboxy methylcellulose; EFF – enhanced freshness formulation; MLE – moringa leaf extracts.

3.4. Results and discussion

3.4.1. Fruit mass loss

The interaction among maturity stage and treatments had no significant effect ($p = 0.334$) on fruit mass loss (Fig. 3.2). EFF + MLE treated fruit at all three maturity stages, rapidly lost marketability (over 50 % at all maturity stages by day 21) and were very poor in quality and visual appearance, thus were terminated after 21 days of storage. The results showed that the postharvest treatments and maturity stage had a significant effect ($p < 0.001$) on mass loss (Fig. 3.2). The treatments significantly reduced mass loss at all the maturity stages, with the turning stage (Fig. 3.2c) exhibiting the greatest mass loss, followed by breaker (Fig. 3.2b) and mature green stage (Fig. 3.2a). At the mature green stage, fruit treated with CMC + MLE had the lowest mass loss followed by those treated with EFF, throughout the storage period. However, at the breaker and turning stage, EFF treated fruit exhibited the lowest mass loss after cold storage even though CMC + MLE treated fruit had lower mass loss during cold storage.

A mass loss of 10 % may render a wide range of horticultural crops unacceptable for sale and is an acceptable threshold for the quality of fresh produce (Robinson et al., 1975; Acedo, 1997). The major cause of mass loss is transpiration followed by respiration. At the end of storage, mass loss of treated fruit at the mature green and breaker stage was less than 10 %, with CMC + MLE registering the lowest mass loss of ~5 % at the mature green stage and EFF registering the lowest mass loss of ~8 % at the breaker stage. Untreated and treated fruit at the turning stage exhibited a mass loss greater than 10 %.

Our results are in accordance with previous studies, which demonstrated that the intensity of mass loss of tomatoes during storage is dependent on the maturity stage at harvest (Lu et al., 2009; Tolesa and Workneh, 2017). As fruit advance in maturity, they lose their structural integrity, leading to a decrease of the fruit's resistance to moisture loss as it advances in

maturity (Bargel and Neinhuis, 2005). A reduction in saleable mass of fresh produce is caused by moisture loss, which results in an economic loss for commodities sold by weight.

The application of EFF delays the lipogenases in fruit skin, which facilitates in delaying the ripening processes, resulting in a lower fruit mass loss (Anusuya et al., 2016). Edible coatings reduce moisture loss by sealing the cuticles and pores by functioning as a barrier against O_2, CO_2 and water vapour transmission (Baldwin et al., 1999). This leads to less O_2 availability and uptake for respiration (Abbasi et al., 2009), thus, reduced transpiration and respiration leading to a reduction in fruit mass loss. Hence, the control (untreated fruit) exhibited the highest mass loss at all stages. This may be attributed to greater transpiration and respiration rates, in comparison to the treated fruit. Our results are also in accordance with those of Qi et al. (2011) and Vyas et al. (2013) who also reported that EFF and CMC minimised mass loss of oriental sweet melons and papaya fruit, respectively. Thus, these treatments have a positive effect during long shipment periods with economic benefits.

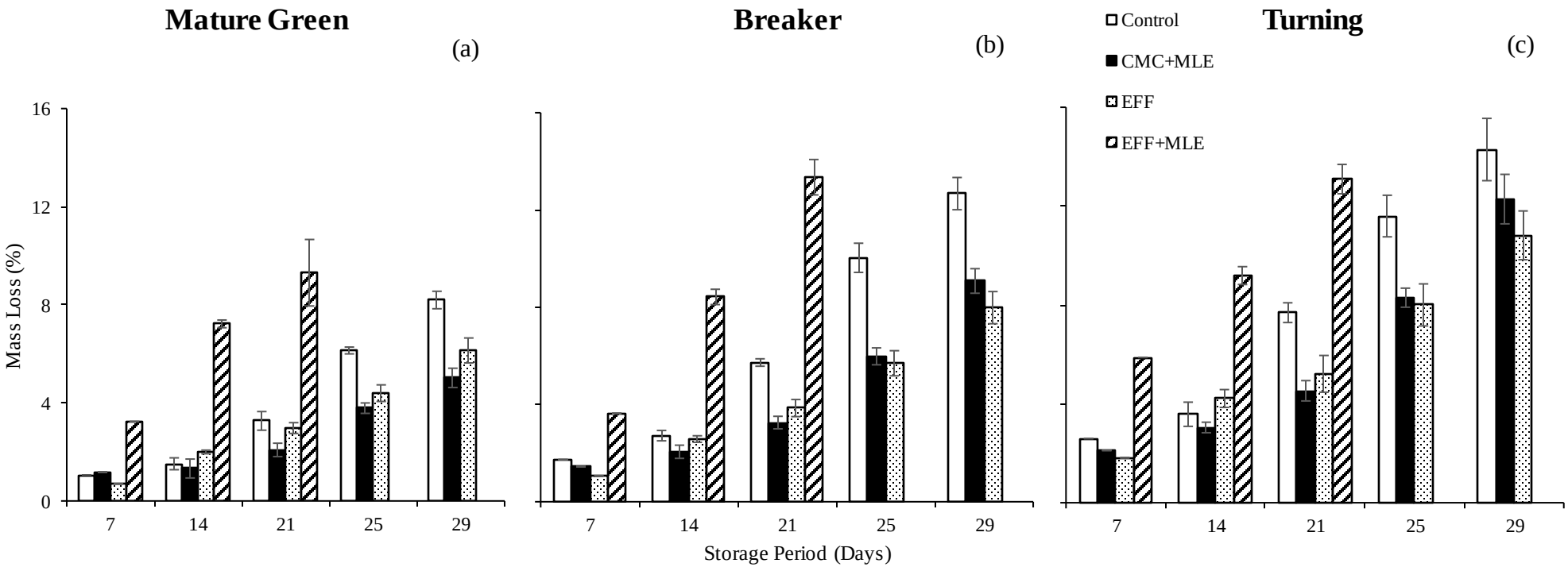

Fig. 3.2. Effect of moringa leaf extract, enhanced freshness formulation and carboxy methylcellulose on the mass loss of tomato fruit at the mature green (a), breaker (b) and turning maturity stage (c). Values are the means ± SE (n = 5).

3.4.2. Fruit firmness

The interaction among maturity stage and treatments had no significant effect ($p = 0.517$) on fruit firmness (Fig 3.3). The maturity stage and treatments had a significant effect on fruit firmness (Fig. 2; $p < 0.001$). Firmness declined during storage, with greater loss exhibited at the turning stage (Fig. 3.3c) followed by the breaker (Fig. 3.3b) and mature green stage (Fig. 3.3a). EFF and CMC + MLE were effective at firmness retention ($p < 0.001$), with greater effect at the mature green stage. In addition, EFF treated fruit registered the highest firmness whereas the untreated fruit registered the lowest firmness during storage at all three maturity stages. Gormley and Maher, (1987), reported that the minimum acceptable firmness level for tomato fruit should be 10 N at retail. At the end of storage, the EFF treatment registered firmness levels above 10 N at the mature green stage (~19 N) and breaker stage (~13 N), whereas the CMC + MLE treatment maintained a firmness level above 10 N at the mature green stage only (~15 N). Firmness levels at the turning stage dropped below 10 N for all treatments.

Pectin substances are responsible for the cohesiveness of the fruit and degradation of cellular material and degradation of pectin results in softening of fruit (Gross and Wallner, 1979). During the fruit ripening process, de-polymerisation (shortening of pectin and other pectic substances) occurs with an increase in pectin-esterase and poly-galactronase activities (Maftoonazad and Ramaswamy, 2005). At elevated CO_2 and low O_2 levels within the fruit, the activity of these enzymes is reduced, which results in enhanced firmness retention of fruit (Salunkhe et al., 1991).

The ability of an edible coating to decrease the internal O_2 and increase the CO_2 concentration allows for firmness retention by retarding the degradation of insoluble pectin and proto-pectin, which are some of the components responsible for the structural rigidity of the fruit (Maftoonazad and Ramaswamy, 2005). In addition, EFF treatment down regulates transcript

levels of polygalacturonase involved in pectin degradation, which facilitates in firmness retention of fruit during postharvest storage (Tiwari and Paliyath, 2011). Our results are in agreement with those reported by Khaliq et al. (2016) of chitosan treated mango fruit and Cheema et al., (2018) of EFF treated sweet bell pepper.

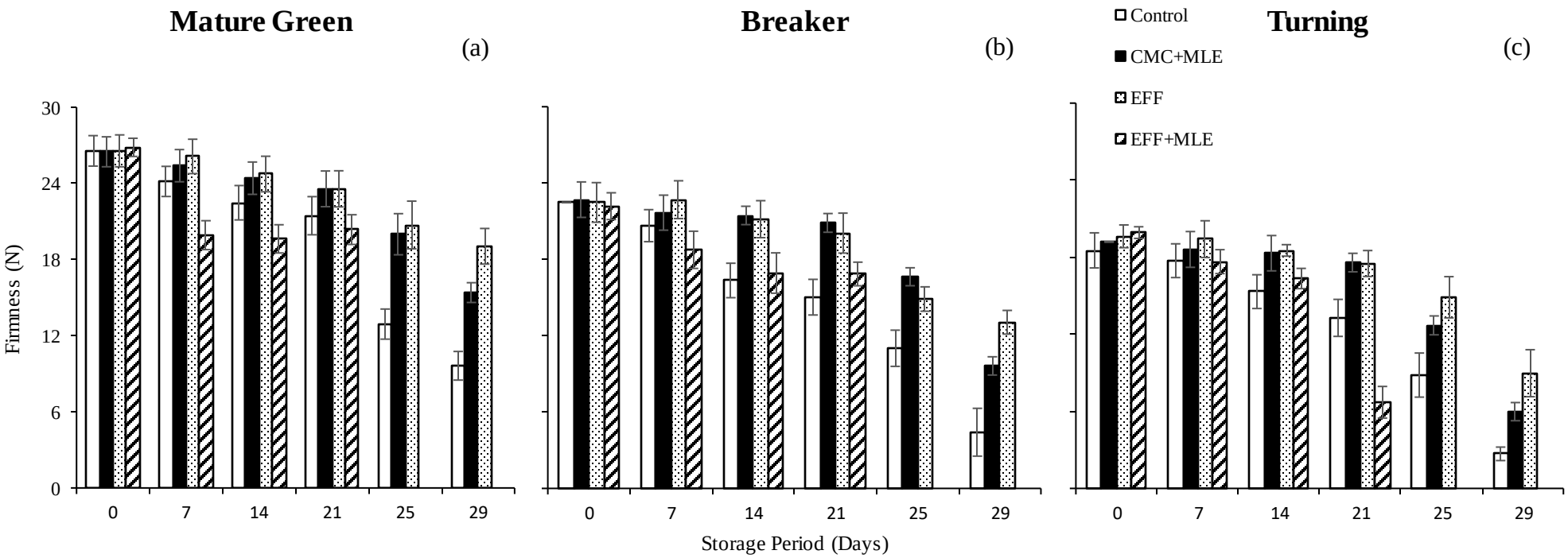

Fig. 3.3. Effect of moringa leaf extract, enhanced freshness formulation and carboxy methylcellulose on the firmness of tomato fruit at the mature green (a), breaker (b) and turning maturity stage (c). Values are the means ± SE (n = 5).

3.4.3. Colour Index

The interaction among maturity stage and treatments had no significant effect ($p = 0.124$) on the colour index (Fig 3.4). The maturity stage and treatments had a significant effect (Fig. 3.4; $p < 0.001$) on colour index throughout the storage period. The colour index increased with increasing storage duration, with control fruit showing a maximum increase. The turning stage (Fig. 3.4c) exhibited the maximum colour index followed by the breaker (Fig. 3.4b) and mature green stage (Fig. 3.4a). CMC + MLE and EFF significantly ($p < 0.05$) delayed colour change, with greater effect at the mature green stage. CMC + MLE registered the lowest colour index at the breaker and turning stage, except for the mature green stage, where EFF registered a lower colour index relative to CMC + MLE.

At the breaker and turning stage, the colour index of treated fruit was similar to the untreated fruit at the end of the storage period, which depicts that the treatments did not have a negative effect on colour development. CMC + MLE and EFF treatment did not impair colour development; hence, the treated tomato fruit were able to reach their full colour potential. At the mature green stage, colour index of CMC + MLE and EFF treated fruit was ~21 % and ~27 % lower than the control, respectively. At the breaker stage, colour index of CMC + MLE and EFF treated fruit was ~2 % lower and ~2 % higher than the control, respectively. At the turning stage, colour index of CMC + MLE and EFF treated fruit was ~8 % and ~6 % lower than the control, respectively.

The comparisons above refer to day 29 of storage. Regardless of the lower values, the visual appearance of these fruit was fully red in colour. Our results are in agreement with those of Athmaselvi et al. (2013) and Cheema et al. (2014), who reported that aloe vera based edible coating and EFF treatment delayed the colour change of ripening tomato fruit, respectively.

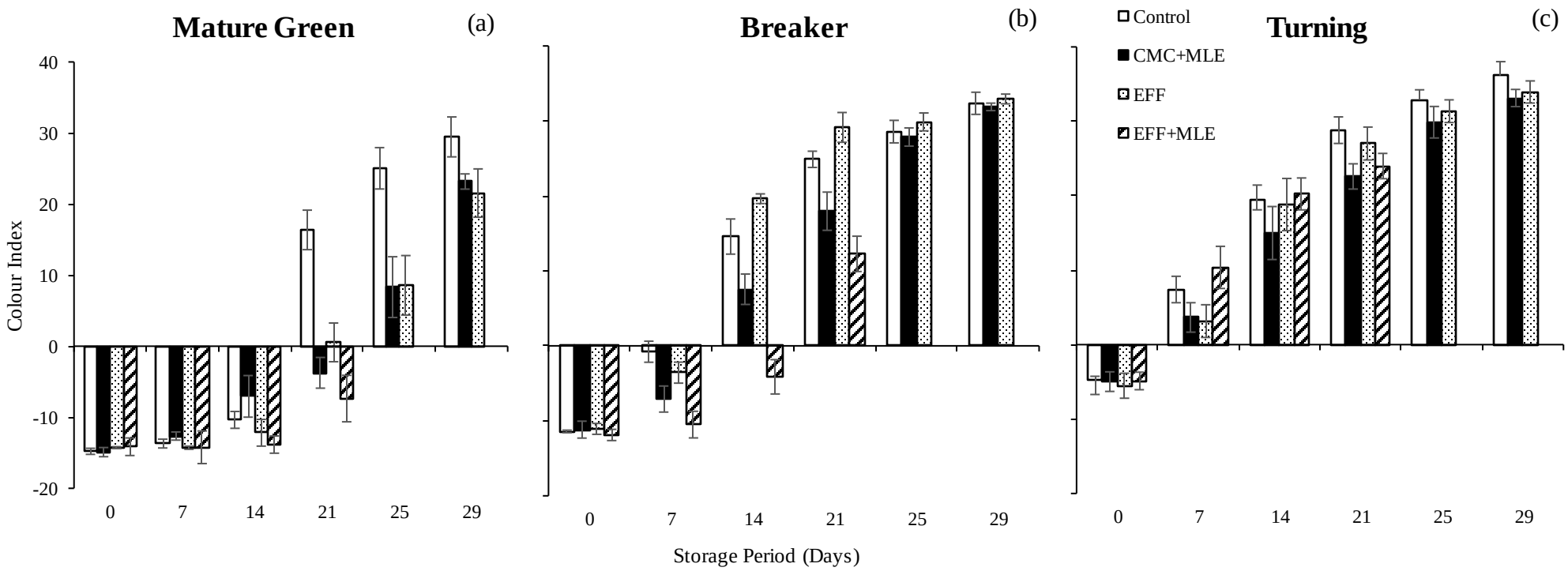

Fig. 3.4. Effect of moringa leaf extract, enhanced freshness formulation and carboxy methylcellulose on the colour index of tomato fruit at the mature green (a), breaker (b) and turning maturity stage (c). Values are the means ± SE (n = 5).

