# EFFECTIVENESS OF HEXANAL AND ITS MODE OF ACTION ON THE POST-HARVEST QUALITY OF BANANA FRUITS (*MUSA* SPP)

SUBMITTED BY

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# A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN HORTICULTURE

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# DECLARATION

This thesis is my original work and has not been presented for award of a degree in any other university.

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# DEDICATION

I dedicate this work to Almighty God for His unlimited grace and mercy, which has kept me up to this far. To my family especially my parents Mr Daudi Mbithi and Mrs Joyce Yumbya for their unconditional support, prayers and motivation throughout my studies.

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## LIST OF ABBREVIATIONS AND ACRONYMS

- AEZ Agro ecological zones DAA Days after anthesis DEPC Diethyl dicarbonate/ Diethyl pyrocarbonate DEGs Differentially Expressed Genes EDTA Ethylenediaminetetraacetic acid GC Gas Chromatography HCD Horticultural crop directorate HPLC High performance liquid chromatography IDRC International development research center 1-Methylcyclopropene 1-MCP` mL Milliliters NGS Next generation sequencing PG Polygalacturonase enzyme PLD phospholipase D enzyme PL Pectate lyase enzyme PME Pectin methylesterase enzyme qPCR quantitative polymerase chain reaction TSS Total soluble solids TTA Total titratable acidity
- XET Xyloglucan endotransglucosylase enzyme

#### GENERAL ABSTRACT

Banana is one of the major fruits produced in Kenya mainly by smallholder farmers for the domestic market. Despite its importance and potential, the banana value chain is faced with several constraints including high post-harvest losses estimated to be 40%. Application of suitable and affordable post-harvest technologies is key in reducing losses and enhancing banana profitability across the value chain. The present study was conducted to assess the effectiveness of hexanal, a naturally occurring compound in prolonging the shelf-life of 'Grand nain' and 'sweet banana' varieties produced under two agro-ecological zones (AEZ) of Kenya, namely Meru County (AEZ II) and Machakos County (AEZ IV). Further, the study sought to validate the effect of hexanal treatments on the fruits' postharvest quality and elucidate the molecular basis of its mode of action. To realize these objectives, three separate but related experiments were conducted between July 2016 and May 2018.

In the first objective, the best method of hexanal application (pre-harvest spray and post-harvest dip); the effective concentration (2% and 3%); and the effective treatment duration (2.5 minutes or 5 minutes) were evaluated. The treated and untreated fruits were compared for postharvest longevity based on ripening-related changes under ambient room conditions ( $25 \pm 1^{\circ}C$  and RH 60  $\pm 5^{\circ}$ ). The ripening changes determined at regular intervals from randomly sampled fruits included rate of respiration, rate of ethylene production, percentage cumulative weight loss, peel color, peel and pulp firmness. A Complete Randomized Design with factorial arrangements was used for the shelf life studies. In the second objective, the best treatments in experiment one were selected for analysis of the effect of hexanal treatment on selected quality attributes including Total Soluble Solids (TSS), Total Titratable Acidity (TTA), Vitamin C and simple sugars (fructose, glucose and sucrose). The TSS level was determined using a digital handheld refractometer while vitamin C and simple sugars were analyzed using the high performance liquid chromatography (HPLC). The TTA content was determined by titration. Sensory evaluation was conducted using untrained panelists to ascertain consumer perception viz a viz machine-determined quality attributes. In the third objective, maturegreen 'Grand nain' banana fruits were treated with either hexanal, ethylene, 1-Methylcyclopropene (1-MCP), a combination of 1-MCP and hexanal or left untreated (control). After the treatments, all the fruits were left to undergo ripening under ambient room conditions. Three fruits from each treatment combination were randomly sampled at two-day interval for analysis of selected physical

(peel color, peel and pulp firmness) and physiological parameters (rate of respiration and ethylene production). The middle portion of both the peel and the pulp of each sampled fruit was diced and quickly frozen in liquid nitrogen and stored at -80°C for quantitative polymerase chain reaction analysis (qPCR) and sequencing using next generation sequencing technique.

Experiment one results showed that hexanal applied twice as a pre-harvest spray significantly (p < p0.05) delayed time to fruit harvesting by 12 days and 18 days in 'Grand nain' variety produced in AEZ IV and AEZ II, respectively and 12 days in 'sweet banana' variety in both zones compared to the untreated fruits. Effectiveness of hexanal was significantly affected by duration of application with the post-harvest dip for 5 minutes enhancing fruit shelf life by 9 and 6 days compared to 6 and 3 days in fruits dipped for 2.5 minutes in 'Grand nain' and 'sweet banana' variety respectively. In the second objective experiments, the results showed that hexanal significantly reduced the rate of increase of the various quality attribute parameters analyzed. The TSS levels in both varieties and AEZs, were not significantly affected by hexanal treatment. However, variety had a significant effect on the TSS levels with 'sweet banana' having higher levels (31 -33.8 ° brix) compared to 'Grand nain' fruits (27-30° brix) regardless of hexanal treatment and AEZs. Hexanal treated fruits maintained relatively higher vitamin C levels throughout the storage period in both AEZs compared to the untreated controls. 'Grand nain' fruits dipped in hexanal had lost only 49% and 22% of the initial vitamin C content in fruits from AEZ IV and AEZ II respectively, 9 days later compared to 54 % and 51 % in the untreated controls. Hexanal treatment significantly delayed the rate of increase of simple sugars in both varieties and AEZs compared to the untreated ones. Results of sensory evaluation showed no significant differences in the various quality attributes scored between the treated and untreated control fruits. Objective three results showed that, hexanal and 1-MCP treatments suppressed the expression of various cell wall genes such as xyloglucan endotransglucosylase, Polygalacturonase, Pectin Lyase and Pectin Methylesterase as well as 1-Aminocyclopropane-1-Carboxylic Acid Oxidase by 4-6 days and 6-10 days respectively compared to fruits treated with ethylene and the controls. However, later expression of these genes occurred 2-6 days earlier in the hexanal treated fruits as compared to those treated with either 1-MCP or a combination of 1-MCP and hexanal. Sequencing results showed that hexanal treatment suppressed the expression of xyloglucan endotransglucosylase, phospholipase D genes, Polygalacturonase and 1aminocyclopropane-1-carboxylate synthase by a fold change of 3.3 - 6.7 in day four of storage contrary to the observed induction of the same genes in fruits treated with ethylene.

The best results were obtained from hexanal applied at a concentration of 2% or 3% as either a preharvest spray at 30 days and 15 days before harvest or as a post-harvest dip for 5 minutes. These results indicate that hexanal technology is effective to delay ripening and extend the shelf-life of banana fruit without affecting the quality attributes of the fruit. These results further suggest that hexanal mode of action may be through temporal suppression of genes involved in the banana ripening process. The technology should be promoted for adoption by banana farmers and traders in Kenya to prolong the post-harvest shelf-life thereby extending the marketing period and reduce postharvest losses in the banana value chain.

#### **CHAPTER ONE**

#### **1.0 INTRODUCTION**

#### **1.1 Background Information**

Horticulture plays a significant role in the Kenyan economy and it's the fastest growing subsector of the agriculture sector. According to HCD (2018), horticulture accounts for about 33% of agricultural GDP with an annual growth rate of about 19%. This sector contributes to the economy through revenue generation, creation of job opportunities, foreign exchange earnings and provision of raw materials to the agro-processing industries. The sub-sector is characterized by diversity in terms of variety of produce, farm sizes and geographical area of production. Most of the horticultural production in Kenya, especially fruits and vegetables is done by small scale farmers majority of them being women.

Fruits are main component of horticultural subsector and come third to flowers and vegetables in terms of income contribution (HCD, 2016). In the year 2018, fruits' total contribution was USD 523 Million compared to USD 649 million and USD 613 million for floriculture and vegetables respectively (HCD, 2018). The major fruits grown in Kenya in order of importance are; Banana (35%), Mangoes (20%), pineapples (15%), Avocado (10%), water melon (6%), pawpaw (5%) and oranges (3.7%) (HCD, 2018).

Banana is widely grown in almost every part of the country and its production is throughout the year unlike most of the other fruits which are seasonal. Banana fruits are rich in calories, healthy antioxidants (flavonoids, polyphenolics), minerals (potassium, magnesium) and vitamins particularly the B6 complex (Natalia *et al.*, 2014). This has made banana production a promising enterprise in Kenya contributing towards food and nutritional security. Bananas are mostly consumed locally with only a lesser quantity of about 7.2% being exported (HCD, 2018). The potential of this crop is therefore largely unexploited, mostly as a result of several constraints along the value chain. One of the critical areas of concern is the huge post-harvest losses of about 40% (FAO, 2014). In most developing countries such as Kenya, lack of good agricultural practice, lack of adequate knowledge on proper harvesting methods and postharvest handling as well as poor infrastructure further exacerbates the post-harvest losses (FAO, 2014).

Harvesting of banana for commercial purpose is done when the fruits are mature green. Ripening is then done before the fruits are marketed. A ccording to Kader (2005), ripe banana fruits are often very delicate with a short shelf life, which limits the marketing period. Once the ripening process is initiated, banana fruits are characterized by rapid softening, typical to climacteric fruits (Duan *et al.*, 2007). Fruit softening is a significant measure of ripening in banana and the softening rate is high during the later stages of ripening. This is a major contributor to banana deterioration and economical losses. The fruit is regarded unmarketable when approximately 20 percent of its surface is covered with brown spots (Tapre *et al.*, 2012). High post-harvest losses can occur if the fruits are not properly handled thus necessitating post-harvest care.

In Kenya, post-harvest losses in fresh horticultural crops such as bananas are estimated at 40% (FAO, 2014). A study by Kitinoja, (2015), estimates the postharvest losses in banana to be between 18% - 45%, mainly due to poor handling, storage, perishable nature and post-harvest diseases. However, there is limited data on how much is lost at specific points in the value chain. In Kenya, a normal banana market is characterized by transportation of fruit bunches on ox-drawn carts, wheelbarrows, bicycles and motorbikes from farms to the rural local markets without any post-harvest treatment (Kahangi *et al.*, 2005). In the market, traders stack whole bunches or hands in gunny bags and this leads to heavy mechanical damage due to compaction. The poor postharvest handling along the banana value chain causes mechanical injuries which accelerates the rate of deterioration. Proper care and handling should therefore, be taken during harvesting and marketing, so that fruits of high quality reach the consumer with farmers getting higher remunerative prices.

Besides proper postharvest handling practices to reduce mechanical injuries which aggravate deterioration, application of appropriate postharvest technologies is critical in minimizing the high post-harvest losses in fruits such as banana. Over the years, several post-harvest technologies have been developed and their use successfully tested in several climacteric fruits such as banana, mango and papaya. Examples of the postharvest technologies that have been developed and tested in banana include Modified Atmosphere Packaging (Basel *et al.*, 2002, Scetar *et al.*, 2010), Controlled Atmosphere Storage (Kader, 1994), low temperature storage (Narayana *et al.*, 2002), 1-

Methylcyclopropene (Bagnato *et al.*, 2003, Lohani *et al.*, 2004), hot water treatments (Mirshekari *et al.*, 2015) among others.

However, the adoption rate of these technologies especially by small scale farmers is very low due to practical difficulties, non-availability and prohibitive costs (Lorevice et al., 2014). While low temperature storage is considered the most effective method for extending the shelflife of most perishable commodities after harvest, majority of the small scale farmers cannot afford the cold storage facilities. Banana being a tropical fruit, should not be kept at temperatures below 13°C as this will result to chilling injury. Both the mature green and ripe banana fruits are vulnerable to chilling injury, however, green fruits are slightly more sensitive than the ripe ones (Kays and Paul), 2004). The application of 1-Methylcyclopropene (1-MCP), an inhibitor of ethylene action, has been used to prolong the shelf life of several climacteric fruits. Some of these fruits are banana (Boonyaritthongchai et al., 2010, Baez- Sanudo et al., 2009), mango (Hofman et al., 2001, Ambuko et al., 2013), pawpaw (Ahmad et al., 2013), avocado (Meyer and Terry, 2010), oranges (Porat et al., 1999), strawberry (Ku et al., 1999) and apples (Watkins et al., 2006). According to Sisler and Serek, (1996), the mode of action of 1-Methylcyclopropene is by binding permanently and irreversibly to ethylene receptor sites present in the particular plant tissue. This in turn leads to slow ripening and softening of fruits, thus facilitating distribution and maintaining their high edible quality conditions for longer periods of time (Blankenship and Dole, 2003; Adkins et al., 2005). However, some undesirable effects of 1-MCP such as abnormal fruit color, uneven softening and inhibited production of essential volatiles and esters have been reported in banana fruits (Fan and Matthesis, 1999). Modified atmosphere and controlled atmosphere storage have both been reported to be effective in enhancing banana fruit shelf life (Scetar et al., 2010). Controlled atmosphere storage however, requires precise control of gases making it expensive and out of reach for majority of the small scale farmers. On the other hand, MAP is a simple technology that uses bags made of polythene. In Kenya, however, the technology is not yet commercialized and has been affected by the country's ban on the use of polythene bags (Ministry of environment and natural resources, 2017). Limitations of the applicable technologies described above calls for further research in other commercially viable technologies and innovations to enhance the shelf life of banana fruits while maintaining its guality. Recently, there has been an increase in consumer awareness on food safety issues. Therefore, most of the new post-harvest innovations are biased toward the use of naturally occurring organic compounds which do not pose any threats to human health and environment. Hexanal, is one such compound which is naturally produced by plants and has the potential to enhance the shelf life of temperate fruits such as apples and strawberries (Sharma *et al.*, 2010; Tiwari and Paliyath, 2011) as well as some tropical fruits such as papaya (Hutchinson *et al.*, 2018, Hewajulige *et al.*, 2019) and mango (Anusuya *et al.*, 2016). Hexanal can be applied as either a pre harvest spray or post-harvest dip (Paliyath and Padmanabhan, 2019).

#### **1.2. Problem statement**

Banana production and marketing in Kenya is faced by several challenges, among them high postharvest losses estimated at 40% (FAO, 2014). Banana is climacteric in nature and once ripening is initiated, it proceeds rapidly resulting into high perishability. A study by Ahmed and Palta, (2015), reported that banana has a very short shelf-life of less than four days once ripe. To address this challenge, there is need for effective and affordable post-harvest technologies. Over the years, various post-harvest technologies have been developed to extend the shelf-life of banana fruits. However, the adoption rate of the existing technologies especially by small scale farmers is low due to various limitations. Some of the limitations are environmental pollution concerns (Lorevice *et al.*, 2014), high cost of acquisition and increased consumer awareness on the use of chemical preservatives on fruits. The limitations of the existing technologies have necessitated research into other commercially viable technologies.

One of the recently developed innovation to enhance the shelf life of fruits is hexanal. The otherwise naturally-occurring compound has been artificially synthesized and used to enhance the shelf-life of various temperate fruits (Paliyath *et al.*, 2008, Sharma *et al.*, 2010, Paliyath and Padmanabhan, 2019). The current formulation of this technology referred to as 'Enhanced Freshness Formulation' combines several active ingredients that can potentially enhance shelf life and maintain postharvest quality through multiple mechanisms (Paliyath *et al.*, 2008, Paliyath and Padmanabhan, 2019). Hexanal has shown great potential in enhancing the post-harvest shelf life of temperate fruits such as strawberries, nectarines, apples and peaches (Sharma *et al.*, 2010) as well as in some tropical fruits such as papaya (Hutchinson *et al.*, 2018), mango (Anusuya *et al.*, 2016) and tomato (Cheema *et al.*, 2014). The exact mechanism by which hexanal works is not clearly known. However, it has been

hypothesized that, hexanal works by inhibiting the activities of phospholipase D (PLD) enzyme, which initiates cell membrane degradation. This leads to the cell membrane remaining fresh, stable and the ripening process slowed down hence fruits remain firmer and fresh-looking for longer period (Paliyath, 2008). Although hexanal has shown promising results in some tropical fruits such as tomato (Cheema *et al.*, 2014), mango (Anusuya *et al.*, 2016), papaya (Hutchinson *et al.*, 2018) and limequats (Debysingh *et al.*, 2018), there is limited work on banana, one of the major fruits produced in Kenya

Therefore, commercial application of hexanal in Kenya calls for extensive research on major fruits such as banana to establish its effectiveness to extend the postharvest shelf life and preserve quality. Further, the best concentration, effective duration of application, the best mode of application, effect of production factors, varietal response as well as its mode of action should be established. Although hexanal has shown promising results in studies conducted in other regions such as Asia and North America, there is limited work on its efficacy on tropical fruits produced under local conditions. Kenya is a major producer of different varieties of diverse tropical fruits which are produced under different agro ecological zones. Since hexanal's effect is deemed to be physiological, it is possible that pre harvest production agro-ecological conditions, commodity factors and treatment conditions may affect its efficacy. A previous study by Cheema *et al.* (2014) on tomatoes, indicated that hexanal effectiveness is dependent on several components such as physiological maturity, concentration, application duration, method of application and nature of the commodity. Therefore, its critical to establish the effective dosing range for various commodities produced under different agro-ecological conditions, and mode of action among other factors.

#### **1.3. Justification of the study**

Successful application of hexanal and its formulations can significantly delay time to fruit harvesting, delay ripening and preserve post-harvest quality. Further, initial research on hexanal has shown that it has antifungal activity and suppresses postharvest pathogens which are a major cause of postharvest deterioration in fruits (Song *et al.*, 1998). This will translate to enhanced availability of the fruits for extended period, thus broadening the market window, enhanced income for the value chain actors and increased nutritional security. The use of hexanal provides a promising alternative to other postharvest technologies which have limited application for smallholder farmers due to various challenges. Hexanal is a simple and easy to use technology and most of the small scale farmers

targeted by this innovation can fully use this technology in their farms with minimal training. The use of hexanal does not require any specialized equipment for its application. For instance, application of hexanal as a pre-harvest spray requires only a knapsack sprayer while for post-harvest dip, a bucket, basin or trough are necessary for dipping the fruits depending on the volumes. Further, hexanal is effective at very low dose levels, making it ecomically viable for use by small-scale farmers. In the recent past, most consumers are skeptical about the use of potentially harmful chemicals and are biased towards the use of naturally occurring compounds. Being an organic compound, hexanal use does not pose health issues to consumers and the environment. Hexanal has been approved by Food and Drug Administration (FDA) agency in the United States for use in processed plant based foods as a safe green flavor compound (Thavong *et al.*, 2010). Ultimately, effective use of hexanal technology can contribute to the reduction of post harvest losses and enhance profitability for all actors in the banana value chain.

# 1.4. Study Objectives

# 1.4.1. General objective

To reduce postharvest losses and extend the marketing period of banana fruits in Kenya through application of hexanal.

# **1.4.2. Specific objectives**

- i. To determine the effective concentration, treatment duration and the best application method of hexanal in banana fruits produced under different agro-ecological conditions.
- ii. To determine the effect of hexanal treatments on the post-harvest quality of banana fruits
- iii. To elucidate the molecular basis of hexanal's mode of action in banana fruit.

# 1.5. Hypothesis

- i. Different hexanal concentrations, treatment durations and application methods have the same effect on banana fruits from the different agro ecological zones.
- ii. Hexanal treatments have no effect on the post-harvest quality of banana fruits from the different agro ecological zones

iii. Hexanal treatments have no significant effect on the expression profile of genes involved in banana ripening process.

#### **CHAPTER TWO**

#### 2.0 Literature Review

#### **2.1. Background information**

Banana is a perennial tropical herbaceous plant that belongs to the *Musa* genus of the *Musaceae* family. It originated from Southeast Asia. The plant has an underground corm and a trunk usually with leaf sheath in concentric layers. All the dessert bananas and plantains are triploid which belong to the family *Musaceae*. It's quite popular especially in local commercial trade and is one of the most traded fruits in the world (Natalia *et al.*, 2014). Banana is ranked as the leading crop in world agricultural production and trade (FAO, 2019). Global banana production rose from an average of 69 million tonnes in the year 2002 to 116 million tonnes in 2019, which is estimated at approximate value of 31 billion (FAO, 2019). The observed increase in production is mainly attributed to rising population in the world's leading producers such as Brazil, Phillipines, India and China (FAO, 2019). Asia is the largest banana producing region while Latin America and Carribean is the largest exporting regions accounting for approximately 80% of the global export (FAO, 2016). In the Africa region, banana is a major source of income and provide jobs for more than 70 million people (FAO, 2016).

#### 2.2. Banana production in Kenya

Small scale farmers dominate Kenya's banana farming with an average holdings of about 0.3 hectares (Qaim, 1999). The sub-sector has an estimated 390,000 farmers engaged in production while approximately 500,000 people are believed to be directly involved in the banana business (FAO, 2014). Banana is the major fruit in order of importance and accounts for 37.4% of the total value of fruits produced in the country with a domestic horticulture value estimated at 17.4 million USD (HCD, 2018). Banana production is mostly an enterprise for small scale farmers for food as well as income from the surplus. Harvesting of banana can be done continuously throughout the year once it is established. Banana is appropriate for intercropping, providing other crops with soil stability and shelter. During the months of October, November, December, January and February demand shifts to other seasonal fruits such as avocado and mango resulting into low prices in banana. However, the prices improve with time and are highest in August and September.

Bananas perform well in altitudes that range from sea level to approximately 1800 meters above sea level. According to HCD (2018) report, the leading counties in banana production in Kenya are Meru (20%), Murang'a (11.7%), Kirinyaga (8.1%), Taita Taveta (6.6%) and Tharaka Nithi counties (5.6%) (Table 1). Banana production is mainly under rain fed conditions, however, according to HCD report (2016), irrigation is occassionally practiced in various parts of the Eastern region. The environmental variability in terms of temperature, light, water availability and soils in the different production zones affect fruit growth, development and postharvest quality (Léchaudel *et al.*, 2006). Fruits from the warmer zones tend to mature and ripen faster with higher total soluble solids and total sugar content levels compared to the ones from the cooler zones.

Bananas can either be eaten as a fruit or cooked, serving as main staple food among different communities, especially in western Kenya and in many urban centres (FAO, 2014). The main dessert varieties include; Grand Nain, sweet banana (*sukari ndisi*), Giant Cavendish, Dwarf Cavendish, Chinese Cavendish, Gros Michel, Kampala, and *muraru* (Spilsbury *et al*, 2002). The cooking varieties include *Kiganda, Uganda green, ng'ombe, nusu ng'ombe, mutahato* and *Gradi Shisikame* (Spilsbury *et al*, 2002). Findings by Mbaka *et al.* (2008) reveal that dual purpose varieties are commonly cultivated in central Kenya where they are considered important. The different varieties may differ slightly in terms of organoleptic quality. For instance, the sweet banana variety is exceptionally sweet making it a perfect choice for use in desserts compared to the other varieties. Additionally, sweet banana does not brown as quickly as the other varieties (Mbaka *et al.*, 2008). The Cavendish variety when fully ripe is most liked by consumers as it has balanced sweetness and texture.

		2015		2016		2017		2018
COUNTY	Area (Ha)	Volume (Tons)	Area (Ha)	Volume (Tons)	Area (Ha)	Volume (Tons)	Area (Ha)	Volume (Tons)
Meru	7,038	251,132	7,503	276,919	11,305	288,266	10,542	308,095
Murang'a	5,757	159,790	5,987	154,172	7,160	166,641	7,214	173,439
Kirinyaga	6,318	142,036	6,670	145,036	6,208	122,850	5 <i>,</i> 485	152,409
Taita Taveta	2,891	67,865	4,288	63,300	2,528	58,420	2,972	74,231
Tharaka Nithi	4,204	75,544	3,734	76,633	3,622	74,940	4,112	87,540
Kiambu	4,856	132,253	4,288	63,300	6,233	117,951	4,262	70,194
Kisii	3,088	60,975	3,919	77,415	4,193	67,986	3,791	64,158
Tana River	1,985	22,692	1,852	23,091	1,861	24,246	1,400	23,550
Machakos	2,206	20,544	2,572	23,334	2,742	20,716	4,419	42,299
Kakamega	3,402	38,929	3,824	34,717	2,210	31,723	2,518	38,380
Nyamira	2,005	30,708	2,259	42,475	1,943	44,000	1,898	37,840
Bungoma	2,057	30,856	1,987	40,098	2,765	30,667	2,829	42,014
Embu	850	28,050	862	27,584	1,706	45,828	1,801	49,628
Nyeri	1,730	39,584	1,876	37,230	1,264	26,597	1,531	35,194
Others	12,332	189,192	13,013	188,254	10,547	175,149	10,744	162,875
TOTAL	60,718	1,290,150	63,299	1,288,588	69,376	1,357,162	68,248	1,419,176

 Table 1: Banana production statistics in selected Counties, 2015-2018

*Source: (HCD reports 2016 & 2018)* 

# 2.3 Nutritional value of Banana fruit

The ripening and plantain banana types are both good source of important nutrients such as vitamin C, dietary fibre, minerals and antioxidant compounds (Pereira and Maraschin, 2015). The main differences between the two types is that in the ripening bananas most of the carbohydrates comes from sugars while in plantain they are from starch (Pereira and Maraschin, 2015). Plantains contains approximately 31g of carbohydrates while ripening banana has on average 23g though this amount might vary slightly depending on the level of ripeness (Natalia *et al.*, 2014). Upon consumption of a ripe banana, the fruit instantly replenishes energy and revitalizes the body due to its soft and easily digestible flesh that is made up of simple sugars such as fructose, glucose and sucrose.

The vitamin-B6 (pyridoxine) in banana plays an important role in the treatment of anemia and neuritis (Zaman *et al.*, 2007). The fruit also contains moderate source of vitamin C which enhaces immunity against infection as well as the oxygen free radicals (Wall, 2006). The flavonoid and polyphenolic compounds in banana scavenge against oxygen-derived free radicals and reactive oxygen species (ROS) responsible for aging and various disease processes (Wall, 2006). Banana fruit is rich in various essential minerals such as potassium, magnesium, copper and manganese (Zaman *et al.*, 2007). Magnesium strengthens the bones and plays a role in protecting the heart. Manganese is required for the antioxidant enzyme, *superoxide dismutase* to function. Copper plays an important role in the synthesis of red blood cells. Compared to other mineral nutrients, the amount of potassium in banana is high, for example 100 g fruit provides approximately 385 mg (Table 2). Potassium is a significant component of cell and body fluids that helps control heart rate and blood pressure, countering bad effects of sodium (Zaman *et al.*, 2007). A detailed nutrient composition of both ripening banana and plantain is shown in Table 2. However, the nutrients can slightly vary depending on the variety and zone of production.

Constituents	Unit	<b>Ripening banana</b>	Plantain
Energy	Kcal	89	122
Water	g	74	65
Protein	g	1.1	1.3
Total lipid	g	0.3	0.4
Carbohydrate	g	21.8	32
Fibre	g	2	3
Sodium	mg	1	4
Potassium	mg	385	500
Calcium	mg	8	3
Magnesium	mg	30	35
Phosphorus	mg	22	30
Vitamin C	mg	12	20
Thiamin (Vitamin B1)	μg	40	80
Riboflavin (Vitamin B2)	μg	70	40
Niacin	μg	610	600
Vitamin B5	μg	280	0
Vitamin B6	μg	470	0
Folic acid	μg	23	0

Table 2: Com	parable com	position of	ripenina	banana and r	olantains

Source: Modified table adopted from Aurore et al., (2009)

## 2.4. Post-harvest physiology of banana fruit

Ripening is a physiological process that involves various physical and biochemical changes that make fruits appealing and edible (Giovannoni, 2001). Bananas are climacteric fruits which are usually harvested unripe but at a mature green stage. The harvested bananas pass through four phases of physiological development which include pre-climacteric, climacteric, ripening and senescence stage (Ahmad et al., 2001). The pre-climacteric phase also known as the "green phase" is characterized by low metabolic activities and the market objective is to increase shelf life as long as possible (Gowen, 1995). This is then followed by the climacteric stage characterized by increase in ethylene production which precedes the respiratory climacteric (Zhu et al., 2015). This occurs concomitantly with physicochemical and biochemical changes which transforms the fruit into an edible state with desirable quality characteristics (Mbeguie et al., 2009). Ripening is associated with changes in peel color, flavor, taste, softening and increase in membrane permeability which leads to ion leakage. Peel color is one of the most important and visible change that affects consumer acceptance and preference in most of the banana varieties. Color changes in banana fruit is due to chlorophyll breakdown in the peel which exposes the carotenoid pigment responsible for the yellowing (Sivakumar et al. 2011). This causes the banana peel to turn from green to yellow and then turns brown from the high enzymatic browning reaction (Ahmad et al., 2001). Seven distinct visual color stages have been distinguished as banana fruit ripens as shown in the ripening chart (Appendix 1). The peel color changes from dark green (stage 1) to yellow (stage 5) followed by dark brown spot appearance (stage 7). Fruits with about 20% brown spots are considered unmarketable. Ripe banana fruit goes from marketable to unmarketable state within 3-4 days depending on the storage conditions (Ahmad et al., 2001).

Flavor is another critical quality attribute that ultimately defines the fruits' acceptance by the consumer. The amount and types of flavor components such as esters and terpenoids emitted by the fruit are majorly determined by the degree of ripeness. According to Baldwin (2010), flavor consists mainly of sweetness, sourness, and aroma which comes as a result of sugars, acids and volatile compounds. Esters, alcohols, aldehydes and ketones are the predominant aroma compounds in banana fruits (Maduwanthi and Marapana, 2017). Generally, genetic and environmental factors are the key determinants of fruits' final flavor composition. Fruits grown in areas with high temperature

and light intensity have been reported to have more accumulations of total soluble solids and total sugar content compared to ones in the cooler zones (Léchaudel *et al.*, 2006). According to Valero and Serrano (2010), the proportions of individual simple sugars is paramount in the perception of taste. Fruits with high levels of fructose tend to be sweeter compared to those with high sucrose and glucose content. Additionally, the proportion of individual acids is also important in determining flavor (Lobit *et al.*, 2003). Generally, citric acid influnces expression of simple sugars such as sucrose and fructose while malic acid increases the perception of the sugars. A study by John and Marchal (1995), revealed that malic is the principle organic acid in banana fruits and increases from 1.8 meq/100 g to 6.2 meq/100 g during the ripening process.

Peel and pulp softening is an essential part of banana ripening process as it improves its palatability. The keeping quality of any fruit during ripening is directly influenced to a larger extent by the rate of softening which enhances physical damage during handling and disease susceptibility (Valero and Serrano, 2010). Therefore, during ripening process, the rate of softening should be delayed in order to enhance fruits' storage life and quality during post-harvest handling and transportation to far-off market.

The end of fruit ripening is accompanied by senescence whereby anabolic process are hindered by tissue breakdown leading to death and decay of the fruit tissue (Valero and Serrano, 2010). After harvest, breakdown of fruit tissues is rapid and more often if appropriate postharvest handling measures are not put in place, banana fruits reach consumers in deterioriated condition. There is therefore, need for use of appropriate post-harvest technologies that slow physiological processes of senescence.

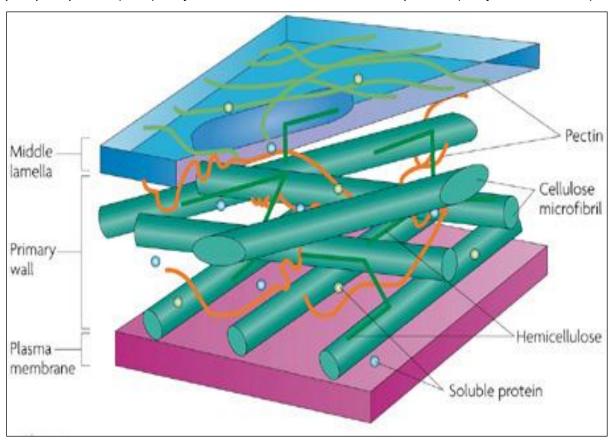
## 2.4.1. Softening in banana fruits during ripening

Banana ripening is characterized by rapid softening which affects quality, consumer acceptance and post-harvest shelf-life. Banana fruit softens mainly due to alteration in texture, disintegration of the primary cell wall and changes in the structure and composition of different polysaccharides (Toivoned and Brummell, 2008). According to Kojima *et al.* (1994), other processes such as breakdown of insoluble starch to soluble sugars may also be involved in determining the overall texture characteristics of banana fruit. Rapid softening reduces the keeping quality of banana fruit,

transportability and storage resulting to high levels of post-harvest losses. According to consumers, market acceptance in banana is greatly influenced by fruit texture. According to Johnston *et al.*, (2002), markets are frequently using fruit firmness as a yardstick to provide customers with banana of good texture. The physiological processes during ripening are the reason for the changes in fruit texture changes. This change in turn is responsible for the loss of turgor pressure which is exacerbated by accumulation of osmotic solutes in the apoplast, degradation of the cell membrane, changes in cell wall structure and starch degradation (Goulao and Oliveira, 2008). According to Valero and Serrano (2010), there is limited information on the role of each process to fruit softening, however, the authors suggest that this could vary depending on the species. Worthy to note from their study, is that alterations of cell wall composition such as the mechanical strength and cell-to-cell adhesion, are important changes to consider. Breakdown of the cell membrane initiated by the phospholipase D enzyme leads to loss of membrane integrity allowing the leakage of cellular osmotic solutes into the apoplastic space (Brummel *et al.*, 2006).