3.4.4. Total soluble solids (TSS)

The interaction among maturity stage and treatments had a significant effect ($p < 0.05$) on TSS (Fig 3.5). The maturity stage had a significant effect (Fig. 3.5; $p < 0.001$) on TSS accumulation throughout the storage period. The treatments significantly ($p < 0.001$) reduced the accumulation of TSS in comparison to the control at all maturity stages, with greater effect at the mature green stage (Fig. 3.5a) followed by the breaker (Fig. 3.5b) and turning stage (Fig. 3.5c). EFF treated fruit registered lower TSS values relative to CMC + MLE treated fruit at the breaker and turning stage, except for the mature green stage, where CMC + MLE treated fruited registered lower TSS values relative to the EFF treated fruit.

At the mature green stage, TSS for control fruit showed a rise in TSS followed by a decline, whereas the TSS of treated fruit did not decline during storage. Untreated and treated fruit at the breaker and turning stages, showed a rise followed by a decline in TSS. However, TSS of treated fruit registered a higher TSS than untreated fruit. However, this was not the case at the breaker stage for EFF treated fruit. The increase in TSS of fruit is due to the increase of soluble sugars (Cheour et al., 1990) caused by the hydrolysis of carbohydrates to soluble sugars such as glucose and fructose (Nath et al., 2012; Waskar et al., 1999). With increased storage, these sugars are used up to maintain growth and senescence, leading to a decline in TSS after the peak rise.

Treated fruit had a lower TSS accumulation, which may be attributed to the reduced activity of the enzymes invertases, sucrose synthase and fructokinase, involved in the hydrolysis of stored carbohydrates into soluble sugars. In addition, an increase in TSS could also be due to moisture loss, which increases the concentration of soluble sugars (Waskar et al., 1999; Nath et al., 2012). It is important to note that the treated fruit had higher TSS at the end of storage in comparison to the control. This may be due to a slower rate of senescence in comparison to the untreated fruit.

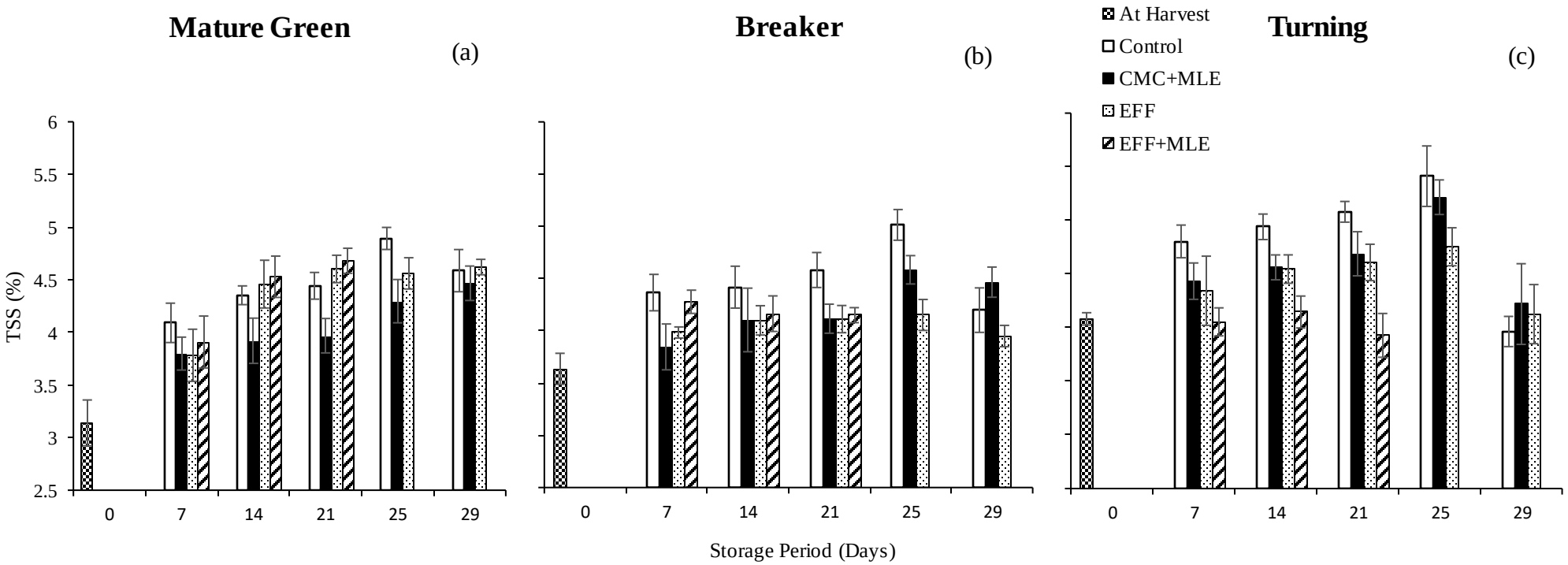

Fig. 3.5. Effect of moringa leaf extract, enhanced freshness formulation and carboxy methylcellulose on the total soluble solids (TSS) of tomato fruit at the mature green (a), breaker (b) and turning maturity stage (c). Values are the means ± SE (n = 5).

3.4.5. Titratable acidity (TA)

Acidity plays an important role in determining tomato fruit flavour. The interaction among maturity stage and treatments had no significant effect (p = 0.960) on TA (Fig 3.6). The findings revealed that the effect of maturity stage and treatments was significant (Fig. 3.6 *;* $p < 0.001$). Treated fruit efficiently ($p < 0.001$) retained TA during storage, with greater effect at the mature green stage (Fig. 3.6a) followed by the breaker (Fig. 3.6b) and turning stage (Fig. 3.6c), relative to the control. This may be due to the slower rate of hydrolysis of organic acids (Bico et al., 2009). A high TA loss is indicative of high respiration rate (Lurie and Klein, 1990), as citric and malic acid (which contribute to the taste of tomato fruit) are the primary substrates for respiration (Yaman and Bayoındırlı, 2002).

At the mature green stage, CMC + MLE and EFF treated fruit, registered ~13 % and ~9 % higher titratable acidity, respectively, relative to the control. At the breaker stage, CMC + MLE and EFF treated fruit, registered ~10 % and ~6 % higher titratable acidity, respectively, relative to the control. At the turning stage, CMC + MLE and EFF treated fruit registered ~11 % and ~8 % higher titratable acidity, respectively, relative to the control.

The quantitative comparisons reported above refer to day 29 of storage. Regardless of the treated fruit registering higher titratable acidity at the end of storage, no significant differences were observed between treatments at all three maturity stages. However, at the end of storage, significant differences ($p < 0.001$) between maturity stages was observed, with the mature green stage registering higher TA, followed by the breaker and turning stage.

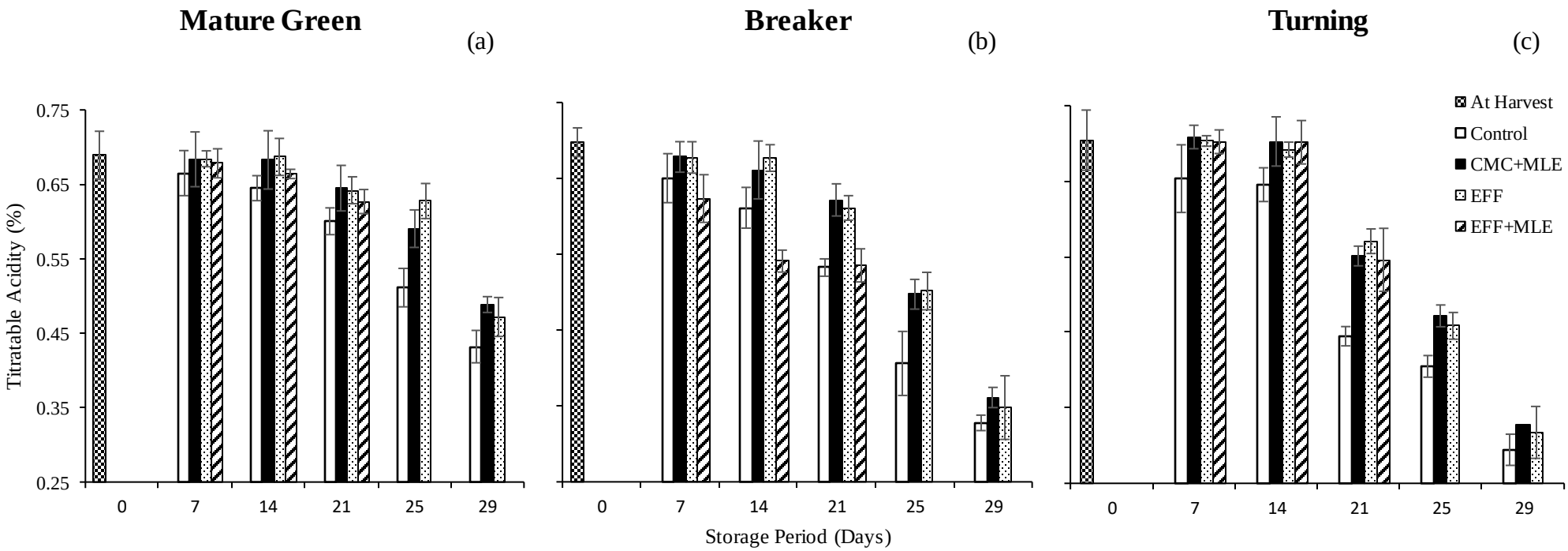

Fig. 3.6. Effect of moringa leaf extract, enhanced freshness formulation and carboxy methylcellulose on titratable acidity (TA) of tomato fruit at the mature green (a), breaker (b) and turning maturity stage (c). Values are the means ± SE (n = 5).

3.4.6. Maturity index (TSS:TA)

The interaction among maturity stage and treatments had no significant effect ($p = 0.757$) on TSS:TA (Fig 3.7). TSS:TA ratio is a useful and important indicator of tomato taste (Suarez et al., 2008) and fruit quality. The maturity stage and treatments had a significant effect (Fig. 3.7; $p < 0.001$) on maturity. The CMC + MLE and EFF treated fruit significantly ($p < 0.001$) delayed the maturation of treated fruit, indicative of better-quality maintenance during storage.

At the mature green stage, CMC + MLE and EFF treated fruit, registered ~14 % and ~7 % lower TSS:TA ratio, respectively, relative to the control. At the breaker stage, CMC + MLE and EFF treated fruit, registered ~3 % and ~7 % lower TSS:TA ratio, respectively, relative to the control. At the turning stage, CMC + MLE and EFF treated fruit registered ~7 % and ~3 % lower TSS:TA ratio, respectively, relative to the control. The quantitative comparisons reported above refer to day 29 of storage.

Our results for TSS, TA and the TSS:TA ratio are in accordance with those of Gill et al. (2015), who demonstrated the efficacy of EFF on reducing TSS accumulation and retaining TA of guava fruit during storage. Abebe et al. (2017), also reported that chitosan reduced the increase of TSS and retained TA of treated tomato at the mature green, turning and light red stage.

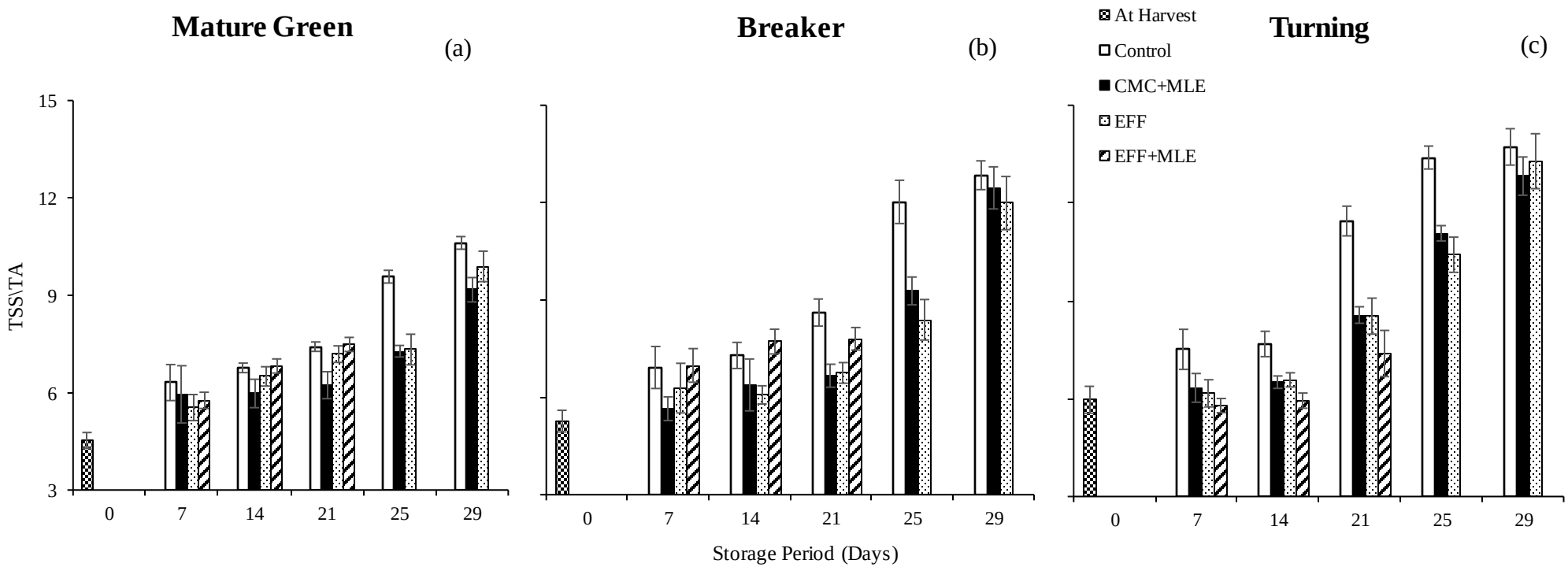

Fig. 3.7. Effect of moringa leaf extract, enhanced freshness formulation and carboxy methylcellulose on the TSS:TA ratio of tomato fruit at the mature green (a), breaker (b) and turning maturity stage (c). Values are the means ± SE (n = 5).

3.4.7. Percentage disease index (PDI)

The EFF and CMC + MLE treatments significantly ($p < 0.001$) reduced postharvest decay, in comparison to the control fruit (Table 3.1) at all the maturity stages, with greater effect at the mature green stage, followed by the breaker and turning stage. At the mature green stage, the CMC + MLE, EFF and EFF + MLE treated fruit registered ~6 %, ~19 % and ~8 % lower PDI, relative to the control, respectively. At the breaker stage, the CMC + MLE, EFF and EFF + MLE treated fruit registered ~15 %, ~38 % and ~24 % lower PDI, relative to the control, respectively. At the turning stage, the CMC + MLE and EFF treated fruit registered ~5 % and ~42 % lower PDI, relative to the control, respectively. The EFF + MLE treated fruit registered ~28 % higher PDI, relative to the control. The quantitative comparisons reported above refer to day 29 of storage.

These findings are in agreement with those of Tesfay et al. (2017), who reported lower decay incidence in avocado fruit coated with CMC + MLE as well as Thavong et al. (2010) who reported that hexanal effectively suppressed decay of longan fruit. Hexanal possesses antifungal properties that suppress postharvest decay. Jabeen et al. (2008) indicated that the antimicrobial activity of moringa is due to the occurrence of lipophilic compounds which permeabilize the fungal cell membrane, thereby inhibiting microbial growth.

Table 3.1: Effect of moringa leaf extract, enhanced freshness formulation and carboxy methylcellulose on tomato fruit decay (%) during storage at three maturity stages.