#### 2.4.1.1. Degradation of the Cell wall during fruit ripening

The cell wall is predominantly made of polysaccharides comprising of glycosidic linkage polymerization of sugar molecules as reported by Toivonen and Brummel, (2008). The cell wall mainly consists of polysaccharides and pectic substances that are integrated into one another to form a network as shown in figure 1 (Brummel, 2006). In addition, there are also some small amounts of structural proteins while middle lamella holds neighbouring cells together and determines the intercellular adhesion (Goulao *et al.*, 2008). The disintegration of the cell wall results from the solubilization of pectins and hemicelluloses (Verma *et al.*, 2017), which are acted upon by polygalacturonase (PG), pectin methylesterase (PME) and pectate lyase (PL) as reported by Amnuaysin *et al.* (2018). Polygalacturonase enzyme causes pectin depolymerization and solubilization, however, it requires the pectin to be de-methyl-esterified by pectin methylesterase (Brummel and Harpster, 2001). Pectin methylesterase action is therefore, a prerequisite to PG's action during ripening. On the other hand, pectate lyases (PLs) promotes the cleavage of glycosidic bounds of unsaturated regions of pectins by a  $\beta$ -elimination reaction (Marin-Rodriguez *et al.*, 2002). Therefore, the softening rate can be significantly reduced by suppressing these key enzymes in the fruit tissues to prolong the post-harvest fruit shelf life. The process of disassembling the cell wall



takes place in conjunction with the initiation of membrane deterioration through the activation of phospholipase D (PLD) enzyme, which drives the senescence process (Paliyath *et al.*, 2008).

Figure 1: Schematic diagram showing the structure of the plant cell wall and cell membrane. Adopted from Sticklen, (2008).

## 2.4.1.2. Cell membrane structure and changes during ripening

The cell membrane is the organelle where senescence-dependent changes occur and is made up of protein and phospholipids (Paliyath *et al.*, 2008). Phospholipids degradation is a critical factor in regulating progression of senescence in fruits (Valero and Serrano, 2010). The deterioration of the cell membrane is the result of phospholipid catabolism in the lipid bilayer and proteolysis of embedded protein as shown in figure 2. With the decline of phospholipids content, the amount of neutral lipids, mainly diacylglycerols, free fatty acids, and fatty aldehydes, progressively increase (Paliyath *et al.*, 2008). Furthermore, the level of sterols may increase resulting in an increase in the sterol-to-phospholipid ratio and such changes in membrane composition may cause non-bilayer lipid structure to form. The changes in the membrane structure may result into leakage of the leading to

the loss of compartmentalization and eventual senescence (Paliyath *et al.*, 1992). Reduction of the lipid content of the membrane results from coordinated action of lipolytic enzymes such as phospholipase D (PLD), phosphatidate phosphatase, lipolytic acyl hydrolase and lipoxygenase (Paliyath *et al.*, 2008). As a result, the products of membrane degradation such as neutral lipids and their oxidation products, initiate physiochemical changes in membrane properties during senescence. The changes further cause gel phase formation, decrease in bulk lipid fluidity, and creation of non-bilayer lipid structures resulting in the loss of compartmentalization (Thompson *et al.*, 2003). According to Wang (2000), phospholipase D is a key enzyme in membrane lipid metabolism as it catalyses the initial step in accelerated phospholipid breakdown producing lipids used in signal transduction, cell proliferation, signaling pathways, and senescence.

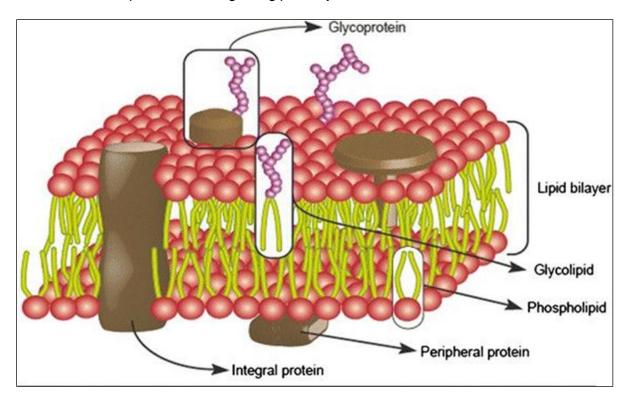


Figure 2: Schematic view of the cell membrane structure, adopted from Lombard, J. (2014).

## 2.4.1.2.1. Role of enzyme phospholipase D in membrane deterioration

During fruit ripening, membrane deterioration is inevitable as is part of the organoleptic quality development. If the deterioration is not controlled, however, the shelf life and quality of horticultural products is significantly reduced (Paliyath *et al.*, 2008). The breakdown of phospholipids found in

the lipid bilayer and proteolysis of embedded protein causes degradation of the cell membrane (Paliyath et al., 2008). Cell membrane lipid degradation occurs due to the action of several enzymes. in which one enzyme acts on the product of the previous enzyme in the sequence (Hong et al., 2000). According to Paliyath et al. (2008), phospholipase D enzyme is said to bind to the membrane resulting in a series of catabolic reactions which in turn releases several neutral lipids which makes the membrane unstable. Hydrolysis of glycerol-phospholipids by phospholipase D enzyme produces phosphatidic acid and a free hydrophilic head group (Paliyath et al., 2008). The removal of the phosphatic group from phosphatidic acid is catalyzed by phosphatidate phosphatase leading to the release of diacylglycerols. The diacylglycerol acyl chain is then de-esterified by the lipolytic acyl hrdrolase enzyme, which releases free fatty acids. Lipoxygenase enzyme acts upon the unsaturated fatty acid with *cis*-1, 4 pentadiene structures (linolenic acid and linoleic acid) causing peroxidation of the fatty acids. Linolenic acid peroxidation products may be 9-hydroperoxylinoleic acid or 13hydroperoxylinoleic acid and are subjected to hydroperoxidelyase cleavage resulting in the formation of short chain aldehydes (C6-C9) as hexenal or (Z)-3-hexenal (Paliyath et al., 2008). Phospholipase D (PLD) is however, the main enzyme involved in membrane deterioration initiation since the subsequent enzymes cannot directly cause phospholipid catabolism (Tiwari and Paliyath, 2011). In view of this metabolic pathway, inhibition of PLD by hexanal is expected to reduce the degradation of banana membrane lipids and improve quality retention by suppressing compounds derived from lipids that alter aroma volatiles.

Various environmental factors such as hormones, heating, freezing and chilling have been reported to regulate the activity of phospholipase D enzyme (Paliyath *et al.*, 1992). The structure of the enzyme is such that the two active sites and several co-factor binding motifs aid in its activatation as reported by Paliyath *et al.* (2008). Studies by Yuan *et al.* (2006) using strawberry have shown that the PLD is confined in cytosol, membrane and cell wall compartments. A study by Paliyath *et al.* (2008), reported that partial inhibition of PLD enzymes in fruits is beneficial since less carbon substrates are required for the synthesis of the lost phospholipids. The spared carbon source can then be assembled through metabolite channeling for biosynthesis of beneficial quality enhancing components such as isoprenoids, carotenoids, anthocyanins and phenolic components.

#### 2.4.2. Applicable technologies to preserve post-harvest quality of banana fruits

Application of best post-harvest technologies is essential in order to slow down the rate of ripening and senescence in banana fruits. This minimizes losses between harvesting and consumption, maintain the best possible quality and ensure food safety (Chun, 2010). Over the years, different types of post-harvest technologies and practices have been developed and are in use in banana fruits. Some of these technologies include controlled atmosphere storage and modified atmosphere storage, low temperature storage and 1-Methylcyclopropane (1-MCP). However, there is need for continued evaluation of emerging new innovations. The use of hexanal and its formulations, is one such innovation which has shown great potential to enhance keeping quality of different temperate fruits (Paliyath *et al.*, 2008).

#### 2.4.2.1. Controlled atmosphere (CA) and Modified atmosphere (MA) storage

These two technologies involve alteration in the composition of gases surrounding the fresh produce. This mostly involves reduction of oxygen concentration, increased carbon dioxide concentration and increased humidity levels which in turn reduces rate of respiration, transpiration and ethylene biosynthesis. Controlled atmosphere storage (CAS) involves the precise control of the concentrations of oxygen and carbon dioxide at storage. Therefore, the gases are periodically measured and adjusted to the predetermined levels. Controlled atmosphere storage has been used successfully in banana fruits where Truter (1990), showed that banana fruits harvested at mature green stage had an extended shelf-life of six weeks when stored at 2% oxygen and 7% carbon dioxide at 12.5°C. Generally, the post-harvest life potential of banana fruits under CAS storage at ambient room conditions is 2 to 4 weeks (Kader, 1994). However, the precise control of gases under CAS condition is guite expensive and not a viable technology for the numerous small-scale banana farmers in a developing country such as Kenya. Modified atmosphere packaging (MAP) is similar to CAS except that the atmospheric composition is obtained passively through the combined effect of respiration, transpiration and the use of sealed semi-permeable plastic bags (Kader, 2005). According to Hewage et al. (1995), green mature banana can be stored up to 30 days under MAP at 8-14°C. However, in Kenya the use of polythene bags for fruit packaging is not yet commercialized and the use of plastic bags in the country is currently prohibited (Ministry of environment and natural resources, 2017).

#### 2.4.2.2. Cold storage

Storage at low temperature is one of the most effective tools for retaining quality and enhancing the post-harvest shelf life of harvested fruits. This is possible because low temperature reduces the fruit metabolism which in turn delays evolution of parameters related to fruit ripening. The harvested produce should be kept at lower temperatures immediately harvesting is done, so as to reduce the metabolic activities. Rate of respiration is one of the main metabolic activities in harvested produce which determines commodity rate of deterioration. Additionally, low temperature storage in climacteric fruits such as banana reduces the production and sensitivity of ethylene hormone which accelerates the ripening and senescence process. A study in banana demonstrated that maximum ethylene is produced at a temperature of 40°C then decreases significantly with a reduction in temperature (Valero and Serrano, 2010). Being a tropical fruit, banana is susceptible to chilling injury if stored at temperatures below 13°C (Satyan *et al.*, 1992). The chilling injury symptoms include retarded peel color development and failure of the fruit to soften.

### 2.4.2.3. 1-Methylcyclopropene (1-MCP)

Climacteric fruit such as banana produce relatively higher amounts of ethylene at the onset of the climacteric rise which initiates the ripening process. The high rate of ethylene production makes banana a very perishable fruit necessitating the use of post- harvest technologies to extend its shelf life. 1-MCP is an antagonist of ethylene responses that binds irreversibly on the receptors that would otherwise be occupied by ethylene and thus inhibiting ethylene from triggering ripening response to the fruit (Sisler and Serek, 1996). However, appearance of new ethylene receptors later in storage results to eventual ripening of the treated fruit (Feng *et al.*, 2000). Over the past few decades, 1-Methylcyclopropene (1-MCP) has successfully been tested and used in banana fruits (Harris *et al.*, 2000, Boonyaritthongchai *et al.*, 2010, Baez- Sanudo *et al.*, 2009). However, some problems were noted following fruits treatments with 1-MCP. It was noted that banana fruits treated with 1-MCP stayed green or ripened with uneven color (Harris *et al.*, 2000). Additionally, it was reported that total volatile production especially esters were inhibited in banana fruits treated with 1-MCP (Golding *et al.*, 1998). A study by Zhu *et al.* (2015), recommended that for better results and even ripening in banana fruits, they should first be subjected to artificial ripening using ethylene before administering 1-MCP.

### 2.4.2.4. Hexanal and Enhanced Freshness Formulation (EFF) Application

Hexanal is a C-6 aldehyde which occurs naturally in plants and it contributes to many fruit and vegetable species' "green" taste (Croteau, 1978). According to Schwab *et al.* (2008), hexanal is formed through the lipoxygenase pathway which consists of four enzymes including lipoxygenase (LOX), Hydroperoxide lyase (HPL), 3Z, 2Eenal isomerase, and alcohol dehydrogenase (ADH) as shown in figure 3. Biogeneration of C6 aldehyde such as Hexanal in plants is due to the formation of 13- and 9-hydroperoxides from C18 fatty acids such as linoleic acid or linolenic acid (figure 3). This is followed by their stereospecific cleavage by fatty acid hydroperoxide lyases (Schwab *et al.*, 2008). Cell walls and membranes may become more permeable during fruit ripening, letting the lipoxygenase pathway to become active without disruption of the tissue (Matsui *et al.*, 1998).

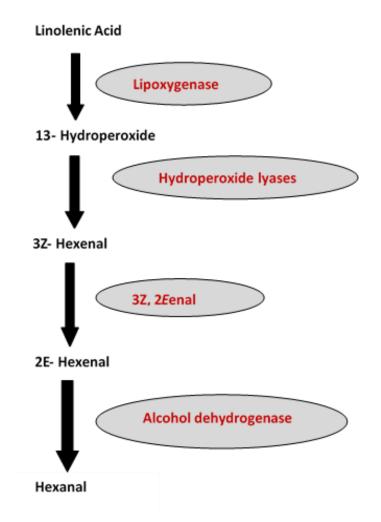


Figure 3: Summary diagram of hexanal biosynthesis pathway, modified from Schwab et al. (2008).

As early research shows, volatiles formed by this pathway in wounded plants have antifungal properties (Song *et al.*, 1998). Hexanal's initial research focused on its antifungal activity and ability on a number of fruits to suppress postharvest pathogens (Song *et al.*, 1998). Hexanal does not remain in the treated tissues after 48 hours of treatment and is also approved for use in processed plant-based foods as a green flavor by the FDA in the United States (Paliyath and Padmanabhan, 2019). Human intake threshold (maximum permitted daily intake) is estimated at 1800µg per day for compounds such as Hexanal. Hexanal is oxidized in the body to hexanoic acid and further oxidized during respiration to carbon dioxide and water through the tricarboxylic acid cycle (Mattia, 1998). Research on several fruits and vegetables has shown that hexanal has an effect on processes of fruit maturation and ripening when applied externally either as a pre-harvest spray or as a post-harvest dip (Paliyath *et al.*, 2008, Paliyath and Padmanabhan, 2019).

Hexanal has been formulated to form an 'Enhanced Freshness Formulation' (EFF) having geraniol, ascorbate, ethanol and calcium chloride (Sharma *et al.*, 2010). Hexanal is immiscible with water and therefore, Tween 20 is added to enhance its solubility (Tiwari and Paliyath, 2011). The application stage is very critical as inhibition of PLD has very little effect once the membrane has started to deteriorate. A study by Paliyath *et al.* (2008), observed that for optimal results in terms of color and flavor development, tomato fruits should be treated at mature green stage while Sharma *et al.*, (2010) reported that a treatment of about 15 days and 7 days before harvest in cherries maximizes keeping quality to 30 days at 4° C. In addition to increasing fruits shelf-life, hexanal treatment has been shown to increase fruits retention on the tree in Mango (Anusuya *et al.*, 2016), papaya (Hutchinson *et al.*, 2018), peach and nectarines (Paliyath *et al.*, 2008). This is quite advantageous as farmers have sufficient time to source market for their produce. In turn, this minimizes overexploitation of farmers by middlemen. Hexanal's mode of action is not fully understood and is currently undergoing further investigations. Previous studies have however, suggested that hexanal works by inhibiting phospholipase D enzyme action which initiates degradation of the cellular membrane (Paliyath *et al.*, 2008, Paliyath and Padmanabhan, 2019).

### 2.4.2.4.1. Effects of hexanal and its formulations on post-harvest shelf life and quality

Being a relatively new technology, several studies are currently underway in different fruits to establish the effect of hexanal on shelf life and post-harvest quality. Studies in cherries, guava and

green house tomatoes have shown considerable improvement in nutritional content and keeping quality (Gill *et al.*, 2015; Cheema *et al.*, 2014, Sharma *et al.*, 2010). Generally, hexanal treatment has shown tremendous success in delaying senescence without compromising colour and flavour development (Tiwari and Paliyath, 2011). A study by Tiwari and Paliyath (2011), revealed that hexanal treatment suppressed expression of genes responsible for ethylene production, cell wall breakdown and lipid metabolism pathways without altering those responsible for characteristic quality development. Hexanal-treated fruits were observed to have better aroma due to enhancement of some flavor compounds such as terpenes and alcohols. In tomato fruits, the color intensity was slightly high in hexanal treated fruits compared to the untreated ones as reported by Cheema *et al.*, (2014). In Cherries, Sharma *et al.* (2010), reported an increase in red color intensity in fruits sprayed with hexanal.

Fruit softening which exacerbates post-harvest losses in fruits is reportedly delayed by hexanal treatment in several fruits such as papaya (Hutchinson *et al.*, 2018), tomatoes (Cheema *et al.*, 2014), mango (Anusaya *et al.*, 2016), Cherries (Sharma *et al.*, 2010), peaches, apples and strawberries (Paliyath *et al.*, 2008). The delayed softening may be as a result of hexanal down regulation of polygal acturonase and  $\beta$ -gal actosidase genes whose manifestation is important for pectin breakdown and fruit softening. Further, hexanal role as a phospholipase D enzyme inhibitor which reduces cell membrane degradation might explain the delayed softening in the hexanal treated fruits.

A study by Utto *et al.* (2008) revealed that hexanal treatment leads to increased vitamin C content and pathogen resistance in fruits. Additionally, hexanal treatment has been shown to improve total soluble solids and sugars in mango (Anusuya *et al.*, 2016), banana (Venkatachalam *et al.*, 2018) and papaya fruits (Hutchinson *et al.*, 2018).

### **CHAPTER 3**

# 3.0. Efficacy of Hexanal on the physical and physiological post-harvest characteristics of Banana Fruits (*Musa* spp) in Kenya

### **3.1 Abstract**

Banana is highly perishable fruit with a short shelf life of less than four days once it is ripe and this leads to high post-harvest losses. The use of appropriate postharvest technologies can reduce these losses considerably along the banana value chain. Hexanal, a naturally occuring compound is one of the recent post-harvest technologies which has shown promising results in various fruits. The objective of this study was to determine the effectiveness of hexanal in enhancing the shelf life of 'Grand nain' and 'sweet banana' fruits as affected by concentration, treatment duration, mode of application and agro-ecological zone (AEZ) of production.

The study was done in Meru County, a high potential agro-ecological zones (AEZ II) and Machakos County, low potential AEZ IV of Kenya. Two methods of hexanal application (pre-harvest spray and postharvest dip) and two concentrations (2% and 3%) were evaluated on 'Grand nain' and 'sweet banana' varieties. In the pre-harvest hexanal treatment experiment, 2% and 3% hexanal concentrations were sprayed either once at 30 days or twice (at 30 and 15 days) before harvest. Once 20% of the fruits per bunch were ripe (based on peel color changes), they were harvested for shelf-life studies. For the post-harvest dip mode of application, fruits harvested at the mature green stage were dipped into either 2% or 3% hexanal, or plain water (control) for 2.5 minutes or 5 minutes. The fruits were left to ripen at ambient room temperature conditions (25  $\pm$  1°C and RH 60  $\pm$  5%). Physiological parameters including rate of respiration and rate of ethylene production and physical parameters including percentage cumulative weight loss, peel color, peel and pulp firmness were evaluated at 3-day intervals.

The results revealed that Hexanal applied twice as a spray significantly (p < .05) delayed time to fruit harvesting by 12 days and 18 days in 'Grand nain' bananas produced in AEZ IV and AEZ II, respectively and 12 days in 'sweet banana' variety in both zones compared to the untreated control fruits. The duration of application significantly (p < .05) affected the effectiveness of hexanal, with the post-harvest dip for 5 minutes enhancing fruit shelf life by 9 and 6 days compared to 6 and 3 days

in fruits dipped for 2.5 minutes in 'Grand nain' and 'sweet banana' respectively. The extended shelf life was evidenced by delayed fruit softening, reduced rate of respiration and ethylene production. The findings of this study reveal that hexanal at 2% or 3% applied either as a pre-harvest spray (30 days and 15 days before harvest) or as a post-harvest dip (5 minutes) has the potential to extend banana shelf life. Applying hexanal as a pre-harvest spray at 30 and 15 days before harvest can also delay time to banana harvesting by approximately 12-18 days which is very beneficial to small scale farmers because of the extended marketing period.

#### **3.2 Introduction**

High post-harvest losses estimated at 40% are a major constraint facing small-holder fruit farmers in developing countries such as Kenya (FAO, 2014). These losses are attributed to the extremely perishable nature of banana fruits and are further compounded by non-use of appropriate post-harvest technologies. Banana is the most important fruit in Kenya since its production is throughout the year in almost all the agro-ecological zones (AEZ) unlike most of the other fruits which are seasonal (HCD, 2018). Banana is harvested commercially at the mature green stage and then ripened before marketing. However, upon ripening, the fruits are highly perishable with a shelf life of only 3-4 days and this limits their marketability (Ahmed and Palta, 2015). Being climacteric in nature, banana produces high levels of ethylene that triggers ripening and senescence. Banana ripening is characterized by rapid softening that predisposes them to other agents of deterioration including water loss and pathological deterioration. Application of postharvest technologies to slow down the deteriorative processes is key to enhance the fruit's shelf life, preserve quality and extend their marketing period, ultimately reducing postharvest losses.

Over the years, numerous post-harvest technologies have been developed to tackle post-harvest losses in perishable commodities. Some of these technologies are, cold storage, Modified Atmosphere Packaging (MAP), Controlled Atmosphere Storage (CAS), 1-Methylcyclopropene (1-MCP) among others. Studies in banana have shown that for best results, the fruits should be stored at temperatures above 10°C- 13°C to avoid chilling injuries which compromises post-harvest quality (Pongprasert *et al.*, 2011). In addition, low temperature storage can be combined with other post-harvest technologies for better perfomance in terms of prolonging shelf life and preserving quality. The use of MAP technology is suitable for use especially by small-scale farmers in developing countries such as Kenya since it does not require any sophisticated equipments. Use of MAP has shown great potential in extending the post-harvest shelf life of various banana varieties such as 'Sucrier' (Romphophak *et al.*, 2004), 'Cavendish' (Chauchan *et al.*, 2006) and 'Robusta' (Kudachikar *et al.*, 2011). However, the adoption rate of MAP technology has been affected by the country's ban on the use of polythene bags (Ministry of environment and natural resources, 2017). Technologies targeting ethylene biosynthesis and action have proven to be very effective in enhancing shelf life of various climacteric fruits. 1-methylcyclopropene (1-MCP) is one such technology which works by binding to the ethylene receptors with a 10-fold higher affinity than ethylene. The use of 1-MCP has successfully been used in various fruits including bananas (Boonyaritthongchai *et al.*, 2010, Baez- Sanudo *et al.*, 2009). However, 1-MCP is not readily available for use especially by small scale banana farmers in Kenya. Limitations for these applicable technologies in developing countries such as Kenya calls for continued research into safe and affordable postharvest technologies that are easily available.

Recent advancements in post-harvest research have yielded new applicable technologies to extend shelf life of perishable commodities. One of the recently developed technology for postharvest management of fruits and vegetables is hexanal. Hexanal, is a naturally-occurring C6 aldehyde produced from the catabolism of unsaturated fatty acids via the lipoxygenase pathway (Croteau, 1978). Hexanal production is stimulated naturally in plants after wounding or during fruit ripening (Schwab et al., 2008). Hexanal use provides an eco-friendly and economically feasible post-harvest preservation product (Paliyath et al., 2008) which is safe to human beings. Once in the human body, hexanal is completely oxidized into carbon dioxide and water through the tricarboxylic acid cycle during respiration process (Paliyath et al., 2008). Studies by Song et al. (1998), have shown that hexanal has antifungal properties against Alternaria alternate and Botrytis Cinerea, which are postharvest pathogens. Previous studies have shown that hexanal has the potential to delay ripening in several fruits such as strawberries, apples and peaches (Paliyath and Padmanabhan, 2019), cherries (Sharma et al., 2010), mango (Anusuya et al., 2016) and papaya (Hutchinson et al., 2018). To enhance its commercial application, Enhanced Freshness Formulation (EFF), which is a biochemical formulation of an artificially synthesized version of hexanal has been developed (Sharma et al., 2010).

Effectiveness of hexanal has been shown to be affected by various factors including species/variety, stage of development of the fruit, mode of application and dosage among other factors. The stage of plant development is crucial in hexanal application as shown by Paliyath *et al.* (2008), who reported that the treatment effect decreased with advancement in fruit development in tomatoes. The best results for color and flavor development were observed when tomatoes were sprayed at mature green stage. In berry fruits such as cherry, maximum extension in shelf life to 30 days was obtained when hexanal was applied at two weeks and one week before harvest (Sharma *et al.*, 2010). According to Paliyath *et al.* (2008), effective concentrations of hexanal vary with commodity, variety and method of application. For example in 'Gala' apple, a concentration of 2% was more effective compared to 1% in 'Delicious' and 'IdaRed' varieties when applied as a pre-harvest spray. A concentration of 1% in strawberry was best applied as a pre-harvest spray, while 0.05% was best applied as a post-harvest vapor (Paliyath *et al.*, 2008). Since hexanal effect is physiological, it is presumed that pre-harvest factors such as temperature and water availability might affect its effectiveness. However, there is no published work on the effect of production zone on the efficacy of hexanal.

Despite the potential benefits of hexanal application in tropical fruits such as banana, there is limited information on hexanal application and therefore no commercial use. The objective of this study was therefore, to establish the effectiveness of hexanal as a postharvest treatment to extend the shelf life of banana fruit as affected by mode of application, dosing, variety and agro-ecological zone of production.

### **3.3 Materials and Methods**

#### 3.3.1 Site description

The study was conducted in two agro-ecological zones (AEZ) of Kenya, namely South Imenti location in Meru County and Kithimani location in Machakos County. Meru County is a high potential AEZ II that lies at an elevation of between 1980- 2700 meters above sea level with an average temperature of 20°C and receives an annual average rainfall of 1500mm. Most of the soils are humic nitisols which are moderately well drained with high nutrient availability (Jaetzold and Schmidt, 1983). On the other hand, Machakos County is a semi-arid zone (AEZ IV) that lies at an elevation of between 1000- 1600 meters above sea level with an average temperature of 25°C and

annual average rainfall of 600mm (FAO, 1996). The soils in Machakos County are a combination of sandy and loamy soils with good drainage and low nutrient availability. A detailed soil analysis of the two study sites is presented below (Table 3). Meru County is currently the leading producer of banana fruits in Kenya (HCD, 2018) while Machakos county is located in a dry region where banana production is practiced on a limited scale. 'Grand nain' and 'sweet banana' varieties are popular dessert bananas in Kenya produced for local consumption. The two varieties differ morphologically and hence appropriate for comparison on their response to hexanal treatment. Choice of farmer selection was based on a baseline survey conducted at the onset of the study. Small to medium-scale farmers were randomly selected in the two AEZs based on their willingness to participate in the study; if they were undertaking good agricultural practices (GAP); producing substantial amount of the two varieties to be used and if they had potential to adopt the technology. Additionally, the selected farmers were members of a common farmer group, in which they are periodically trained on good agricultural practices. To avoid biasness, farmers conducting almost similar agronomic pratices were chosen for this study. The fruit trees were chosen randomly in the selected farmers' fields and tagged for the experimental work. The experiment were conducted in two successive seasons, July to November 2016 and January to April 2017.

Soil parameters	AEZ II	AEZ IV
Soil type	Humic nitisols	Sandy Ioam
рН	6.4	6.96
Potassium (ppm)	1.48	1.4
Nitrogen (%)	0.12	0.09
Phosphorous (mg/kg)	22.6	17.3
Organic Carbon (%)	1.42	0.97
Magnesium (g/100g)	3.24	2.93

 Table 3: Soil analysis results for the two study sites (AEZ II and AEZ IV)

### **3.3.2.** Experimental designs and treatments

A preliminary study was conducted prior to the actual study to establish the dosing range for hexanal. During the preliminary study, different hexanal concentrations were evaluated including 1%, 2%, 3%, 4% and 5% applied at three timings (30, 15, 7 days before harvest). The results revealed that the hexanal concentration of 1% and the timing of 7 days had no significant effect on delaying time to banana harvesting and ripening compared to untreated fruits. On the other hand, concentrations of 2%, 3%, 4% and 5% applied either at 30 days or at 30 and 15 days extended the time to fruit harvesting and delayed ripening but did not have any significant differences among them. Therefore, 2% and 3% were selected as low but effective hexanal concentrations for further evaluation.

For the pre-harvest spray mode of application experiment, a total of 160 plants were randomly selected at flowering stage and used for the whole study. The selected banana trees were tagged with strings of different colors for ease of identification; 'Grand nain' variety was tagged with Red strings while 'sweet banana' was tagged with yellow strings. The experiment was arranged in a randomized complete block design (RCBD), blocking was done by variety. Two different hexanal concentrations (2% and 3%) as well as control (clean plain water) were sprayed on two banana varieties ('Grand nain' and 'sweet banana') at either 30 days, or 30 and 15 days before harvest. Tween 20 and ethanol were added to enhance hexanal's solubility in water (Tiwari and Paliyath, 2011). The stock solutions were mixed with water and diluted accordingly inorder to obtain the required hexanal concentrations. The fruits were sprayed to the point of dripping with the hexanal solution by use of a knapsack sprayer. To avoid spray contamination, alternate rows of trees for the experiment were used and a five tree gap in the same row of trees between the treatments. There were three replications per treatment. The fruits were monitored twice every week for changes in peel color and fruit retention. The fruits were left on the tree until about 20% of the total fruits per bunch had their peel color change from green to yellow. The fruits were then harvested for post-harvest analysis. In the laboratory, the fruits were washed, dried in open shelves and left to undergo normal ripening at ambient room temperature conditions  $(25 \pm 1^{\circ}C \text{ and } RH 60 \pm 5^{\circ})$ .

For the post-harvest dip mode of application experiment, approximately 400 banana fruits were harvested from unsprayed trees at mature green stage based on angularity of the fingers and the number of days after anthesis (approximated at 104 days). However, only 360 fruits were utilized as more fruits were harvested to compensate for any injuries during transportation. In each banana bunch, only the middle hands were used in the analysis. The harvested fruits were packed in cushioned crates and transported immediately to the postharvest laboratory at Jomo Kenyatta University of Agriculture and Technology. The experimental design used was a Complete

Randomized Design (CRD) with factorial arrangements. The factors included variety, agroecological zone of production, mode of application, hexanal concentration and application time.

# **3.3.3 Sample preparation**

In the laboratory, the fruits were washed in clean water, dried and selected for uniformity and freedom from mechanical injuries. All the harvested fruits were pooled together to ensure homogeneity. Fruits for the various treatments were randomly selected from the pooled homogenous samples to ensure uniformity of the starting samples. Pre-harvest sprayed fruits were left to undergo normal ripening under ambient room conditions. Fruits for post-harvest dip treatment were dipped into one of the two hexanal concentrations (2%, 3%) or plain water (control) for either 2.5 minutes or 5 minutes. All the fruits were left to undergo normal ripening under ambient combination were randomly sampled at 3-day intervals to evaluate respiration rate, ethylene evolution rate, and cumulative weight loss. Three fruits were also randomly sampled to evaluate other ripening-related parameters such as peel color, peel and pulp firmness.

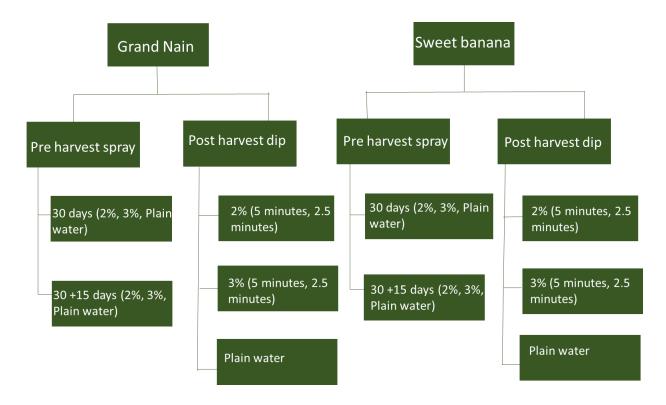


Figure 4: Summary of the experimental layout used for objective one experiment

### 3.3.4 Evaluation of Physical and Physiological Attributes

# 3.3.4.1. Number of days to fruit harvesting

For pre-harvest spray treatment, the number of days to fruit harvesting was monitored by observing how long the treated and untreated fruits took for 20 % of the fruits per bunch to change peel color from green to yellow. Peel color was determined in the field using a NF-333-Color spectrophotometer (Nippon Denshoku Industries, Japan) on three fruits per bunch at 6-day intervals. The L\*, a\*, and b\* coordinates were recorded and a\* and b\* values converted to hue angle (H°).