Treatments	Fruit decay incidence (%)				
	Storage time (days)				
	7	14	21	25	29
MG Control	1.96±0.98^{a}	3.92±0.98^{ab}	5.88±1.70^{bcd}	9.80±1.96^{cd}	18.63±1.55^{bc}
MG CMC+MLE	1.96±1.52^{a}	1.96±1.38^{a}	4.90±1.40^{abc}	6.86±0.76^{abc}	12.75±2.01^{ab}
MG EFF	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
MG EFF+MLE	0.00^{a}	0.00^{a}	6.67±1.76^{cd}	8.67±1.76^{bcd}	10.67±1.76^{ab}
BR Control	4.90±2.59^{a}	8.82^{b}	18.63±3.53^{f}	28.43±5.46^{f}	43.14±5.96^{d}
BR CMC+MLE	2.94±1.70^{a}	4.90±3.53^{ab}	12.75±0.98^{e}	17.65±1.70^{e}	28.43±2.59^{c}
BR EFF	0.00^{a}	0.00^{a}	0.63±0.63^{ab}	1.89^{ab}	5.03±1.26^{a}
BR EFF+MLE	0.00^{a}	5.03±2.27^{ab}	13.21±3.93^{e}	15.72±3.83^{de}	19.5±5.15^{bc}
TN Control	10.78±2.59^{b}	26.47±3.40^{c}	35.29±1.70^{g}	46.08±0.98^{h}	50.98±0.88^{d}
TN CMC+MLE	4.90±2.01^{a}	9.80±0.76^{b}	13.73±1.52^{ef}	36.27±3.04^{g}	46.08±0.76^{d}
TN EFF	0.00^{a}	0.00^{a}	2.72±1.8^{abc}	6.12±2.04^{abc}	9.52±1.36^{ab}
TN EFF+MLE	0.00^{a}	8.67±1.76^{b}	10.67±2.67^{de}	37.33±1.33^{g}	78.67±10.41^{e}
LSD ($p < 0.05$)	4.56	5.35	5.08	6.76	11.83
Significant level ($p<0.05$)					
Maturity (A)	<0.05*	<0.001**	<0.001**	<0.001**	<0.001**
Treatments (B)	<0.001**	<0.001**	<0.001**	<0.001**	<0.001**
A x B	0.134^{ns}	<0.001**	<0.001**	<0.001**	<0.001**

MG – Mature Green stage; BR – Breaker stage; TN – Turning stage; CMC + MLE – carboxymethyl cellulose and moringa leaf extract; EFF – Enhanced freshness formulation. Means with the same letters within columns are not significantly different at $p < 0.05$ using LSD. Each value is the mean ± SE for three (n = 3) replicates. ns, not significantly different. * $p < 0.05$. ** $p < 0.001$.

3.5. Conclusion

The study illustrated that the moringa based edible coating and enhanced freshness formulation optimised the postharvest quality of fresh tomato by significantly delaying changes in physicochemical attributes, compared to the control treatment. Mature green fruit exhibited the longest shelf life followed by the breaker and turning stage. The treatment comprising of enhanced freshness formulation incorporated with moringa leaf extracts had adverse effects on tomato fruit. Due to rapid deterioration and loss in quality, fruit were not marketable after 21 days of storage and were terminated thereafter.

We hypothesize that the methanol solvent containing the moringa leaf extract may have damaged the fruit surface. Thus, further research needs to investigate safer methods of extracting the phytochemicals of moringa in order to reduce or eliminate the toxic solvent while retaining the active phytochemicals.

Based on these findings, the suitable stage for treating tomato fruit with the moringa based edible coating is the mature green and breaker stage as the important quality attributes are maintained during storage. Our findings demonstrate that the EFF treatment is suitable for application at all three maturity stages, as this treatment efficiently maintained physicochemical properties and effectively suppressed decay at all maturity stages.

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Chapter 4: Effect of moringa leaf extracts, enhanced freshness formulation and carboxy methylcellulose on bioactive compounds and antioxidant attributes of tomato fruit (*Solanum lycopersicum* L.) harvested at different maturity stages

4.1. Abstract

Rapid ripening of tomato fruit (*Solanum lycopersicum* L.) during postharvest handling results in the loss of bioactive compounds, antioxidants and the overall nutritional quality. This study evaluated the efficacy of postharvest treatments of enhanced freshness formulation (EFF), and carboxyl methylcellulose (CMC) infused with moringa leaf extract (MLE) on maintaining antioxidant capacity of tomato fruit (cv. 'Star 9037'). Four treatments, namely, control (untreated fruit), 1 % CMC + 10 % MLE, 0.02 % (v/v) EFF and 0.02 % (v/v) EFF + 10 % MLE were applied to tomato fruit at three maturity stages (mature green, breaker and turning). Fruit were stored for 21 days in a cold room with a delivery air temperature of 11.5 ºC and 90 % relative humidity (RH). Thereafter, fruit were transferred to ambient conditions at 22 ± 2 ºC for 8 days, to simulate shelf life at retail conditions. Bioactive compounds including total phenolics, ascorbic acid, total flavonoids, lycopene, and β-carotene as well as the antioxidant activities using DPPH and FRAP assays were evaluated. The findings showed that CMC + MLE and EFF significantly ($p < 0.05$) maintained the concentrations of total phenolics and ascorbic acid, delayed lycopene accumulation and enhanced the antioxidant activity of tomato fruit at all maturity stages. Compared to the control treatment, EFF + MLE at the turning stage had the lowest total phenolics, ascorbic acid content and antioxidant activity. The mature green stage recorded the minimum loss of bioactive compounds, followed by the turning and breaker stage. These findings suggest that moringa based edible coating and the

enhanced freshness formulation may be used as postharvest treatments to delay ripening and improve the maintenance of nutritional quality of tomato fruit during postharvest storage and transit.

Keywords: *Phenolic content, edible coating, lycopene, hexanal, ripening stages*

4.2. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important worldwide agricultural crops, which is rich in antioxidant compounds, such as lycopene, beta carotene, phenolics, flavonoids and ascorbic acid. These bioactive compounds contribute to the antioxidant capacity of fresh tomatoes (Lenucci et al., 2006). These antioxidants have the capacity to inhibit the initiation of oxidation chain reactions, thus delaying the oxidation of lipids and certain molecules (Yahia et al., 2007). In addition, carotenoids, flavonoids and lycopene have the ability to protect cells from reactive oxygen species by detoxifying free radicals (Clinton, 1998; Spencer et al., 2005). Tomato fruit have beneficial effects on human health due to their antioxidant compounds that reduce oxidative damage in the human body. For instance, carotenoids in tomato fruit reduce the risk of certain types of cancer, arteriosclerosis and cataract formation (Sandstrom et al., 1994; Weisburger, 1998).

Lycopene exhibits the highest physical quenching rate constant with singlet oxygen (Di Mascio et al. 1989). Phenolic compounds found in plant extracts are known for reducing the risk of cardiovascular diseases in humans due to their free radical-scavenging properties (Velioglu et al., 1998). The increase in consumption of fresh fruit and vegetables and the interest in the roles of antioxidants in human health, has promoted research in the field of horticulture and food science to evaluate antioxidants of horticultural produce. As well as to determine how their content and antioxidant activity can be maintained or enhanced during postharvest storage, by improving storage and transit conditions (Ayala-Zavala et al., 2004; Tzortzakis et al., 2007).

Rapid ripening of tomato fruit during postharvest storage has been shown to lead to the reduction of bioactive compounds and the overall antioxidant activity of tomato fruit, leading to a loss in fruit quality during storage (Wang et al., 2008). As a result, the high perishable nature of tomato fruit requires postharvest technologies that can delay ripening, reduce deterioration and ultimately maintain or enhance nutritional quality and antioxidant activity of tomato fruit. The use of postharvest treatments such as edible coatings and hexanal formulations have been reported to delay ripening and deterioration of horticultural produce by enhancing nutritional quality and antioxidant activity (Qi et al., 2011; Gol et al., 2013).

The hexanal formulation known as enhanced freshness formulation (EFF), has been reported to enhance the activity of antioxidant enzymes, resulting in reduced levels of reactive oxygen species and improved quality of strawberry fruit during postharvest storage (Yuan et al., 2009). Edible coatings have also been reported to increase the antioxidant capacity of fruit by enhancing total phenolics and ascorbic acid content and optimize overall quality during storage (Baraiya et al., 2012). These treatments have the potential to prolong storage of fresh tomatoes along the supply chain by maintaining and enhancing antioxidant activity while preserving nutritional quality.

The use of treatments that have the capacity of preserving nutritional content and bioactive compounds that contribute to antioxidant activity are of importance. This is attributed to the fact that such treatments maintain the value of fresh tomatoes as well as improve its beneficial effects on human health (Pataro et al., 2015). Tomato fruit are harvested at various maturity stages. The maturity stage at which tomato fruit are harvested is regulated by consumer and market preference (Ilahy et al., 2011).

The health promoting compounds in tomato fruit is influenced by the maturity stage at harvest (Helyes et al., 2007). Therefore, the aim of this study was to determine the potential of a

moringa based edible coating (carboxy methylcellulose infused with moringa) and enhanced freshness formulation on optimising the concentration of bioactive compounds and enhancing the antioxidant activity of tomatoes harvested at three maturity stages.

4.3. Materials and methods

4.3.1. Fruit source

Tomato fruit (*Solanum lycopersicum* L.) cv. 'Star 9037' were harvested from TRISBN Sibani farming (Pty) Ltd, a commercial tomato farm located at Henley Dam (Latitude: 29°37′03.8″S, Longitude: 30°15′10.5″E), Pietermaritzburg, KwaZulu-Natal Province, South Africa. Fruit were harvested at three maturity stages, namely, mature green, breaker and turning stage. Harvested fruit were immediately transported in a ventilated vehicle to the postharvest laboratory of the University of KwaZulu-Natal, where postharvest treatments were applied, and storage experiments conducted.

4.3.2. Preparation of treatment formulations

4.3.2.1. Enhanced freshness formulation

Tomato fruit were dipped in an aqueous solution containing 0.02 % (v/v) EFF for 2.5 min, containing 2 mM hexanal. The basic ingredients of the stock formulation include 1 % (v/v) hexanal, 1 % (v/v) geraniol, 1 % (w/v) α-tocopherol, 1 % (w/v) ascorbic acid, 0.1 % (w/v) cinnamic acid, 10 % (v/v) Tween 80 dissolved in ethanol (10 % v/v). The stock solution was mixed in water (1 L to 50 L final, with hexanal concentration at 0.02 % v/v).

4.3.2.2. Carboxy methylcellulose

CMC coating was prepared by solubilising 1 g of CMC powder in 100 mL of distilled water at 75 °C under magnetic stirring for 15 min.

4.3.2.3. Plant tissue extraction

Thirty grams of moringa plant tissues (leaf extracts) were extracted with 300 mL of methanol/HCl 1 % (v/v) for 2 h with constant agitation at 4 °C. Extracts were concentrated in a rotary evaporator and 20 mL of distilled water was added. Finally, crude extract was subjected to sequential liquid - liquid extraction with hexane, chloroform and finally ethyl acetate.

4.3.3. Postharvest treatments and storage

Tomato fruit were subjected to four postharvest treatments, namely, control (untreated) and 1 % CMC + 10 % MLE, 0.02 % (v/v) EFF and 0.02 % (v/v) EFF + 10 % MLE. Fruit were thereafter stored for 21 days in a cold room with temperature set at 11.5 °C and 90 % RH. Thereafter, stored fruit were transferred to ambient conditions at 22 (± 2) °C for 8 days, to simulate shelf life at retail conditions.

4.3.4. Bioactive compounds and antioxidant activities

4.3.4.1. Total Phenolic content

Phenolic content was analysed spectrophotometrically using the modified Folin–Ciocalteu method (Singleton et al., 1999; Eberhardt et al., 2000). Each sample (2 g) was extracted with 10 mL methanol for 30 min. Thereafter, 125 µL of the diluted extract was mixed with 500 µL distilled water in a test tube followed by the addition of 125 µL of Folin-Ciocalteu reagent and allowed to stand for 3 min. Then, 1250 µL of 7 % sodium carbonate solution was added and the final volume was made up to 3 mL with distilled water. Each sample was allowed to stand for 90 min at room temperature and measured at 760 nm against the blank on a spectrophotometer (Beckman DU 650). The linear reading of standard curve was from 0 to 300 mg of gallic acid per mL. Results were expressed as mg gallic acid equivalent per kg of tomato FW (mg GAE/kg FW).

4.3.4.2. Ascorbic acid

Ascorbic acid was measured by titration using phenolindo-2,6-dichlorophenol (DCPIP) (Sood and Malhotra, 2001). Three grams of frozen fruit sample was homogenized with 10 mL of 3 % metaphosphoric acid. The fruit sample was centrifuged at 10,000 rpm for 15 min at 4 ºC. An aliquot fraction of the extract was titrated against a solution containing 50 mg of DCPIP dissolved in 150 mL of hot distilled water, containing 42 mg of sodium bicarbonate, cooled and diluted to 200 mL. Results were expressed as mg/100 g FW, using Eq. 1:

$$\text{Ascorbic acid } \left(\frac{\text{mg}}{\text{100g}}\right) = \frac{\text{titre x dye factor x volume made up x 100}}{\text{(aliquot of extract taken for estimation) x (weight of sample taken for estimation)}} \quad (1)$$

4.3.4.3. Lycopene and β-carotene

Lycopene from frozen pericarp tissues was measured by the method adapted from Fish et al., (2002). Approximately 0.6 g of tomato sample was homogenized in 5 mL of 0.05 % (w/v) BHT in acetone, 5 mL of 95 % ethanol, and 10 mL of hexane (10:5:5 v/v). The solution in glass tube under ice was covered with aluminium foil and mixed thoroughly for 15 min using an orbital shaker at 180 rpm. After 15 min of shaking, 3 mL of deionized water were added to each vial, and the samples shaken for another 5 min. Shaking was stopped, and vials were left at room temperature for 5 min to allow for phase separation. The absorbance of the hexane (upper) layer was measured in a 1 cm path length quartz cuvette at 503 nm for lycopene and 451 nm for β-carotene (Lime et al., 1957). The concentrations of lycopene and β-Carotene were determined spectrophotometrically using Eq. 2 and 3, respectively:

$$\text{Lycopene } \left(\frac{\text{mg}}{\text{kg tissue}}\right) = \frac{A_{503} \text{ x } 0.0312}{\text{kg tissue}} \quad (2)$$

$$\beta\text{-carotene (mg/100 g)} = 4.624 \text{ x } A_{451} - 3.091 \text{ x } A_{503} \quad (3)$$

4.3.4.4. Total Flavonoid content

The total flavonoid content was determined by adding 1 mL of methanolic extract of tomato into 4 mL of distilled water and 0.3 mL of 5 % sodium nitrite. After 5 min of rest, 0.3 mL of 10 % aluminium chloride was added. After 5 min, 2 mL solution of 1 M sodium hydroxide was added and was diluted with distilled water up to the final volume of 10 mL. Flavonoid concentration was estimated using the calibration curve of rutin. The absorbance was measured at 510 nm and the results were expressed as mg rutin equivalent (RE) 100 g^{-1} FW.

4.3.4.5. Antioxidant capacity

Total antioxidant capacity was measured through determining the free radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. For DPPH, tomato fruit were homogenized in methanol: water (60:40), and centrifuged at 6000 × g for 10 min. This determination was based on the method described by Odriozola-Serrano et al. (2008). Fruit extract (0.2 mL) was mixed with 2.8 mL of 60 µM ethanolic DPPH. The homogenate was shaken vigorously and kept in darkness for 30 min. The absorption of the sample at 515 nm was measured with a spectrophotometer against a blank of water. Results were expressed as percentage of DPPH radical inhibition (DPPH %) using Eq. 4:

$$\text{DPPH (\%)} = [1 - (A_{515}\ \text{sample} / A_{515}\ \text{blank})] \times 100 \quad (4)$$

Ferric Reducing Antioxidant Power (FRAP) assay was used to measure the total antioxidant capacity in tomato fruit. The FRAP assay was performed following the method described by Alothman et al. (2009). Briefly, a 40 µL aliquot of diluted fruit extract was mixed with 3.6 mL FRAP reagent and the reaction mixture was incubated at 37 ºC for 30 min. Absorbance then was determined at 593 nm against a blank of water. FRAP reagent was freshly prepared by mixing a 10 mM 2,4,6-tris (1-pyridyl)-5-triazine (TPTZ) solution in 40 mM HCl with a 20 mM $FeCl_3 \cdot 6H_2O$ solution and 0.3 M acetate buffer (pH 3.6) in the ratio of 1:1:10. A calibration

curve was prepared using an aqueous solution of ferrous sulphate $FeSO_4 \cdot 7H_2O$. The findings were expressed as µM/g FW.

4.3.5. Statistical analysis

The data were subjected to the analysis of variance (ANOVA) using the 18th version of GenStat (VSN International, Hemel Hempstead, United Kingdom). Fischer's least significant differences were calculated and used to separate means at 5% significance level.

4.4. Results and Discussion

4.4.1. Total phenolic content

The interaction among maturity stage and treatments had no significant effect ($p = 0.171$) on phenolic content (Fig. 4.1). The stage of maturity had a significant effect ($p < 0.05$) on total phenolic content throughout the storage period, with the turning stage (Fig. 4.1c) registering the highest phenolic content followed by the breaker (Fig. 4.1b) and mature green stage (Fig. 1a). The treatments significantly ($p < 0.001$) enhanced the total phenolic content relative to the control at all maturity stages, with CMC + MLE recording the highest phenolic content followed by EFF and EFF + MLE at all maturity stages. A peak in total phenolic content was observed on day 25 followed by a decrease until the end of the storage period for treated and untreated fruit at all maturity stages. However, for the untreated fruit at the turning stage, the total phenolic content peaked on day 21 followed by a decrease.

At the mature green stage, CMC + MLE, EFF and EFF + MLE treated fruit, registered ~46 %, ~37 % and ~29 % higher total phenolic content, respectively, relative to the control. At the breaker stage, CMC + MLE, EFF and EFF + MLE treated fruit, registered ~6 %, ~10 % and ~3 % higher total phenolic content, respectively, relative to the control. At the turning stage, CMC + MLE and EFF treated fruit registered ~27 %, ~31 % higher total phenolic content, respectively, relative to the control. The total phenolic content of EFF + MLE treated fruit was

~24 % lower relative to the control. The quantitative comparisons reported above refer to day 29 of storage.

Our results are in accordance with those of Ali et al. (2013), who demonstrated the ability of gum arabic edible coating to enhance total phenolic content of tomato fruit and Abebe et al. (2017) who reported that chitosan enhanced phenolic content of tomato fruit at the mature green and turning stage. As well as Gol et al. (2013) who demonstrated the efficacy of chitosan in reducing the decrease in phenolic content during storage. Results obtained for the EFF treatment are in accordance with those of Gill et al. (2015), who illustrated the efficacy of EFF to reduce the decrease in phenolic content of guava fruit. In addition, our results are in disagreement with those of Sharma et al. (2010), who reported that EFF treatment had no major effects on phenolic content of sweet cherry.

Phenolic compounds have the capacity to provide cells with protection against oxidative damage by scavenging free radicals, thus providing beneficial effects for horticultural produce (Wada and Ou, 2002; Chun et al., 2003). Phenylalanine ammonia-lyase (PAL) is an enzyme that uses phenylalanine to synthesise phenolic compounds (Ke and Salveit, 1989). Previous studies have reported that low O_2 and high CO_2 levels increase the activity of the PAL enzyme, resulting in enhanced production of phenolic compounds (Wu and Lin, 2002; Odriozola-Serrano et al., 2008).

Edible coatings limit gaseous exchange by forming a semi-permeable barrier on the fruit, thus modifies the endogenous O_2 and CO_2 (El Ghaouth et al., 1991). Edible coatings have the ability to reduce internal O_2 levels and increase internal CO_2 levels, leading to an increase in the activity of PAL (Romanazzi et al. 2002), resulting in enhanced production of phenolic compounds (Oms-Oliu et al., 2008).

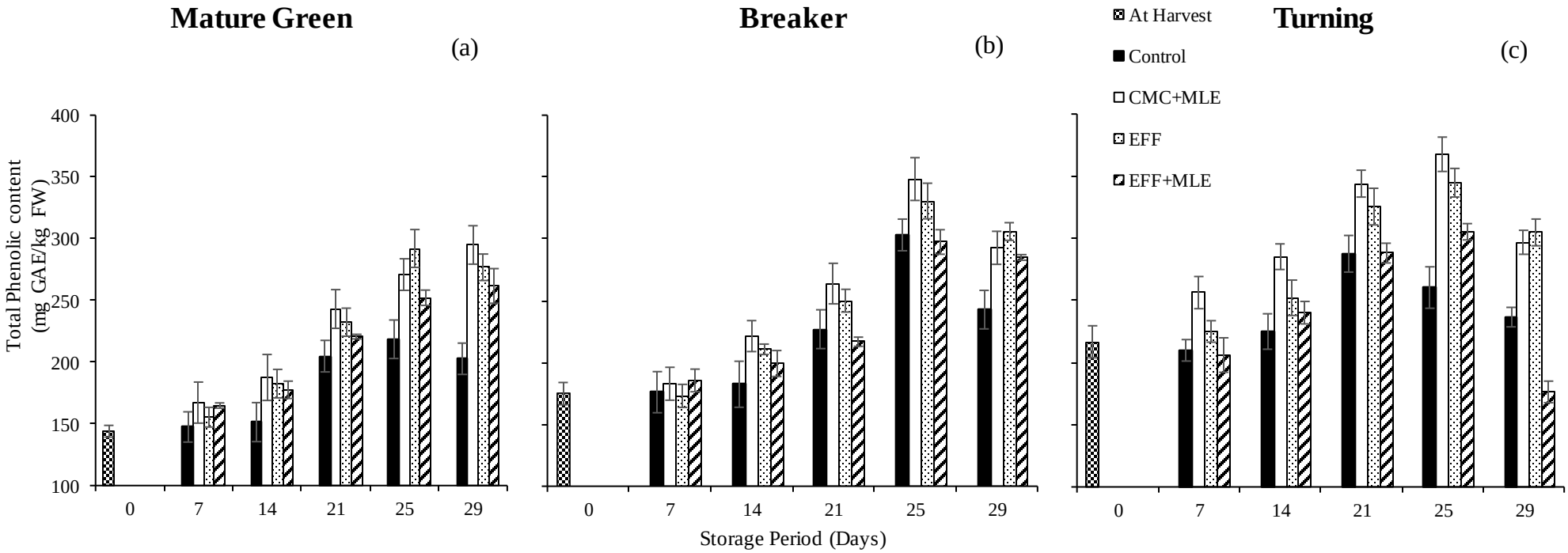

Fig. 4.1. Effect of moringa leaf extract, enhanced freshness formulation and carboxy methylcellulose on the total phenolic content of tomato fruit harvested at the mature green (a), breaker (b) and turning maturity stage (c). Values are the means ± SE (n = 3).

4.4.2. Ascorbic acid

The interaction among maturity stage and treatments had a significant effect ($p < 0.001$) on ascorbic acid content (Fig. 4.2). The turning stage (Fig. 4.2c) exhibited the greatest ascorbic acid content, followed by the breaker (Fig. 4.2b) and the mature green stage (Fig. 4.2a). The untreated fruit registered higher ascorbic acid content relative to the treated fruit, followed by a decrease after day 21 at all the maturity stages. The CMC + MLE and EFF treatments significantly ($p < 0.001$) enhanced ascorbic acid synthesis and retention, at all maturity stages relative to the control and EFF + MLE, after day 21 of the storage period. Tomato fruit showed an increase in ascorbic acid content followed by a decline.

The ascorbic acid content of untreated and EFF + MLE treated fruit declined after day 21 at all maturity stages, whereas ascorbic acid content of CMC + MLE and EFF treated fruit declined after day 25 of storage. At the turning stage, the EFF + MLE treated fruit had the lowest ascorbic acid content at the end of storage. At the end of storage, the CMC + MLE and EFF treatments registered significantly ($p < 0.001$) higher ascorbic acid content, relative to the untreated and EFF + MLE treated fruit. These results demonstrate the efficacy of CMC + MLE and EFF treatment at retaining ascorbic acid of tomato fruit. Thus, making them suitable treatments for optimising ascorbic acid concentrations of tomato fruit during postharvest storage along the supply value-chain.

At the mature green stage, CMC + MLE, EFF and EFF + MLE treated fruit, registered ~44 %, ~54 % and ~17 % higher ascorbic acid content, respectively, relative to the control. At the breaker stage, CMC + MLE, EFF and EFF + MLE treated fruit, registered ~67 %, ~73 % and ~10 % higher ascorbic acid content, respectively, relative to the control. At the turning stage, CMC + MLE and EFF treated fruit registered ~29 % and ~53 % higher ascorbic acid content, respectively, relative to the control. The ascorbic acid content of EFF + MLE treated fruit was

~37 % lower, relative to the control. The quantitative comparisons reported above refer to day 29 of storage.

Results obtained in this study are in agreement with those of Baraiya et al. (2012) and Vyas et al. (2013), who demonstrated that CMC reduced ascorbic acid synthesis, delayed the decrease in ascorbic acid content and maintained higher ascorbic acid content at the end of storage, relative to untreated tomato and papaya fruit, respectively. However, our results are in disagreement with those reported by Ali et al. (2010), where ascorbic acid content of gum arabic treated tomato fruit were lower than that of the untreated fruit at the end of storage. The contradicting results may be due to a difference in cultivars. In addition, results obtained in this study are in accordance with those of Cheema et al. (2014), who demonstrated the efficacy of EFF to retain ascorbic acid of tomato fruit during storage, registering ascorbic acid content approximately 141% higher than that of untreated fruit at the end of storage.

Ascorbic acid is a low molecular weight metabolite, which serves as an antioxidant agent in plants. The role of ascorbic acid in cells is to reduce H_2O_2 and reacts rapidly with radical species to preserve cell integrity against reactive oxygen species (Davey et al., 2000; Kleszczewska, 2000; Mittler, 2002). Ascorbate oxidase is involved in degradation of ascorbic acid in the presence of oxygen (Saari et al., 1995). The semi-permeable membrane formed by an edible coating, may slow down the synthesis and delay oxidation of ascorbic acid content, by regulating oxygen diffusion, thus reducing availability of oxygen for oxidation of ascorbic acid.

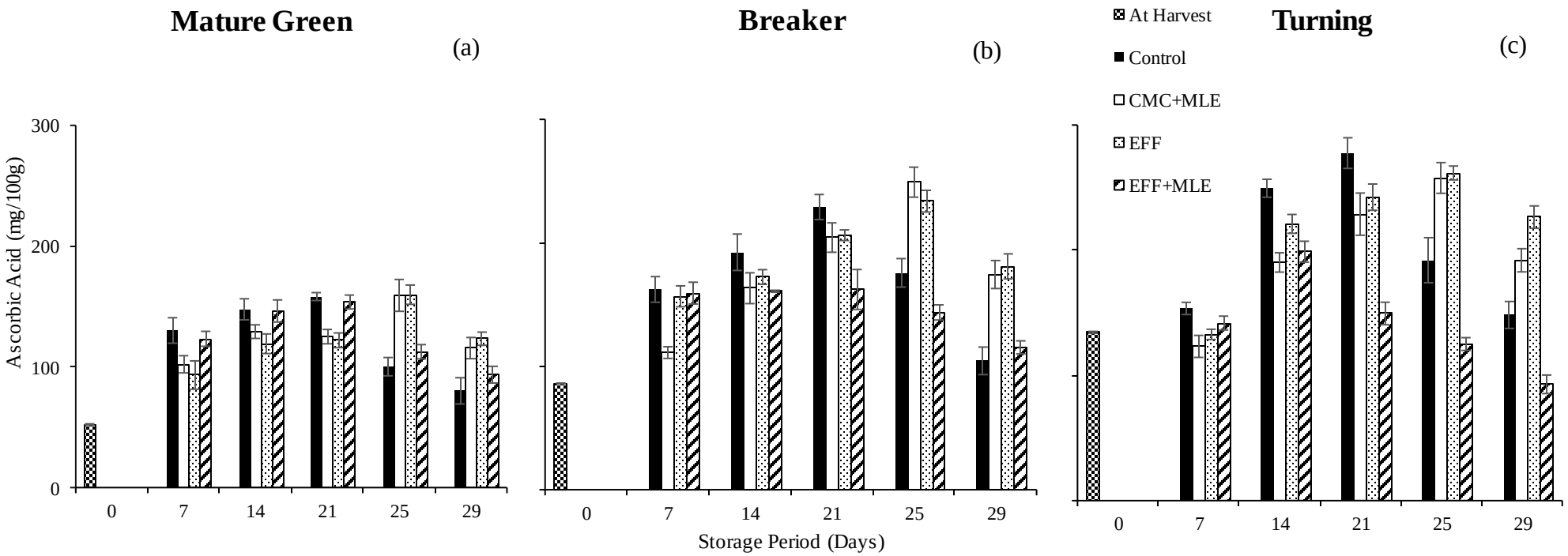

Fig. 4.2. Effect of moringa leaf extract, enhanced freshness formulation and carboxy methylcellulose on the total ascorbic acid content of tomato fruit harvested at the mature green (a), breaker (b) and turning maturity stage (c). Values are the means ± SE (n = 3).

4.4.3. Lycopene and β-carotene

The interaction among maturity stage and treatments had no significant effect ($p = 0.071$) on lycopene and β-carotene content ($p = 0.748$). The maturity stage significantly ($p < 0.001$) influenced the development of lycopene (Fig. 4.3) and β-carotene (Fig. 4.4) during storage. The turning stage (Fig. 4.3c and 4.4c) registered the highest lycopene and β-carotene content followed by the breaker (Fig. 4.3b and 4.4b) and mature green stage (Fig. 4.3a and 4.4a). The treatments significantly ($p < 0.001$) influenced the development of β-carotene at all three maturity stages, with EFF + MLE registering the lowest β-carotene content, followed by EFF and CMC + MLE at the breaker and turning stage at the end of storage, relative to the control. However, at the mature green stage, EFF +MLE registered the lowest β-carotene content followed by CMC + MLE and EFF treatment at the end of storage, relative to the control. At the end of storage, the β-carotene content of the treated fruit was significantly lower at all maturity stages, relative to the control.

From day 21, treatments had a significant effect ($p < 0.05$) on lycopene development. CMC + MLE recorded the lowest lycopene content at the mature green and breaker stage, whereas EFF + MLE registered the lowest lycopene content at the turning stage, at the end of storage. On day 29, there was no significant difference between treatments at the mature green stage, whereas lycopene content at the breaker and turning stage was significantly lower ($p < 0.05$) relative to the control. At the end of storage, untreated and treated fruit from all three maturity stages developed full red colour.

At the mature green stage, CMC + MLE, EFF and EFF + MLE treated fruit registered ~16 %, ~15 % and ~16 % lower lycopene content, respectively, relative to the control. At the breaker stage, CMC + MLE, EFF and EFF + MLE treated fruit registered ~22 %, ~11 % and ~32 % lower lycopene content, respectively, relative to the control. At the turning stage, CMC + MLE, EFF and EFF + MLE treated fruit registered ~24 %, ~19 % and ~43 % lower lycopene content,

respectively, relative to the control. The quantitative comparisons reported above refer to day 29 of storage.