# 3.3.4.2. Shelf-life

This was determined by counting the number of days from initial day of harvest to the optimal, edible ripe stage (Standard Banana Ripening Chart Stage 7, see Appendix 1).

# 3.3.4.3. Rate of respiration and ethylene production

Five banana hands randomly selected from each of the treatment combination were sampled, numbered and initial weight measured. Each of the five hands were placed in a four litre air-tight containers fitted with self-sealing rubber septa for gas sampling and incubated for one hour at room temperature. Gas samples were taken from the headspace using an airtight 1 ml hypodermic syringe and injected into gas chromatographs (Models GC-8A and GC-9A, Shimadzu Corp., Kyoto, Japan) for measurement of respiration and ethylene production rates respectively. The gas chromatograph for carbon dioxide determination were fitted with a thermal conductivity detector (TDC) and a Poropak N column while that of ethylene determination were fitted with an activated alumina column and a flame ionization detector (FID). Rate of ethylene and respiration was calculated using the formula  $k \times 1/r \times h \times (v-w)/t/w$ . The rate of ethylene production was expressed as nl/g/hr while rate of carbon dioxide production (used to estimate respiration rate) was expressed as ml/Kg/hr.

Where: k = Calibration value (nm equivalent to 1cm peak height on gas chromatograph)

r = Volume of gas injected for sample (ml)

h = Weight of sample (g in case of ethylene and kg for respiration)

t = Incubation time (hr).

### 3.3.4.4. Percentage weight loss (PWL)

Five banana hands (cluster of six fingers) were randomly sampled from each treatment and clearly numbered. The initial weight of the selected hands was measured using a digital balance (Model Libror AEG-220, Shimadzu Corp. Kyoto, Japan). The same fruits were weighed at three day interval during the storage period. The initial weight (W<sub>1</sub>) of each hand at day 0 and the new weight of the same hand on the subsequent days (W<sub>2</sub>) was noted. The following formula was used to calculate the % physiological loss in weight:

Percentage weight loss (%) =100 x (W<sub>1</sub> - W<sub>2</sub>)/W<sub>1</sub>

## 3.3.4.5. Peel and pulp firmness

Firmness was measured along the equatorial region of individual fruit using a penetrometer (CR-100D, Sun Scientific Co. Ltd, Japan) fitted with an 8 mm probe. Four locations along the equatorial zone of the fruit were used and average value of firmness calculated. For the peel firmness, a pointed probe was used to penetrate the fruit peel and the force required recorded to determine firmness. For pulp firmness, banana was peeled first, the probe was allowed to penetrate the flesh to a depth of 1cm and the corresponding force required to penetrate this depth determined. Firmness was expressed as Newtons (N/mm) (Jiang *et al.*, 1999).

### 3.3.4.6. Peel Color

Color was measured at three different spots of three fingers randomly selected from each treatment using a Minolta color difference meter (Model CR-200, Osaka, Japan) calibrated with a white and black standard tile. The L\*, a\* and b\* coordinates were recorded and a\* and b\* values converted to hue angle (H°) according to Mclellan *et al.* (1995).

# 3.3.5 Statistical analyses

The data collected was subjected to analysis of variance (ANOVA) using the Genstat statistical package (version 15). The means were separated by Least Significance Difference (LSD) at  $p \le .05$  using Fisher's protected test.

#### **3.4 Results**

### 3.4.1 Days to fruit harvesting and shelf life

Application of hexanal as a pre harvest spray significantly (p < 0.05) delayed the duration to fruit harvesting to different extends based on the number of sprays done, variety and AEZs (Table 4) as compared to the untreated ones. Application of hexanal delayed fruit harvesting by 6 and 12 days in fruits sprayed once at 30 days and those sprayed twice at 30 and 15 days before harvest respectively, in AEZ IV in both varieties and in 'sweet banana' from AEZ II. However, hexanal, delayed fruit harvesting by 18 days in 'Grand nain' fruits from AEZ II sprayed twice at 30 and 15 days before harvest. This was determined by measuring changes in peel color of the fruits while on the tree. The banana bunches were harvested when at least 20% of the fruits had changed color from green to yellow. Overall, hexanal treatment significantly delayed changes in peel color while on the tree in both AEZs compared to the controls (Table 4). Generally, fruits from the colder AEZ II maintained significantly higher peel hue angle compared to those from drier AEZ IV. The hue angle in 'Grand nain' fruits decreased from an initial of 141.8° to 119° (day 54) and 132° (day 60) in fruits sprayed twice compared to 112° and 125° in the control fruits at day 42 in AEZ IV and AEZ II respectively (Table 4). A similar trend was observed in 'sweet banana' sprayed fruits (double spray) which had hue angle of 115°-121° (day 42) and 124°-129° (day 48) compared to 108° (day 30) and 115° (day 36) in the untreated fruits from AEZ IV and AEZ II respectively (Table 4). A significant varietal differences were observed were changes in peel color were more drastic in 'sweet banana' variety as compared to 'Grand nain' in both AEZs.

Upon harvesting the fruits, shelf life studies were done in both modes of hexanal application (preharvest spray and post-harvest dip). Results of this study showed that, banana shelf life was significantly affected by the interaction between mode of hexanal application, treatment duration and variety. Pre-harvest spray treated 'Grand nain' fruits had a shelf life of 6 days and 3 days incase of double and single sprays, respectively in both AEZs (Figure 6). However, pre-harvest sprayed 'sweet banana' fruits had a shelf life of 3 days irrespective of AEZ and hexanal time of application (Figure 5). On the other hand, post-harvest dip treated 'Grand nain' fruits for 5 minutes had a longer shelf life of 9 days compared to 6 days in 'sweet banana' variety in both AEZs (Figures 7 and 8).

Table 4: Fruit peel color (Hue Angle <sup>0</sup>) in 'Grand nain' and 'sweet banana' fruits from AEZ IV (Machakos County) and AEZ II (Meru County) exposed to different pre-harvest Hexanal spray regimes.

DAYS	·(	Grand naii	n'				· 5	sweet bar	nana'	
ZoneIV	2 <b>%-S</b>	2%-D	3%-S	3%-D	Control	2 <b>%-</b> S	2%-D	3%-S	3%-D	Control
0	141.8b	141.8b	141.8a	141.8b	141.8b	141.8a	141.8b	141.8a	141.8a	141.8a
6	145.1a	145.5a	143.4a	140.3b	143.5a	145.6a	140.7b	137.3b	143.3a	143.3a
12	142.8a	140.8b	142.1a	145.8a	147.5a	140.2b	143.5a	141.5a	144.8a	139.0a
18	136.9b	140.3b	139.4a	141.5a	135.0c	135.7b	138.1b	140.1b	140.0b	139.3a
24	139.2b	143.4ab	141.5a	144.5a	124.0d	127.2c	132.9c	129.3c	138.0b	130.3b
30	130.0c	134.7c	137.2b	138b	125.1d	120.9d	132.9c	124.0d	133.9c	<b>108.</b> 2d
<b>3</b> 6	125.1d	137.7c	132.6c	139.3b	12 <b>3.9</b> d	114.7e	132.1c	115.4e	131.9c	
42	123.7d	<b>131</b> .7d	126.2d	136.2b	110.9e		121.5d		115.1e	
48	111.1e	134.7c	114.4e	127c						
54		116.6e		119.7e						
60										
ZoneII										
0	141.8b	141.8a	141.8a	141.8b	141.8b	141.8a	141.8a	141.8a	141.8a	141.8a
6	142.9ab	145.3a	140.3a	142.7a	141.1b	142.9a	144.0a	144.7a	142.7a	143.2a
12	141.6ab	143.3a	142.6a	142.4a	141.2b	143.7a	142.1a	142.0a	143.6a	141.5a
18	140.1b	140.9b	142.6a	141.7a	140.0b	138.3b	146.6a	140.4a	142.7a	141.2a
24	140.2b	142.8a	141.3a	142.6a	135.0c	138.5b	142.1a	140.7a	139.2b	138.8a
30	137.1b	141.0b	138.5b	140.6b	132.5c	136.8b	138.5b	137.5b	138.8b	130.5b
<b>3</b> 6	133.7b	141.9a	135.5b	140.4b	124.6d	134.5bc	138.1b	129.8c	137.2b	114.6c
42	131.1cb	136.3c	131.0cb	137.9b	12 <b>3.0</b> d	117.8d	137.3b	121.7d	135.1bc	
48	124.9d	139.5cb	12 <b>7.8</b> d	134.7bc			128.5c		12 <b>3.8</b> d	
54		136.6c		132.9c						
60		130.0d		131.6c						
Mean	134.8	137.9	135.6	137.9	132.8	134.3	137.5	135.1	137.4	133.1
LSD*(T)	1.2									
$LSD^{*}(T^{*}V^{*}Z))$	2.5									
% CV	3.9									

S= Single spray at 30 days before harvest, D= double spray at 30 and 15 days before harvest, T= Treatment, V= Variety and Z= Agro ecological zone of production. Values followed by different letters within the same column differ significantly at (p < 0.05).

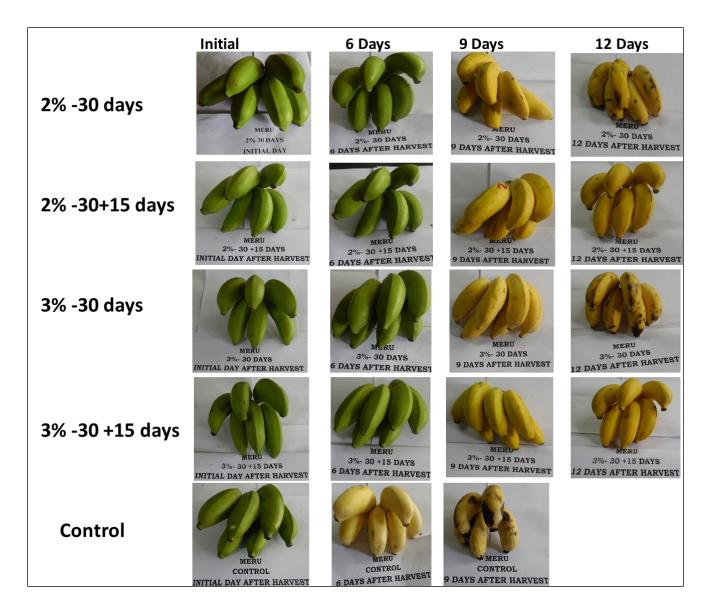


Figure 5: Progression of ripening in 'sweet banana' fruits sprayed with hexanal (2 % and 3 %) either once at 30 days or twice at 30 and 15 days before harvest or left untreated and allowed to ripen under ambient room conditions.

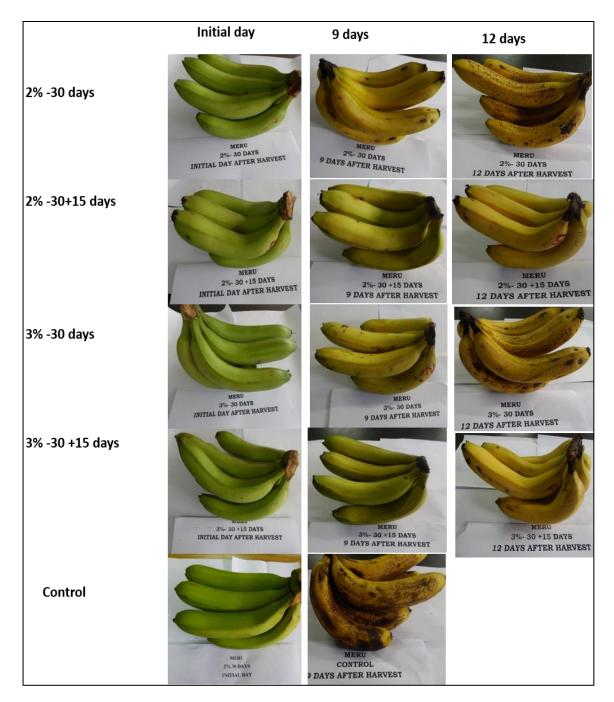


Figure 6: Progression of ripening in 'Grand nain' banana fruits sprayed with hexanal (2 % and 3 %) either once at 30 days or twice at 30 and 15 days before harvest or left untreated and allowed to ripen under ambient room conditions.

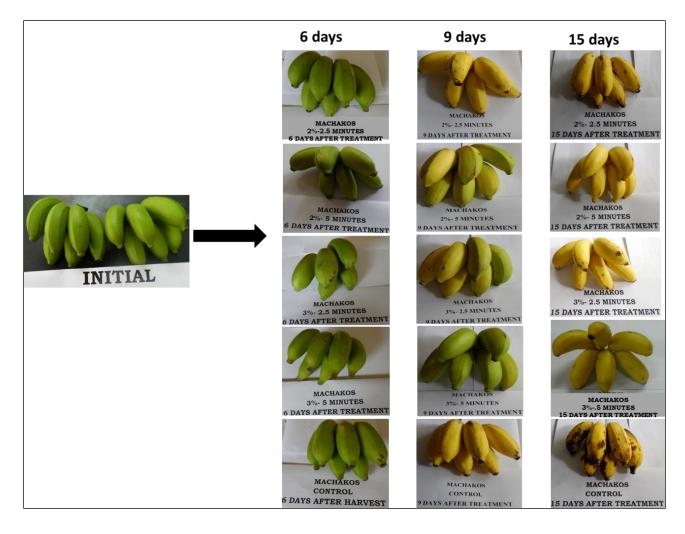


Figure 7: Progression of ripening in 'sweet banana' fruits post-harvest dip treated in hexanal (2 % and 3 %) either for 2.5 minutes, 5 minutes or left untreated and allowed to ripen under ambient room conditions

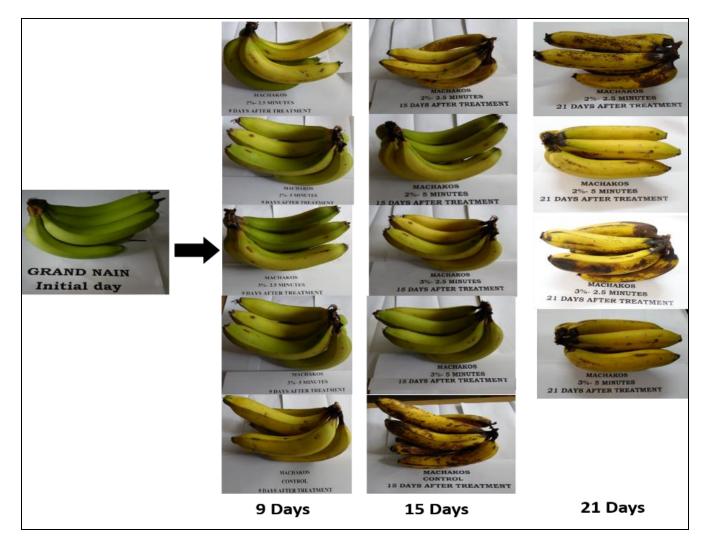


Figure 8: Progression of ripening in 'Grand nain' banana fruits post-harvest dip treated in hexanal (2 % and 3 %) either for 2.5 minutes, 5 minutes or left untreated and allowed to ripen under ambient room conditions

# **3.4.2. Ethylene production Levels**

Levels of ethylene production increased gradually to peak levels and drastically declined till the end of storage in all the fruits exhibiting a true climacteric pattern (Figures 9A-D and 10A-D). Hexanal treatments significantly (p < 0.05) reduced ethylene evolution rate irrespective of AEZ, mode of application, concentration and variety. The rate of ethylene production was significantly affected by the interaction between mode of application, hexanal concentration, variety and AEZ. Overall, fruits from the drier AEZ IV had significantly (p < 0.05) higher rate of ethylene production compared to those from colder AEZ II, irrespective of mode of application of hexanal (Figure 9). Generally, post-

harvest dip treatment (Figures 9A and C, 10A and C) significantly delayed and reduced rate of ethylene production compared to the pre-harvest spray treatment (Figure 9B and D, 10B and D). However, post-harvest dip for 5 minutes and a double spray at 30 days and 15 days maintained low levels of ethylene throughout the storage period in fruits of both AEZs and varieties. No significant differences were observed between the two varieties analyzed.

In the untreated 'Grand nain' fruits, ethylene levels increased drastically to peak levels of 6 nL/kg/h (day 6) and 5.9 nL/kg/h (day 3) for the pre-harvest spray mode of treatment compared to 12 nL/kg/h (day 9) and 8.7 nL/kg/h at day 12 of storage for post-harvest dip mode of application in Machakos and Meru Counties respectively (Figure 9 and 10). Similarly, in 'sweet banana' variety, ethylene peaked at day 3 (10.58 nL/kg/h, 9.9 nL/kg/h) in the controls for pre-harvest mode of application as compared to day 9 (10.2 nL/kg/h, 8.2 nL/kg/h) in the post-harvest mode of application in Machakos and Meru Counties respectively. Although hexanal treatment significantly delayed the climacteric peaks in pre-harvest spray treatment, a double spray in 'Grand nain' variety (Meru County) (Figure 9C) delayed the peaks by 6 days compared to 3 days in Machakos County and 'sweet banana' in both zones. 'Grand nain' fruit in Machakos County sprayed with hexanal had significantly reduced climacteric peaks of 3.7 nL/kg/h and 4.2 nL/kg/h sprayed twice with 2% and 3% hexanal, respectively, compared to 8.5 - 8.8 nL/kg/h in fruits dipped in hexanal for 5 minutes (Figures 9A and B). A similar trend was observed in 'sweet banana' variety, where a double spray with 2% and 3% hexanal, respectively, reduced the climacteric peaks to 4.7 nL/kg/h and 6.4 nL/kg/h as compared to 8.1 nL/kg/h and 7.7 nL/kg/h in single spray at 30 days before harvest (Figures 10A and B).

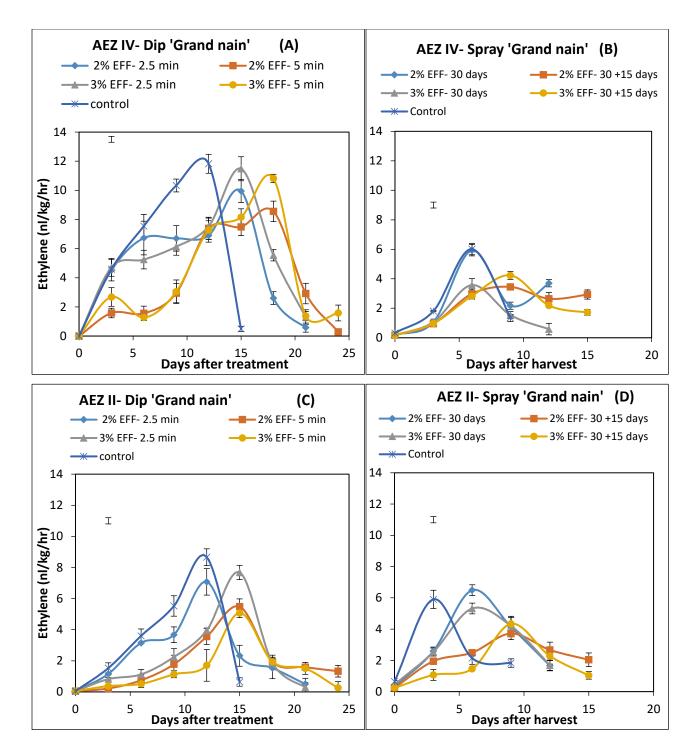


Figure 9: Effect of post-harvest dip and pre-harvest spray modes of hexanal application on the rate of ethylene production in 'Grand nain' banana fruits from AEZ IV (A, B) and AEZ II (C, D). The top bar in each graph represents the least significant difference (LSD) of interaction between treatment, mode of application and AEZ at p < 0.05. The vertical bars represents SE of the means (p < 0.05).

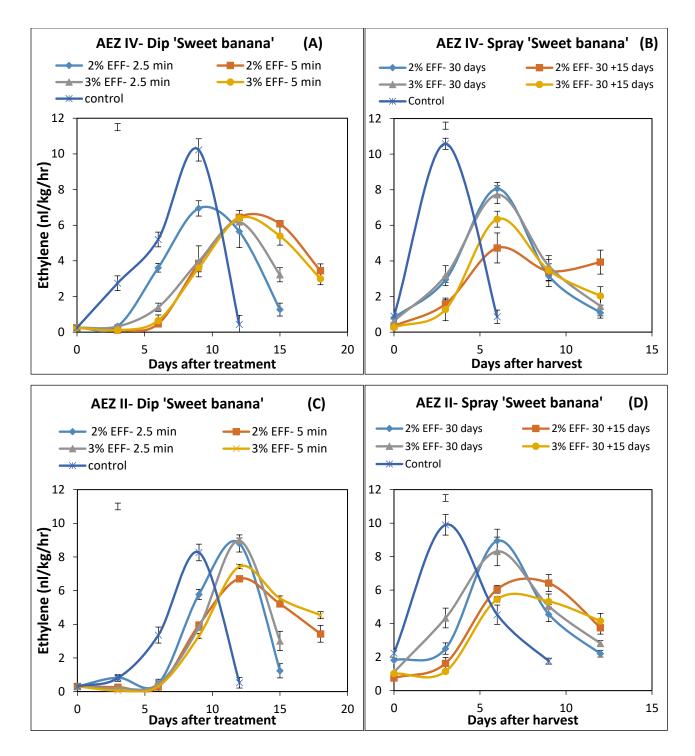


Figure 10: Effect of post-harvest dip and pre-harvest spray modes of hexanal application on the rate of ethylene production in 'Sweet banana' fruits from AEZ IV (A, B) and AEZ II (C, D). The top bar in each graph represents the least significant difference (LSD) of interaction between treatment, mode of application and AEZ at p < 0.05. The vertical bars represents SE of the means (p < 0.05).

#### **3.4.3. Respiration rates**

The rate of respiration increased gradually to peak levels and then drastically declined until the end of storage in all banana fruits irrespective of variety, AEZ, hexanal treatments and mode of application (Figures 11A-D and 12A-D). However, hexanal treatment significantly (*p* < 0.05) reduced respiration rate in both varieties and AEZs. The mode of application of hexanal and variety had a significant effect on the respiration rate with pre-harvest spray treated fruits (Figures 11B and D, 12B and D) having high respiration rate compared to post-harvest dip treatment (Figures 11A and C, 12A and C). A varietal difference was observed with 'Grand nain' fruits having significantly low rate of respiration (Figure 11) compared to 'sweet banana' variety (Figure 12) in both AEZs. The AEZ had a significant effect on the rate of respiration, with fruits from the drier AEZ IV having higher respiration rate compared to those from wetter AEZ II regardless of the mode of application of hexanal and variety.

The duration of hexanal application had a significant (*p* < 0.05) effect on the rate of respiration. Fruits sprayed twice at 30 days and 15 days before harvest or dipped for 5 minutes had lower rates of respiration compared to those sprayed once at 30 days, dipped for 2.5 minutes and the untreated controls in both varieties and AEZ. Respiration peaks were significantly reduced and delayed by 3 days in fruits sprayed twice at 30 and 15 days with either 2% or 3% hexanal as compared to the controls. In sprayed 'Grand nain' fruits, respiratory peaks of 38 mg/kg/h (2%), 43 mg/kg/h (3%) ) and 34 mg/kg/h (2%), 44 mg/kg/h (3%) occurred at day 9 of storage in AEZ IV and AEZ II fruits respectively. A similar trend was observed in the untreated fruits where the high respiratory peaks of 52.6 mg/kg/h and 44.3 mg/kg/h, occurred 3 days earlier (day 6 of storage) in the drier AEZ IV and wetter AEZ II respectively. 'Sweet banana' variety had high respiratory peaks of 61 mg/kg/h and 44 -48 mg/kg/h (occurred at day 3) in the untreated fruits compared to peaks of 41 -47 mg/kg/h and 44 -48 mg/kg/h in fruits sprayed twice, 3 days later in AEZ IV and AEZ II fruits respectively.

In the post-harvest dip mode of application experiment, the treated fruits had lower levels of respiration compared to the pre-harvest spray experiment with respiratory peaks of 34.8 mg/kg/h, 44.5 mg/kg/h in 'Grand nain' variety and 42 mg/kg/h, 58 mg/kg/h in 'sweet banana' variety in AEZ IV and AEZ II, respectively, occurring 6 days later.

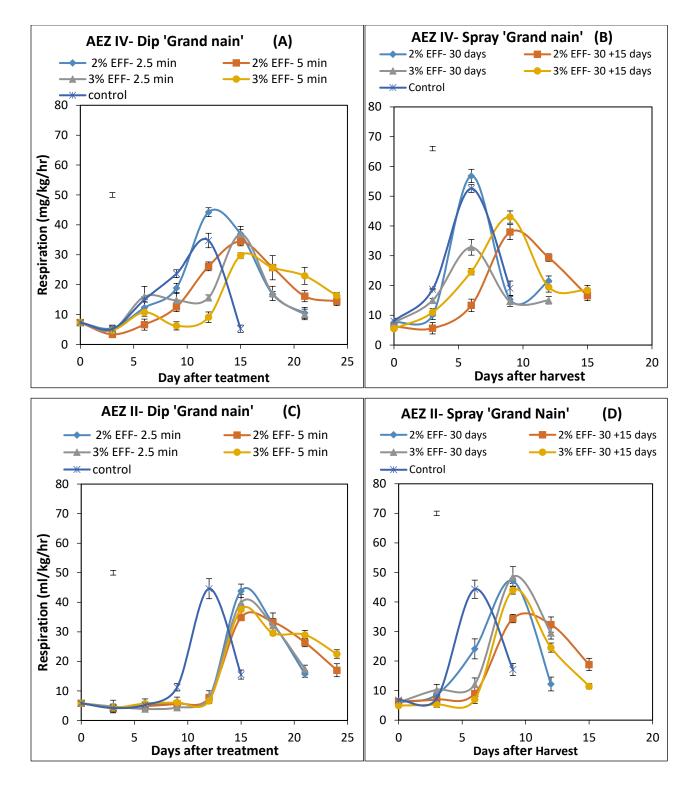


Figure 11: Effect of post-harvest dip and pre-harvest spray modes of hexanal application on the rate of ethylene production in 'Grand nain' banana from AEZ IV (A, B) and AEZ II (C, D). The top bar in each graph represents the least significant difference (LSD) of interaction between treatment, mode of application and AEZ at p < 0.05. The vertical bars represents SE of the means (p < 0.05).

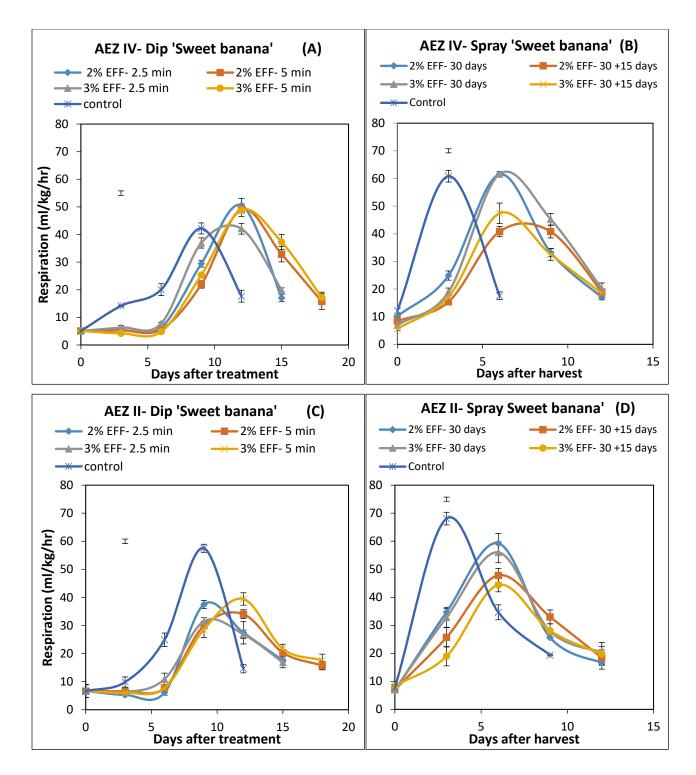


Figure 12: Effect of post-harvest dip and pre-harvest spray modes of hexanal application on the rate of ethylene production in 'Sweet banana' fruits from AEZ IV (A, B) and AEZ II (C, D). The top bar in each graph represents the least significant difference (LSD) of interaction between treatment, mode of application and AEZ at p < 0.05. The vertical bars represents SE of the means (p < 0.05).

# 3.4.4. Cumulative weight loss (%)

As ripening progressed, all the fruits gradually lost weight irrespective of the variety, treatment, mode of application and zone of production (Tables 5 and 6). At the end of storage period, the fruits had approximately lost between 10% - 20.9% of the original weights depending on the variety treatment, and mode of hexanal application. In 'Grand nain' variety, hexanal treatment did not have significant effect on the percentage cumulative weight loss except towards the end of storage (between day 15 and 18) in fruits from AEZ II (Table 5). However, a significant treatment effect was only observed in 'sweet banana' fruits (post-harvest dip experiment) from the drier AEZ IV as from day 6 of storage (Table 6). Variety had a significant effect on weight loss with 'sweet banana' fruits losing more weight (20.9%) at the end of storage compared to 'Grand nain' variety which had lost approximately 18.9-19.8% of the original weight. Mode of hexanal application and AEZ did not have any significant effect on percentage cumulative weight loss in both varieties (Tables 5 and 6).

DAYS	Po	stharves	t Dip		Preharvest spray							
Zone IV	2%-A	2%-В	3%-A	3%-В	Ctrl	2%- S	2%-D	3%-S	3%-D	Ctrl		
0	0.0	0.0k	0.0m	0.0m	0.0g	0.0i	0.0i	0.0g	0.0h	0.0g		
3	2.5j	2.2j	2.6k	2. <b>4k</b>	2.8f	5.2g	4.7h	5.6 <b>e</b>	6.4f	5.5e		
6	5.5h	5.3h	5. <b>3</b> i	5. <b>3</b> i	5.9d	8.9e	8.1f	<b>8.3</b> d	9.4e	9.5c		
9	7.7g	7.1g	7.6h	7.6g	8.3c	13.6c	12.1d	12.4b	12.9d	14.7a		
12	9.1f	8.7f	9.2g	9.3f	10.1b	17.0a	15.7b	15.4a	16.2b			
15	11.6d	11.3e	11.6e	11.0e	13.1a		17.1a		17.7a			
18	14.6b	<b>13.8</b> d	13.7c	<b>13</b> .5d								
21	17.4a	16.4b	16.7a	16.5c								
24		19.8a		19.9a								
Zone II												
0	0.0	0.0k	0.0m	0.0m	0.0g	0.0i	0.0i	0.0g	0.0h	0.0g		
3	1.4k	1.6j	1.51	1.51	1.7f	3.9h	3.9h	3.7f	3.6g	4.0f		
6	4.0i	4.2i	3.7j	4.0j	<b>4.2e</b>	6.2f	6.2g	5.6e	5.7f	6.2d		
9	6.1h	6.1h	5.9i	6. <b>4</b> h	6.2d	11.1d	10.8e	9.3c	10.1e	13.1b		
12	7.5g	7.7g	7.3h	8.0g	7.7c	14.7b	14.0c	12.1b	12.5d			
15	10.6e	11.5e	10.6f	11.5e	12. <b>3</b> a		17.2a		14.9c			
18	12.7c	14.9c	12.8d	<b>13.4</b> d								
21	14.8b	17.2b	15.2b	15.7c								
24		19.8a		19.0b								
Mean	7.8	9.3	7.7	9.2	6	8.1	9.2	7.2	9.1	6.6		
LSD* (T)	0.2											
LSD* (Z)	0.1											
LSD* (M)	0.1											
LSD* (TxZx	M) 0.8											
% CV	9.5											

Table 5: Effect of postharvest dip and preharvest spray application of hexanal on percentage cumulative weight loss of 'Grand nain' fruits from AEZ IV (Machakos County) and AEZ II (Meru County).

Values followed by different letters within the same column differ significantly at (p < 0.05) while means with a similar letter in a column do not differ significantly at (p < 0.05). A= post-harvest for 2.5 minutes, B= post-harvest for 5 minutes, D= double spray at 30 and 15 days before harvest, Ctrl= Controls, T= Treatment, V= Variety, M= mode of application and Z= Agro ecological zone of production.