These findings are in accordance with those of Baraiya et al. (2012), Ali et al. (2013), Tiwari and Paliyath, (2011) and Pak Dek et al., (2018), who demonstrated the efficacy of CMC, gum arabic edible coating, EFF and hexanal, respectively, in delaying the production of lycopene and β-carotene of tomato fruit. Control fruit ripened at a quicker rate than their treated counterparts, thus exhibiting higher production of lycopene and β-carotene relative to treated fruit. As tomato fruit ripen, chloroplasts are converted to chromoplasts and lycopene accumulates in the internal membrane system (Khudairi, 1972; Grierson and Kader, 1986). In addition, red colour development of tomato fruit occurs when sesquiterpene cyclase is inhibited. Initiation of ripening reduces sesquiterpene cyclase levels, leading to the accumulation of lycopene and red colour (Ronen et al., 1999; Srivastava and Handa, 2005). Therefore, a delay in lycopene development in the treated fruit is indicative of a slower rate of ripening and better-quality maintenance.

Lycopene and β-carotene comprise of approximately 78 and 7 % of the total carotenoid content of tomatoes, respectively (Rao et al., 1998). Carotenoids provide protection against some types of cancer, which may be owed to their antioxidant properties. In addition, β-carotene may also promote health through its pro-vitamin A activity. The consumption of lycopene has previously been demonstrated to be inversely associated with cardiovascular disease and coronary heart disease incidence (Jacques et al., 2013).

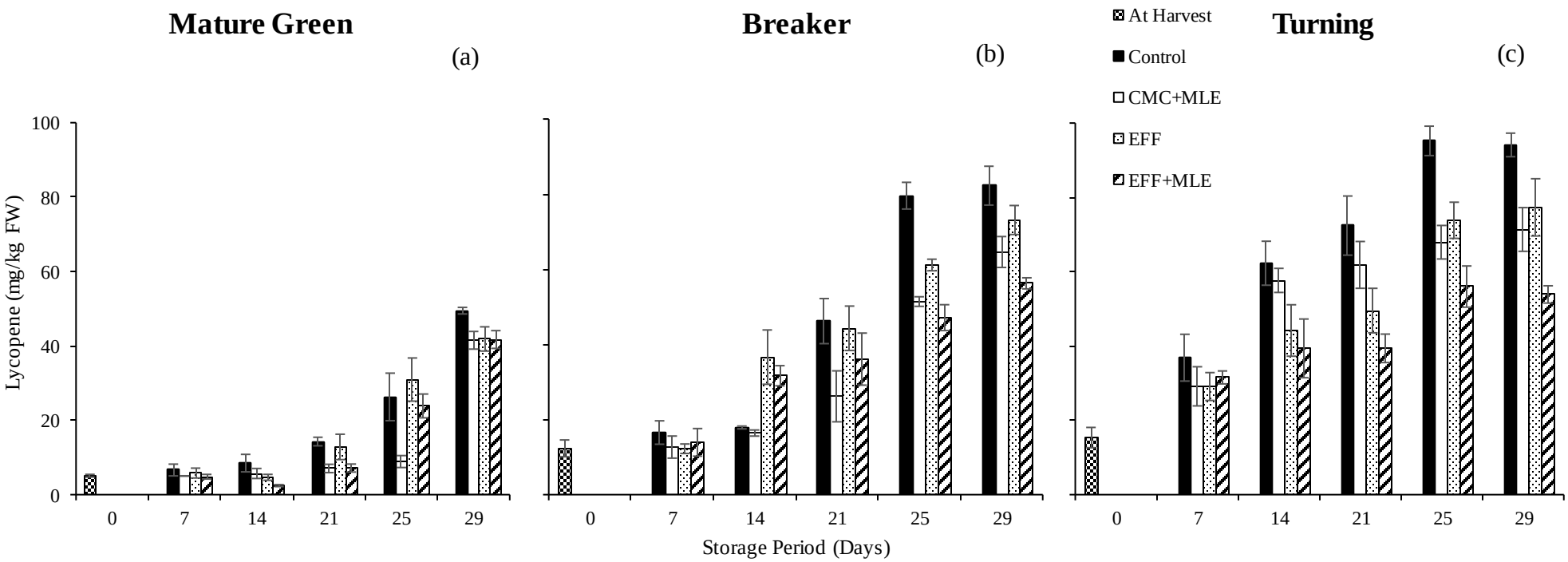

Fig. 4.3. Effect of moringa leaf extract, enhanced freshness formulation and carboxy methylcellulose on the lycopene content of tomato fruit harvested at the mature green (a), breaker (b) and turning maturity stage (c). Values are the means ± SE (n = 3).

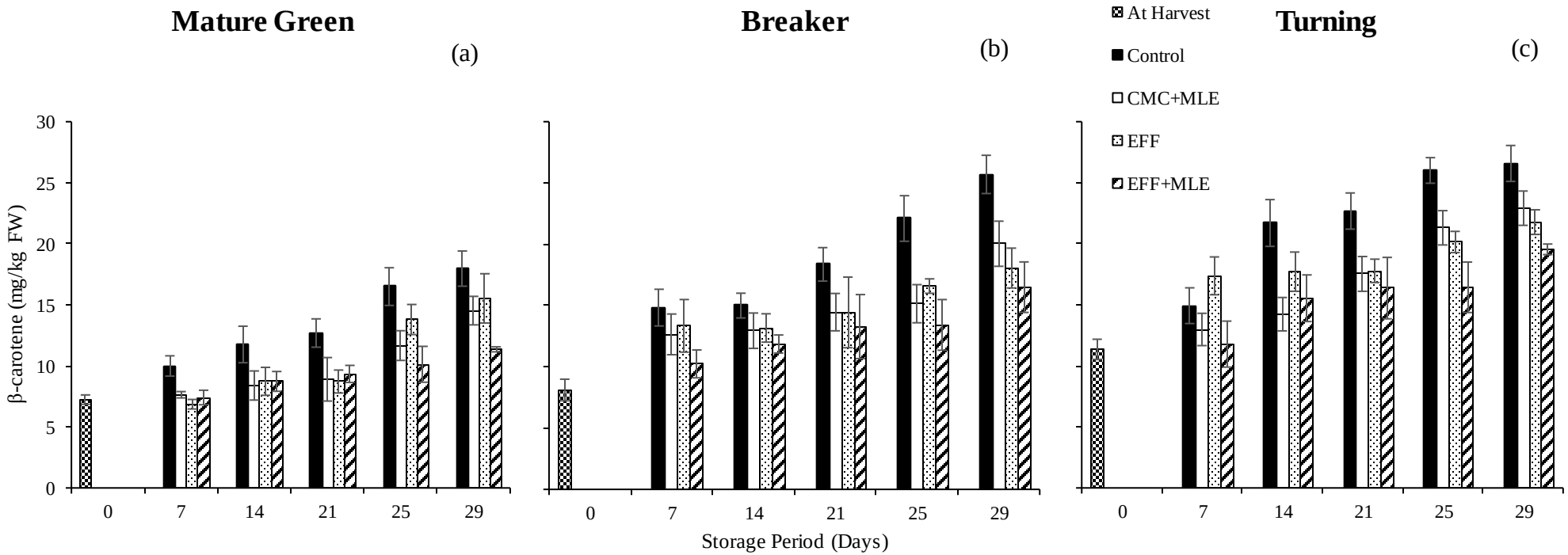

Fig. 4.4. Effect of moringa leaf extract, enhanced freshness formulation and carboxy methylcellulose on the β-carotene content of tomato fruit harvested at the mature green (a), breaker (b) and turning maturity stage (c). Values are the means ± SE (n = 3).

4.4.4. Total flavonoid content

The interaction among maturity stage and treatments had no significant effect ($p = 0.068$) on flavonoid content. The maturity stage had a significant ($p < 0.05$) effect on the total flavonoid content (Fig. 4.5), with the turning stage (Fig. 4.5c) exhibiting the greatest flavonoid content, followed by the breaker (Fig. 4.5b) and mature green stage (Fig. 4.5a). The postharvest treatments significantly ($p < 0.05$) influenced flavonoid content, with CMC + MLE treated fruit exhibiting lower flavonoid content relative to the control, EFF and EFF + MLE treatment at the end of the storage period. At the turning stage, CMC + MLE registered higher flavonoid content relative to EFF + MLE but differences were not significant.

CMC + MLE treated fruit registered ~4 % lower flavonoid content at both the mature green and breaker stage and ~9 % lower flavonoid content, relative to the control at the turning stage. EFF and EFF + MLE treated fruit, registered similar flavonoid content relative to the control, at the mature green, breaker and turning stage. The results obtained in this study are similar to those reported by Robles-Sánchez et al. (2013), Davila-Avina et al. (2014) and Guerreiro et al. (2017), who demonstrated that fresh cut mangoes, tomato and fresh cut apples treated with edible coatings, recorded lower flavonoid content relative to the untreated fruit. Sharma et al. (2010) reported no significant effect on flavonoid content of EFF treated sweet cherries. Similar results were observed in our study for EFF treated tomatoes.

Flavonoids possess free radical scavenging activity and are associated with reduced risk of certain types of cancer (Shahidi and Wanasundara, 1992; Hertog, 1994). The flavonol quercetin-rutinoside (rutin), accumulates in ripening tomato peel (Verhoeyen et al., 2002). In addition, analysis of flavonol accumulation during fruit ripening, demonstrated that flavonols are formed with colouring of the fruit (Muir et al., 2001). Therefore, we hypothesise that the lower flavonoid content exhibited by CMC + MLE treated fruit may be due to a delay in ripening, thus lower accumulation of flavonoid content.

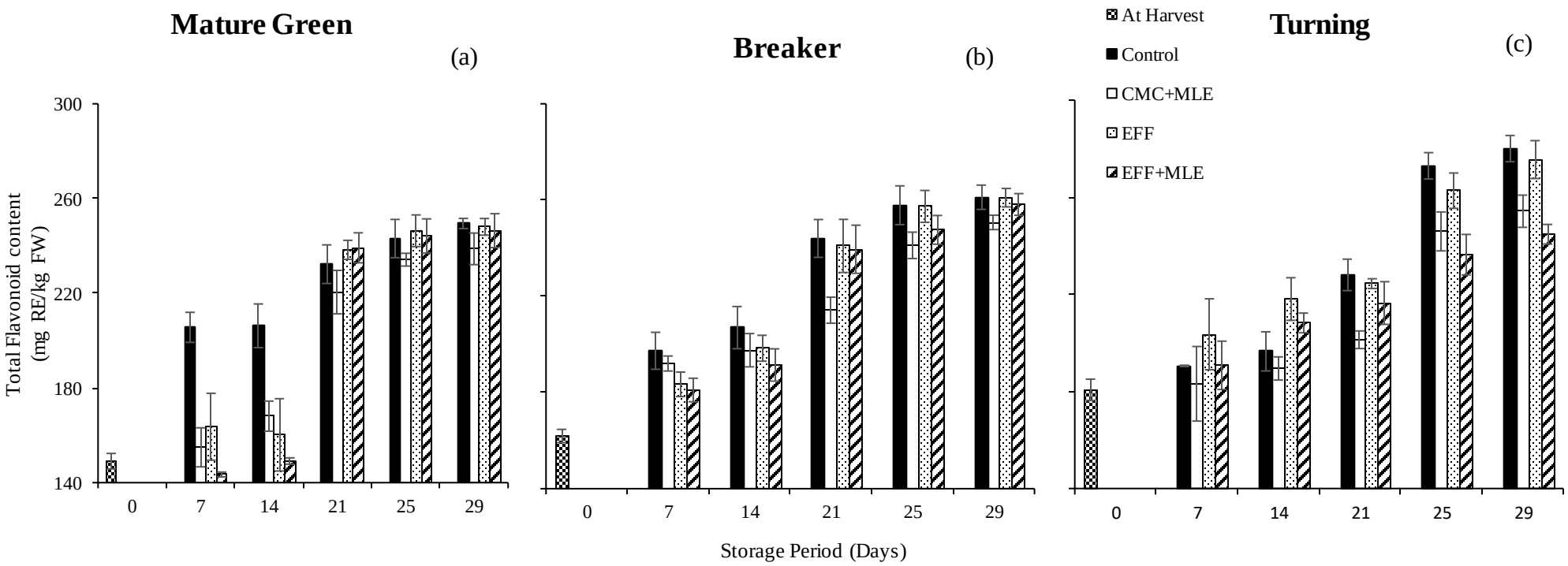

Fig. 4.5. Effect of moringa leaf extract, enhanced freshness formulation and carboxy methylcellulose on the total flavonoid content of tomato fruit harvested at the mature green (a), breaker (b) and turning maturity stage (c). Values are the means ± SE (n = 3).

4.4.5. Antioxidant capacity

The antioxidant activities of tomato fruit were evaluated using DPPH radical scavenging and FRAP assays. The interaction among maturity stage and treatments had a significant effect ($p < 0.05$) on FRAP (Fig. 4.6) and DPPH (Fig. 4.7), at the end of the storage period. The turning stage (Fig. 4.6c) exhibited the highest FRAP value, followed by the breaker (Fig. 4.6b) and mature green stage (Fig. 4.6a). The breaker stage (Fig. 4.7b) registered higher DPPH scavenging activity, followed by the turning (Fig. 4.7c) and mature green stage (Fig. 4.7a). The treatments significantly ($p < 0.001$) enhanced antioxidant activity at all maturity stages, with CMC + MLE registering higher FRAP and DPPH scavenging ability followed by EFF, relative to the control.

The EFF + MLE treatment exhibited the lowest FRAP and DPPH scavenging activity, but registered higher scavenging activity at the end of storage, relative to the control at only the mature green and breaker stage, whilst the turning stage registering significantly lower scavenging activity relative to the control. FRAP and DPPH scavenging ability peaked at day 25 at all maturity stages for treated fruit, with CMC + MLE and EFF treated fruit registering significantly higher ($p < 0.001$) antioxidant activity relative to the control and EFF + MLE treated fruit, at day 25 and at the end of the storage period.

At the mature green stage, CMC + MLE, EFF and EFF + MLE treated fruit registered ~69 %, ~42 % and ~19 % higher FRAP, respectively, relative to the control. At the breaker stage, CMC + MLE, EFF and EFF + MLE treated fruit registered ~37 %, ~44 % and ~3 % higher FRAP, respectively, relative to the control. At the turning stage, CMC + MLE and EFF treated fruit registered ~42 % and ~30 % higher FRAP respectively, relative to the control. The FRAP of EFF + MLE treated fruit was ~14 % lower, relative to the control. The quantitative comparisons reported above refer to day 29 of storage.

At the mature green stage, CMC + MLE, EFF and EFF + MLE treated fruit registered ~23 %, ~25 % and ~17 % higher DPPH scavenging activity, respectively, relative to the control. At the breaker stage, CMC + MLE, EFF and EFF + MLE treated fruit registered ~34 %, ~25 % and ~18 % higher DPPH scavenging activity, respectively, relative to the control. At the turning stage, CMC + MLE and EFF treated fruit registered ~28 % and ~39 % higher DPPH scavenging activity, respectively, relative to the control. The DPPH scavenging activity of EFF + MLE treated fruit was ~28 % lower, relative to the control. The quantitative comparisons reported above refer to day 29 of storage.

These findings are in agreement with those reported by Ali et al. (2013) and Jincy et al. (2017), who demonstrated that gum arabic edible coating and hexanal significantly enhanced the antioxidant activity of tomato and mango fruit, respectively. Antioxidant activity is an important parameter to establish the health functionality of fruit. In tomato fruit, hydrophilic antioxidants (hydrophilic phenolics, ascorbic acid and total flavonoids) contribute 91 – 93 % towards the total antioxidant activity of tomatoes, whereas lipophilic antioxidants (lycopene and lipophilic phenolics) contribute 7 – 9 % towards the total antioxidant activity of tomato fruit (Toor and Savage, 2005).