DAYS	Po	Postharvest Dip Preharvest spray								
Zone IV	2%-A	2%-В	3%-A	3%-В	Ctrl	2%- S	2%-D	3%-S	3%- D	Ctrl
0	0.0i	0.0k	0.0g	0.0j	0.0i	0.0e	0.0e	0.0h	0.0e	0.0d
3	3.4g	3.4i	2 <b>.9</b> f	3.7h	3.5g	5.1d	5.0d	6.0f	5.7d	5.8c
6	5.8f	5.6h	5.5e	5.2g	6.6 <b>e</b>	8.9c	8.9c	9.4d	9.0c	10.0b
9	<b>7</b> .6e	7.4g	<b>7.4</b> d	6.8f	10.0c	16.0b	16.6b	15.6c	15.0b	
12	11.4c	10.8e	11.1c	11.1e	15.3a	18.2a	18.9a	18.8a	16.9a	
15	14.7b	14.2d	14.7b	15.1d						
18		18.3b		19.7b						
Zone II										
0	0.0i	0.0k	0.0g	0.0j	0.0i	0.0e	0.0e	0.0h	0.0e	0.0d
3	1.7h	2.2j	2.0f	1.7i	2. <b>1</b> h	<b>4.1</b> d	4.0d	4.3g	5.1d	5.2c
6	4.2g	5. <b>1</b> h	4.5e	4.7gh	5.1f	8.3c	8.7c	7.9e	9.1c	9.3b
9	6.6 <b>e</b>	8.7f	7.5d	7.9f	8.0d	15.8b	15.9b	14.4c	15.8b	16.4a
12	9.5d	11.1e	10.3c	11.0e	12. <b>7</b> b	18.4a	17.6b	17.5b	17.3a	
15	16.1a	16.8c	16. <b>3</b> a	17.0c						
18		2 <b>0</b> .2a		2 <b>0.9a</b>						
Mean	6.8	8.8	6.9	8.9	6.3	10.5	9.6	9.4	9.5	6.7
LSD* (T)	0.3									
LSD* (Z)	0.2									
LSD* (M)	0.1									
LSD* (TxZ	xM) 1.1									
%CV	6.7									

Table 6: Effect of postharvest dip and preharvest spray application of hexanal on percentage cumulative weight loss of 'Sweet banana' fruits from AEZ IV (Machakos County) and AEZ II (Meru County).

Values followed by different letters within the same column differ significantly at (p < 0.05) while means with a similar letter in a column do not differ significantly at (p < 0.05). A= post-harvest for 2.5 minutes, B= post-harvest for 5 minutes, D= double spray at 30 and 15 days before harvest, Ctrl= Controls, T= Treatment, V= Variety, M= mode of application and Z= Agro ecological zone of production.

#### **3.4.5. Peel firmness**

Gradual decrease in peel firmness resulted in softening of all the banana fruits. However, all hexanal treatments (different modes, concentrations and time of application) slowed down the rate of softening irrespective of AEZ and variety. Peel firmness was significantly (p < 0.05) affected by the interaction between mode of application, AEZ, treatment duration and variety. Hexanal treatment applied either as a preharvest spray or postharvest dip significantly delayed peel softening in both AEZs. However, the postharvest dip mode of application (Figures 13A and C, 14A and D) was more effective compared to the preharvest spray mode of application (Figures 13B and D, 14B and D).

The untreated fruits softened very quickly from the initial firmness of 38N to 3N at the end of storage (day 15) for the post-harvest dip mode of application, compared with 4N in the hexanal-treated fruits at day 24 (i.e. 9 days later) in ' Grand nain' variety (Figures 13A and C). On the other hand, peel firmness in 'sweet banana' fruits decreased at the end of storage from an initial of 39N to 2-3N, which occurred at day 12 and 18 for the untreated and hexanal treated fruits respectively. Comparing the two varieties, hexanal application was more effective in 'Grand nain' variety as compared to ' sweet banana' in both AEZs. Duration of hexanal application had a significant effect on the rate of softening in both varieties, with a 5-minute dip being more effective compared to 2.5 minutes dip regardless of the concentration and AEZ. By day 21 of storage, peel firmness of fruits treated with a 2.5 minutes dip had decreased from initial of 38N to a final of 3.3- 5.4N and 2.1- 4N in AEZ II and AEZ IV, respectively, compared to 10N and 7.2N in fruits dipped for 5 minutes, respectively, in the same AEZs in case of 'Grand nain' variety.

In the pre-harvest spray mode of application, spraying twice at 30 and 15 days was significantly more effective in maintaining peel firmness as compared to a single spray and untreated controls in 'Grand nain' variety in both AEZ. Fruits sprayed once at 30 days had lost their peel firmness from 34- 36N to 4.2- 7N and 3.3- 5N in AEZ IV and AEZ II, respectively at the end of storage (day 12), compared to 4.2- 5N and 2.6N in the double-sprayed 'Grand nain' fruits, respectively at day 15 (Figures 13B and D). In 'sweet banana' variety, no significant differences were observed between the single and double spray treatments. 'Sweet banana' fruits sprayed with hexanal maintained higher firmness of 2.7N and 3.5N at the end of storage (day 12) compared to 1.9N and 1.7N in the untreated fruits by end stage which occurred at day 6 and 9 in AEZ IV and AEZ II respectively (Figures 14B and D).

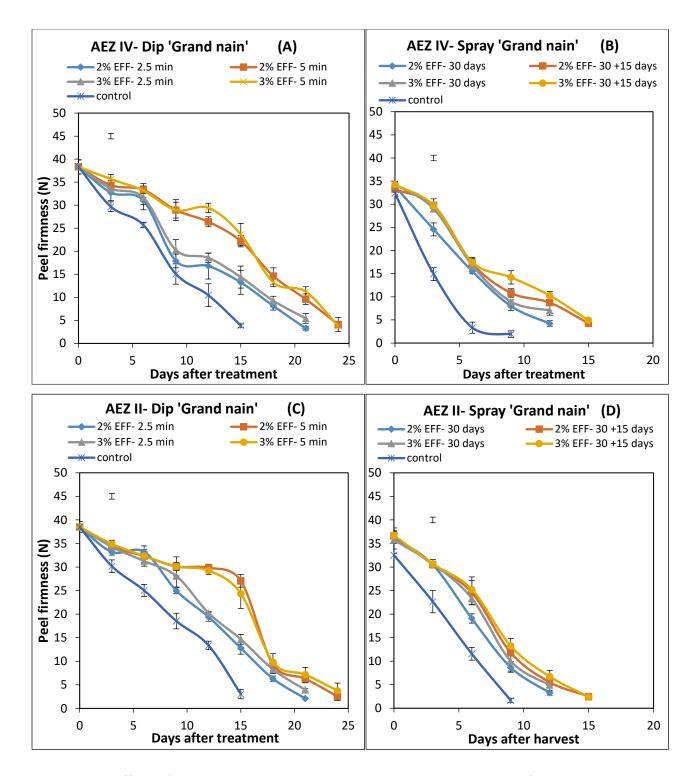


Figure 13: Effect of post-harvest dip and pre-harvest spray application of hexanal on the peel firmness in 'Grand nain' fruits from AEZ IV (A, B) and AEZ II (C, D). The top bar in each graph represents the least significant difference (LSD) of interaction between treatment, mode of application and AEZ at p < 0.05. The vertical bars represents SE of the means (p < 0.05).

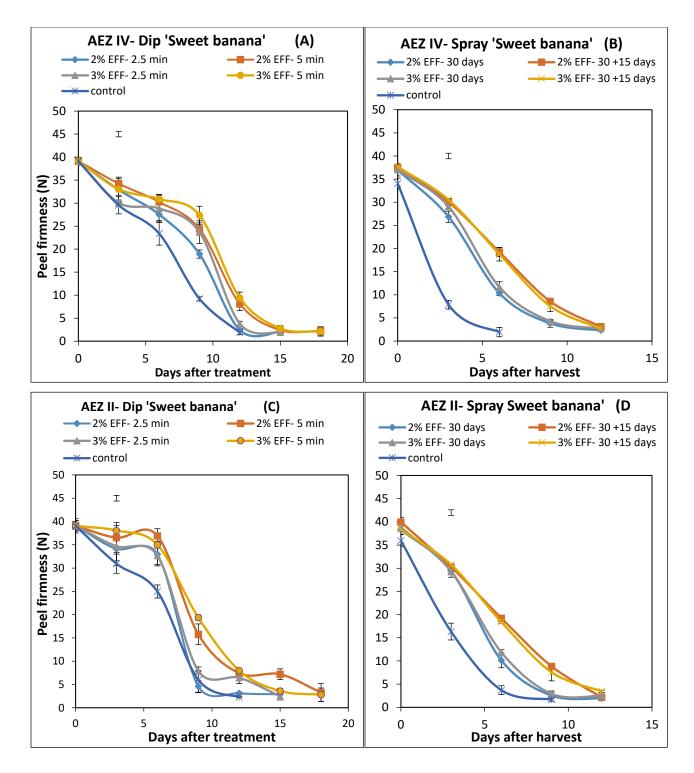


Figure 14: Effect of post-harvest dip and pre-harvest spray application of hexanal on the peel firmness in 'Sweet banana' fruits from AEZ IV (A, B) and AEZ II (C, D). The top bar in each graph represents the least significant difference (LSD) of interaction between treatment, mode of application and AEZ at p < 0.05. The vertical bars represents SE of the means (p < 0.05).

### 3.4.6. Pulp firmness

Pulp firmness exhibited a similar trend to peel firmness with a gradual decrease in all the fruits during the storage period (Tables 7 and 8). Pulp firmness was significantly (p < 0.05) affected by hexanal treatment and the mode of application used. The post-harvest dip mode of application was more effective in delaying pulp softening in fruits harvested from both zones compared to the pre-harvest spray-treated ones. However, zone of production did not have any significant effect on the rate of pulp softening.

The untreated control fruits drastically lost their pulp firmness, to 0.93N in fruits produced in both AEZs, after 9 days of storage in the pre-harvest spray mode of application compared to 1.8N and 2N, in AEZ IV and AEZ II fruits, respectively in the post-harvest dip experiment (Table 7) after 15 days of storage in 'Grand nain' variety. On the hand, the controls in 'sweet banana' had lost their pulp firmness to 1.4- 1.6N at the end of storage (day 12) in both AEZs in the pre-harvest spray mode of application compared to 1.4N after day 6 and 9 in AEZ IV and AEZ II, respectively in the postharvest dip mode of application (Table 8).

In 'Grand nain' variety, pulp firmness was maintained up to day 15 and day 18 in post-harvest dipped fruits compared to day 9 and day 6 in pre-harvest sprayed fruits in AEZ II and AEZ IV, respectively (Table 7). Fruits dipped in hexanal for 5 minutes had pulp firmness of 10.3- 11.3N and 12.7- 13.8N by day 15 of storage in AEZ II and AEZ IV, respectively, compared to 1.5- 1.8N and 1.8N in doubled-sprayed fruits in AEZ II and AEZ IV, respectively. A similar trend was observed in 'sweet banana' variety fruits were 5-minutes (2% & 3%) post-harvest dip treatment delayed pulp softening up to day 9 of storage in AEZ II while only 3% concentration was effective in AEZ IV fruits (Table 8). A pre-harvest at 30 and 15 days before harvest maintained significantly (p < 0.05) high pulp firmness up to day 6 of storage compared to single spray and controls in 'sweet banana' variety in both AEZs (Table 7). By the 6<sup>th</sup> day of storage, pulp firmness of hexanal sprayed fruits had declined to 9.4-12.3N and 11.3- 11.9N in AEZ II and AEZ IV fruits, respectively, compared to 1.4- 1.7N in the untreated controls in 'sweet banana' variety.

DAYS	Pos	tharvest	Dip				Preł	narvest s	pray	
Zone IV	2%-A	2%-В	3%-A	3%-В	Ctrl	2%- S	2%-D	3%-S	3%-D	Ctrl
0	20.3a	2 <b>0.3a</b>	20.3a	20.3a	20.3ab	16.6a	17.3a	16.4a	16. <b>8a</b>	16.4a
3	18.5b	17.3b	17.7c	17.6c	16.9d	12.1c	14.7c	10.9c	13.0c	8.1c
6	16.2d	16.4c	15. <b>3</b> d	17.3c	14.0e	6. <b>8</b> d	10.5d	10.1c	9.8d	2. <b>7</b> d
9	12.5e	16.1c	13.7e	16.9c	9.7g	4.9e	9.8d	5.1d	9.3d	0.9
12	11.6f	15.1d	11.6g	16.8c	7.1h	1.1i	2. <b>7e</b>	1.9f	2.2 <b>ef</b>	
15	10.6g	13.9e	10.6h	12.7d	1.8i		1.7f		1.8f	
18	8.5h	9.5f	7.6j	9.6f						
21	1.9i	5.1g	3.1k	6.2g						
24		1.7i		1.7i						
Zone II										
0	2 <b>1.0a</b>	21.0a	2 <b>1.0a</b>	2 <b>1.0a</b>	2 <b>1.0a</b>	16.4a	16.4b	16.4a	16.8a	16.0a
3	20.7a	2 <b>0.8a</b>	19.2b	20.2a	19.6b	14.6b	16.0b	16.5a	15.2b	13.6b
6	19.2b	20.2a	17.4c	19.2b	17.8c	4.0f	14.4c	13.3b	14.7b	1.9d
9	17.4c	18.4b	17.0c	18.6b	12.2f	2 <b>.1g</b>	2. <b>4e</b>	2.8e	3.0e	0.9e
12	13.1e	16.9c	12. <b>7</b> f	17.5c	7.9h	1.3g	2.8e	1.6f	2.8e	
15	8.5h	10.3f	9.0i	11.3e	2.0i		1.8f		1.5f	
18	1.8i	2.6h	3.1k	3.2h						
21	1.4i	2.2h	1.8	2.4hi						
24		1.5i		1.6i						
Mean	12.7	12.8	12.7	13	12.5	8.0	9.2	9.5	8.9	7.6
LSD* (T)		0.5								
LSD* (Z)		0.2								
LSD* (M	)	0.2								
LSD* (Tx	ZxM)	0.8								
% CV		9.5								

Table 7: Effect of postharvest dip and preharvest spray application of hexanal pulp firmness of 'Grand nain' fruits from AEZ IV (Machakos County) and AEZ II (Meru County).

Values followed by different letters within the same column differ significantly at (p < 0.05) while means with a similar letter in a column do not differ significantly at (p < 0.05). A= post-harvest for 2.5 minutes, B= post-harvest for 5 minutes, D= double spray at 30 and 15 days before harvest, Ctrl= Controls, T= Treatment, V= Variety, M= mode of application and Z= Agro ecological zone of production.

DAYS	Pos	tharvest	Dip				Pre	harvest s	pray	
Zone IV	2%-A	2%-В	3%-A	3%-В	Ctrl	2%- S	2%-D	3%-S	<b>3%-D</b>	Ctrl
0	32.4a	32.4a	32.4a	32.4a	32.4a	2 <b>9.1</b> a	<b>30</b> .2a	2 <b>9.4</b> b	30.5a	2 <b>7.8</b> b
3	21.2e	2 <b>7.8c</b>	25.9c	26.7d	15.7e	12.1c	17.0b	15.0c	19.1b	3.3c
6	20.7e	22.6d	21.2d	22. <b>3e</b>	9.0f	6.8d	11.3c	9.5d	11.9c	1.4d
9	4.5f	7.8f	6. <b>0e</b>	16.2f	2.6gh	2. <b>3e</b>	<b>8</b> .6d	3.5f	7.5d	
12	2.2g	2.0h	2.2g	3.0i	1.4h	1.8e	2 <b>.8</b> f	1.4g	2.5f	
15	1.7g	1.9h	1.9hg	2. <b>1</b> i						
18		1.6h		2.0i						
Zone II										
0	31.2b	31.2b	31.2b	31.2b	31.2b	2 <b>9.7</b> a	30.6a	30.6a	30.1a	2 <b>9.1a</b>
3	28.9c	30.8b	2 <b>7.8</b> b	30.9b	25.9c	16.6b	18.0b	17.5b	20.2b	4.4c
6	27.5d	28.8c	25. <b>7c</b>	29.6c	22 <b>.1</b> d	4.5e	9.4d	5.6e	12.3c	1.7d
9	<b>3</b> .6f	9.2e	4.8f	12.5g	3.4g	1.6e	4.2e	<b>3</b> .2f	5. <b>3e</b>	<b>1.3</b> d
12	2.1g	5. <b>3</b> g	3.3g	7.0h	1.6h	1.4e	1.7f	1.6g	2.2f	
15	1.4g	1.9h	1.9h	2.9i						
18		1.6h		1.3j						
Mean	14.8	14.6	15.4	15.7	13.3	10.6	13.4	11.7	14.2	9.9
LSD* (T)		0.7								
LSD* (Z)		0.2								
LSD* (M	)	0.3								
LSD* (Tx	(ZxM	1.1								
% CV		11.8								
/aluesfollo	wed by d	ifferentle	tterswithi	n the sam	ecolumn	differsion	ificantly	at(n < 0.0)	5) while n	neans wit <sup>i</sup>

**Table 8:** Effect of post-harvest dip and pre-harvest spray application of hexanal pulp firmness of 'Sweet banana' fruits from AEZ IV (Machakos County) and AEZ II (Meru County).

Values followed by different letters within the same column differ significantly at (p < 0.05) while means with a similar letter in a column do not differ significantly at (p < 0.05). A= post-harvest for 2.5 minutes, B= post-harvest for 5 minutes, D= double spray at 30 and 15 days before harvest, Ctrl= Controls, T= Treatment, V= Variety, M= mode of application and Z= Agro ecological zone of production.

#### 3.4.7. Peel color

Peel color expressed as hue angle decreased gradually as the fruits ripened. Peel color was significantly affected (p < 0.05) by the interaction between area of production, mode of application, and hexanal treatment. Hexanal applied either as a pre-harvest spray or post-harvest dip significantly delayed peel color change with the effect being more pronounced in fruits sprayed twice (at 30 days and 15 days) before harvest and those dipped for 5 minutes (Tables 9 and 10). Changes in peel color were more drastic in the sprayed fruits compared to the post-harvest dipped ones.

In the pre-harvest spray experiment, the peel hue angle in the untreated fruits decreased drastically from initial values of 104° and 108° to 77° and 79° in fruits from the drier Machakos and wetter Meru Counties, respectively at the end of storage (day 9) compared to the control in the post-harvest dip experiment where hue angle decreased from 120° to 78° and 79° in Machakos and Meru Counties, 6 days later (day 15). A 5-minute dip was significantly more effective in delaying peel color change as from day 6 and 12 of storage in fruits from Machakos and Meru Counties, respectively. However, the two hexanal concentrations (2% and 3%) used were not significantly different from each other. Zone of production had a significant effect (p < 0.05) on peel color only in 'Grand nain' variety with fruits harvested from Machakos County having low hue angle compared to those harvested from Meru County irrespective of hexanal mode of application (Table 9). Interestingly, at the end of storage both the sprayed and dipped fruits attained almost the same hue angle values as the controls.

<b>2%-A</b>  20.8a  16.3b  13.3d  10.2d  08.6d	<b>2%-B</b> 120.8a 118.9ab 116.1c 115.3d	<b>3%-A</b> 120.8a 117.1b 114.4c	<b>3%-B</b> 120.8a 118.0b 115.2c	Ctrl 120.8a 115.4c	<b>2%-S</b> 114.5a 105.0b	<b>2%-D</b> 116.7a	<b>3%-S</b> 114.3a	<b>3%-D</b> 114.7b	Ctrl 103.8b
16.3b  13.3d  10.2d  08.6d	118.9ab 116.1c 115.3d	117.1b 114.4c	118.0b				114.3a	114.7b	103.8b
13.3d  10.2d  08.6d	116.1c 115.3d	114.4c		115.4c	105 Ob				100100
10.2d  08.6d	115. <b>3</b> d		115.2c		105.00	104.5	102.7c	<b>103</b> .5d	95.7d
1 <b>08</b> .6d		444.01		107.1f	87.8e	95.6e	91.9f	96.0f	84.8f
		111.3d	115.6c	103.1g	85.0f	92.0f	88.3g	92.9g	77.4h
	112.5e	110.8d	111.9d	85.7h	83.0f	84.7g	82.3h	85.9i	
<b>)1.2e</b>	107.5f	90.8g	108.1e	77.7i		79.3h		79.7j	
38.5f	99.1g	88.8g	98.2g						
34.7g	87.7i	82.1h	92. <b>3</b> i						
	85.3j		83.9k						
120.8a	120.8a	120.8a	120.8a	120.8a	115.2a	117.1a	116.3a	117.0a	108.4a
1 <b>18</b> .2b	119.2ab	119.6a	119.6 <b>a</b> b	118.6b	106.3b	110.9b	107.9b	106.7c	99.8c
15.6c	118.2bc	119.2a	117.8bc	<b>113.3</b> d	102.1c	105.3c	101.8d	103.8e	91.2e
12.2d	114.3de	115.0bc	116.1c	110.2e	92.5d	<b>99.1</b> d	96.0e	97.9f	79.3g
1 <b>08.8</b> d	114.0de	111.1d	113.3d	106.5f	80.3g	86.2g	81.2h	88.1h	
92. <b>7e</b>	106.3f	102.6e	103.6f	78.9i		79.7h		78.3j	
3 <b>8</b> .6f	95.0h	93.3f	94.6h						
30.9h	88.9i	82.0h	87.3j						
	83.7j		84.6k						
04.5	106.9	106.2	106.8	104.8	97.2	97.6	98.3	97.0	92.6
	0.3								
	0.3								
	0.6								
xM)	2.1								
	9.9								
	1.2e 8.5f 4.7g 20.8a 18.2b 15.6c 12.2d 08.8d 2.7e 8.6f 0.9h 04.5	1.2e       107.5f         8.5f       99.1g         4.7g       87.7i         85.3j         20.8a       120.8a         18.2b       119.2ab         15.6c       118.2bc         12.2d       114.3de         08.8d       114.0de         2.7e       106.3f         86.6f       95.0h         0.9h       88.9i         83.7j       0.3         0.3       0.3         0.6       2.1         9.9       9.9	1.2e       107.5f       90.8g         1.2e       107.5f       90.8g         1.2e       99.1g       88.8g         8.5f       99.1g       88.8g         4.7g       87.7i       82.1h         85.3j       87.7i       82.1h         20.8a       120.8a       120.8a         18.2b       119.2ab       119.6a         15.6c       118.2bc       119.2a         12.2d       114.3de       115.0bc         08.8d       114.0de       111.1d         2.7e       106.3f       102.6e         8.6f       95.0h       93.3f         0.9h       88.9i       82.0h         83.7j       0.4.5       106.9       106.2         0.3       0.3       0.6         xM)       2.1       9.9	1.2e       107.5f       90.8g       108.1e         8.5f       99.1g       88.8g       98.2g         4.7g       87.7i       82.1h       92.3i         85.3j       83.9k         20.8a       120.8a       120.8a       120.8a         18.2b       119.2ab       119.6a       119.6ab         15.6c       118.2bc       119.2a       117.8bc         12.2d       114.3de       115.0bc       116.1c         08.8d       114.0de       111.1d       113.3d         2.7e       106.3f       102.6e       103.6f         86.f       95.0h       93.3f       94.6h         0.9h       88.9i       82.0h       87.3j         83.7j       84.6k       04.5       106.9       106.2       106.8         0.3       0.3       0.6       0.3       0.3       0.6         xM)       2.1       9.9       9.9       9.9       9.9	1.2e       107.5f       90.8g       108.1e       77.7i         8.5f       99.1g       88.8g       98.2g         4.7g       87.7i       82.1h       92.3i         85.3j       83.9k         20.8a       120.8a       120.8a       120.8a         18.2b       119.2ab       119.6a       119.6ab       118.6b         15.6c       118.2bc       119.2a       117.8bc       113.3d         12.2d       114.3de       115.0bc       116.1c       110.2e         08.8d       114.0de       111.1d       113.3d       106.5f         2.7e       106.3f       102.6e       103.6f       78.9i         86.6f       95.0h       93.3f       94.6h       94.6h         0.9h       88.9i       82.0h       87.3j       84.6k         04.5       106.9       106.2       106.8       104.8         0.3       0.3       0.6       0.4       9.9	1.2e       107.5f       90.8g       108.1e       77.7i         8.5f       99.1g       88.8g       98.2g         4.7g       87.7i       82.1h       92.3i         85.3j       83.9k         20.8a       120.8a       120.8a       120.8a       115.2a         18.2b       119.2ab       119.6a       118.6b       106.3b         15.6c       118.2bc       119.2a       117.8bc       113.3d       102.1c         12.2d       114.3de       115.0bc       116.1c       110.2e       92.5d         08.8d       114.0de       111.1d       113.3d       106.5f       80.3g         27.7e       106.3f       102.6e       103.6f       78.9i         88.6f       95.0h       93.3f       94.6h       94.6h         00.9h       88.9i       82.0h       87.3j       83.7j       84.6k         04.5       106.9       106.2       106.8       104.8       97.2         0.3       0.3       0.6       0.3       0.6       0.3       0.6         kM)       2.1       9.9       9.9       9.9       9.9       9.9	1.2e       107.5f       90.8g       108.1e       77.7i       79.3h         8.5f       99.1g       88.8g       98.2g       77.7i       79.3h         8.5f       99.1g       88.8g       98.2g       77.7i       79.3h         8.7g       87.7i       82.1h       92.3i       79.3h         20.8a       120.8a       120.8a       120.8a       115.2a       117.1a         18.2b       119.2ab       119.6a       118.6b       106.3b       110.9b         15.6c       118.2bc       119.2a       117.8bc       113.3d       102.1c       105.3c         12.2d       114.3de       115.0bc       116.1c       110.2e       92.5d       99.1d         08.8d       114.0de       111.1d       113.3d       106.5f       80.3g       86.2g         2.7e       106.3f       102.6e       103.6f       78.9i       79.7h         8.6f       95.0h       93.3f       94.6h       94.6h       94.6h       94.6h       94.6h       94.6h       94.6h       94.6h       95.0h       93.3f       94.6h       94.6h       95.0h       94.6h       94.6h       94.6h       94.6h       94.6h       94.6h       94.6h       94.6h	1.2e       107.5f       90.8g       108.1e       77.7i       79.3h         8.5f       99.1g       88.8g       98.2g       77.7i       79.3h         8.5f       99.1g       88.8g       98.2g       79.3h         4.7g       87.7i       82.1h       92.3i       79.3h         85.3j       83.9k       83.9k       110.2a       117.1a       116.3a         18.2b       119.2ab       119.6a       119.6ab       118.6b       106.3b       110.9b       107.9b         15.6c       118.2bc       119.2a       117.8bc       113.3d       102.1c       105.3c       101.8d         12.2d       114.3de       115.0bc       116.1c       110.2e       92.5d       99.1d       96.0e         08.8d       114.0de       111.1d       113.3d       106.5f       80.3g       86.2g       81.2h         2.7e       106.3f       102.6e       103.6f       78.9i       79.7h       84.6k         0.9h       88.9i       82.0h       87.3j       84.6k       97.2       97.6       98.3         0.3       0.3       0.3       0.6       0.4       99.9       99.9       99.9	1.2e       107.5f       90.8g       108.1e       77.7i       79.3h       79.7j         8.5f       99.1g       88.8g       98.2g       79.7j       79.3h       79.7j         8.77i       82.1h       92.3i       83.9k       79.7j       79.3h       79.7j         20.8a       120.8a       120.8a       120.8a       115.2a       117.1a       116.3a       117.0a         18.2b       119.2ab       119.6a       119.6ab       118.6b       106.3b       110.9b       107.9b       106.7c         15.6c       118.2bc       119.2a       117.8bc       113.3d       102.1c       105.3c       101.8d       103.8e         12.2d       114.3de       115.0bc       116.1c       110.2e       92.5d       99.1d       96.0e       97.9f         08.8d       114.0de       111.1d       113.3d       106.5f       80.3g       86.2g       81.2h       88.1h         2.7e       106.3f       102.6e       103.6f       78.9i       79.7h       78.3j         8.6f       95.0h       93.3f       94.6h       97.2       97.6       98.3       97.0         0.3       0.3       0.3       0.4       06.8       104.8

Table 9: Effect of postharvest dip and preharvest spray application of hexanal on peel color changes of 'Grand nain' fruits from AEZ IV (Machakos County) and AEZ II (Meru County).

Values followed by different letters within the same column differ significantly at (p < 0.05) while means with a similar letter in a column do not differ significantly at (p < 0.05). A= post-harvest for 2.5 minutes, B= post-harvest for 5 minutes, D= double spray at 30 and 15 days before harvest, Ctrl= Controls, T= Treatment, V= Variety, M= mode of application and Z= Agro ecological zone of production.

DAYS	Pos	Postharvest Dip					Pre	harvest s	pray	
Zone IV	2%-A	2%-В	3%-A	3%-В	Ctrl	2%- S	2%-D	3%-S	3%-D	Ctrl
0	120.6a	120.6a	120.6a	120.6a	120.6a	110.4b	111.8c	108.1c	110.9c	100.8b
3	116.6bc	117.9b	117.1bc	117.4b	111.1c	102.6c	<b>109.3</b> d	<b>104.7</b> d	106.3d	95.5c
6	114.3d	117.5b	116.1cd	118.0b	94.8e	92.6e	97.7f	94.9f	99.0f	77.7e
9	107.7e	113.7c	110.5e	112.1c	92.7f	84.2f	88.0g	82.9g	<b>8</b> 6.7h	
12	93.0g	94.3f	92.2g	95.4e	81.1h	79.9g	81.0h	79.9h	79.5i	
15	81.8i	87.5g	84.0h	84.5f						
18		83.8h		82.0g						
Zone II										
0	121.2a	121.2a	121.2a	121.2a	121.2a	114.4a	116.6a	114.5a	114.8a	111.2a
3	118.2b	117.6b	118.4b	118.1b	114.4b	109.3b	113.9b	110.4b	112.8b	95.2c
6	115.2 <b>c</b> d	116.6b	115.1d	117.6b	103.8d	<b>97.0</b> d	100.1e	98.6e	101.1e	<b>90.1</b> d
9	95.2f	102.4d	95.7f	109.9d	92.9f	93.9e	96.0f	95.8f	93.9g	77.7e
12	93.9fg	98.3e	92.6g	94.1e	81.5h	85.7f	86.4g	84.5g	86.3h	
15	84.4h	83.8h	84.0h	85.8f						
18		<b>81</b> .6i		82.2g						
Mean	105.2	104.1	105.6	104.2	101.4	97	100.1	97.4	99.1	92.6
LSD* (T)	0.5									
LSD* (Z)	0.3									
LSD* (M)	) 0.2									
LSD* (Tx	ZxM) 1.8									
% CV	13.4									
Values	followed by a	lifferent let	terswithin	the same c	olumn dif	fersignifi	cantly at (p	v < 0.05) v	vhile mean	swith

Table 10: Effect of post-harvest dip and pre-harvest spray application of hexanal on peel color changes of 'Sweet banana' fruits from AEZ IV (Machakos County) and AEZ II (Meru County).

Values followed by different letters within the same column differ significantly at (p < 0.05) while means with a similar letter in a column do not differ significantly at (p < 0.05). A= post-harvest for 2.5 minutes, B= post-harvest for 5 minutes, D= double spray at 30 and 15 days before harvest, Ctrl= Controls, T= Treatment, V= Variety, M= mode of application and Z= Agro ecological zone of production.

### **3.5. Discussion**

Hexanal is a relatively new technology that has been found to be effective in extending the shelf life of several temperate fruits such as apples, peaches, strawberries, and sweet cherries (Paliyath *et al.*, 2008). Several studies have recently been carried out to investigate the ability of hexanal-based technologies to enhance post-harvest characteristics of tropical fruits such as mangoes and papayas (Anusaya *et al.*, 2016, Hutchinson *et al.*, 2018). However, there are research gaps regarding hexanal application in some tropical fruits such as banana. Specifically, there is limited information regarding the best method of application and performance of hexanal application (pre-harvest spray and post-harvest dip) were evaluated on banana varieties 'Grand nain' and 'sweet banana' produced under two different AEZs in Kenya: Meru (a high potential AEZ II) and Machakos (a low potential AEZ IV).

Hexanal, applied as a pre-harvest spray twice at 30 days and 15 days before harvest significantly delayed fruit harvesting while in the field by 18 days and 12 days in AEZ II and AEZ IV respectively in 'Grand nain' variety compared to 12 days in 'sweet banana' variety in both AEZs. Climactic differences between the two study sites might have caused the differences in delayed fruit harvesting time observed in 'Grand nain' variety. Agro ecological zone II is relatively cool with mean annual temperature ranging between 18°C and 21°C while AEZ IV is relatively hot with a mean annual temperature of 30°C. Hexanal treatment coupled with the cool temperatures in AEZ II could have led to the longer time taken by the fruits while in the field.