Results from this study showed that enhanced phenolic and ascorbic acid content was associated with enhanced antioxidant activity. Our results are in agreement with those obtained by Wang et al. (2008), Liu et al. (2011) and Ahmed et al. (2013), who demonstrated that enhancement of soluble antioxidant activity resulted in enhanced total antioxidant activity of tomato fruit. Correlation analyses conducted by Liu et al. (2012), further demonstrated that gallic acid (a phenolic compound), was significantly correlated with antioxidant activity ($r = 0.85$ and 0.93 corresponding to FRAP and DPPH, respectively, $p < 0.01$).

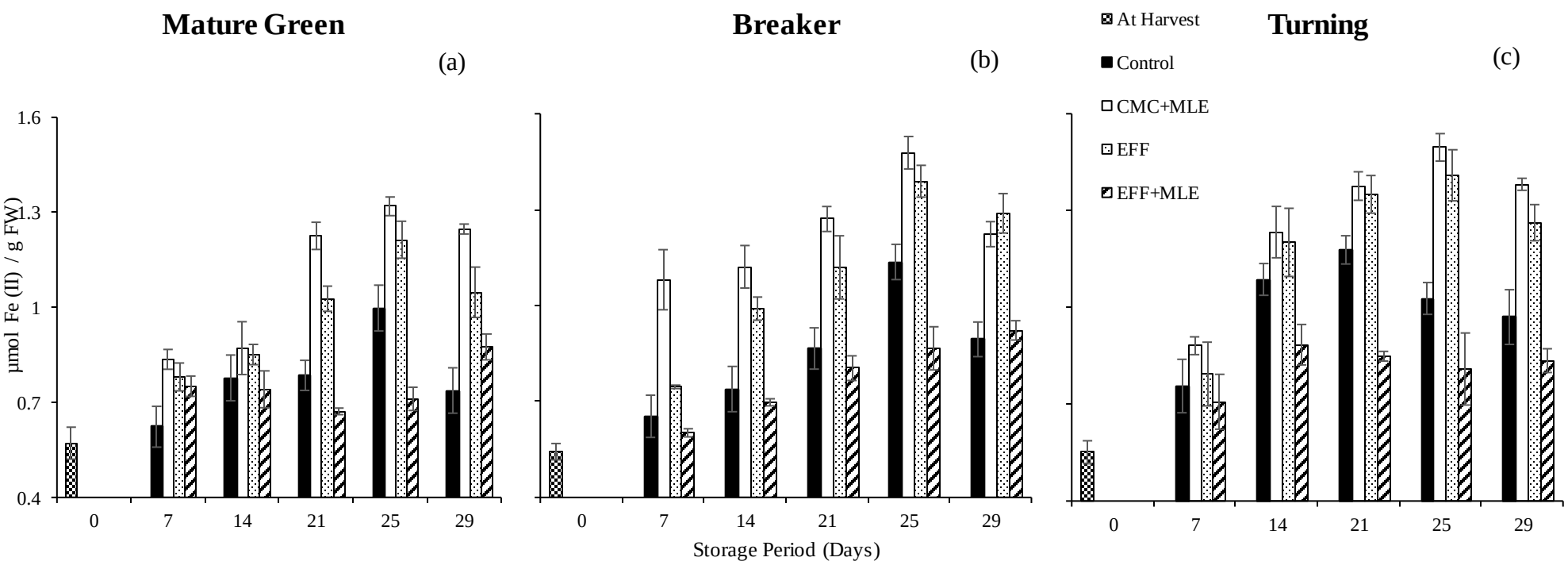

Figure 4.6. Effect of moringa leaf extract, enhanced freshness formulation and carboxy methylcellulose on FRAP assay of tomato fruit harvested at the mature green (a), breaker (b) and turning maturity stage (c). Values are the means ± SE (n = 3).

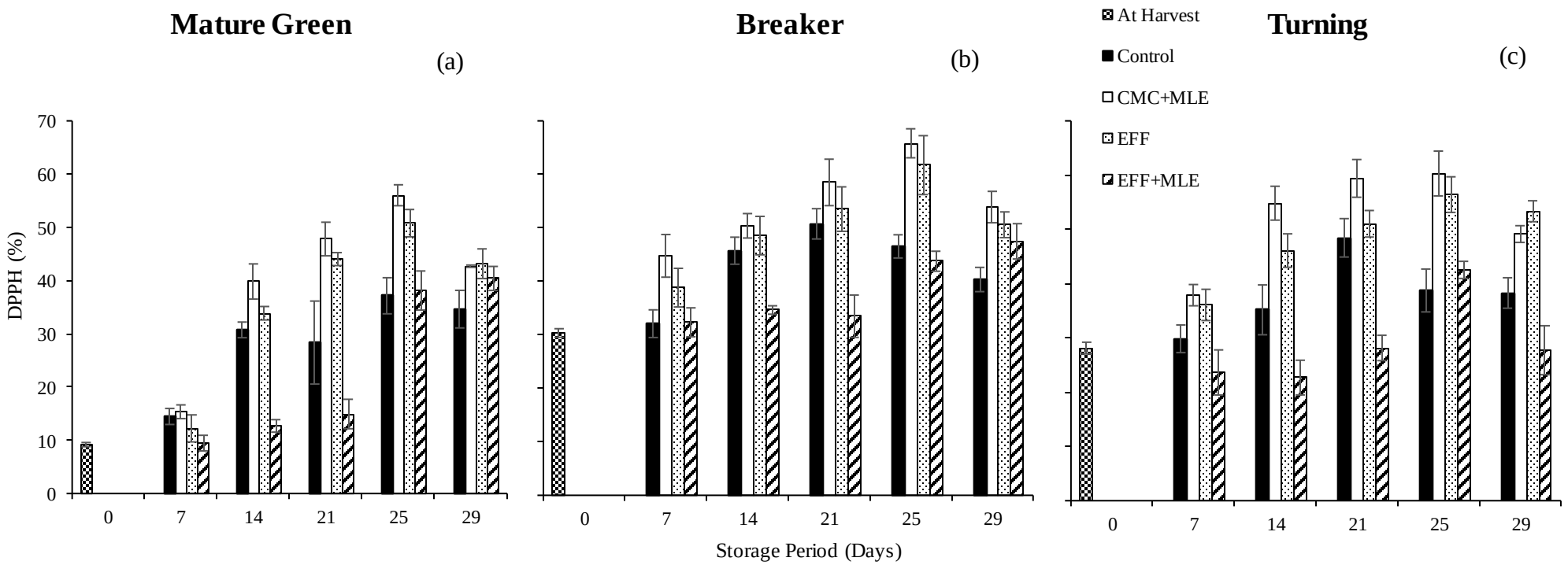

Fig. 4.7. Effect of moringa leaf extract, enhanced freshness formulation and carboxymethyl cellulose on the DPPH scavenging activity of tomato fruit harvested at mature green (a), breaker (b) and turning maturity stage (c). Values are the means ± SE (n = 3).

4.5. Conclusion

The present study demonstrated the efficacy of the enhanced freshness formulation and the moringa based edible coating at preserving and enhancing bioactive compounds and antioxidant activity of tomato at the mature green, breaker and turning stage. The ability of these treatments to delay ripening while maintaining antioxidant capacity, shows their potential of being adopted as commercial postharvest treatments to optimise bioactive compounds of tomato fruit.

The results demonstrated that the mature green stage exhibited a minimal loss of total phenolic and ascorbic acid content, followed by the turning and breaker stage. At the mature green stage CMC + MLE treatment recorded the highest phenolic content (6.13 % > EFF) and EFF treatment recorded the highest ascorbic acid content (6.31 % > CMC + MLE), at the end of storage. Further research is needed to gain an in-depth understanding as to how hexanal affects the synthesis of bioactive compounds, such as investigating whether it has an effect on the PAL and ascorbate oxidase enzymes.

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Chapter 5: Calibration and validation partial least square regression models for rapid determination of quality and sweetness attributes of tomato fruit (*Solanum lycopersicum* L.) using Vis/NIR spectroscopy

5.1. Abstract

Total soluble solids (TSS) and titratable acidity (TA) of tomato are important quality attributes used for determining the quality of tomato fruit. However, TSS is not an accurate measure of tomato sweetness. Measurement of sugar content and total sweetness index (TSI) provides accurate measurements of tomato fruit quality and sweetness. Current methods used to quantify sugar content is laborious and require specialised sample preparation. Therefore, the study aimed to develop visible to near infrared (Vis/NIR)-based spectroscopic method for rapid and non-destructive quantification of TSS, TA, TSS:TA ratio, sugar content, TSI and TSI:TA ratio of tomatoes. The Vis/NIR spectral data was acquired using a laboratory bench-top monochromator NIR Systems. Reference measurements and spectral data sets were subjected to partial least square (PLS) regression analysis. The results obtained provided robust and reliable models for parameters such as glucose (R^2 = 0.963, RMSEP = 0.373, RPD = 5.281), fructose (R^2 = 0.965, RMSEP = 0.318, RPD = 5.431) and TSI (R^2 = 0.969, RMSEP = 0.067, RPD = 5.770). The results obtained in this study demonstrated the feasibility of Vis/NIRS to predict quality and sweetness attributes of tomato fruit. The Vis/NIRS technology may be recommended for rapid assessment of tomato fruit quality and sweetness along the postharvest supply value-chain.

Key words: *Near infrared spectroscopy, total sweetness index, sugar content, sensory quality*

5.2. Introduction

Tomato is one of the most consumed horticultural crops in the world, making the crop a key product in the global agricultural market. In many countries, including South Africa, the majority of tomato fruit is destined for the fresh-produce market, therefore it requires comprehensive monitoring of internal quality parameters along the supply value-chain (Alvés De Oliveira et al., 2014). Total soluble solids (TSS) is one of the key components of tomato fruit quality and serves as a suitable substitute for sugar content (Beckles, 2012). TSS is a refractometric index, indicating the amount of dissolved solids in a solution. It consists of sugars (sucrose and hexoses; 65%), acids (citrate and malate; 13%) and minor components (phenols, amino acids, soluble pectins, ascorbic acid, and minerals) in the tomato fruit pulp (Balibrea et al., 2006).

In premium markets of the South African tomato supply chain, TSS is used to determine the internal quality of fresh tomato produce (Sibomana et al., 2016). Measuring internal quality such as TSS and titratable acidity (TA), provides an estimation of the shelf life capacity of the produce. In addition, TSS and TA provide an estimation of the sweetness of a tomato fruit, which is an attribute valued by consumers of fresh tomatoes (Gough and Hobson, 1990). The flavour of tomato fruit, results from the interaction between taste and aroma. Concerning tomato taste, sugars make the largest contribution to the taste of tomato fruit (Kader, 2008).

Sugars and their influence on taste are measured using three common methods, namely TSS, TSS:TA ratio as well as the total sweetness index (TSI). The TSI is used to indicate sweetness, however, TSS is the most commonly used method as it is rapid, inexpensive and has good correlation with tomato sugar levels. However, TSS is not an accurate measurement of tomato sweetness or sugar content. In fact, Baldwin et al. (1998), demonstrated that TSI:TA ratio was a better predictor of tomato taste in comparison to TSS and TSS:TA ratio, because it involves the specific measurement of sugars (such as glucose, fructose and sucrose). Similarly, Farneti

et al. (2013) also emphasised the importance of measuring sugars in order to determine tomato quality.

There is general consensus amongst postharvest physiologists and food scientists regarding an over-reliance on TSS and TA as the methods of assessing the quality of fresh tomatoes. Beckles, (2012) proposed that tomato sugar content should be measured in order to compliment TSS. Thus, providing an accurate description of the occurring biochemical changes and an improved indication of fruit quality and sweetness.

Measurement of these parameters has the potential to facilitate quality management and determine produce class along the supply value-chain. However, the conventional methods used are destructive, costly and time-consuming. Therefore, the use of rapid and non-destructive technologies such as near-infrared spectroscopy (NIRS) to determine these parameters in intact tomato fruit may be beneficial for the tomato industry. The use of NIRS will allow for rapid determination of sugar content, TSI and TSI:TA ratio, which are recommended parameters for the tomato industry (Beckles, 2012), because the sugar content stored in tomato fruit are a major component of postharvest quality, that affects tomato taste and overall fruit quality.

NIRS is a readily available analytical technique, which has been used for the analysis of agricultural and food samples (Cen and He, 2007). Conversely, NIR absorption bands are regularly broad and lack detailed structure required for analysis. Such spectra can be resolved with the use of chemometrics data analysis (Munck et al., 2010). The use of sophisticated multivariate statistical techniques facilitates the extraction of valuable information from an NIR spectrum. Partial least squares (PLS) regression are one of the regression techniques used in this regard (Dupuy et al., 2010).

PLS regression, which defines the latent variables based on the covariance between the spectral data and the component of interest, has been demonstrated to be one of the most successful multivariate model development methods, especially in NIR spectroscopy (Næs et al., 2004; Nicolaï et al., 2014). Therefore, the objective of this study was to develop visible to near infrared (Vis/NIR)-based spectroscopic method for rapid measurement and non-destructive quantification of tomato fruit TSS, TA, TSS:TA ratio, sugar content, TSI and TSI:TA ratio of tomato fruit during storage, using Vis/NIRS technology.

5.3. Methods and materials

5.3.1. Fruit samples

Tomato fruit (*Solanum lycopersicum* L.) cv. 'Star 9037' were harvested from TRISBN Sibani farming (Pty) Ltd, a commercial tomato farm located at Henley Dam (Latitude: 29°37′03.8″S, Longitude: 30°15′10.5″E), Pietermaritzburg, KwaZulu-Natal Province, South Africa. Fruit were harvested at three maturity stages, namely, mature green, breaker and turning. Harvested fruit were immediately transported in a ventilated vehicle to the Postharvest Research Laboratory of the Department of Horticultural Science at University of KwaZulu-Natal. Upon arrival at the laboratory, fruit were equilibrated at room temperature (21 ± 1 ºC; 65 ± 1 % RH) for 24 h before Vis/NIR spectra were acquired.

5.3.2. Acquisition of VIS/NIR spectroscopy

Vis/NIR 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. To reduce baseline shift of spectral data, the system was calibrated by scanning a 100 % white reference tile prior to fruit scanning and after every 30 min of scanning fruit. The Vis/NIR reflectance spectrum ranging from 400 to 2500 nm was acquired from two opposite sides along the fruit equator and

recorded as log 1/reflectance (log 1/R). Each spectrum was recorded using Vision software (Vision TM, version 3.5.0.0, Tidestone Technologies Inc., KS, USA).

5.3.3. Destructive reference data

5.3.3.1. Total Soluble Solids (TSS) and Titratable acidity (TA)

TSS of tomato juice was determined using a desktop refractometer (Bellingham + Stanley Ltd, Model: RFM340+, UK). Titratable acidity was determined by titrating 8 mL of tomato juice with 0.1 M NaOH to a pH value of 8.1, using a Mettler Toledo Potentiometric compact titrator (Model G20S, Greifensee, Switzerland). Acidity was expressed as the percentage of citric acid equivalent on a fresh weight basis, using Eq. 1.

$$\text{TA (\% citric acid)} = (0.0064 \text{ x titre (NaOH) mL})/(8 \text{ mL juice}) \; x \; 100 \quad (1)$$

The ratio of TSS to TA, also known as maturity index, was calculated using Eq. 2:

$$\text{TSS to TA ratio (maturity index)} = \text{TSS/TA} \quad (2)$$

5.3.3.2. Sugar content

The concentration of sugars was determined using the HPLC-refractive-index detector (RID) according to Liu et al. (1999), with slight modifications. Briefly, 1 g sample of ethanolic extract was mixed with 10 mL 80 % (v/v) ethanol and homogenized for 1 min. Thereafter, the mixture was incubated in an 80 ºC water bath for 60 min to extract the soluble sugars. Subsequently, the mixture was kept at 4 ºC overnight. After centrifugation at 12,000 rpm for 15 min at 4 ºC, the supernatant was filtered through glass wool and taken to dry in a vacuum concentrator. Dried samples were re-suspended in 2 mL ultra-pure water, filtered through a 0.45 mm nylon filter and analysed using an isocratic HPLC system equipped with a RID on a Phenomenex® column (Rezex RCM–Monosaccharide). The concentration of individual sugars was determined by comparison with authentic sugar standards (0 – 2.5 mg/L).