The observed delayed time of 12–18 days in the hexanal-sprayed fruits could be attributed to its effect on the fruit abscission process. According to Anusaya *et al.* (2016), hexanal slows down peroxidase, RNA, and protein synthesis activities in the abscission zone and thus extends fruit retention in hexanal-sprayed trees. Similar results have been reported in mango where trees sprayed with hexanal had retained approximately 20% of the total fruits at the end of a 33-day retention study period compared to the untreated fruits which had shed almost all the fruits (Anusaya *et al.*, 2016). The observed delayed time to banana harvesting of approximately 2- 3 weeks as result of hexanal preharvest spray application could be very beneficial to small-scale fruit farmers in Kenya, because of the extended marketing period. Previous studies in temperate fruits such as berries, have shown that hexanal efficacy is affected by the stage of application. For instance, in cherries, optimum results were obtained when hexanal was applied twice at 15 and 7 days before harvest (Sharma *et al.*, 2010) whereas in tomatoes, optimum results were obtained when hexanal was applied at the mature green stage (Cheema *et al.*, 2014). In the present study, hexanal was more effective as a pre-harvest spray when applied twice at 30 and 15 days before harvest. Phospholipase D enzyme activities has been reported to increase during fruit maturation and at the onset of ripening (Paliyath *et al.*, 2008). In the present study, it is possible that application of hexanal at 30 and 15 days before harvest coincided with the temporal increase in phospholipase D activity that occurs during fruit maturation as suggested by Pinhero *et al.* (2003). A previous molecular study in tomatoes showed that hexanal suppresses the expression of PLD genes responsible for expression of phospholipase D enzyme that initiates cell membrane degradation leading to loss of integrity and accelerated senescence.

Hexanal prolonged shelf life by 9 days and 6 days in post-harvest dip treatments for 5 minutes and 2.5 minutes, respectively in 'Grand nain' compared to only 3 days in 'sweet banana' variety regardless of the concentration. The effect of exposure time could be because banana fruit has a thick and fibrous peel that could have possibly affected the penetration of hexanal solution. Therefore, the longer duration of exposure of 5 minutes during the post-harvest dip treatment is likely to have allowed enough penetration of the treatment solution compared to 2.5 minutes resulting to its effectiveness. On the other hand, pre-harvest spray treatment applied twice before harvest extended shelf life by 6 days in 'Grand nain' variety compared to 3 and 6 days in 'sweet banana' fruits from the semi-arid AEZ IV and wetter AEZ II respectively. The banana fingers are tightly arranged adjacent to each other in a bunch. This morphological arrangement hinders the penetration of the hexanal solution to the surface of all the fruit fingers. Therefore, a double spray might have increased the chances of the hexanal penetration to most of the fingers making it more effective compared to a single spray. Further, the reduced rates of ethylene production and respiration observed in the hexanal-treated fruits could partly explain the observed enhanced shelf life of the treated fruits. An increase in respiration rate contributes faster deterioration and senescence of climacteric fruits. Similar findings have been reported in papaya (Hutchinson et al., 2018) and 'Grand nain' banana fruits in India (Venkatachalam *et al.*, 2018), where a post-harvest dip in 2% hexanal for 5 minutes at ambient room temperature conditions enhanced the fruits shelf life by 6 days.

In the present study, the rate of ethylene production was significantly affected by the interaction between mode of application and zone of production. 'Grand nain' fruits from warmer AEZ IV had a higher rate of ethylene production compared to those from cooler AEZ II. This could be attributed to the high temperatures of over 30°C, in AEZ IV that could have accelerated the ripening process. Generally, hexanal treatment slowed down the rate of ethylene evolution and delayed the climacteric peaks in both modes of application and varieties. Molecular studies conducted by Tiwari and Paliyath (2011) in tomato fruit, indicated that hexanal treatment caused temporal suppression of 1aminocyclopropane-1-carboxylate synthase 6 (ACS6) and 1-aminocyclopropane-1-carboxylate synthase (ACS) genes. The expression of both genes is responsible for the biosynthesis of 1-Aminocyclopropane-1-carboxylic acid (ACC) synthase enzyme, which is a key enzyme in the ethylene biosynthesis pathway. The inhibition of ACS genes by hexanal will therefore lead to a reduction in ethylene evolution, and this may explain the low levels of ethylene production observed in this study. The present results corroborates the observations by Hutchinson et al. (2018), who reported that hexanal treatment reduced the rate of ethylene production and delayed the climacteric peaks by 3 days in papaya fruits. Further, a study by Jakubowicz et al. (2010), reported that hexanal could be an effective inhibitor of the ethylene biosythensis pathway.

Respiration is a central physiological process in ripening of climacteric fruits such as banana and is inversely proportional to the shelf life. Hexanal treatment significantly reduced the rate of respiration throughout the storage period in both modes of application and varieties in this study. A reduction in the rate of respiration leads to a reduction in utilization of substrates such as free sugars, leading to an increase in post-harvest life (Saltveit, 2004). The rate of respiration can therefore be used to gauge the rate of metabolism in a commodity and in this study, it correlated positively with other ripening changes including peel and pulp softening and peel color changes.

Fruit softening is a major determinant of ripening in banana and the rate of softening is rapid in the later stages of ripening (Mirshekar *et al.*, 2015). A decline in banana firmness during ripening is largely due to the result of disassembly of the cell wall, deterioration of the cell membrane, and

breakdown of starch into simple sugars (Mirshekar et al., 2015). However, other mechanism such as loss of turgor and breakdown of starch to sugar may also be involved in determining the overall texture characteristics of banana fruit (Kojima et al., 1994). Results of this study show that hexanal significantly delayed peel and pulp softening to different extents. For instance, untreated 'Grand nain' fruits initial firmness decreased by approximately 94% at day 15 of storage compared to 90% in hexanal treated fruits, 9 days later (day 24). The delayed softening in hexanal treated fruits could be attributed to preservation of fruit cell wall structures and cell membrane as previously reported by Sharma et al. (2010). Decrease in firmness during ripening is associated with activities of the enzymes involved in cell wall metabolism such as polygalacturonase (PG), pectin methylesterase (PME) and pectate lyase (PL) activities (Cheng et al., 2011). A previous research by Tiwari and Paliyath (2011) in tomato, showed that hexanal treatment suppressed the expression of genes involved in pectin and hemicellulose degradation which are the main components of the plant cell wall. In addition, the delay in fruit softening may also be as a result of the observed low rate of ethylene production and respiration in the hexanal-treated fruits. Similar results have been reported in India, where hexanal treated 'Grand nain' fruits under ambient room conditions remained firmer compared to the untreated fruits after 18 days of storage (Venkatachalam et al., 2018). Further, Hutchinson et al. (2018), reported similar observations in papaya where hexanal treatment retained fruit firmness by up to 38% at the end of storage which occurred 12 days later compared to the untreated fruits. Fruits from the semi-arid AEZ IV softened faster compared to those from the cooler and wetter AEZ II regardless of the treatment. This could be attributed to temperatures and rainfall differences in both zones, both having been reported to have an impact on fruit softening (Ferguson et al., 1999). These findings concur with the observations of Ambuko et al. (2006), who reported that banana fruits produced in drier zones and during the dry periods of the year, softened faster. A varietal difference was observed where 'sweet banana' fruits softened faster compared to the 'Grand nain' in both modes of application. This could be attributed to the physiological differences between the two varieties with 'sweet banana' variety ripening faster compared to 'Grand nain'. In the present study, 'sweet banana' variety softened faster compared to 'Grand nain' irrespective of AEZ, treatment and mode of application. The observed varietal differences could be attributed to morphological differences between the two varieties with 'sweet banana' variety having a thinner peel compared to 'Grand nain'.

Physiological weight loss in fruits is as a result of respiration, transpiration and other biological changes during ripening. In banana, the peel loses water to both the pulp and the environment during ripening process as reported by Burdon *et al.* (1994). In the present study, all the fruits were observed to lose significant amount of weight during the storage process. Hexanal treatment, however, had no significant effect on percentage weight loss except in post-harvest dip treated 'sweet banana' fruits from the drier AEZ IV as from day 6 of storage. These results are in contrast with the superior delayed weight loss observed in hexanal treated mango fruits in India (Anusuya *et al.*, 2016). It was expected that hexanal would facilitate skin thickening that results to reduced water loss, and this along with the observed reduced rate of respiration could have caused low weight loss (Anusuya *et al.*, 2016).

Peel color expressed as hue angle decreased gradually as the fruits ripened indicating color changes from green to yellow and followed by the appearance of dark brown spots. Color changes in banana fruit during ripening are as a result of the breakdown of chlorophyll by chlorophyllase enzyme in the peel which unmasks the yellow color of the carotenoid pigment (Sivakumar *et al.*, 2011). In the present study, hexanal treatments applied either as a pre-harvest spray or post-harvest dip significantly delayed changes in peel color in both varieties. The observed high hue angle in the hexanal-treated fruits is a clear indication of a delayed ripening process. Delayed color changes in hexanal-treated fruits have been reported in tomatoes (Cheema *et al.*, 2014), mangoes (Anusuya *et al.*, 2016), and sweet cherries (Sharma *et al.*, 2010). In the present study, although hexanal treatment delayed color change, at the end of storage the fruits attained almost the same hue angle as the untreated controls. This shows that, hexanal treatment does not affect the processes that lead to color changes during banana ripening.

In conclusion, this study established that, hexanal applied at 2% and 3% has the potential to enhance fruit shelf life to different extends depending on the variety, mode of application and time/duration of application. Hexanal effectiveness was significantly affected by variety where 'Grand nain' responded better compared to 'sweet banana' in both modes of application. However, the AEZs did not significantly influence hexanal effectiveness in both varieties. The study revealed that, the time/duration and mode of hexanal application greatly influenced hexanal effectiveness in both varieties. Post-harvest dip treatment for 5 minutes extended fruits' shelf life by 6 and 9 days in 'Grand

nain' and 'sweet banana' varieties respectively, irrespective of the AEZ. On the other hand, preharvest spray treated 'Grand nain' fruits had a shelf life of 3 and 6 days incase of single and double spray respectively in both AEZs. However, pre-harvest sprayed 'sweet banana' fruits had a shelf life of only 3 days irrespective of AEZ and hexanal time of application. The observed delayed time to fruit harvesting of approximately 2 -3 weeks in the hexanal's pre harvest spray treated fruits might be very beneficial to small scale farmers as it extends the marketing period thereby giving them opportunity to find good markets for their fruit. Additionally, the two modes of application of hexanal are easy to use and most of the small scale farmers targeted by this technology can fully apply hexanal in their farms. Therefore, the use of hexanal and its formulations can be promoted as an alternative post-harvest technology for use especially by small scale banana farmers in Kenya.

#### **CHAPTER 4**

# 4.0 Effects of Hexanal Treatments on the Post-Harvest Quality Characteristics Banana Fruits

# 4.1 Abstract

Hexanal, is a naturally-occurring compound that has been shown to extend the postharvest shelf life of perishable fruits. However, for a successful commercialization of any post-harvest technology, it should not only prolong the shelf-life but also maintain the fruits quality. This study was therefore, conducted to evaluate the effect of hexanal treatments on the post-harvest quality of 'Grand Nain' and 'Sweet banana' varieties in Kenya. In this study, samples from the best performing treatments from each mode of application in chapter 3 were selected to evaluate the effect of the hexanal treatments include hexanal concentrations of 2% and 3% applied as a post-harvest dip for 5 minutes or a pre-harvest spray for 30 +15 days. The analysis was conducted on various quality parameters including total soluble solids, total titratable acidity, vitamin C and simple sugars. In addition, sensory analysis was conducted on fruits samples which were harvested at mature green stage from the two agro-ecological zones, subjected to the various hexanal treatments and left to ripen at ambient room conditions. The experimental design used was a Complete Randomized Design (CRD) with factorial arrangements. The factors included variety, AEZ, hexanal concentration and mode of application.

The results showed that hexanal delayed ripening of the fruits, it significantly reduced the rate of increase in most of the quality attribute parameters such as TSS, total titratable acidity, vitamin C and simple sugars. Hexanal treatment did not have any significant effect on TSS levels in both varieties and AEZs. However, in both seasons, variety had a significant (p < .05) effect on the TSS levels with 'sweet banana' having higher levels compared to 'Grand nain' fruits regardless of hexanal treatment and AEZs. Hexanal treatment had a significant (p < .05) effect on Vitamin C, where treated fruits mantained relatively higher levels throughout the storage period in both AEZs compared to the untreated controls. Grand nain' fruits dipped in hexanal had lost only 49 % and 22 % of the initial vitamin C content in fruits from AEZ IV and AEZ II respectively, 9 days later compared to 54 % and 51 % in the untreated controls. Changes in simple sugar levels followed a similar trend to that of TSS

with fruits from the drier AEZ IV having high sugar content compared to those from the more humid AEZ II. Hexanal treatment significantly (p<0.05) delayed the rate of increase of simple sugars (fructose, glucose, sucrose) in both varieties and AEZs compared to the untreated ones. At the end of storage period, hexanal treated fruits mantained high levels of glucose compared to the untreated controls. Results of the sensory evaluation showed no significant differences in the various quality attributes scored between the hexanal-treated and untreated control fruits. The findings of this study reveal that use of hexanal is a potential technology that could be adopted by banana farmers in Kenya to enhance shelf life without compromising on the postharvest quality attributes.

# 4.1. Introduction

Banana is a climacteric fruit which is often harvested at the physiological maturity stage and then ripened before marketing. The fruit undergoes various biochemical and physiological changes during ripening that transforms the fruit into edible state. Some of these changes include fruit softening, changes in peel color, degradation of starch to sugars, changes in concentration of aroma volatiles and acids. According to Maduwanthi et al. (2017), sugar levels increases from an initial of 2% in green banana to approximately 15% -20% in the ripe fruit making it sweeter. Similarly, different set of volatile constituents such as esters, alcohols, ketones and aldehydes which are responsible for the unique aroma of banana increases during ripening process (Maduwanthi et al., 2017). However, once the fruit is fully ripe, senescence sets in leading to decline in the nutritional and sensory quality of the fruit. There is no doubt that the quality of any produce is determined majorly by pre-harvest factors and appropriate postharvest handling across the entire value chain. Ideally, banana fruits should be harvested at mature green stage for optimum quality upon ripening. If the fruits are harvested too young before the attainment of physiological maturity, the fruits don't ripen fully and lack appropriate quality characteristics. More often than not, farmers and traders are faced with the dilemma of harvesting their fruits at the right maturity stage for optimum quality or early before achieving physiological maturity for prolonged shelf life.

Changes in banana quality is experienced across the value chain during handling, storage and marketing. End of fruit ripening is followed by senescence whereby anabolic reactions are suppressed by degradative changes leading to death and decay of the fruit tissue (Valero and Serrano, 2010).

This renders the fruit very perishable and in some cases do not reach consumers at optimal quality. In order to increase storage life of the fruits, appropriate post-harvest technologies aimed at reducing the deterioration rate have been developed. These technologies are used to slow down fruits metabolic processes to provide optimal quality without compromising on the consumer safety. Recently, efforts are being made to develop new post-harvest technologies for extension of banana shelf life while maintaining its quality. Use of hexanal is one of the new innovations which have been proved effective in enhancing the post-harvest shelf life of banana fruits (Venkatachalam *et al.*, 2018). Being a relative new technology, there is need to test its suitability to preserve banana post-harvest quality while extending its shelf life in Kenya. The objective of this study was therefore, to determine the effect of hexanal treatments on the post-harvest biochemical characteristics and sensory quality of banana fruits.

# 4.2. Materials and methods

# 4.2.1. Fruit Samples

Fruit samples from the best treatment combinations in chapter 3 were analyzed for various selected quality attributes. These included; Total soluble solids (Brix), Total Titratable Acidity (TTA), Vitamin C, sensory analysis and simple sugars (sucrose, fructose and glucose). The middle portion of banana samples used for destructive sampling (peel and pulp firmness) in chapter 3 were peeled, diced and stored in a freezer at -20<sup>o</sup>C in zip lock bags. The quality parameters were analyzed once all the samples had been obtained at the end of each season. The experiments were conducted in two successive seasons, July to November 2016 and January to April 2017. For sensory analysis, fresh fruit samples were obtained from each AEZ at mature green stage. The fruits were either dipped in 2 % hexanal for 5 minutes or in plain water (control) and allowed to undergo normal ripening at ambient room conditions ( $25 \pm 1^{\circ}$ C and RH 60  $\pm 5^{\circ}$ ). A complete randomized design (CRD) with factorial arrangements was used for this study. The factors included variety, AEZ and mode of application.

### 4.2.2. Measurement of biochemical attributes of banana

### 4.2.2.1. Total Soluble Solids (TSS/°Brix)

Total soluble solids content of banana fruit pulp was determined using digital hand held refractometer (Model 500, Atago, and Tokyo, Japan). Approximately, 5 grams of banana paste extracted from three different fruits in each treatment by use of mortar and pestle was placed on the prism of the refractometer and TSS content was recorded as % Brix from direct reading of the instrument. The % Brix was converted and expressed in dry weight basis using the formula below;

$$\% Brix (dry weight basis) = \left(\frac{\% Brix in wet weight basis * 100}{100 - Moisture content}\right)$$

# **4.2.2.2. Total Titratable Acidity (TTA)**

Total titratable acidity was determined by titration where 5 grams of the fruit pulp was macerated and diluted with 20ml of distilled water. Ten milliliters of the diluted solution was obtained, mixed with 2-3 drops of phenolphthalein indicator and titrated with 0.1N Sodium hydroxide until the solution changed color to faint pink. The titer volume was recorded and the results expressed as percent malic acid, the predominant organic acid in banana fruits according to the method of Ranganna, (1991). The TTA value was converted and expressed in dry weight basis using the formula below;

$$TTA (dry weight basis) = \left(\frac{TTA \ value \ in \ wet \ weight \ basis * 100}{100 - Moisture \ content}\right)$$

# 4.2.2.3. Ascorbic acid content

The ascorbic acid content in the samples was determined by HPLC method according to Mamun *et al.* (2012) with slight modifications. About 5g of sample was weighed and extracted with 0.8% metaphosphoric acid under subdued light conditions. The extract was made to 20 mL of juice and centrifuged at 10000 rpm at 4°C for 10 minutes. The supernatant was filtered and diluted with 10 mL of 0.8% meta-phosphoric acid. This was passed through 0.45  $\mu$  micro filters. The samples were then set as a post-run into HPLC machine (Model LC- 10AS, Shimadzu Corp., Kyoto, Japan) where 20  $\mu$ L of the micro filtered sample was automatically injected into the HPLC machine on the same day of extraction. The ODS C-18 column of size 250 mm x 4.6mm x 0.5µl was used for the analysis. Various concentrations of ascorbic acid standards were prepared at 10, 20, 40, 60, 80 and 100 ppm and a blank containing only degassed meta-phosphoric acid and used to obtain a calibration curve. HPLC analysis was done using Shimadzu UV-VIS detector. The mobile phase was 0.8% meta-phosphoric acid, at 1.2 mL/min flow rate and wavelength of 266.0 nm. The quantity of ascorbic acid was calculated using AOAC, (1996) method where standard vitamin C regression curve was obtained with the freshly prepared Vitamins C standards as follows;

Ascorbic acid, 
$$\left(\frac{mg}{100ml}\right) = \left(\frac{Peak \ area \ from \ graphs}{y}\right) * \left(\frac{Dilution \ volume}{Sample \ weight \ (g)}\right) * \left(\frac{100}{1000}\right)$$

Where y =Calibration coefficient obtained from standard regression curve when y-intercept is zero (AOAC, 1996). Since the ascorbic acid content obtained above was in fresh weight basis, the values were converted and expressed in dry weight basis as follows;

Ascorbic acid (dry weight basis) = 
$$\left(\frac{Ascorbic acid value in wet weight basis * 100}{100 - Moisture content}\right)$$

### 4.2.2.4. Determination of simple sugars (Glucose, fructose and Sucrose) contents

Total soluble sugars were analyzed using AOAC (1996) method. Approximately 10g of the banana pulp was completely blended and 96% ethanol added. Refluxing was done for one hour at 100°C and then cooled under running water. The solution was then filtered using 42mm whatman filter paper. Rinsing was done using 5ml of 96% ethanol. The solution was rotary evaporated to dryness at 60°C. 5ml of 50% acetonitrile was then added and finally micro-filtered (0.45µ). The individual sugars were analyzed using a high performance liquid chromatography (HPLC) (Model LC-20AS, Shimadzu Corp., Kyoto, Japan) fitted with a refractive index detector (RID) and running under the following conditions: oven temperature: 30°C, Flow rate: 0.5-1.0 ml/min, Injection volume: 20 uL, Column: NH<sub>2</sub> (5.0µl) Mobile phase: Acetonitrile: water (75:25). Sugars present in the solution including sucrose, glucose and fructose were identified and their individual concentration calculated

using the standards. A graph was plotted for the concentration of the standard (X-axis) versus absorbance (Y-axis). Carbohydrate concentration was calculated as:

Amount of Carbohydrate present in sample (% mg)=  $\frac{Sugar value from graph (mg)}{Aliquot sample used} X \frac{Total volume of extract (ml)}{Weight of sample (mg or ml)} X 100$ 

Each individual sugar was expressed in dry weight basis as follows;

$$Sugar value (dry weight basis) = \left(\frac{Sugar value in wet weight basis * 100}{100 - Moisture content}\right)$$

#### 4.2.2.5. Sensory quality evaluation

The sensory quality attributes were done on hexanal-treated and untreated control fruits of both varieties ripen naturally to a predetermined stage 6 according to the standard banana ripening chart (appendix 1). The ripe fruits were washed with clean water, dried and diced into approximately equalsized slices, avoiding the extreme ends. Three slices were placed on white plates which were anonymously coded based on treatment, AEZ and variety to ensure objectivity. A panel of 36 untrained judges drawn from the University faculty of agriculture student population was guided on the scoring procedure of the various sensory attributes which included; fruit color, aroma, texture, flavor, mouth feel and the general acceptability. The panelists scored for these attributes on five point hedonic scale where 1 = dislike (worst), 2 = (dislike moderately), 3 = (neither like nor dislike), 4 = (like moderately) and 5 = Like extremely (Best). This was adapted from Galan Sauco *et al.* (1984), but with few modifications.

#### 4.2.3. Statistical analyses

Data collected was subjected to analysis of variance (ANOVA) using Genstat statistical package (version 15). The means were separated by Least Significance Difference (LSD) at  $p \le .05$  using Fisher's protected test. The sensory quality evaluation data was analyzed using SPSS statistical package version 21.

# 4.3. Results

### **4.3.1.** Total soluble solids (<sup>0</sup>Brix)

In both seasons, TSS levels increased gradually with ripening in all the fruits (Figures 15 and 16), irrespective of treatment, variety, mode of application and AEZ. The increase in TSS was significantly affected (p < 0.05) by hexanal mode of application only in season 1 experiment (Figure 15), where the increase was greater and drastic for the pre-harvest spray mode of application compared to post-harvest dip. However, hexanal treatment did not have any significant effect on TSS levels in both varieties and AEZs. In season 1 and 2, variety had a significant (p < 0.05) effect on the TSS levels. Overall, 'sweet banana' had higher TSS levels compared to 'Grand nain' fruits in both modes of hexanal application and AEZs.

In post-harvest dip mode of application (season 1), TSS levels in 'Grand nain' fruits increased from an initial 0.96 °Brix and 0.63 °Brix to 30 °Brix and 29 °Brix in the control fruits in AEZ IV and AEZ II, respectively by the end of storage (day 15). However, increase in TSS was less rapid in the diptreated fruits, compared to the sprayed fruits and controls. In the 'Grand nain' dip treated fruits, TSS increased to approximately 27.5 °Brix and 28.2 °Brix in AEZ IV and AEZ II, respectively, 9 days later compared to the untreated fruits. A similar trend was observed in 'sweet banana' dip treated fruits which had TSS levels of 30 °Brix and 28-29 °Brix in AEZ IV and AEZ II respectively at the end stage (day 18), which occurred 6 days earlier than in dip treated 'Grand nain' fruits.

In the pre-harvest spray mode of application (season 1), TSS levels increased rapidly to peak levels of 27 °Brix – 29 °Brix in 'Grand nain' spray treated fruits in AEZ IV and AEZ II, respectively at the end stage which occurred 9 days earlier than in fruits dipped post-harvest. In the 'sweet banana' fruits, TSS increased from an initial 9.5° brix and 8.1° brix to peak levels of 33.8 ° brix (day 3) and 31.7 ° (day 6) in Machakos and Meru Counties, respectively then declined gradually until the end of storage. On the other hand, the increase in TSS was less rapid in sprayed fruits which had a peak of 27.4° brix at day 9 and 12 in fruits treated with 2 % and 3 % hexanal respectively in AEZ IV and AEZ II, compared to 26.6 ° brix and 25.3 ° in 2 % and 3 % in Meru County at day 15 of storage. In 'sweet banana', sprayed fruits attained almost the same TSS level (29° - 31° brix) compared to the controls whose end stage occurred three days earlier.

Overall, fruits in season 1 experiment (Figure 15) had significantly (p < .05) high TSS levels irrespective of variety and zone of production compared to those in season 2 (Figure 16).

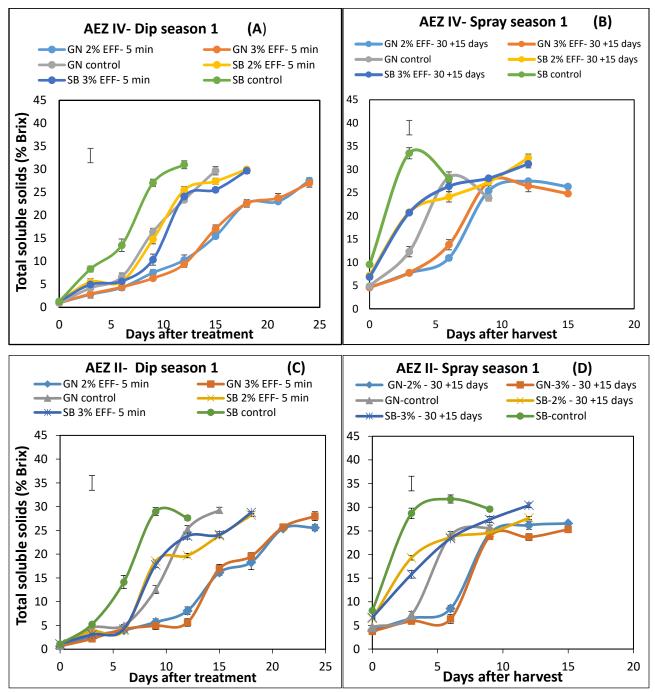


Figure 15: Effect of post-harvest dip and pre-harvest spray modes of hexanal application on total soluble solids in 'Grand nain' (GN) and 'Sweet banana' (SB) fruits from AEZ IV (A, B) and AEZ II (C, D) season 1. The top bar in each graph represents the least significant difference (LSD) of

interaction between treatment, mode of application and AEZ at p<0.05. The vertical bars represent SE of the means (p<0.05).

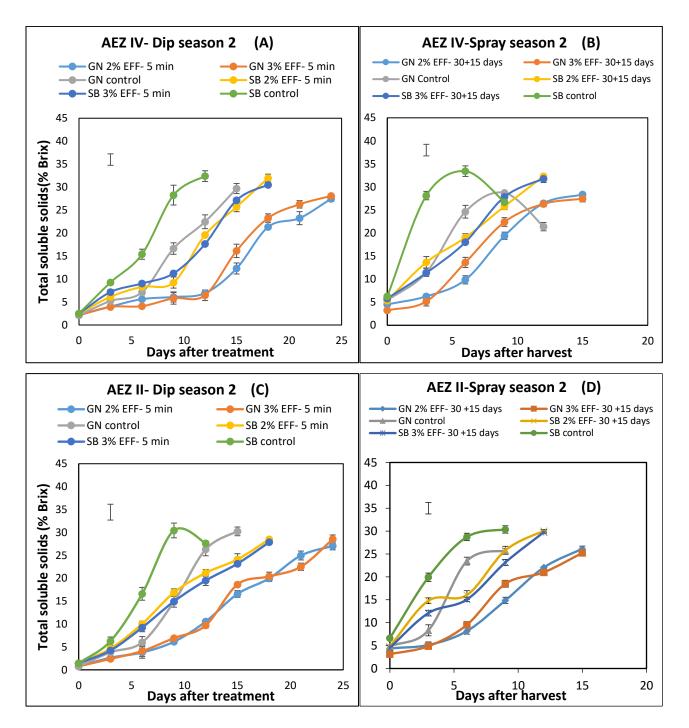


Figure 16: Effect of post-harvest dip and pre-harvest spray modes of hexanl application on total soluble solids in 'Grand nain'(GN) and 'Sweet banana' (SB) fruits from AEZ IV (A, B) and AEZ II (C, D) season 2. The top bar in each graph represents the least significant difference (LSD) of

interaction between treatment, mode of application and AEZ at p<0.05. The vertical bars represent SE of the means (p<0.05).

# **4.3.2.** Total titratable acidity (TTA)

The total titratable acidity levels increased gradually as ripening progressed in all the banana fruits in both AEZs (Figures 17 and 18). There was no significant differences between the two seasons. The TTA levels were significantly (*p*<0.05) affected by the interaction between mode of hexanal application, treatment and variety. Hexanal treatment delayed the rate of TTA increase in both AEZs, with pre-harvest spray-treated fruits having high TTA levels throughout storage compared to the post-harvest dip treatment in both zones (Figures 17 and 18). 'Sweet banana' had high TTA levels compared to the 'Grand nain' variety in both AEZs and seasons.

In season 1, post-harvest dip treated 'Grand nain' fruits retained lower TTA levels of approximately 2% - 2.3% at the end of storage which occurred 9 days later compared to the controls in Machakos and Meru Counties respectively (Figure 17). However, the spray treated fruits maintained significantly (p<0.05) high TTA values of 2.8% - 3.1% at the end of storage (day 15). In season 2, a similar trend to the one in season one was observed in both modes of application. In the dip mode of application, TTA levels in the untreated 'sweet banana' fruits (Machakos county) increased drastically from 1.4% to peak value of 2.8% (day 9) before declining till the end of storage. However, in Meru county fruits, the TTA values in the controls increased from 0.7% to 1.8% at the end of storage, (day 12).

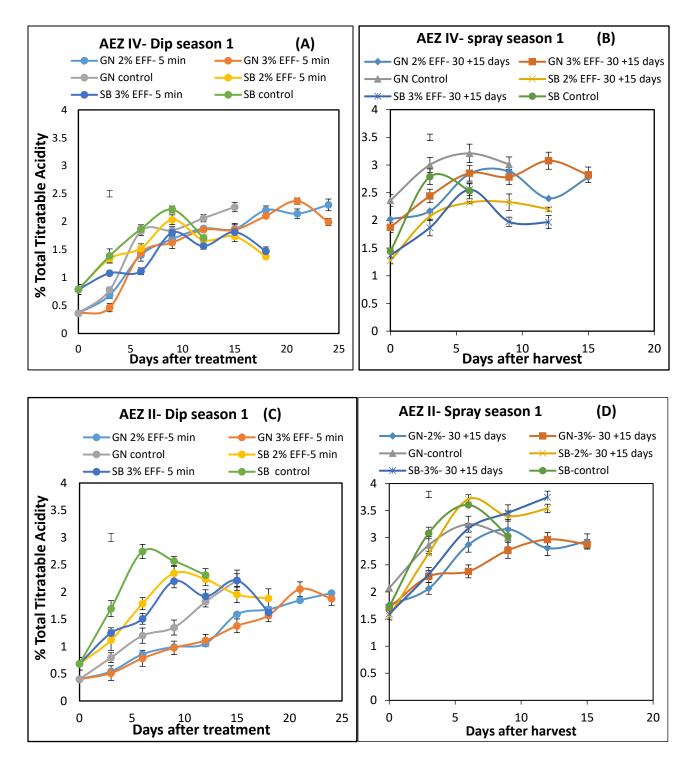


Figure 17: Effect of post-harvest dip and pre-harvest spray modes of hexanal application on Total titratable acidity (TTA) in 'Grand nain' (GN) and 'Sweet banana' (SB) fruits from AEZ IV (A, B) and AEZ II (C, D) season 1. The top bar in each graph represents the least significant difference (LSD) of interaction between treatment, mode of application and AEZ at p<0.05. The vertical bars represent SE of the means (p<0.05).