5.3.4. Determination of total sweetness index (TSI) and TSI:TA

TSI and TSI:TA ratio was calculated using Eq. 3 and 4, respectively:

$$TSI = [(1.00 \times sucrose) + (0.76 \times glucose) + (1.50 \times fructose)] \quad (3)$$

$$TSI{:}TA = TSI/TA \quad (4)$$

5.3.5. Statistical analysis

The data were subjected to the analysis of variance (ANOVA) using the 18th version of GenStat (VSN International, Hemel Hempstead, United Kingdom). Fisher's least significant differences were calculated and used to separate means at 5 % significance level.

5.3.6. Chemometric analysis

The chemometric analysis was performed using the Unscrambler® X chemometric software (The Unscrambler® X version 10.2, CAMO SOFTWARE AS, Oslo Science Park, NORWAY). The spectral data was subjected to principal component analysis (PCA) to compare spectral characteristics. Spectral outliers were evaluated using full cross-validation of PCA (Magwaza et al., 2014). After PCA analysis, spectral data were related with reference data using PLS (Sáiz-Abajo et al., 2005) regression which ensured that latent variables (LV) were ordered based on their relevance for predicting the Y – variable (Nicolaï et al., 2007). A calibration model was developed by applying partial least squares (PLS) regression using the spectral data and reference measurement.

Samples were divided into calibration and validation sets, using 60 samples for calibration and 39 samples for validation. The Vis/NIRS data obtained was pre-processed using a combination of different methods, such as Savitzky-Golay smoothing, multivariate scatter correction (MSC), which modifies the additive and multiplicative effects, Savitzky-Golay first and second-order derivatives, which remove the influence of any baseline variations. The spectral outliers were detected using the residual and leverage plots on the PCA and PLS models

regression (Magwaza et al., 2014). Samples located far from the zero line of the residual variance plot were identified outliers. Two spectral outliers were detected and removed.

5.3.7. Evaluation of the performance of models

Model performance was evaluated by the statistical terms of the root mean square error of calibration (RMSEC, Eq. (5)), the root mean square error of prediction (RMSEP, Eq. (6)), the correlation coefficients for calibration (Rc, Eq. (7)), correlation coefficients of prediction (Rp, Eq. (8)), bias Eq. (9) and residual predictive deviation (RPD, Eq. (10)).

$$\mathrm{RMSEC} = \sqrt{\sum(\mathrm{y_c} - \mathrm{y})^2/\mathrm{n}} \quad (5)$$

$$\mathrm{RMSEP} = \sqrt{\sum(\mathrm{y_p} - \mathrm{y})^2/\mathrm{n}} \quad (6)$$

$$\mathrm{R_C} = \frac{\mathrm{n}(\sum \mathrm{y_c y}) - (\sum \mathrm{y_c})(\sum \mathrm{y})}{\sqrt{\sum \mathrm{y_c^2} - (\sum \mathrm{y_c})^2}\sqrt{\mathrm{n}\sum \mathrm{y}^2 - (\sum \mathrm{y})^2}} \quad (7)$$

$$\mathrm{R_p} = \frac{\mathrm{n}(\sum y_p \mathrm{y}) - (\sum y_p)(\sum \mathrm{y})}{\sqrt{\sum y_p^2 - (\sum y_p)^2}\sqrt{\mathrm{n}\sum \mathrm{y}^2 - (\sum \mathrm{y})^2}} \quad (8)$$

$$\mathrm{Bias} = \frac{1}{n}\sqrt{\sum(\mathrm{y}_p - \mathrm{y})^2} \quad (9)$$

$$\mathrm{RPD} = \frac{\mathrm{SD}}{\mathrm{RMSEP}} \quad (10)$$

Where, n is the number of fruit samples used in calculation; y is the measured value of fruit quality parameters; y_c is the calculated value of quality parameters of calibration samples; y_p is the predicted value of fruit quality parameters.

5.4. Results and discussion

5.4.1. Spectra description

The raw average spectra of the tomato fruit acquired by the FOSS NIRSystem, is shown in Fig. 5.1. The pattern of the mean relative absorbance for the three maturity stages acquired was consistent with overlapping over the entire wavelength range. The spectra showed absorption

peaks around the 450, 570, 670, 970, 1200, 1450, 1790 and 1930 nm regions. The spectra were similar to the ones of tomato fruit acquired by Flores et al. (2009) and Huang et al. (2018). In addition, the wavelength range of 900 to 1050, which corresponds to the absorption of –OH groups, has been of particular interest in the prediction of TSS (Cayuela, 2008; Camps and Christen, 2009).

For fruit and vegetables such as tomato, which have high water content (± 90 %), spectral peaks at 970, 1450 and 1930 nm are associated with the first and second overtones of water absorption (Lammertyn et al., 2000; Lestander and Geladi, 2005). The spectral peak at 1200 nm corresponded to a sugar-related absorption band (Osborne et al., 1993; Flores et al., 2009).

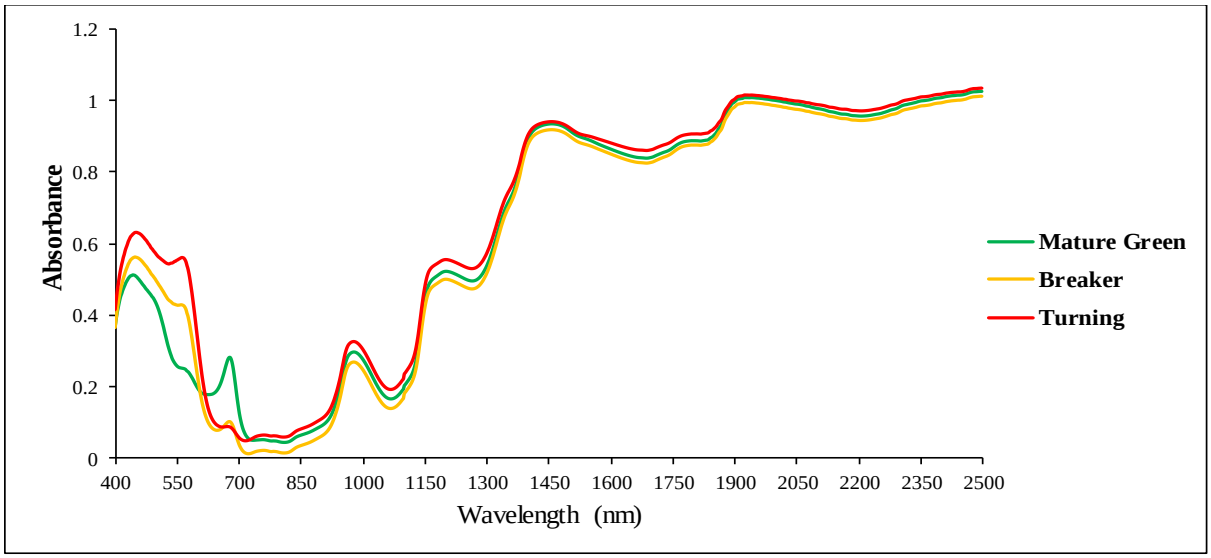

Fig. 5.1. Mean absorbance of intact tomato fruit of three maturity stages.

5.4.2. Description of reference data sets

The models developed using the NIR spectral region from 850 to 2500 nm, were superior to those developed using the 400 to 2500 nm spectral region. The models developed using the 850 to 2500 nm region had a higher R^2, lower RMSEP and higher RPD values compared to the models developed using the 400 to 2500 nm spectral region (data not shown). Notably, the bias of these models did not exceed the confidence limit (± 0.55 x SEC (standard error of calibration)). Therefore, the models developed using the 850 to 2500 nm spectral region were

used. In addition, the range from 850 to 2500 nm is suitable for the prediction of sweetness and flavour parameters, such as sugar content (Guo et al., 2016) as well as TSS, TA and TSS:TA ratio (Ncama et al., 2017).

The distributional statistics of the calibration and validation data sets are presented in Table 5.1. The reference measurements for calibration and validation data sets were normally distributed around the mean. The data sets had enough variation, exhibited by CV%. High variation of the reference data is useful in the development of reliable prediction models for Vis/NIRS (Clément et al., 2008).

The mean values of TSS for calibration and validation sets were 5.500 and 5.490, respectively, with CV% of 12.876 % and 12.443 %. The mean values of TA for calibration and validation were 0.791 and 0.851, respectively, with CV% of 24.451 % and 26.235 %. The mean values of TSS:TA ratio for calibration and validation were 15.411 and 14.461, respectively, with CV% of 32.753 % and 35.413 %. The mean values of glucose for calibration and validation were 13.189 and 12.848 mg/g FW, respectively, with CV% of 20.239 % and 22.75 %.

The mean values of fructose for calibration and validation were 12.633 and 11.769 mg/g FW, respectively, with CV% of 21.083 % and 21.7681 %. The mean values of TSI for calibration and validation were 3.273 and 2.696, respectively, with CV% of 20.834 % and 20.288 %. The mean values of TSI:TA ratio for calibration and validation were 6.167 and 6.054, respectively, with CV% of 32.363 % and 27.975 %.

Table 5.1: Descriptive statistics for calibration and validation subsets for quality parameters of tomato fruit

Parameter	Data set	Max	Min	Mean	SD	CV (%)
TSS	Calibration set	5.500	2.630	4.345	0.559	12.876
	Validation set	5.490	3.130	4.408	0.548	12.443
TA	Calibration set	0.791	0.275	0.571	0.14	24.451
	Validation set	0.851	0.299	0.567	0.149	26.235
TSS:TA	Calibration set	15.411	4.503	8.204	2.687	32.753
	Validation set	14.461	4.135	8.517	3.016	35.413
Glucose	Calibration set	13.189	4.873	8.804	1.782	20.239
	Validation set	12.848	3.771	8.658	1.969	22.75
Fructose	Calibration set	12.633	4.385	8.082	1.683	21.083
	Validation set	11.769	4.597	7.933	1.727	21.768
TSI	Calibration set	3.273	1.028	1.823	0.379	20.834
	Validation set	2.696	0.976	1.905	0.387	20.288
TSI:TA	Calibration set	6.167	1.748	3.406	1.102	32.363
	Validation set	6.054	1.506	3.558	0.995	27.975

TSS (total soluble solids), TA (titratable acidity), TSS:TA (maturity index), TSI (total sweetness index), Max (maximum of the calibration and validation data set), Min (minimum of the calibration and validation data set), Mean (average of the calibration and validation data set), SD (standard deviation of the calibration and validation data set), CV (%) (the coefficient of variation for the calibration and validation data set).

5.4.3. Summary statistics of actual and predicted quality parameters

The statistics of the reference and prediction data sets are summarised in Table 5.2. Besides TSS, the standard deviation of the predicted data was lower than that of the reference data, for the measured parameters. A similar trend was observed for the coefficient of variation (CV%), where the CV% of the predicted data was lower than that of the reference data. The results for TSS, TA and TSS:TA ratio were similar to those reported by Hong and Tsou (1998), He et al. (2005), Ncama et al. (2017) and Radzevičius et al. (2016).

For the predicted data to be considered valuable, it should fall within the actual variation, thus having a deviation lower than that of the reference data (Ncama et al., 2017). Therefore, the predicted data of the measured parameters, may be considered valuable as they registered lower deviation values, in comparison to the reference data.

Table 5.2: Summary statistics of actual and predicted quality parameters of tomato fruit

Parameter	Data set	Max	Min	Mean	SD	CV (%)
TSS	Reference	5.490	3.130	4.408	0.548	12.443
	Predicted	5.459	3.197	4.391	0.562	12.801
TA	Reference	0.851	0.299	0.567	0.149	26.235
	Predicted	0.832	0.322	0.568	0.135	23.697
TSS:TA	Reference	14.460	4.135	8.517	3.016	35.413
	Predicted	14.608	4.049	8.421	2.965	35.213
Glucose	Reference	12.848	3.771	8.632	2.003	23.201
	Predicted	12.372	3.627	8.586	1.963	22.863
Fructose	Reference	11.769	4.597	7.933	1.727	21.768
	Predicted	11.518	4.913	7.911	1.681	21.254
TSI	Reference	2.696	0.976	1.905	0.387	20.288
	Predicted	2.582	0.911	1.884	0.370	19.618
TSI:TA	Reference	6.054	1.506	3.558	0.995	27.975
	Predicted	5.362	1.505	3.505	0.899	25.649

TSS (total soluble solids), TA (titratable acidity), TSS:TA (maturity index), TSI (total sweetness index), Max (maximum of the reference and predicted data), Min (minimum of the reference and predicted data), Mean (average of the reference and predicted data), SD (standard deviation of the reference and predicted data), CV (%) (the coefficient of variation for the reference and predicted data).

5.4.4. PLS models: Prediction of internal compositions

The prediction models for all seven parameters yielded adequate results. Scatter plots of the predicted values against the analysed values of TSS, TA, TSS:TA ratio, glucose, fructose, TSI and TSI:TA ratio are presented in Fig. 5.2. For TSS, from the prediction model, the $R^2 = 0.905$, RMSEP = 0.167, RPD = 3.284 and bias = -0.017. For the TA prediction model, $R^2 = 0.952$, RMSEP = 0.032, RPD = 4.645 and bias = 0.002. For the TSS:TA model, $R^2 = 0.972$, RMSEP = 0.496, RPD = 6.081 and bias = -0.045. For the glucose prediction model, $R^2 = 0.963$, RMSEP = 0.373, RPD = 5.281 and bias = -0.072. For the fructose prediction model, $R^2 = 0.965$, RMSEP = 0.318, RPD = 5.431 and bias = -0.047. For the TSI prediction model, $R^2 = 0.969$, RMSEP = 0.067, RPD = 5.770 and bias = -0.021. For the TSI:TA prediction model, $R^2 = 0.938$, RMSEP = 0.246, RPD = 4.046 and bias = -0.053.

RPD is a measure of the model robustness. The RPD values between 1.5 to 2 indicate that the model can distinguish between low and high response variables. RPD values between 2 to 2.5 indicate that the model is suitable for coarse quantitative predictions. On the other hand, RPD values of 2.5 to 3 or above are indicative of good and excellent predictive accuracies of the model (Nicolaï et al. (2007). The models for the prediction for all the parameters, exhibited an outstanding ability for future predictions. The bias is the difference between the mean of the predicted versus the mean of the actual values. In this study, the bias values of all the prediction models were below 1, indicative of stability and high robustness of these models.

The limit control or confidence limit of the bias of a model is ± 0.55 x SEC (Shenk et al., 1989). To ensure accurate predictions, the bias of a model should not exceed this confidence limit. The bias of prediction models for all seven parameters, were below the confidence limit, indicative of accurate predictions. Results obtained in this study are similar to those of Flores et al. (2009), Camps and Gilli (2017), as well as Huang et al. (2018), for intact tomato fruit.

Table 5.3: Results for calibration and prediction of the partial least squares (PLS) models

Parameter	LV	Calibration		Validation			
		R^2_C	RMSEC	R^2_p	RMSEP	Bias	RPD
TSS	5	0.962	0.108	0.905	0.167	-0.017	3.284
TA	7	0.965	0.026	0.952	0.032	0.002	4.645
TSS:TA	4	0.989	0.201	0.972	0.496	-0.043	6.081
Glucose	4	0.983	0.261	0.963	0.373	-0.072	5.281
Fructose	6	0.989	0.209	0.965	0.318	-0.047	5.431
TSI	4	0.988	0.042	0.969	0.067	-0.021	5.77
TSI:TA	3	0.981	0.150	0.938	0.246	-0.053	4.046

TSS (total soluble solids), TA (titratable acidity), TSS:TA (maturity index), TSI (total sweetness index), LV (latent variable), R^2, correlation coefficient between Vis/NIRS predicted and measured values, RMSEC (root mean square error of calibration), RMSEP (root mean square error of prediction), RPD (residual predictive deviation).