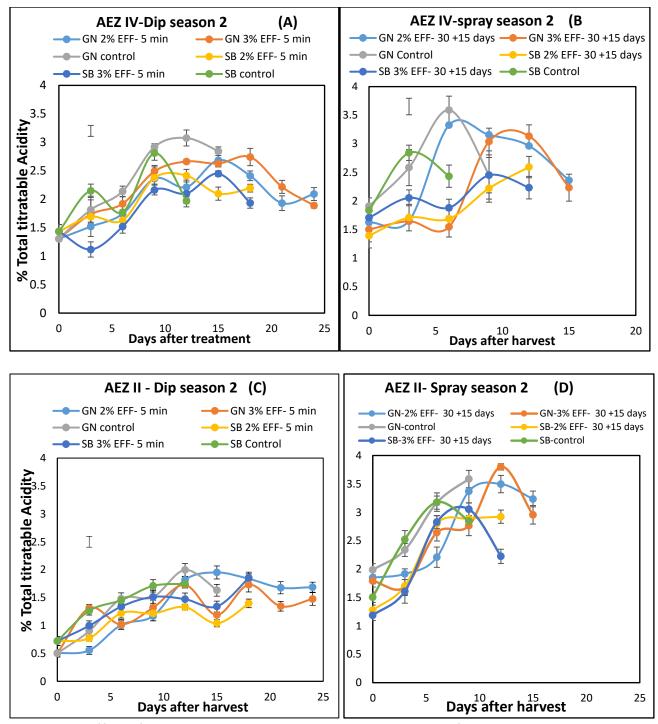


Figure 18: Effect of post-harvest dip and pre-harvest spray modes of hexanal application on Total titratable acidity (TTA) in 'Grand nain' (GN) and 'Sweet banana' (SB) fruits from AEZ IV (A, B) and AEZ II (C, D) season 2. The top bar in each graph represents the least significant difference (LSD) of interaction between treatment, mode of application and AEZ at p<0.05. The vertical bars represent SE of the means (p<0.05).

#### **4.3.3.** Ascorbic acid (vitamin C)

As the fruits ripened, vitamin C levels decreased gradually in all the fruits irrespective of zone of production and hexanal treatment (Figures 19 and 20). Vitamin C content was significantly (*p*< 0.05) affected by the interaction between hexanal treatment, mode of application and AEZ in both seasons. Generally, fruits from the cooler AEZ II zone had higher ascorbic acid levels compared to those from the drier AEZ IV. Hexanal treatment, applied as either a pre-harvest spray or post-harvest dip significantly delayed the rate of vitamin C reduction in both AEZs as compared to the untreated controls. However, both hexanal concentrations (2 % and 3 %) were equally effective with no significant differences between them in 'Grand nain' variety. In 'sweet banana', contrasting results were observed where 2 % concentration was more effective in the drier AEZ IV fruits whereas in the cooler AEZ II, 3 % concentration was more effective. In both season 1 and 2, 'Grand nain' fruits maintained significantly high vitamin C levels compared to 'sweet banana' irrespective of the AEZ of production (Figures 19 and 20).

In season 1, Vitamin C levels reduced drastically by 54 % and 51 % by the end of storage (day 15) in the untreated control ('Grand nain' fruits) in post-harvest dip experiment, compared to 49 % and 22 % in AEZ IV and AEZ II respectively, in the hexanal-treated fruits. In the dip treated fruits, (Figures 19A and 19C), vitamin C levels decreased from initial levels of 68.9 mg/100g and 73.9 mg/100 g to an average of 29 – 34 mg/100g and 33 – 37 mg/100g in AEZ IV and AEZ II, respectively, by the end of storage (day 24) in the 'Grand nain' variety. In the 'sweet banana' variety, the ascorbic acid levels decreased from initial values of 51.4 mg/100g and 52.3 mg/100g to 24 mg/100g and 24 – 31 mg/100g in the treated fruits at the end of storage (day 18), 6 days later compared to the controls in AEZ IV and AEZ II, respectively.

A similar trend was observed in season 2 experiment where vitamin C levels decreased gradually during storage in all the fruits. In the untreated 'Grand nain' fruits, vitamin C levels decreased drastically from an initial of 75.7 mg/100g and 47.7mg/100g to 30.2 mg/100g and 38 mg/100g in AEZ IV and AEZ II, respectively, by day 15 of storage in case of post-harvest dip mode of application (Figure 20). On the other hand, rate of vitamin C decrease was slow in the hexanal treated fruits irrespective of variety and AEZ. By day 24 of storage, 'Grand nain' fruits treated with 2 % hexanal had vitamin C content of 33.1 mg/100g and 33.5 mg/100g in AEZ IV and AEZ II, respectively, in post-harvest dip mode of hexanal application.

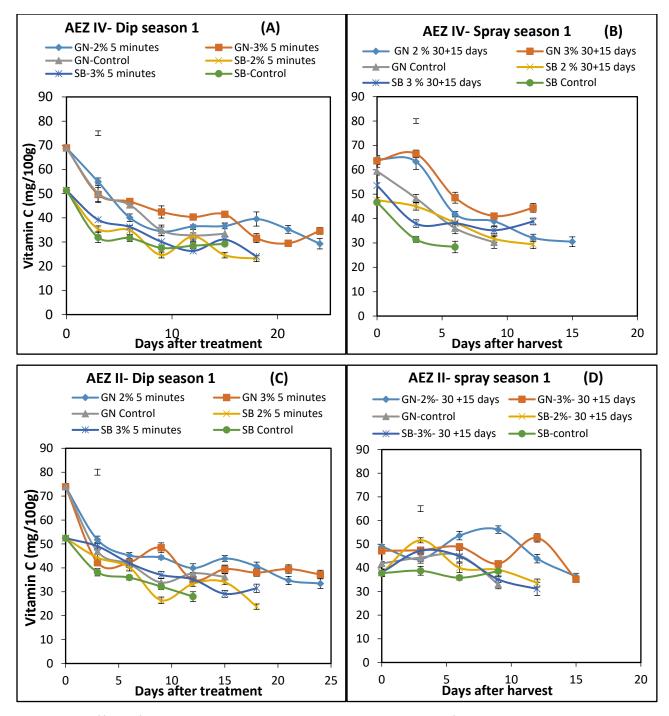


Figure 19: Effect of post-harvest dip and pre-harvest spray modes of hexanal application on Vitamin C in 'Grand nain' (GN) and 'Sweet banana' (SB) fruits from AEZ IV (A, B) and AEZ II (C, D) season 1. The top bar in each graph represents the least significant difference (LSD) of interaction between treatment, mode of application and AEZ at p<0.05. The vertical bars represent SE of the means (p<0.05).

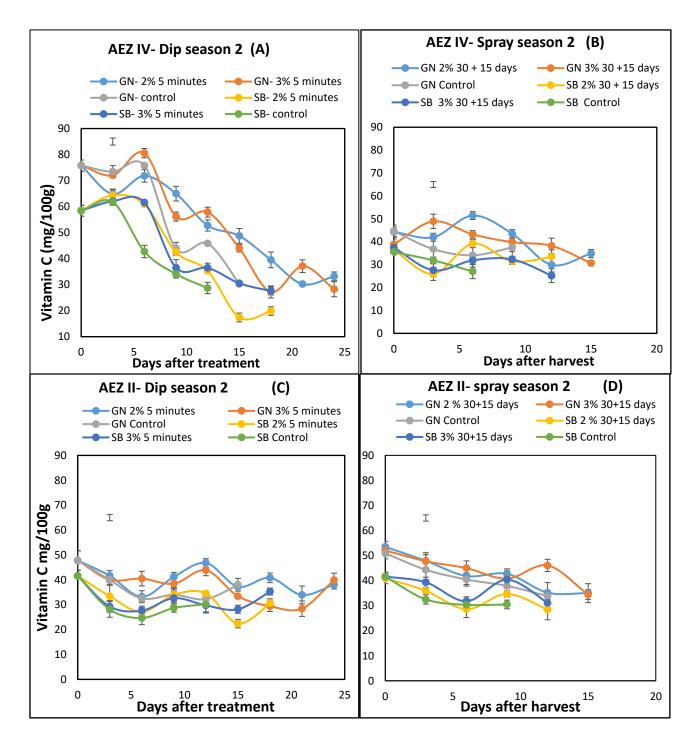


Figure 20: Effect of post-harvest dip and pre-harvest spray modes of hexanal application on Vitamin C in 'Grand nain' (GN) and 'Sweet banana' (SB) fruits from AEZ IV (A, B) and AEZ II (C, D) season 2. The top bar in each graph represents the least significant difference (LSD) of interaction between treatment, mode of application and AEZ at p<0.05. The vertical bars represent SE of the means (p<0.05).

### 4.3.4. Changes in simple Sugars

#### 4.3.4.1. Fructose

Fructose levels increased gradually with ripening in all the fruits irrespective of hexanal treatment, variety, mode of application used and AEZ (Tables 11A and B). The increase of fructose content was significantly (p < 0.05) affected by interaction between mode of application and AEZ. Changes in fructose levels were more rapid in the pre-harvest spray treatments, where peaks of 217.2 mg/100g (day 6) and 164 mg/100g (day 3) occurred in the untreated 'Grand nain' and 'sweet banana' fruits respectively from AEZ IV (Tables 11A and B). A similar trend was observed in fruits from AEZ II, where fructose levels in 'sweet banana' peaked high (148.6 mg/100g) at day 6, compared to a slightly lower average peak (99.2 – 113.9 mg/100g) for the hexanal treated fruits which occurred six days later (Table 11B). Fruits from the drier AEZ IV had significantly high fructose levels compared to those from the wetter AEZ II.

Hexanal treatment significantly delayed the rate of fructose increase in both varieties and AEZs compared to the untreated ones. In the untreated 'Grand nain' fruits, fructose levels increased drastically from initial of 5.5 mg/100g and 6.3 mg/100g to peaks of 201.1 mg/100g and 186.8 mg/100g ,12 days earlier compared to the hexanal treated fruits (Tables 11A). On the other hand, fructose levels increased to 207.6 mg/100g and 194.3 mg/100g in fruits treated with 2 % hexanal at the end of storage (day 24) in AEZ IV and AEZ II respectively, incase of post-harvest dip treatment. Similarly, in the pre-harvest spray treatment, fructose levels of the hexanal treated fruits increased to levels of 197- 206 mg/100g and 146- 167 mg/100g in AEZ IV at day 12 and AEZ II at day 15 of storage, respectively.

The trend was not different in the 'sweet banana' variety. The fructose levels increased rapidly to 184.7 mg/100g and 149 mg/100g in the untreated control fruits at the end of storage (day 12) in the AEZ IV and AEZ II respectively in the post-harvest dip experiment (Table 11B). Fructose increase in the hexanal treated fruits assumed a similar trend to that in 'Grand nain' variety with levels of 99.2-113.9 mg/100g recorded in AEZ II fruits as compared to 139 mg/100g in AEZ IV, at the end of storage (day 12), incase of preharvest spray mode of application. Generally, there were no significant differences between 2 % and 3 % hexanal concentrations used.

Mode of application		Post-harv	est Dip	Pre-harvest spra			
ZONE	DAYS	Control	2%	3%	Control	2%	3%
AEZ IV	0	5.5h	5.5k	5.51	15.8e	9.0i	12.6i
	3	18.5g	10.1k	8.3k	59.0c	19.4h	18.7h
	6	49.3e	25 <b>.4</b> j	18.0k	2 <b>17</b> .2a	65.4f	90.8f
	9	<b>104</b> .5d	56.8g	50.1i		151.5b	176.2b
	12	2 <b>01.1a</b>	48.4h	61.7h		<b>197</b> .2a	206.3a
	15	185.1b	67.2f	70.9g		132.3c	115.3e
	18		140.1c	122.9d			
	21		2 <b>03</b> .5a	183.3ab			
	24		207.6a	189.9a			
AEZ II							
	0	6. <b>3</b> h	6. <b>3k</b>	6. <b>3</b> I	18.0e	10.2i	8.1i
	3	12.9g	10.7k	8.0k	5 <b>0.1</b> d	2 <b>3.8gh</b>	2 <b>3</b> .5h
	6	26. <b>8</b> f	22.1j	15.4k	122.5c	<b>30</b> .6g	42.0g
	9	5 <b>3</b> .2e	2 <b>8</b> .2j	31.9j	192.2b	98.8e	108.1e
	12	186.8b	<b>38</b> .6i	5 <b>7.3</b> h		120.8d	141.8d
	15	138.8c	6 <b>8</b> .2f	81.8f		146.0b	167.3c
	18		92.3e	102.0e			
	21		<b>131</b> .6d	142.0c			
	24		194.3b	180.3b			
	Mean	82.4	75.4	74.2	96.4	83.8	92.6
	LSD* (T)	4.9					
	LSD* (M)	2.7					
	LSD* (Z)	3.1					
	LSD* (T * Z* M)	7.3					
	CV (%)	17.6					

Table 11A: Effect of post-harvest dip and pre-harvest spray application of hexanal on Fructose content (mg/100g) of 'Grand nain' bananas from AEZ IV (Machakos County) and AEZ II (Meru County).

Values followed by different letters within the same column differ significantly at (p<0.05) while means with a similar letter in a column do not differ significantly at (p<0.05). T= Treatment, V= Variety, M= mode of application and Z= Agro ecological zone of production.

Mode of application		Post-harvest	Dip		Pre-harvest spray		
ZONE	DAYS	Control	2%	3%	Control	2%	3%
AEZ IV	0	15.5f	15.5f	15.5g	53.5f	21.3g	13.5i
	3	17.7f	9.3g	7.2h	164.0a	29.2f	20.5h
	6	59.2d	14.7g	16. <b>3</b> g	129.2c	96.0b	77.9e
	9	134.1c	22.2f	24.6f		132.4a	121.9b
	12	184.7a	<b>80.4</b> d	102.9d		88.1c	139.0a
	15		121.9b	120.3c			
	18		135.3a	145.5a			
AEZ II							
	0	14.1f	14.1g	14.1g	13.9g	9.1h	4.5j
	3	14.4f	9.8g	11.6g	81.6e	66.6d	47.7g
	6	50.7e	21.1f	11.8g	148.6b	52 <b>.3e</b>	59.1f
	9	133.7c	60.8e	90.4e	99.2d	<b>88.6c</b>	<b>107.3</b> d
	12	149.0b	104.3c	116.4c		99.2b	113.9c
	15		102.7c	136.7b			
	18		117.9b	<b>108</b> .6d			
	Mean	77.3	59.3	65.9	98.6	68.3	70.5
	LSD* (T)	7.2					
	LSD* (M)	4.1					
	LSD* (Z)	4.6					
	LSD* (T * Z* M)	6.2					
	CV (%)	16.4					

Table 11B: Effect of post-harvest dip and pre-harvest spray application of hexanal on Fructose content (mg/100g) of 'Sweet banana' from AEZ IV (Machakos County) and AEZ II (Meru County).

Values followed by different letters within the same column differ significantly at (p<0.05) while means with a similar letter in a column do not differ significantly at (p<0.05). T= Treatment, V= Variety, M= mode of application and Z= Agro ecological zone of production

# 4.3.4.2. Glucose

Increase in glucose content followed a similar trend with that of fructose, were it increased with progression in ripening (Tables 12A and B). However, the increase was less rapid in the hexanal treated fruits as compared to the untreated controls. The increase in glucose content was significantly (p < 0.05) affected by the interaction between mode of application of hexanal and variety. Pre-harvest spray treated fruits retained significantly (p<0.05) high glucose levels at the end of storage compared to the post-harvest dip treated fruits and untreated controls in both AEZs and varieties. Generally, 'sweet banana' fruits had significantly high levels of glucose compared to 'Grand nain' variety (Tables 12A and B).

In the pre-harvest spray experiment, glucose levels increased to a peak level of 214.1 mg/100g by day 3 in the untreated 'Grand nain' fruits, compared to approximately 140 -164 mg/100g in the hexanal treated fruits from AEZ IV at the end stage, which was 9 days later (Table 12A). Similarly, in 'sweet banana' variety, glucose levels increased rapidly in the untreated fruits from initial value of 47.5 mg/100g to a peak level of 179.6 mg/100g at day 3 before declining to 142 mg/100g by day 6 of storage in the preharvest sprayed AEZ IV fruits. Unexpectedly, the untreated control 'Grand nain' fruits from AEZ II in the post-harvest dip experiment, had slightly high glucose levels of 87.4 mg/100g on average compared to those from AEZ IV (79.7 mg/100g).

Table 12A: Effect of post-harvest dip and pre-harvest spray application of hexanal on Glucose content (mg/100g) of 'Grand nain' bananas from AEZ IV (Machakos County) and AEZ II (Meru County).

Mode of application		Post-harve	st Dip		Pre-harvest spray		
ZONE	DAYS	Control	2%	3%	Control	2%	3%
AEZ IV	0	14.8hi	14.8j	14.8j	56.7d	18.2h	16.7i
	3	19.9h	9.4jk	13.6j	2 <b>14.1</b> b	59.9f	43.5g
	6	36.6g	2 <b>3</b> .8i	27.1i	169.1c	87.6e	65.7f
	9	99.5d	33.6h	41.5h		147.8d	132.0e
	12	156.7c	6 <b>8.9</b> f	75.4g		163.6c	139.9d
	15	150.8c	84.5e	111.7e			
	18		124.5d	147.7c			
	21		142.5c	170.5b			
	24		160.0b	174.6b			
AEZ II							
	0	11.8i	11.8jk	11.8j	20.6e	7.4i	10.5i
	3	15.1hi	7.7k	15.2j	5 <b>7.0</b> d	22 <b>.4</b> h	33.9h
	6	52. <b>8</b> f	2 <b>4.9</b> i	2 <b>3.8</b> i	166.2c	44.5g	61.7f
	9	65.2 <b>e</b>	2 <b>7.9</b> h	<b>30</b> .2h	2 <b>3</b> 5.7a	166.4c	152.7c
	12	2 <b>11.9a</b>	44.6g	<b>93</b> .6f		184.5b	2 <b>13.1a</b>
	15	167.2b	66.1f	99.1f		204.9a	198.0b
	18		84.0e	122.2d			
	21		159.0b	169.6b			
	24		2 <b>43</b> .2a	22 <b>4.4a</b>			
	Mean	83.5	73.9	87.0	131.3	100.7	97.1
	LSD* (T)	8.1					
	LSD* (M)	4.6					
	LSD* (Z)	4.2					
	LSD* (T * Z* M)	6.5					
	CV (%)	10.5					

Values followed by different letters within the same column differ significantly at (p<0.05) while means with a similar letter in a column do not differ significantly at (p<0.05). T= Treatment, V= Variety, M= mode of application and Z= Agro ecological zone of production

Mode of application		Post-harve	est Dip		Pre-harvest spray		
ZONE	DAYS	Control	2%	3%	Control	2%	3%
AEZ IV	0	17.3f	17.3h	17.3h	47.5e	15.3e	14.0g
	3	22. <b>3e</b>	11.7i	9.9i	179.6a	50.2f	<b>36.4e</b>
	6	<b>49.1</b> d	22.9g	21.6g	141.8b	73.4e	55.1d
	9	95.3c	59.9f	6 <b>3</b> .2f		12 <b>3.9</b> b	110.7a
	12	164.9a	107.0d	126.5d		137.2a	117.3a
	15		136.9c	143.7c			
	18		146.5b	140.4c			
AEZ II							
	0	13.2f	13.2i	13.2i	32.8f	10.5h	9.9g
	3	2 <b>4.3e</b>	16.2hi	18.1gh	110.7d	47.0f	25.0f
	6	93.9c	19.0gh	12.0i	132.8c	<b>89.0</b> d	74.9c
	9	91.8c	75.0e	104.6e	106.5d	100.8c	94.2b
	12	144.5b	109.6d	159.1b		139.8a	113.3a
	15		110.0d	172.1a			
	18		166.7a	104.0e			
	Mean	71.6	72.3	79.0	107.5	78.7	65.1
	LSD* (T)	6.2					
	LSD* (M)	3.5					
	LSD* (Z)	3.5					
	LSD* (T * Z* M)	5					
	CV (%)	12.1					

Table 12B: Effect of post-harvest dip and pre-harvest spray application of hexanal on Glucose content (mg/100g) of 'Sweet bananas' from AEZ IV (Machakos County) and AEZ II (Meru County).

Values followed by different letters within the same column differ significantly at (p< .05) while means with a similar letter in a column do not differ significantly at (p< .05). T= Treatment, V= Variety, M= mode of application and Z= Agro ecological zone of production

### 4.3.4.3. Sucrose

Sucrose was the most abundant sugar in banana fruit compared to fructose and glucose in both varieties. Sucrose content increased gradually as ripening progressed in both varieties irrespective of zone of production, variety and mode of application (Tables 13A and B). Hexanal treatment had a significant (p < 0.05) effect on the sucrose content. The rate of sucrose increase was slower in hexanal treated fruits compared to the untreated controls in both varieties. However, variety, mode of hexanal application and AEZ did not have any significant effect on the sucrose content. Additionally, there was no significant (p < 0.05) difference between the two concentration of hexanal tested (2 % and 3 %).

In the untreated 'sweet banana' fruits (post-harvest experiment), sucrose levels increased from initial of 20.6 mg/100g and 12.3 mg/100g to peak levels of 245 mg/100g and 249 mg/100g by day 9 of storage in AEZ IV and AEZ II respectively (Table 13B). The controls in the pre-harvest experiment peaked to 150 mg/100g (day 3) and 161mg/100g (day 6) in 'sweet banana' fruits from AEZ IV and AEZ II respectively. The increase in sucrose content in the hexanal treated fruits was less rapid, peaking to 247.4 mg/100g and 200.6 mg/100g in AEZ IV and AEZ II respectively at day 15 of storage incase of post-harvest dip mode of application using 2% hexanal.

The trend was not different in the 'Grand nain' variety which had sucrose levels increasing to a peak level before gradually declining till the end of storage (Table 13A). In AEZ IV fruits, sucrose content increased from initial of 12.7 mg/100g to a peak value of 276.2mg/100g (day 18) and 272.7 mg/100g (day 15) in post-harvest dip treated fruits with 2 % and 3 % hexanal respectively. A similar trend was observed in AEZ II fruits which peaked to 204 mg/100g and 266 mg/100g at the end of storage, 9 days later compared to the controls in 2 % and 3 % hexanal concentration respectively (Table 13A).

Mode	of application	Post-harv	est Dip		Pre-harve	st spray	
ZONE	DAYS	Control	2%	3%	Control	2%	3%
AEZ IV	0	12.7d	12.7g	12.7g	13.8e	<b>8.8</b> d	6.2f
	3	27.7d	10.0g	8.1g	<b>98.8</b> d	50.0c	6 <b>7.7e</b>
	6	92.7c	26.5g	2 <b>3.8g</b>	168.8bc	138.1b	114.6c
	9	252 <b>.7</b> b	84.2f	103.8ef	192.7ab	130.8b	105.4dc
	12	281.5a	149.6e	194.2cd		155.8ab	163.5b
	15	250.4b	254.2b	2 <b>7</b> 2.7a		178.8a	2 <b>03.1a</b>
	18		276.2a	2 <b>41.9</b> b			
	21		242.3c	222.7bc			
	24		22 <b>7.3c</b> d	168.5d			
AEZ II							
	0	<b>11.</b> 2d	11.2g	11.2g	4.6e	<b>3</b> .5d	3.1f
	3	15.4d	9.2g	16.2g	11.9e	<b>3.8</b> d	17.3f
	6	71.2c	16.2g	2 <b>1.2g</b>	140.8b	60.8c	49.6e
	9	2 <b>41.9b</b>	75.0f	83.5f	146.5c	142.7b	136.2bc
	12	2 <b>49.</b> 2b	163.8e	127.7e	201.5a	145.0b	200.4a
	15	245.0b	175.4e	150.0de		179.2a	195.0a
	18		163.1e	171.2d			
	21		175.4e	207.3c			
	24		2 <b>04.</b> 2d	265.8a			
	Mean	146.0	126.5	127.9	97.3	<b>99.8</b>	105.2
	LSD* (T)	25.8					
	LSD* (M)	16.7					
	LSD* (Z)	16.5					
	LSD* (T * Z*M)	20.5					
	CV (%)	<b>3</b> 6.4					

Table 13A: Effect of post-harvest dip and pre-harvest spray application of hexanal on Sucrose content (mg/100g) of 'Grand nain' banana from AEZ IV (Machakos County) and AEZ II (Meru County).

Values followed by different letters within the same column differ significantly at (p<0.05) while means with a similar letter in a column do not differ significantly at (p<0.05). T= Treatment, V= Variety, M= mode of application and Z= Agro ecological zone of production

Mode	of application	Post-har	vest Dip		Pre-harve	st spray	
ZONE	DAYS	Control	2%	3%	Control	2%	3%
AEZ IV	0	20.6e	20.6g	20.6f	12.9c	6.1d	8.4e
	3	<b>104</b> .5d	16.8g	21.0f	149.7a	57.4c	63.5c
	6	147.4c	62. <b>8</b> f	92.6e	112.9b	116.5b	104.8b
	9	2 <b>44</b> .5a	144.2d	<b>137.7</b> d		133.9a	110.6b
	12	22 <b>3.</b> 2a	209.4b	198.7bc		143.9a	148.4a
	15		2 <b>47.4a</b>	2 <b>08.7</b> b			
	18		207.1b	194.2bc			
AEZ II							
	0	12. <b>3e</b>	12. <b>3</b> g	12. <b>3</b> f	19.7c	<b>11.3</b> d	16.5e
	3	<b>88.7</b> d	11.0g	18.7f	120.0b	50.6c	<b>37.1</b> d
	6	132.6c	38.7f	2 <b>4</b> .5f	161.0a	153.5a	119.0b
	9	206.1b	101.6e	121. <b>3</b> d	114.8b	120.0b	163.2a
	12	199.7b	170.6c	2 <b>3</b> 5.2a		98.4b	123.5b
	15		200.6b	196.5b			
	18		190.6bc	175.5c			
	Mean	138.0	116.7	118.4	<b>98.7</b>	89.2	89.5
	LSD* (T)	29.1					
	LSD* (M)	18.4					
	LSD* (Z)	18					
	LSD* (T * Z* M)	16.1					
	CV (%)	42.9					

Table 13B: Effect of post-harvest dip and pre-harvest spray application of hexanal on Sucrose content (mg/100g) of 'Sweet bananas' from AEZ IV (Machakos County) and AEZ II (Meru County).

Values followed by different letters within the same column differ significantly at (p<0.05) while means with a similar letter in a column do not differ significantly at (p<0.05). D= double spray at 30 and 15 days before harvest, T= Treatment, V= Variety, M= mode of application and Z= Agro ecological zone of production

# 4.3.5. Sensory quality evaluation

The sensory scores presented here are of banana fruits harvested in AEZ IV (Machakos County) and AEZ II (Meru County) and either treated with 2 % hexanal for 5 minutes or dipped in plain clean water to act as the control. The panelists scored all the fruits on a 5-point hedonic scale. The sensory parameters evaluated include; peel color, texture, taste/flavor, mouth feel, aroma and general acceptability.

Generally, untreated control 'Grand nain' fruits from Meru County scored relatively higher for taste/flavor, aroma and general acceptability compared to hexanal treated fruits though the variation was not significantly (p<0.05) different (Figure 21). In Machakos County, hexanal treated fruits scored relatively higher for texture, flavor, mouth feel and general acceptability compared to the controls in 'Grand nain' variety (Figure 21).

In 'sweet banana' variety, there was no significant (p<0.05) differences observed in all the quality attributes scored in both zones between the hexanal treated and control fruits (Figure 22). The treated and control fruits from both zones had almost the same scores for peel color, texture (Machakos county) and aroma (Meru County). On the other hand, hexanal treated fruits scored slightly high for taste/flavour in both zones while general acceptability and aroma scored highest in Machakos fruits though this was not significantly (p<0.05) different (Figure 22).

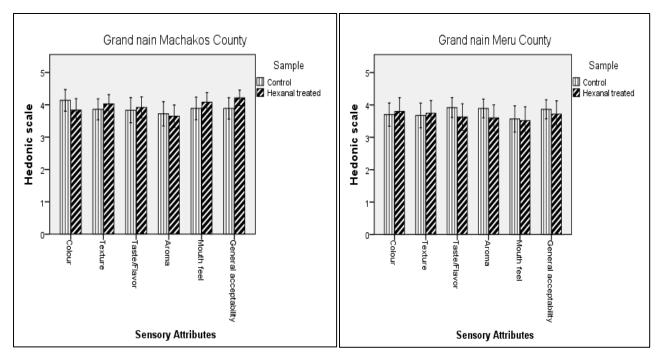


Figure 21: Hedonic scores for sensory quality attributes of 'Grand nain' bananas harvested from Meru and Machakos Counties and treated with Hexanal or left untreated to serve as the control. The values on Y-axis represent scores on a 5-point hedonic scale (1 = dislike (worst), 2 = (dislike moderately), 3 = (neither like nor dislike) 4 = (like moderately) and 5 = (Like extremely/best)). The vertical bars represent means  $\pm$  SE.

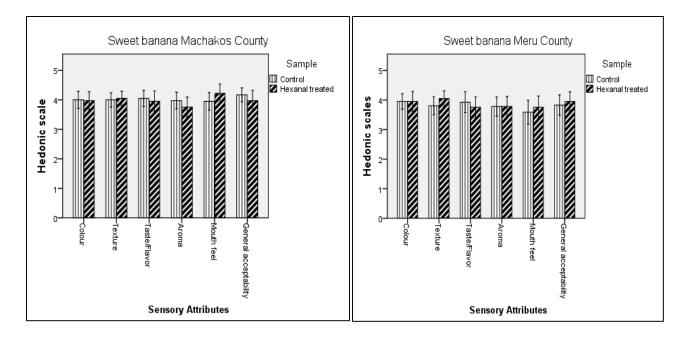


Figure 22: Hedonic scores for sensory quality attributes of 'sweet banana' variety harvested from Meru and Machakos Counties and treated with Hexanal or left untreated to serve as the control The values on Y-axis represent scores on a 5-point hedonic scale (1 = dislike (worst), 2 = (dislike moderately), 3 = (neither like nor dislike) 4 = (like moderately) and 5 = (Like extremely/best)). The vertical bars represent means  $\pm$  SE.

# 4.4 Discussions

Application of suitable post-harvest technologies in banana fruits is of paramount importance to minimize post-harvest losses while maintaining the best possible quality. Over the past decades, different post-harvest technologies have been developed and tested in various fruits. In the recent past, most of the consumers and other actors in the value chain have high affinity for naturally occurring post-harvest preservative compounds which are environment friendly, safe and are easy to use. Hexanal, is one such compound which is naturally occurring and has been shown to enhance shelf life of several fruits such as papaya (Hutchinson *et al.*, 2018), tomatoes (Cheema *et al.*, 2014), mango (Anusuya *et al.*, 2016) and sweet cherries (Sharma *et al.* 2010). For commercialization and use of any post-harvest technology/compound, it should not only have the potential to enhance shelf-life but preserve the post-harvest quality. In this study, the quality of banana fruits was not affected negatively by hexanal treatment, although differences were observed between the two varieties and production zones. This can be attributed to variations between the genotypes, prevailing

environmental conditions such as light and temperatures as well as cultural practices, all of which have been previously reported to have an effect on the post-harvest quality of fruits (Léchaudel and Joas, 2006).

In the present study, TSS levels increased gradually during ripening in all the fruits, however, the increase was less drastic in the hexanal treated fruits. The observed increase in TSS during ripening is associated with the breakdown of starch into simple sugars during ripening (Siddigui and Dhua 2010). Further, the increased TSS levels in banana fruit could also be as a result of the partial breakdown of pectins and cellulose as reported by De Lima and Lima 2001. The less rapid change in TSS level in hexanal-treated fruits in this study could be due to the reduced activity of the enzymes such as invertase and amylase involved in the hydrolysis of stored carbohydrates into soluble sugars (Kumar et al., 1994). Similar results have been reported in tomato and mango fruits (Cheema et al. 2014; Anusuya et al. 2016). In general, fruits from the semi-arid AEZ IV had high TSS levels compared to those from AEZ II, regardless of hexanal treatments. This could be attributed to the longer periods of sunlight and higher temperatures found in semi-arid zones, such as Machakos County, which tend to favor photosynthetic activity and carbon accumulation. Similar findings have been reported in papaya (Hutchinson et al., 2018), banana (Ambuko et al. 2006), avocado fruits (Ferguson et al., 1999), passion fruit (Baraza et al., 2012) and Mango (Mendoza et al., 1972). The seasonal variation observed with season 1 experiment having significantly high TSS levels compared to season 2 could be attributed to variations in the environmental factors such as temperature, light intensity and water supply. The experiments were conducted in two successive seasons one being drier (July to November, 2016) and another being moderately wet (January to April, 2017). The dry season is characterized by high temperatures, high solar intensity and limiting water supply which all have a positive effect on TSS levels.

Results of this study have indicated that TTA in banana fruits increased with the advancement of ripening. This agrees with Siriboon and Banlusilp (2004) who reported that the TTA levels of banana fruit increased with ripening. The observed increase in TTA during ripening may be due to the increase in malic acid. Malic acid content has been shown to rise from 1.8 meq/100 g to 6.2 meq/100 g during ripening in bananas as reported by John and Marchal (1995). In general, hexanal treatments significantly delayed the rate of TTA increase. This could be attributed to reduced activities of

enzymes such as malate dehydrogenase, which influence the level of malic acid in banana (John and Marchal, 1995).

Vitamin C is an important quality trait in fruits. In the present study, vitamin C levels decreased gradually in all the fruits with storage time in both the hexanal-treated and untreated control fruits. These findings agree with Opara *et al.* (2012) who found ascorbic acid decreased with ripening in banana fruits due to its oxidative degradation during respiration or its transformation to other metabolites such as sugars and amino acids. However, hexanal treatment retarded degradation of ascorbic acid in this study in both varieties. The higher retention of ascorbic acid observed in the treated fruits may be because of reduced enzymatic oxidation by hexanal. This has been previously reported in tomatoes (Cheema *et al.* 2014) and sweet cherries (Sharma *et al.* 2010). According to De Souza *et al.* (2014), Vitamin C levels have been reported to vary across genotypes, pre-harvest climactic conditions, cultural practices and post-harvest handling. This might explain the observed variations in Vitamin C levels between the two varieties tested and the modes of hexanal application. Similar findings have been reported in papaya fruit (Hutchinson *et al.*, 2018) where hexanal treatment delayed the rate of Vitamin C decrease from 70.12 mg/100 g to 26.11 mg/100 over the 15 days storage period.

In banana, starch forms approximately 25% of the unripe fruits' fresh weight (Mawaduwathi *et al.*, 2017). During the ripening process, sugar levels in the pulp increases from an initial of 1-2% to approximately 20% though this may vary depending on the variety and production zone (Mawaduwathi *et al.*, 2017). On the other hand, starch content has been shown to decrease from 15.7 g/100g to 3.40 g/100g during ripening. This is because during the ripening process, starch is hydrolyzed into simple sugars such as glucose, fructose and sucrose by activities of various enzymes such as invertase, amylase and sucrose synthase (Kumar *et al.*, 1992). In the present study, glucose, fructose and sucrose were the dominant sugars present during ripening of 'Grand nain' and 'sweet banana' fruits. Levels of these individual sugars increased drastically during the ripening process in all the fruits irrespective of treatment, variety and production zone. Hexanal treatment however, significantly slowed down the rate of increase of these sugar levels in all the fruits. This might be because of the observed delayed ripening and reduced rate of respiration in the treated fruits. During ripening process, starch is usually catabolized into sugars, which enters the metabolic pool where

they are used as respiratory substrates or further transformed to other metabolites. Similar findings have been reported in hexanal treated banana fruits by Venkatachalam *et al.* (2018), who reported an increase in percentage total sugars from 10 % to 19 % by day 27 of storage.

Various quality attributes such as peel color, firmness, aroma, taste, mouth-feel and general acceptability were evaluated during the sensory evaluation analysis. The findings of the sensory evaluation showed that, hexanal treatment did not have any significant effect on the various sensory quality parameters analysed. Further, there was no significant difference on the general acceptability of the treated and the control fruits. This indicates that hexanal's effect on shelf life of banana fruit did not have any detrimental effects on the various quality parameters. These results are in agreement with an ealier studies by Tiwari and paliyath (2011), who reported that hexanal treatment does not affect the expression of genes involved in quality development pathway of tomato fruit. The sensory evaluation results by the panelists correlates positively with results of the various quality attribute parameters analyzed in the laboratory.

# 4.5 Conclusion

The results of this study show that, use of hexanal was effective in maintaining the post-harvest quality of banana fruits. Hexanal treatment significantly delayed the rate of vitamin C decrease during storage period in both AEZs compared to the untreated controls. Hexanal treatment significantly delayed the rate of increase of simple sugars (fructose, glucose, sucrose) in both varieties and AEZs compared to the untreated ones. At the end of storage period, hexanal treated fruits mantained high levels of glucose compared to the untreated controls. Further, results of the sensory analysis showed no significant differences in the general acceptability by the panelist between the hexanal treated and untreated fruits. However, the post-harvest quality attributes of banana fruits were significantly affected by the choice of variety and zone of production. Generally, fruits from the drier AEZ IV had higher TSS and simple sugar content, which are key attributes influencing consumer acceptability. The use of hexanal and its formulation can therefore, be promoted for commercialization in Kenya for use by both banana farmers and retailers to enhance post-harvest shelf-life while maintaining quality.

#### **CHAPTER FIVE**

# 5.0 The molecular basis of hexanal's mode of action in preserving the post-harvest shelf life of banana fruits

#### 5.1 Abstract

Banana is climacteric fruit whose ripening process is controlled by the ripening hormone, ethylene. Once the ripening process has been initiated, it is irreversible and proceeds very quickly making the fruits highly perishable. Several post-harvest technologies have been developed over the years to control the fast rate of ripening in climacteric fruits such as banana. Recently, hexanal use has shown promising results in delaying ripening process and softening in banana fruits. Being a relatively new compound, various studies are being conducted to understand the mode of action of hexanal in delaying fruit ripening. In the present study, gene expression studies were conducted to better understand the molecular mechanisms behind the ripening process in banana fruits and the role of hexanal in regulating the process. Mature green 'Grand nain' banana fruits were harvested from Meru county and treated with either hexanal, ethylene perception inhibitor, 1-Methylcyclopropene (1-MCP), combination of hexanal with 1-MCP, ethylene or left untreated to serve as control. Three fruits were randomly sampled from each treatment combination at two day interval for analysis of physiological and physical parameters including rate of ethylene production, respiration rate, changes in peel color as well as peel and pulp firmness. The middle portion of both the peel and the pulp of each of the three randomly sampled fruits were diced quickly, frozen in liquid nitrogen and stored in -80°C for subsequent RNA extraction. Gene expression analyses of genes encoding various cell wall degradation enzymes and ethylene biosynthesis pathway in both banana peel and pulp was carried out by use of quantitative polymerase chain reaction (qPCR). These genes include; MaPG1, MaPME, MaXET1, MaPL1 and MaACO coding for Polygalacturonase (PG), Pectin methylesterase (PME), Xyloglucan endotransglycosylase (XET), Pectin lyase (PL) and 1-Aminocyclopropane-1-Carboxylic Acid Oxidase (ACO) enzymes respectively. In addition, a transcriptome analysis using RNA sequencing (next generation sequencing technique) was carried out to understand changes in expression of various ripening related genes in banana pulp tissue induced by hexanal treatment over the storage period.

Results of the various physiological and physical parameters analysed showed that hexanal and 1-MCP treatments delayed fruit ripening to different extents compared to the controls and those treated with ethylene. Results of the quantitative polymerase chain reaction showed that hexanal treatment suppressed the expression of four genes coding for different cell wall degrading enzymes which are XET, PG, PL and PME as well as ACO involved in ethylene biosynthesis as compared to fruits treated with ethylene and the controls. However, expression of these four cell wall degrading genes occurred 2-4 days earlier in the hexanal treated fruits as compared to those treated with 1-MCP. Expression of cell wall degrading enzymes occurred earlier in the peel than the pulp tissues regardless of the treatments. Results of the transcriptomic analysis showed that hexanal treatment suppressed the expression of phospholipase D gene, xyloglucan endotransglucosylase, Expansin, Pectate lyase and Polygalacturonase by day four of storage contrary to the observed induction of the same genes in fruits treated with ethylene. In addition, genes involved in starch degradation such as fructosebisphosphate aldolase were also suppressed. Later on, at days 18-24 of storage, genes involved in ethylene biosynthesis, cell wall and cell membrane degradation, aroma synthesis and glucose synthesis were induced. Further, 1-Aminocyclopropane-1-Carboxylic Acid Oxidase (ACO) and 1-Aminocyclopropane-1-Carboxylic Acid Synthase (ACS) genes involved in ethylene biosynthesis pathway were supressed in the hexanal treated fruits in day four of storage.

Findings of this study reveal that, hexanal works by temporarily suppressing the expression of phospholipase D genes which codes for phospholipase D, a key enzymes involved in cell membrane degradation. Hexanal also transiently suppressed the expression of xyloglucan endotransglucosylase, Expansin, Pectate Lyase and polygalacturonase genes which are known to play a major role in cell wall degradation as well as ACO and ACS genes involved in ethylene biosynthesis pathway.

# **5.2 Introduction**

Ripening is a crucial phase in the maturation process of fruits. As the fruit ripens, expression of various genes involved in different metabolic pathways are triggered leading to several physiological and biochemical changes which transforms the fruit into an edible state (Asif *et al.*, 2014). Key among them include, softening, color change, sugar metabolism, synthesis of aroma volatiles and increased susceptibility to phytopathogens (Valero and Serrano, 2010). Banana is a climacteric fruit and once ripening is initiated, it is irreversible and proceeds very rapidly making the fruit highly perishable.

This in turn causes postharvest deterioration which results in economical losses for all the banana value chain actors. Fruit softening during ripening is one of the critical changes which enhances banana acceptability by consumers. In banana, softening is high during the later stages of ripening and is due to extensive cell wall degradation associated with the disassembly of primary cell wall and the middle lamella. Additionally, other mechanisms such as loss of turgor, degradation of starch into sugars and degradation of the cell membrane also contributes to softening in banana fruits. However, significant softening in banana fruit is the result of degradation of the cell wall and the cell membrane (Asif and Nath, 2005).

Degradation of the cell wall is caused by additive action of pectolytic enzymes such as polygalacturonase (PG), pectin methylesterase (PME) and pectate lyase (PL) (Brummell and Harpster, 2001). The polygalacturonase and pectin methylesterase enzymes regulates the breakdown of pectins polysaccharides (Amnuaysin *et al.*, 2018). However, the action of PME is reported to be a prerequisite for PG activity during fruit ripening as the pectins have to be de-esterified first before PG action can commence (Duan *et al.*, 2007). On the other hand, Pectate lyases (PLs) operate by catalyzing the cleavage of glycosidic bonds of unsaturated regions of pectins by a  $\beta$ -elimination reaction (Payasi *et al.*, 2006). Therefore, the suppression of these enzymes in fruit tissues will considerably reduce the rate of softening, improve fruit quality and extend the post- harvest shelf life (Brummell *et al.*, 1999).

Extensive research for the last 20 years, has shown that hexanal and its formulations has the potential to enhance the shelf-life of various temperate fruits (Paliyath *et al.*, 2008). Recently, studies have shown hexanal can enhance shelf-life of tropical fruits such as mango (Anusuya *et al.*, 2016), tomato (Cheema *et al.*, 2014) and papaya (Hutchinson *et al.*, 2018). Additionally, earlier results of the present study (chapters 3 and 4), have shown that hexanal has the potential to enhance banana fruit shelf life by up to 9 days without compromising its quality. Being a relatively new technology, hexanal's mode of action is not very clear. Several studies have suggested that it works by inhibiting the action of the enzyme phospholipase D which catalyzes hydrolysis of membrane phospholipids and initiates membrane deterioration and thus, fruit softening (Paliyath and Padmanabhan, 2019). Further, a study by Tiwari and Paliyath (2011), showed that hexanal is a weak inhibitor of ethylene, a hormone which

triggers ripening in climacteric fruits such as banana. In chapter 3 of this study, fruits treated with hexanal showed a consistent trend of delayed softening, reduced rate of respiration and ethylene production throughout storage compared to the untreated controls. It is therefore, possible that hexanal works by down regulating the expression of various genes regulating cell wall and cell membrane degradation as well as those involved in the ethylene biosynthesis pathway. A study by Tiwari and Paliyath, (2011), showed that treatment of fruits with hexanal was more advantageous as compared to other existing post-harvest technologies such as 1-MCP. Tomato fruits treated with hexanal had higher levels of lycopene,  $\beta$ -carotene and major volatile components as compared to those treated with 1-MCP (Tiwari and Paliyath, 2011). Further, studies in banana fruits reported that 1-MCP treatment caused poor peel color and flavor development (Kondo *et al.*, 2005; Cliff *et al.*, 2009) which is not the case in hexanal treated fruits (Venkatachalam *et al.*, 2018). The mode of action of 1-MCP, an inhibitor of ethylene perception is well known (Watkins and Miller, 2003).

In this study, the regulation of gene expression during ripening in relation to hexanal, 1-MCP, ethylene and a combination of 1-MCP and hexanal treatments were examined in four genes coding for various cell wall degrading enzymes and one gene involved in ethylene biosynthesis pathways using quantitative polymerase chain reaction. Further, transcriptome analysis was done on the banana pulp tissues using RNA sequencing technique in order to understand changes in expression profiles of various ripening genes induced following hexanal treatment over the storage period.

#### **5.3.** Materials and methods

#### 5.3.1. Plant material and post-harvest treatment

Banana fruits variety 'Grand nain' were obtained from a small scale farm in Meru County, which is located in AEZ II of Kenya. The fruits were harvested at mature green stage based on the disappearance of angularity as well as number of days after flowering (approximated at 104 days). Once harvested, the fruits were quickly transported to the post-harvest laboratories at JKUAT in cushioned crates.

In the laboratory, the fruits were washed in clean water, dried, selected for uniformity and batched into five for various treatments. The first batch was dipped in hexanal's most effective concentration and application duration of 2% for 5 minutes (Yumbya *et al.*, 2018). It is hypothesized that hexanal

is a strong inhibitor of Phospholipase D enzyme which initiates membrane degradation and will suppress the PLD genes. The second batch was treated with ethylene, at the recommended commercial rate of 1ppm 24 hours in an airtight container to serve as the positive control. Ethylene is a ripening hormone which promotes ripening in climacteric fruits such as banana and will enhance expression of the ethylene dependent genes. The third and fourth batches were treated with 1-MCP (2ppm for 24 hours) and a combination of 1-MCP and hexanal respectively. The 1-MCP will block the ethylene receptors and suppress ethylene dependent genes such as the cell wall softening genes. A combination of hexanal and 1-MCP treatment will suppress both the ethylene dependent genes such as cell wall softening genes and PLD genes found in the cell membrane. The final batch was left untreated to serve as the control. After the treatments, all the fruits were left to undergo normal ripening under ambient room conditions. Three fruits from each treatment combination were randomly sampled at two-day intervals for analysis of ripening-related physical and physiological parameters including peel and pulp firmness, peel color, rate of respiration and rate of ethylene production. The middle portion of both the peel and the pulp were diced very fast, frozen in liquid nitrogen and stored in -80°C for subsequent RNA extraction. The molecular studies were conducted at Biosciences eastern and central Africa (BecA) laboratories based at International Livestock Research Institute (ILRI) in Nairobi, Kenya.

#### 5.3.1. Analysis of physical and physiological parameters

Rate of ethylene production, rate of respiration, peel color change, peel and pulp firmness were measured according to procedures listed in thesis sections; 3.4.2, 3.4.3, 3.4.7, 3.4.5 and 3.4.6 respectively.

#### **5.3.2. Extraction of total RNA**

Total RNA was extracted from both the treated and untreated control banana peels and pulp sampled at two day interval according to Asif *et al.*, 2014. Approximately 2 grams of fruit tissue was ground into fine powder in liquid nitrogen and homogenized in a tube containing 1 ml extraction buffer with 100mM Tris-HCl (pH 8.0), 2 M NaCl, 25 mM EDTA (pH 8.0), 2% CTAB, and 1% Dithiothreitol (DTT). 1 ml of chloroform: Isoamyl alcohol (24:1) was added, vortexed and incubated at 50°C for 15 minutes. The homogenate was cooled to room temperature and extracted twice with equal volumes of chloroform: isoamyl alcohol (24:1). The aqueous phase was separated each time using centrifugation at 13,000×g (RC5C Plus centrifuge: Sorvall, Newtown, USA) at room temperature for 15 minutes. The supernatant was transferred into a new eppendorf tube in which a quarter volume of 10 M Lithium chloride (LiCl) was added to a final concentration of 2 M LiCl in the total solution. After overnight incubation at 4°C, RNA was pelleted using centrifugation at 13,000×g for 20 minutes at 4°C. The supernatant was discarded and the RNA pellet dried for 15 minutes before resuspending in 250 µL of sterile water and 50 µL of 10 M LiCl. The homogenate was incubated at 4°C for three hours and the RNA was pelleted using centrifugation at 13,000×g for 10 minutes at 4°C. After resuspending the RNA pellet in 250 µL of nuclease free water, 25 µL of 3M sodium acetate and 1 mL 100% ethanol was added and incubated at -20°C for 1 hour before centrifuging at 13,000×g for 10 minutes at 4°C. The supernant was discarded, RNA pellet was vacuum dried for 10 minutes then resuspended in 50 µL of RNase free sterile water. Total RNA was quantified by Nanodrop<sup>®</sup>-1000 spectrophotometer (Nanodrop technologies, Delaware, USA) while the integrity was verified using 1.5% agarose gel electrophoresis run at voltage of 70 for 45 minutes. Inorder to remove any possible DNA contamination, DNase 1 treatment was done. One microgram of the extracted RNA was allowed to react in a volume containing 1 µL of reaction buffer containing magnesium chloride, 1 µL of the Dnase 1 enzyme and nuclease free water at 37°C for 30 minutes. After this, 1 µL of 50 mM EDTA was added and incubated at 65°C for 10 minutes. The RNA integrity was verified again using 1.5% agarose gel electrophoresis. The prepared RNA was aliquoted into two for quantitative polymerase chain reaction (qPCR) and transcriptome analysis using next generation sequencing (NGS).

#### 5.3.3. Complementary DNA (cDNA) synthesis and quantitative PCR

First strand cDNA synthesis was done from 1µg of total RNA using Invitrogen superscript III first strand synthesis system as per the manufacturer's protocol. Random hexamer, 10 mM dNTP mix and diethylprocarbonate (DEPC) treated water were added into 1 µg of total RNA and incubated at 65°C for 5 minutes followed by addition of 10 µL of cDNA Synthesis mix. This was mixed gently before centrifuging briefly for 30 seconds. The mixture was incubated at 25°C for 10 minutes followed by 50 minutes at 50°C. The reaction was terminated by incubating at 85°C for 5 minutes followed by incubation at 37°C for 20 minutes after the addition of 1 µL of RNase H. The cDNA synthesis reaction

was stored at -20°C until use. Quantitative PCR was performed on ABI 7500 using Maxima SYBR qPCR master mix (Thermo scientific). The reaction mixture contained 2  $\mu$ L of template cDNA, 5  $\mu$ L of SYBR qPCR master mix, 0.2  $\mu$ L each of the forward and reverse primers and 2.4  $\mu$ L of nuclease free water. All qPCRs were performed in three biological replicates. Quantitative polymerase chain reaction (qPCR) was conducted for four genes encoding cell wall degrading enzymes and one gene encoding ethylene biosynthesis enzymes. Gene specific primers obtained from literature (Inaba *et al.*, 2007, Amnuaysin *et al.*, 2012) were used as shown in Table 12. Banana β-actin, was used as a reliable internal reference gene for qPCR as previously reported by Amnuaysin *et al.* (2012). The qPCR program included one cycle of 10 minutes at 95°C for template denaturation, followed by 40 cycles of 15 seconds at 95°C for template denaturation, 45 seconds at 60°C for primer annealing, and 30 seconds at 72°C for primer elongation.

Primer		
name	Sequences	Gene
MaPG1(F)	CGGATGAGCAATGTTTCCAACCCA	Polygalacturonase enzyme
MaPG1(R)	CATGGAGAACTGTCGCTGCAAGA	
MaPME (F)	TGTCCAATGTGTCAAAGCCAGTGC	Pectin methylesterase enzyme
MaPME (R)	TGGAATGCAAATCCGGAATGGTGG	
MaXET1(F)	GCTGGCTGTTGGGGAAGGCGATGCC	Xyloglucan endotransglycosylase
MaXET1(R)	CAATGTGTTCACCCAGGGAAAGGGA	
MaPL1(F)	AAGACCTGGTTCAGAGGATGCCAA	Pectin Lyase enzyme
MaPL1(R)	ATGATAGTCCTGAAAGTTGG	
MaACO(F)	AAGCTCTACGTCGGGCATAA	ACC Oxidase enzyme
MaACO(R)	GACAGCTTCCTAACGCGAAG	
MaActin (F)	TGCTAGCGGACGTACCACAGGTATCGTG	Actin gene
MaActin (R)	GAATCTTCATGAGGGAATCTGTCAGGTC	

Table 14: Genes and gene specific primers used for qPCR

# 5.3.4. Transcriptome analysis/ Next generation sequencing (NGS)

Due to the high cost involved in the library preparation and sequencing, only the pulp tissue and three treatments were selected for this analysis. The treatments were; hexanal applied at the most effective dosing rate of 2% for 5 minutes, ethylene which is a ripening hormone applied at the recommended commercial rate of 1ppm for 24 hours and the untreated control fruits. The ethylene treatment was selected to serve as the positive control while the untreated fruits served as the negative control. The samples were obtained at four different time points which included; initial day, day 4, day 18 and day 24 of storage. The already extracted RNA from the pulp of three biological replications was used per treatment. 500 ng of Dnase 1 treated total RNA was used for library preparation using the Truseq Stranded Total RNA sample preparation protocol with Ribo zero depletion as per the manufacturer's instruction. Ambion® ERCC Spike-in Control was used as the internal control check which is necessary in gene expression profiling studies (Tonui et al., 2018). The concentration of the prepared libraries was checked using Qubit (Fisher Scientific) high sensitivity parameters. The integrity of the library fragments was checked using Agilent Bioanalyzer and 1.2% agarose gel electrophoresis. The libraries were then normalized to 10nm and sent to Genohub Company (USA) for sequencing using Illumina Hiseq 4000. The reads obtained were exported to the International Livestock Research Institute (ILRI) high performance cluster (HPC) server for subsequent bioinformatic analysis as discussed below.

# 5.3.5. Data analysis

# 5.3.5.1. Quantitative polymerase chain reaction (qPCR) analysis

Relative changes in gene expression were quantified using the  $2^{-\Delta Ct}$  method (Livak and Schmittgen, 2001) and normalized using actin as the housekeeping gene (Amnuaysin *et al.*, 2012, Inaba *et al.*, 2007). Samples at the initial day before any treatment was done were set as calibrators for calculating relative expression levels.

# 5.3.5.2. Next generation sequencing analysis/ Bioinformatics analysis

The quality control of the raw reads was performed using fastQC v0.11.7 software. The fastQC results were then aggregated using MultiQC v1.4 for visualization. The raw reads were trimmed and filtered using trimmomatic v0.33 to remove adapter sequences, low quality reads with base quality smaller

than 20 ( $Q \le 20$ ) and a minimum length of 100 bases. Quality control was repeated on the trimmed reads using fastQC v0.11.7 to ensure only high quality reads were used for the downstream analysis.

The trimmed reads of high quality were mapped to banana (*Musa acuminata*) reference genome, ASM31385v1 (<u>https://plants.ensembl.org/Musa\_acuminata/Info/Index</u>) obtained from ensembl database using STAR aligner 2.5.3a. The mapped reads were counted using feature count v1.6.2. In general, a higher ratio of gene alignment indicated a closer genetic relationship of the study samples and the reference genome. Screening of the Differentially Expressed Genes (DEGs) was done by use of EdgeR version 3.8 software to determine the differential expressed genes between the different treatments and days in storage. The differential expressed genes were determined statistically using p < 0.05 and a log2fold ratio  $\ge 1$  as the threshold. The significance of the DEGs was determined by using the false discovery rate (FDR) method to justify the p <0.05 value (Gul *et al.*, 2019).

# 5.4. Results

#### 5.4.1. Changes in physical and physiological parameters

#### 5.4.1.1. Rate of ethylene production

All the treatments had a significant effect (p<0.05) on the rate of ethylene production throughout the storage period (Figure 23). Fruits treated with external ethylene and untreated control had significantly (p<0.05) high levels of ethylene compared to hexanal treatment. However, fruits treated with hexanal produced significantly (p<0.05) high levels of ethylene compared to hexanal treatment to ones treated with either 1-MCP alone or a combination of 1-MCP and hexanal. No significant differences were observed between fruits treated with 1-MCP and a combination of 1-MCP and hexanal.

In all the treatments, levels of ethylene production increased gradually to peak levels and then declined till the end of storage. In the ethylene treated fruits, the rate of internal ethylene production increased drastically from an initial of 0.15 nl/kg/hr to a peak of 4.0 nl/kg/hr at day four of storage compared to a peak of 3.7 nl/kg/hr at day 12 in the untreated control fruits. Fruits treated with either 1-MCP, a combination of 1-MCP and hexanal or hexanal, produced low levels of ethylene throughout storage, with a significantly reduced peaks of 0.5, 1.4 and 2.4 nl/kg/hr occurring at day 18 of storage, respectively.

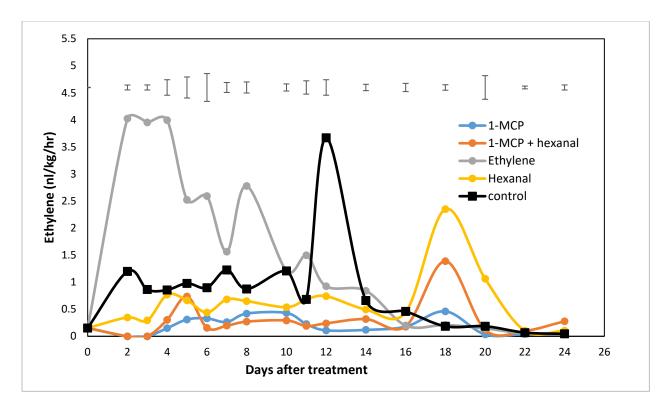


Figure 23: Effect of 1-MCP, hexanal, combination of 1-MCP + hexanal and Ethylene treatments compared to control on the rate of ethylene production in 'Grand nain' bananas. Top bars indicate least significant difference (LSD) between means at p<0.05 for each day of storage.

# 5.4.1.2. Rate of Respiration

Rate of respiration followed a similar trend to that of ethylene production. Fruits treated with ethylene and the untreated ones had significantly (*p*<0.05) high rate of respiration compared to those treated with hexanal, 1-MCP or both (Figure 24). Rate of respiration in the fruits treated with ethylene, hexanal or the untreated controls, increased gradually to a peak level which occurred at different days before drastically declining. In the ethylene treated fruits, rate of respiration increased drastically from an initial of 3.8 ml/kg/hr to a peak level of 27.1 ml/kg/hr at day 7 of storage before gradually decreasing till the end of storage. Similarly, in the untreated control fruits, the rate of respiration peaked to 22.3 ml/kg/hr at day 14 of storage, which was 7 days later after the ethylene treated fruits. The rate of respiration in fruits treated with hexanal followed a similar pattern to that of the untreated fruits but maintained significantly low levels which peaked to 18.6 ml/kg/hr by day 20 of storage. Fruits treated with 1-MCP or a combination of both 1-MCP and hexanal, had relatively low rate of respiration throughout the storage period with no definite respiratory peaks.

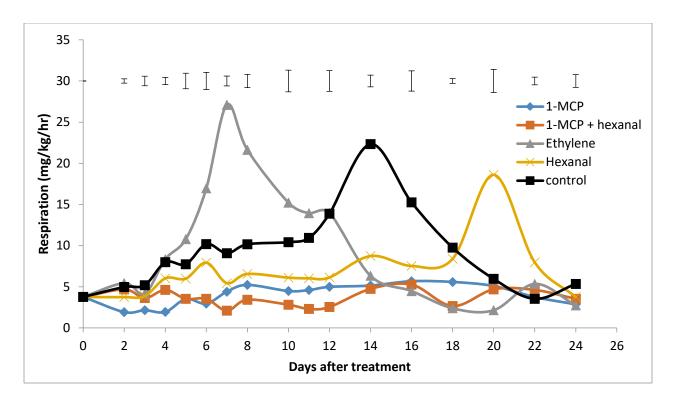


Figure 24: Effect of 1-MCP, hexanal, combination of 1-MCP + hexanal and Ethylene treatments compared to control on the rate of respiration in 'Grand nain' bananas. Top bars indicate least significant difference (LSD) between means at p<0.05 for each day of storage.

# 5.4.1.3. Changes in peel firmness

Peel firmness decreased in all the fruits over the storage time irrespective of the treatment (Figure 25). Fruits treated with either 1-MCP, hexanal or a combination of 1-MCP and hexanal had significantly (p<0.05) reduced rate of peel softening each sampling day compared to the untreated controls and ethylene treated fruits. However, there was no significant differences between 1-MCP and combined 1-MCP and hexanal treatments. Fruits treated with ethylene lost their peel firmness very fast from an initial of 40.7N to 1.8N at the end of storage compared with 1-MCP and hexanal treated fruits which maintained a final firmness of 7.5- 12.2N on average. Peel softening in the untreated controls followed a similar trend to that of ethylene treated fruits though the loss in firmness was less drastic. Peel firmness of the control fruits declined from the initial of 40.7N to 3.1N at the end of storage. Both the ethylene treated and untreated control fruits softened drastically as from day 8 of storage.

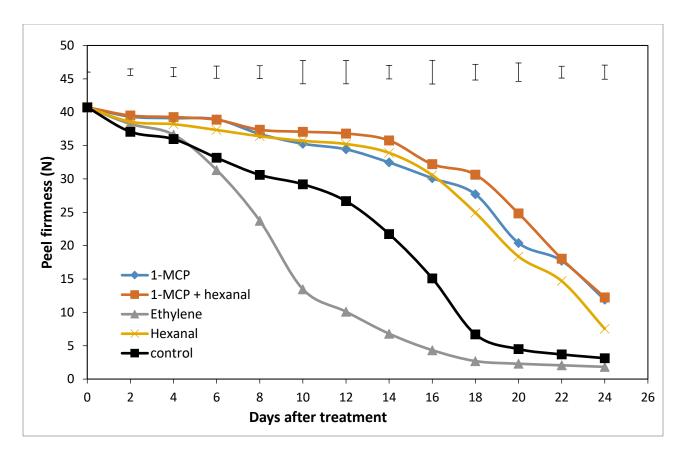


Figure 25: Effect of 1-MCP, hexanal, combination of 1-MCP + hexanal and Ethylene treatments compared to control on peel firmness in 'Grand nain' bananas. Top bars indicate least significant difference (LSD) between means at p<0.05 for each day of storage.

#### 5.4.1.4. Changes in pulp firmness

Pulp firmness decreased gradually in all the fruits during the storage period, exhibiting a similar trend to that of peel firmness (Figure 26). Pulp firmness was significantly (*p* <0.05) affected by the various treatments administered. Hexanal, 1-MCP and a combination of both 1-MCP and hexanal significantly slowed the rate of change of pulp firmness compared to the ethylene treated and control fruits. Overall, ethylene treated fruits lost their pulp firmness faster compared to the untreated controls. By the 12<sup>th</sup> day of storage, ethylene treated fruits had lost their pulp firmness to 2.3N as compared to 4.3N, in the untreated controls, 4 days later. On the other hand, fruits treated with 1-MCP, hexanal or both remained firmer throughout the storage period, with a significant difference observed as from day 20 of storage. By the 20<sup>th</sup> day of storage, the pulp firmness of thehexanal treated fruits had decreased to 6.6N compared to 9.4N and 10.8N in fruits treated with 1-MCP and a combination of 1-MCP and hexanal respectively.

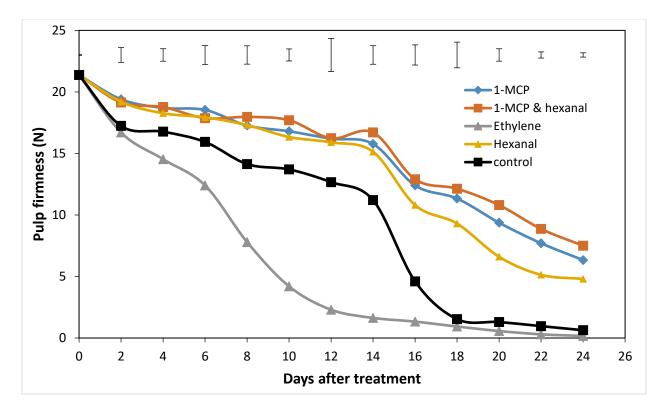


Figure 26: Effect of 1-MCP, hexanal, combination of 1-MCP + hexanal and Ethylene treatments compared to control on pulp firmness in 'Grand nain' bananas. Top bars indicate least significant difference (LSD) between means at p<0.05 for each day of storage.