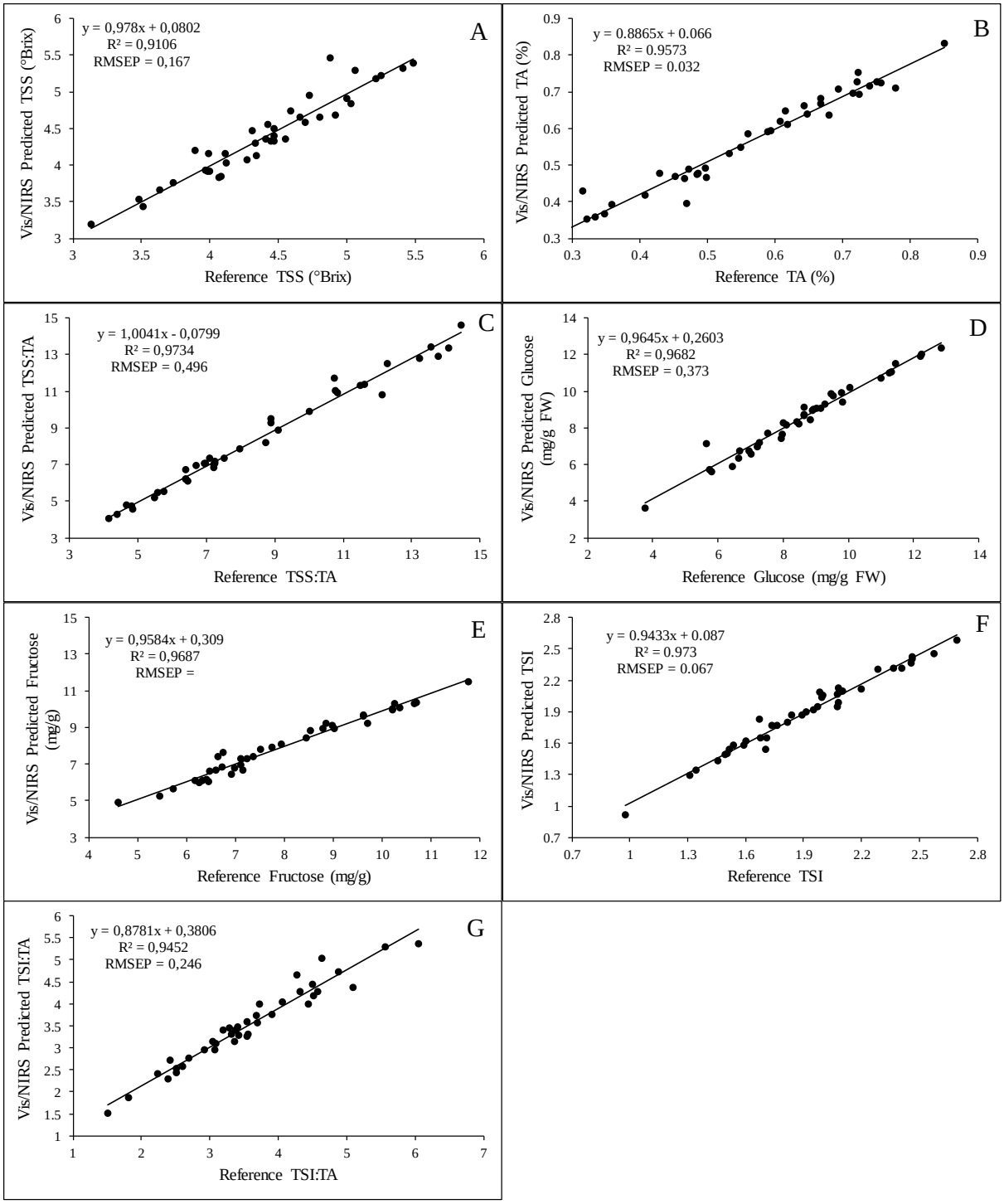

Fig. 5.2. Scatter plot of Vis/NIRS predicted versus measured values of tomato fruit for TSS (A), TA (B), TSS:TA (C), glucose (D), fructose (E), TSI (F) and TSI:TA (G).

5.5. Conclusion

The results obtained in this study demonstrated that Vis/NIRS technology together with chemometrics, can be accurately used to predict quality and flavour parameters of tomato fruit. This will allow for the management and monitoring changes of internal quality parameters, during postharvest handling and supply chain of tomato fruit. The ability of Vis/NIRS to predict TSI and TSI:TA ratio will be particularly beneficial to the tomato industry, as these parameters are aligned with consumer perception for quality and flavour. In addition, the rapid determination of TSI and TSI:TA ratio ensures an accurate guarantee of the tomato's fruit quality. Thus, enabling fast grading of tomato produce to increase market value.

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Chapter 6: General discussion, conclusion and recommendations

6.1. Introduction

Chlorinated water is used as a fungicide treatment in South African pack houses to disinfect tomatoes. In addition to chlorinated water being a threat towards human health and the environment, it is not an efficient treatment as it causes off-flavours in tomato fruit (Hassenberg et al., 2008). The increase in demand for fresh tomatoes without any hazardous residues by both consumers and regulatory agencies, puts producers under pressure to come up with eco-friendly surface disinfectants that can improve shelf life while providing health benefits to the consumer (Chereno, 2016).

The present study was aimed at reducing the postharvest losses of tomato fruit, using non-chemical postharvest treatments that can retain quality and optimise shelf life, without causing adverse effects on the fruit. Tomato producers in South Africa (emerging, small holder and commercial farmers), harvest tomatoes at different maturity stages based on their target market. Thus, the present study was an attempt to determine if these treatments can maintain quality and reduce losses of tomatoes of different maturity stages.

The of use TSS as a predictor of tomato quality and taste has proven to be inaccurate. Measuring more accurate predictors of tomato quality and sweetness such as sugar content, total sweetness index (TSI) and the TSI:TA ratio is costly and laborious. Hence the use of TSS, which is a quick and cost-effective measurement. However, it does not provide an accurate description of the biochemical changes of tomato fruit. Therefore, the present study investigated the use of a rapid and non-destructive technology to determine sugar content, TSI and the TSI:TA ratio. This was done to accurately predict quality and sweetness of tomato fruit, as an attempt to provide a precise description of fruit biochemical changes. Thus, facilitating better quality management of tomato fruit along the supply value-chain.

6.2. Literature review on the effect of hexanal on horticultural produce

The objective of the literature review (chapter 2), was to discuss the effect of hexanal treatment on physiological and biochemical attributes of fresh produce and to provide an understanding of the mode of action of hexanal formulation in relation to improving fruit quality and shelf life. One of the key findings was the versatility of hexanal in terms of mode of application. Hexanal can be applied at the preharvest or postharvest stage, in order to preserve fruit quality, optimise shelf life and reduce postharvest losses. Literature demonstrated the efficacy of hexanal formulations to optimise quality of fresh produce by suppressing enzymes that are responsible for rapid deterioration of fresh produce.

Another key finding was the efficacy of enhanced freshness formulation (EFF) to suppress postharvest decay. There were two research gaps that were identified in the review. The first research gap was related to previous studies focusing on hexanal treatment at the mature green stage only of tomato fruit. The maturity stage at which tomato producers harvest their tomatoes is dictated by their individual target markets. Thus, it would be interesting to know whether or not EFF treatment is effective across tomato fruit of different maturity stages. The present study was set out to address this research gap.

The second research gap was related to the development of physiological disorders of fresh produce during postharvest storage. The review discusses the effect hexanal has on the membrane of fruit and the ability of hexanal to improve membrane stability of fruit. Even though rapid ripening leads to membrane deterioration and postharvest losses, membrane associated physiological disorders contribute to postharvest losses as well. Conducting future research on the effect of hexanal on reducing membrane associated physiological disorders of fruit, can make a great contribution to research in the postharvest science field.

6.3. Moringa leaf extracts, enhanced freshness formulation and carboxy methylcellulose as postharvest treatments to optimise postharvest quality of tomato fruit

The change in physicochemical and biochemical parameters, such as mass loss, firmness, colour, TSS, TA and maturity index (TSS:TA), are used to evaluate fruit quality. In addition to fruit quality evaluation, these parameters have a major effect on fruit marketability, organoleptic properties and consumer acceptability. The results presented in chapter 3, demonstrated the capacity of the moringa based edible coating (CMC + MLE) and enhanced freshness formulation (EFF) on optimising marketability of tomato fruit.

The treatments had a positive and significant effect on the reduction of mass loss (Fig. 3.1.), retention of fruit firmness (Fig. 3.2.), fruit colour development (Fig. 3.3.), and reduction of postharvest decay (Table 3.1.). These are important parameters related to consumer acceptance and market value of tomato fruit. CMC + MLE and EFF, serve as good alternatives to chemical treatments and other proposed postharvest treatments for fresh tomatoes.

In the present study, the CMC + MLE and EFF treatments did not cause any physiological disorders on the tomatoes. In addition, the treatments yielded positive results across all three maturity stages. Thus, making the adoption of these treatments applicable to tomato producers that harvest tomato fruit at the mature green, breaker and turning maturity stage.

6.4. Influence of moringa leaf extracts, enhanced freshness formulation and carboxy methylcellulose on bioactive compounds and antioxidant attributes of tomato fruit

Bioactive compounds and antioxidant activity are important parameters used to establish the health functionality of fruit and have beneficial effects on human health. The high perishable nature of tomato fruit, owed to its rapid ripening, results in the loss of these bioactive

compounds. This reduces the nutritional quality, antioxidant activity and shelf-life of tomato fruit.

The postharvest treatments significantly delayed ripening, enhanced antioxidant activity and maintained the concentration of bioactive compounds of the tomato fruit (chapter 4). EFF and the moringa based edible coating, improved nutritional composition and quality of tomato during shelf-life. Thus, showing the potential of these treatments to increase the market value of tomato fruit.

Based on the results obtained in the present study, the aim and objectives of the study were achieved. The treatments effectively retained fruit quality and reduced the postharvest losses of tomato (chapter 3 and 4). This can be explained by the treatments ability to optimise physiochemical and biochemical attributes, enhance bioactive compounds, delay ripening and suppress postharvest decay. These results are in alignment with those previously reported (Baraiya et al., 2012; Cheema et al., 2014; Tiwari and Paliyath, 2011).

6.5. Development of Vis/NIRS models to predict sugar content, maturity and sweetness indices of tomato fruit

The adoption of Vis/NIRS technology by the tomato industry, may be instrumental in monitoring quality and fast grading of tomato produce, by the rapid prediction and determination of quality and sweetness parameters of tomato fruit. Vis/NIRS successfully and accurately predicted quality and sweetness parameters. The parameters which were of importance in this specific experiment were TSI (Fig. 5.2F) and TSI:TA ratio (Fig. 5.2G), which are more accurate predictors of tomato quality and sweetness (an attribute valued by consumers). Prediction models of these parameters were excellent, exhibiting the ability of Vis/NIRS to rapidly predict quality and sweetness parameters.

The Vis/NIRS results showed the possibility of developing accurate models to rapidly predict maturation indices and biochemical parameters, which relate to postharvest quality. The results for TSS, TA, TSS:TA ratio and sugar content are in alignment with those of Flores et al. (2009), Huang et al. (2018) as well as Pedro and Ferreira, (2007). There has not been literature found that demonstrate the ability of Vis/NIRS to predict sweetness indices of tomato, such as TSI and the TSI:TA ratio. The results obtained in the present study, demonstrated the possibility of Vis/NIRS to accurately predict and determine sugar content and sweetness indices (TSI and TSI:TA), for quality assessment along the supply value-chain.

6.6. Conclusion

This dissertation/study was conducted as an attempt to address the problems experienced by the tomato industry. These problems include finding alternative treatments to currently used chemical products, that leave hazardous residues and cause physiological disorders of tomato fruit. The measurement of internal quality parameters that facilitate in determining the produce class of tomato fruit is laborious, costly and time-consuming. Thus, presents a challenge in the tomato supply value-chain with regard to determining the shelf-life capacity of the tomato produce. Investigating the use of Vis/NIRS technology to measure and predict the internal quality of intact tomatoes, will allow rapid determination and quality assessment of tomato produce along the supply value-chain.

This dissertation/study yielded positive results with regards to addressing the problems experienced by the tomato industry. The proposed treatments significantly optimised physicochemical and biochemical attributes that are important for market value and consumer acceptance of tomato fruit. In addition, the proposed treatments significantly improved the antioxidant capacity of the tomatoes, without causing any adverse effects on the fruit. Thus, fruit quality was optimised, maintained and postharvest decay was reduced, in an eco-friendly

manner. Lastly, the development of prediction models, to determine internal quality parameters such as sugar content, TSI and the TSI:TA ratio was successful. Therefore, the results of this study demonstrate the potential impact of the adoption of moringa based edible coating and enhanced freshness formulation, to resolve problems associated with the use of postharvest chemical treatments.

The enhanced freshness formulation can be applied as dip, spray or vapour treatment. Thus, making the applicability of this formulation possible to incorporate in the tomato industry. EFF can be applied as a postharvest dip treatment by submerging fruit in the formulation for three to five minutes, this makes EFF possible to incorporate in tomato packhouses. Thus, serving as an alternative to the currently used chemical treatment in South African packhouses.

EFF can also be applied as a spray for preharvest fruit treatment, with an application rate of 50 – 100L per acre (0.4 hectares). The versatility of hexanal formulations makes it applicable to commercial farmers that can use EFF as postharvest treatment either by dip or vapour treatment. The formulation is also applicable to emerging and small holder farmers that do not have access to expensive packhouse facilities, as EFF can be applied at the preharvest stage and continue to optimise fruit quality after harvest.

At the cost of $15 per litre, hexanal is a low-cost treatment that can be used to preserve quality and reduce postharvest losses of tomato. The impact of the adoption of this technology in the tomato industry, may be generating more income for farmers by reducing loss of produce at the pre-consumer level. This can be achieved by reducing postharvest losses of tomatoes during long distance transportation for emerging, small holder and commercial farmers that use non-refrigerated vehicles for transport.

The adoption of a rapid and non-destructive tool such as Vis/NIRS, has the potential to provide accurate and precise description of internal quality and biochemical changes of tomato fruit.

Thus, allowing cost effective, faster and accurate grading of produce and better estimation of the shelf-life capacity of the specific tomato produce, based on maturation and sweetness indices.

6.7. Recommendations

As highlighted in the literature review, EFF can be applied at both the postharvest and preharvest stage. Additional research would be essential to find out if preharvest application of EFF would yield the same results of tomato fruit harvested at different maturity stages. This information would be beneficial for small scale and emerging farmers with no packhouse facilities. In addition, future research based on hexanal formulations should be conducted in an attempt to reduce if not inhibit physiological disorders of fruit, in order to further minimise postharvest losses.

In previous studies, the use of CMC and hexanal formulations were only applied at the mature green stage. The results of this present study showed the efficacy of these treatments to preserve quality and extend shelf life of tomato fruit at three different maturity stages. This means that the application/use of these treatments may be recommended for tomato producers that harvest their produce at the mature green, breaker and turning stage.

In addition, these treatments do not pose a threat to the environment, human health nor cause adverse effects on the tomato produce. Thus, may be recommended as an alternative to chemical treatments currently used in the tomato industry. To add value to the results relating to postharvest decay, future research would be vital to analyse the capacity of these treatments to suppress the fungal infections and microbial growth of at least three common preharvest and postharvest pathogens of tomato fruit.

The adoption of Vis/NIRS to predict sweetness indices (TSI and TSI:TA) in the tomato industry may be recommended for quality assessment along the supply value-chain. This would

be beneficial to the tomato industry as it will facilitate in providing a better indication of sweetness, quality and provide better alignment with the prediction of human perception of good taste (Baldwin et al., 1998).

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