#### 5.4.1.5. Changes in peel color

A gradual decrease in hue angle was observed in all the fruits, irrespective of the treatment (Figure 27). Peel color was significantly affected (p< 0.05) by the various treatments. Hexanal, 1-MCP and a combination of 1-MCP and hexanal significantly delayed changes in peel color, with the effect being more pronounced in the 1-MCP and 1-MCP plus hexanal treated fruits. The peel hue angle in the ethylene and untreated control fruits drastically declined from an initial of 121° to 73° and 77° respectively, by the 24<sup>th</sup> day of storage. For the hexanal treated fruits, the hue angle decreased to 83.3° by day 24 of storage as compared to 96° in both 1-MCP and a combination of 1-MCP and hexanal treatments. Overall, 1-MCP treatment maintained significantly high peel hue angle as compared to a combination of 1-MCP and hexanal as well as hexanal treatment.

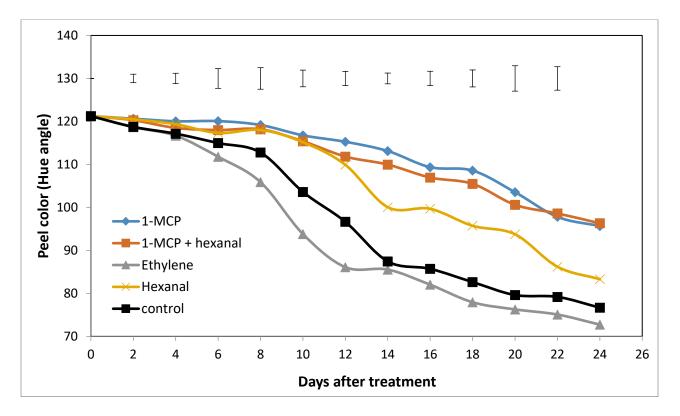


Figure 27: Effect of 1-MCP, hexanal, combination of 1-MCP + hexanal and Ethylene treatments compared to control on peel color in 'Grand nain' bananas. Top bars indicate least significant difference (LSD) between means at p<0.05 for each day of storage.

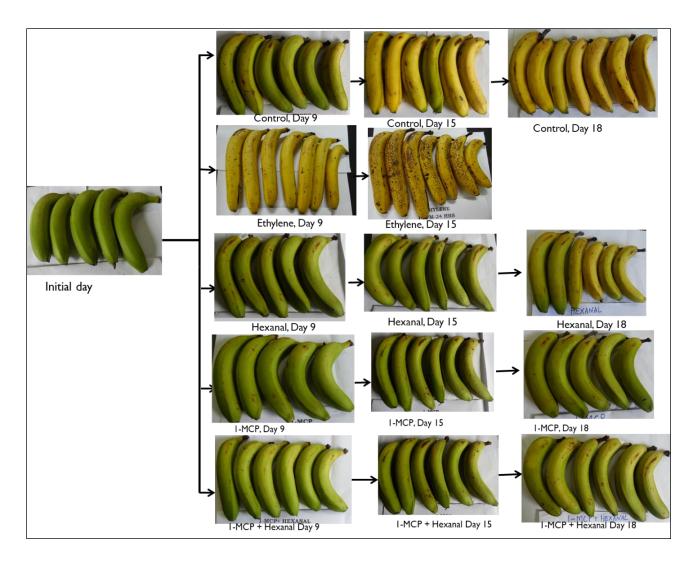
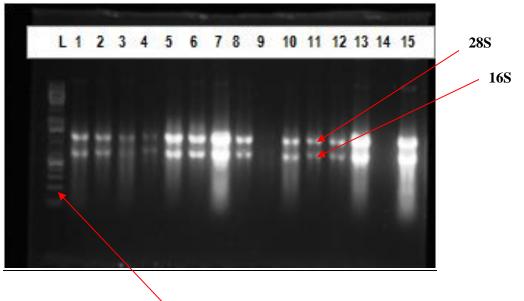


Figure 28: Progression of ripening in 'Grand nain' banana fruits treated with hexanal, ethylene, 1-MCP, a combination of 1-MCP and hexanal or left untreated and allowed to ripen under ambient room conditions.

# 5.4.2. Quantitative polymerase chain reaction (qPCR) and Transcriptome analysis

# 5.4.2.1. Quality Check of the extracted RNA

Figure 29 shows a photo of a gel run for samples 1 to 15. RNA of good quality should have intact two bands of the 28S and 16S. In sample 9 and 14 the two bands were not visible in the gel, a sign of degradation hence the RNA was of poor quality and the extraction process was repeated. Samples 7, 13 and 15 had very thick 28S and 16S bands a sign of very highly concentrated RNA. This was confirmed by quantifying their concentration by Nanodrop<sup>®</sup>-1000 spectrophotometer.



Ladder

Figure 29: Gel electrophoresis photo of the extracted RNA from samples 1-15. L means one kilo base ladder used to determine the size of the RNA

# 5.4.2.2 Quantitative PCR Results

# i. Polygalacturonase gene (PG gene)

The differential expression of PG gene in the peels increased remarkably in the ethylene treated fruits and controls as from day 2 and 4 of storage respectively (Figure 30). The maximum expression was at day 6 of storage with a 6 and 3 fold increase in the ethylene-treated and untreated control fruits respectively in the peel tissue (Figure 30). On the other hand, PG gene expression in the pulp tissues occurred 2 days later in both the controls and hexanal treated fruits (Figure 31). Generally, the highest expression of PG gene in the pulp tissues was at day 8 of storage, with the expression being abundant in ethylene treated fruits (3 fold) as compared to the controls (2 fold). In both the peel and pulp tissues of hexanal treated fruits, PG expression was highest at days 10-12 of storage. However, inconsistent trends were observed in fruits treated with either 1-MCP or a combination of 1-MCP and hexanal. In the peel tissue, PG expression in the 1-MCP treated fruits increase in expression was at day 12 (Figures 30 and 31). For fruits treated with both 1-MCP and hexanal, PG expression increased as from day 14 up to day 18 of storage in the peel tissues while in the pulp the expression occurred slightly high between day 8 and 16 of storage.

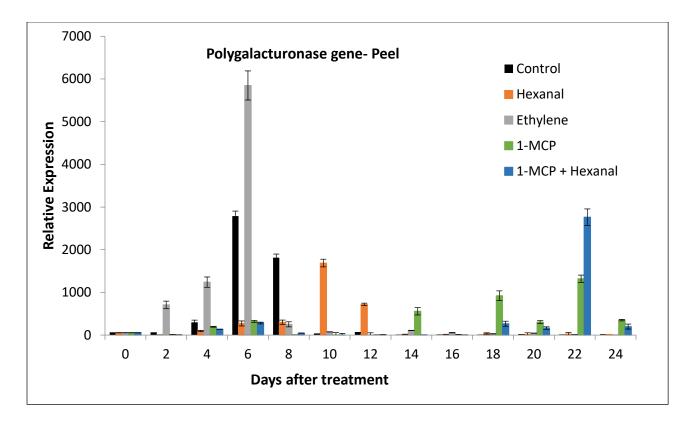


Figure 30: Effect of Hexanal, Ethylene, 1-MCP, combination of hexanal and 1-MCP and untreated control on polygalacturonase gene expression in banana peel during fruit ripening. The relative transcript abundance was normalized using banana actin gene

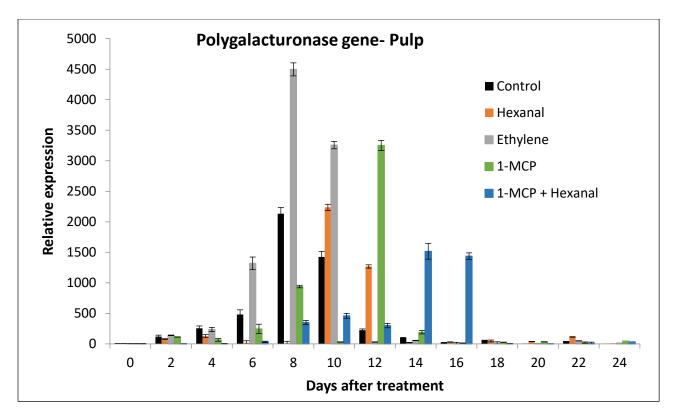


Figure 31: Effect of Hexanal, Ethylene, 1-MCP, combination of hexanal and 1-MCP and untreated control on polygalacturonase gene expression in banana pulp during fruit ripening. The relative transcript abundance was normalized using banana actin gene

# ii. Pectate Lyase gene (PL gene)

Pectate lyase was the highly expressed gene with a fold change of upto 12 in the ethylene treated fruits as compared to all the other genes expressed in both the peel and pulp tissues (Figures 32 and 33). The PL gene initially showed a down-regulation in all the fruits at initial day of storage, but a significant increase was observed in the ethylene treated fruits as from day 2 of storage till day 10 in the pulp while in the peel tissue, the gene was expressed up to day 20. A similar trend was observed in the control fruits, where PL expression increased remarkably between days 10 to 14 of storage. The expression of PL increased gradually as from day 8 up to day 22 in peel of hexanal treated fruits as compared to sharp increase as from day 18 -22 in the pulp tissue (Figures 32 and 33). Expression of PL gene in fruits treated with either 1-MCP or a combination of 1-MCP and hexanal was down-regulated throughout storage with an exception of day 22 in the peel tissue of fruits treated with 1-MCP.

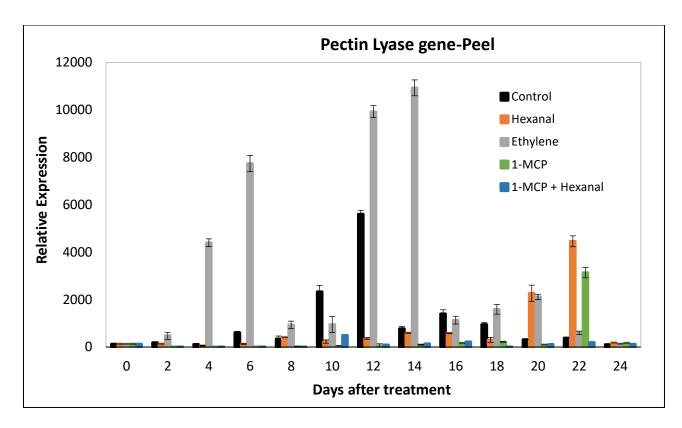


Figure 32: Effect of Hexanal, Ethylene, 1-MCP, combination of hexanal and 1-MCP and untreated control on pectate lyase gene expression in banana peel during fruit ripening. The relative transcript abundance was normalized using banana actin gene

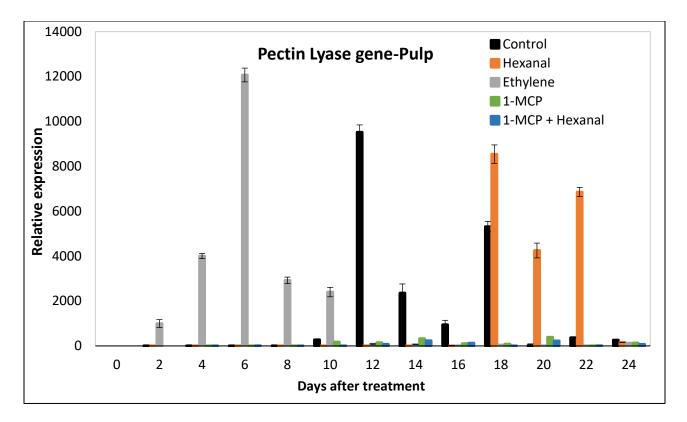


Figure 33: Effect of Hexanal, Ethylene, 1-MCP, combination of hexanal and 1-MCP and untreated control on pectate lyase gene expression in banana pulp during fruit ripening. The relative transcript abundance was normalized using banana actin gene

# iii. Pectin methylesterase gene (PME gene)

Expression of the PME gene occurred earlier in the peel as compared to the pulp tissue irrespective of the treatment. In the peel tissue, expression of the PME gene in the ethylene treated and control fruits increased between day 4 and 6 of storage, with ethylene treated fruits having the highest expression (Figure 34). Despite low expression of PME gene in 1-MCP and hexanal treated fruits, the highest expression was observed between day 12 and 14 of storage. However, 1-MCP treated fruits showed significantly high expression compared to hexanal treatment. On the other hand, the highest expression was observed at day 16 of storage in fruits treated with a combination of hexanal and 1-MCP. Inconsistent trends were observed in the pulp tissue, however, there was a slight expression of PME gene at day 2 which remained constantly low in the ethylene treated and control fruits until day 8 and 10 respectively (Figure 35). The highest expression was observed between days 10-14 in all the treatments before declining gradually until the end of storage.

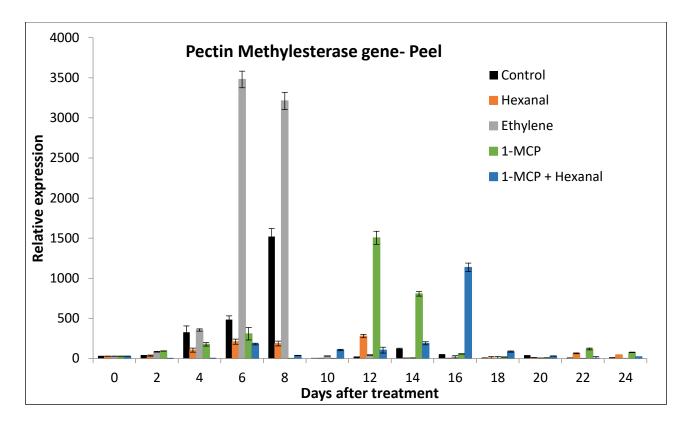


Figure 34: Effect of Hexanal, Ethylene, 1-MCP, combination of hexanal and 1-MCP and untreated control on pectin methylesterase gene expression in banana peel during fruit ripening. The relative transcript abundance was normalized using banana actin gene

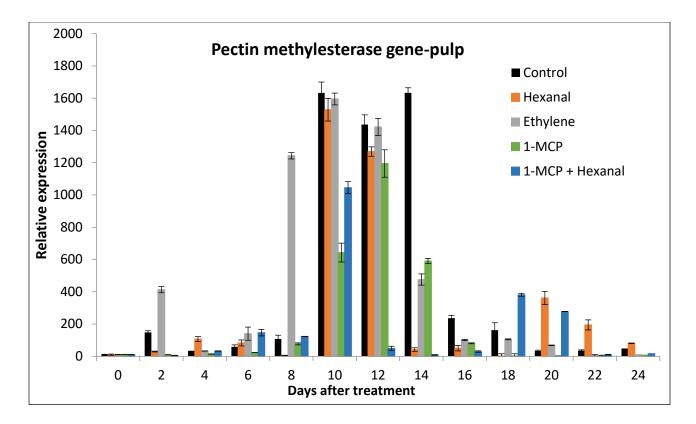
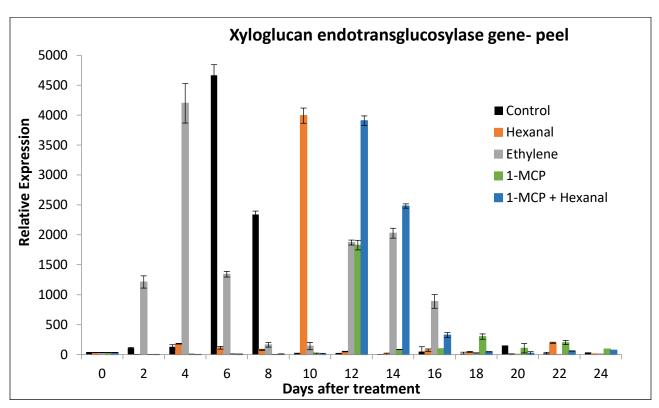


Figure 35: Effect of Hexanal, Ethylene, 1-MCP, combination of hexanal and 1-MCP and untreated control on pectin methylesterase gene expression in banana pulp during fruit ripening. The relative transcript abundance was normalized using banana actin gene

# iv. Xyloglucan endotransglucosylase gene (XET gene)

A similar trend to PME gene was observed with the expression of XET gene occurring earlier in the peel as compared to the pulp tissue. In ethylene treated fruits, expression of XET gene increased gradually as from day 2 and peaked at day 4 in the peel while in the pulp tissue the expression peaked at day 8 before decreasing gradually (Figures 36 and 37). In the untreated control fruits, expression of XET gene was highest in the peel tissues between day 6 and 8 of storage while in the pulp, the expression was slightly high between days 10 and 16. In the hexanal treated fruits, expression of XET gene sharply increased at day 10 in the peels as compared to a gradual increase as from day 12 to day 20 of storage in the pulp (Figures 36 and 37). In fruits treated with either 1-MCP or a combination of 1-MCP and hexanal, highest expression was observed in day 12 of storage in the peel, before decreasing gradually. However, in the pulp tissue, the expression increased gradually as from day 12



to a peak at day 20 of storage in fruits treated with 1-MCP while those treated with a combination of both 1-MCP and hexanal had significantly low expression (Figure 37).

**Figure 36:** Effect of Hexanal, Ethylene, 1-MCP, combination of hexanal and 1-MCP and untreated control on Xyloglucan endotransglucosylase gene expression in banana peel during fruit ripening. The relative transcript abundance was normalized using banana actin gene

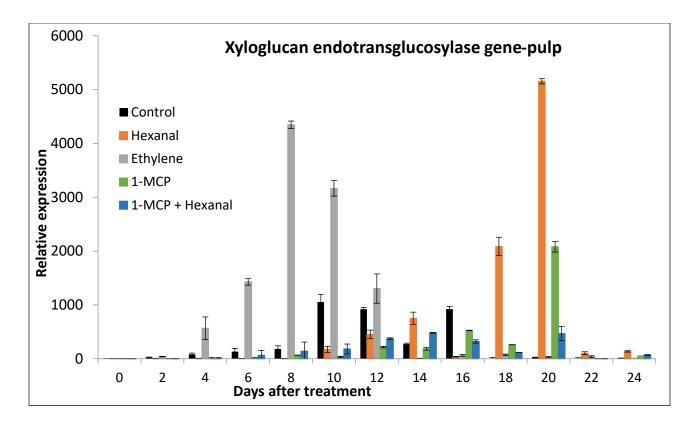


Figure 37: Effect of Hexanal, Ethylene, 1-MCP, combination of hexanal and 1-MCP and untreated control on Xyloglucan endotransglucosylase gene expression in banana peel during fruit ripening. The relative transcript abundance was normalized using banana actin gene

# v. 1-Aminocyclopropane-1-Carboxylic Acid Oxidase gene (ACO gene)

The differential expression of ACO gene increased gradually in the ethylene treated fruits and untreated controls as from day 2 of storage with no significant difference between the peel and pulp tissues (Figures 38 and 39). Expression of ACO gene peaked at day 8 and 12 of storage in the peel and pulp tissues respectively in the ethylene treated fruits as compared to day 12 in both tissues in the untreated controls. Unexpectedly, untreated fruits had higher expression especially in the pulp tissue as compared to the ethylene treated fruits. Hexanal treatment, transiently down-regulated the expression of ACO gene was down-regulated in the 1-MCP treated fruits upto day 10 in peel and day 14 in the pulp tissue. Generally, ACO gene was highly expressed in the 1-MCP treated fruits towards the end of storage with the maximum expression being on days 20 and 22 in pulp and peel tissues respectively (Figures 38 and 39). On the other hand, in fruits treated with a combination of 1-MCP and hexanal

had low expression profile in peel tissues throughout storage period while in the pulp the highest expression was recorded on day 20.

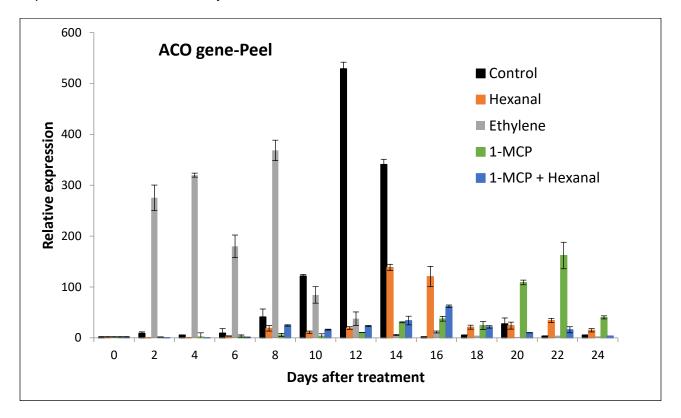


Figure 38: Effect of Hexanal, Ethylene, 1-MCP, combination of hexanal and 1-MCP and untreated control on ACO gene expression in banana peel during fruit ripening. The relative transcript abundance was normalized using banana actin gene

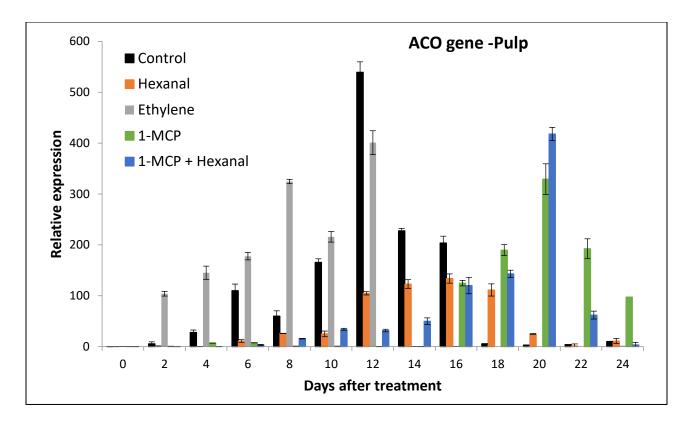


Figure 39: Effect of Hexanal, Ethylene, 1-MCP, combination of hexanal and 1-MCP and untreated control on ACO gene expression in banana pulp during fruit ripening. The relative transcript abundance was normalized using banana actin gene

# 5.4.3. Next Generation sequencing / Transcriptome analysis

# **5.4.3.1.** Quality control of the prepared libraries

The integrity of the prepared libraries was checked by use an Agilent Bio analyzer while the concentration was determined by use of qubit<sup>4</sup> fluorometer machine. The library is termed to be of good quality when only one fragment size of between 250 and 300bp is obtained in addition to the lower and upper peak markers (Figure 40). The figure is a sample photo of an agilent bio-analyzer reading of a library prepared of sample 3 before being send for sequencing. The library peak of this sample is 291bp with no diamers occurring before or after it. In addition to a single fragment size of between 250 and 300bp, the library concentration should be above 4 ng/µl.

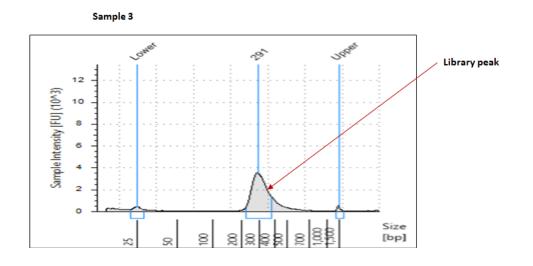


Figure 40: Agilent Bio-analyzer reading for checking integrity of the library fragments before sequencing for sample 3. The library fragment peak size was 291bp with no diamers

#### **5.4.3.2.** Differentially Expressed Genes (DEGs)

After sequencing each sample on average, had a total of one hundred million raw reads with an exception of two samples which had very few reads of less than five hundred thousand. After trimming to remove the poor reads, on average 80% of the total reads mapped to the banana genome. The DEGs were analyzed at day 1, day 4, day 18 and day 24 of storage between the treated and untreated samples. Below is a bar graph summarizing the number of DEGs across the various treatments over storage period (Figure 41). However, since so many DEGs were obtained, this study focused majorly on genes involved in banana ripening and senescence process. Therefore, genes involved in ethylene biosynthesis, softening, respiration, flavor and aroma will be discussed in respect to hexanal and ethylene treatment as well as in the untreated control fruits. Further, a summary of the topmost DEGs genes was generated through a heatmap (Figure 42). Some of these genes are involved either in plant growth, biotic stress, protein biosynthensis or they are housekeeping genes. Interestingly, some of the genes involved in cell wall degradation such as Expasins (GSMUA\_Achr5G07480\_001, GSMUA\_Achr5G07470\_001, GSMUA\_Achr11G22960\_001), pectate lyase 22 (GSMUA\_Achr6G28260\_001) and mannan endo-1,4-beta-mannosidase 9 (GSMUA\_Achr3G12210\_001) were identified. Additionally, genes involved in respiration (GSMUA Achr4G17840 001) and synthesis of aroma volatiles such as 4-coumarate--CoA ligaselike 6 (GSMUA\_Achr11G06230\_001), Anthocyanin 5-aromatic acyltransferase (GSMUA\_Achr4G26740\_001), O-methyltransferase ZRP4 (GSMUA\_Achr4G16570\_001) and Polyneuridine-aldehyde esterase (GSMUA\_Achr11G11790\_001) were also among the top 50 DEGs (Figure 42).

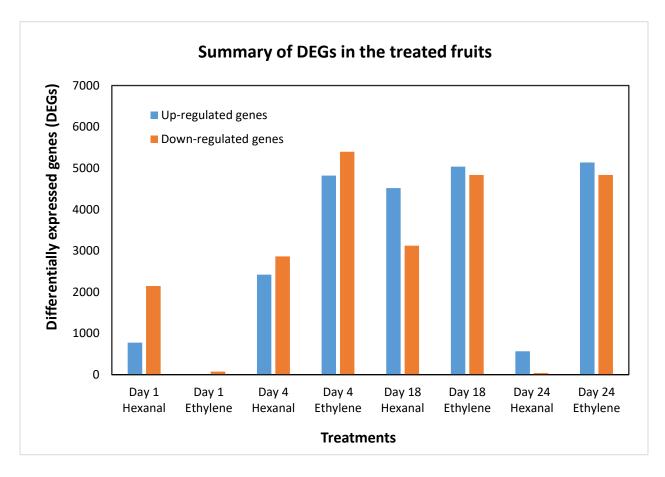


Figure 41: Summary of the differentially expressed genes in the hexanal and ethylene treated fruits in each day of storage.

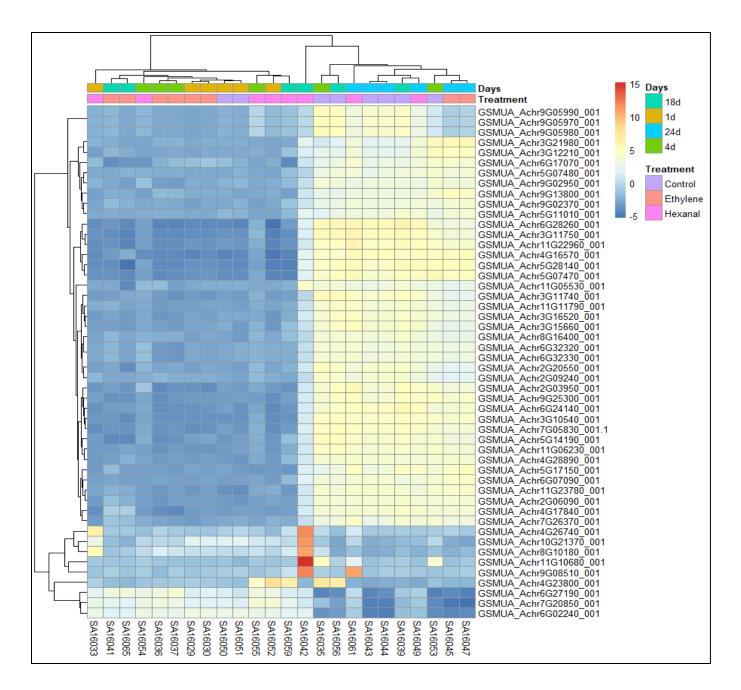


Figure 42: Heatmap showing the topmost 50 genes differentially expressed across the treatments (hexanal, ethylene and untreated controls) in all the days (day 1, day 4, day 18 and day 24) of storage

#### 5.4.3.2.1 Differentially Expressed Genes (DEGs) at day one of storage

At day one of storage, a total of 776 and 2146 were up-regulated and down-regulated respectively in the hexanal treated fruits (Figure 43). On the other hand, 4 genes were up-regulated while 76 genes were down-regulated in the ethylene treated fruits (Figure 44). Most of the down-regulated genes in both hexanal and ethylene treatments are involved in various ripening processes such as starch and sucrose metabolism, biosynthesis of secondary metabolites and cell wall degradation pathways (Figures 43 and 44). GSMUA\_Achr1G14590\_001 which codes for phospholipase D gene, the key enzyme in the cell membrane degradation pathway was significantly down-regulated (3.3 fold) in the hexanal treated fruits. Two different Expansin genes (GSMUA\_Achr1G02650\_001, GSMUA\_Achr1G00120\_001) coding for expansin enzyme and one Mannan endo-1,4-betamannosidase genes (GSMUA Achr3G09750 001) coding for endo-1,4-β-mannanase enzymes which are all involved in cell wall degradation were significantly down-regulated in the hexanal treated fruits at day I post treatment (Figure 43). Additionally, genes coding for Polygalacturonase, Endoglucanase 9 and xyloglucan endotransglucosylase were down-regulated by 3.3, 5.2, 3.6 folds respectively following hexanal treatment (Figure 44). Genes involved in synthesis of aroma volatiles such as 4-coumarate-CoA ligase-like 3, flavonoid 3',5'-hydroxylase, putative O-methyltransferase ZRP4, glycosyltransferase and uncharacterized acetyltransferase were also down regulated in the hexanal treated fruits.

A similar trend was observed in the ethylene treated fruits, were some cell wall degradation genes such as GSMUA\_Achr8G14160\_001 coding for xyloglucan endotransglucosylase and GSMUA\_Achr2G05670\_001 coding for Endoglucanase 12 were down-regulated by 3.2 and 2.3 folds respectively (Figure 44).

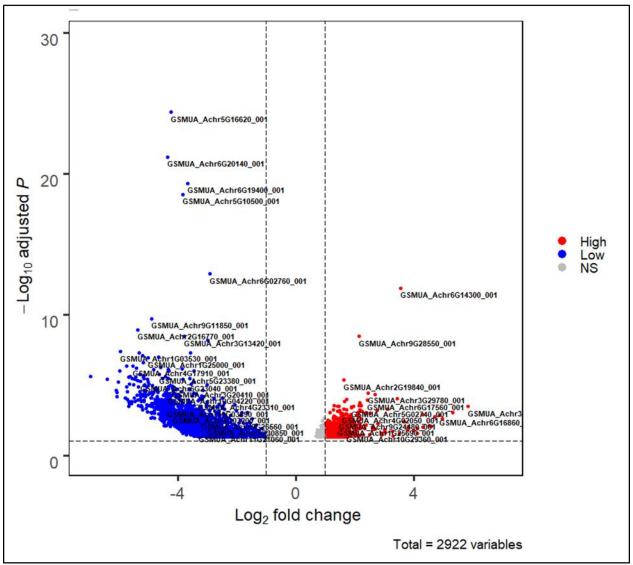


Figure 43: Enhanced volcano plot showing differentially expressed genes (DEGs) at p < 0.05 and a log2fold ratio  $\ge 1$  at day one of storage in the hexanal treated fruits

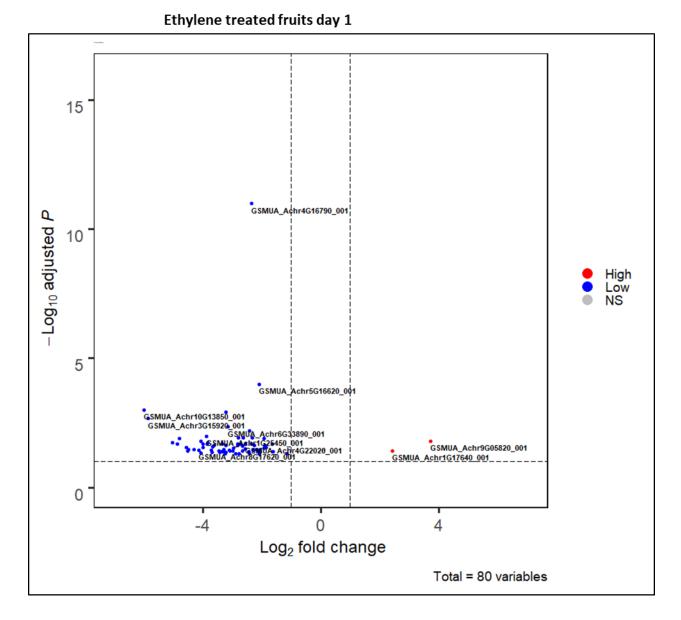
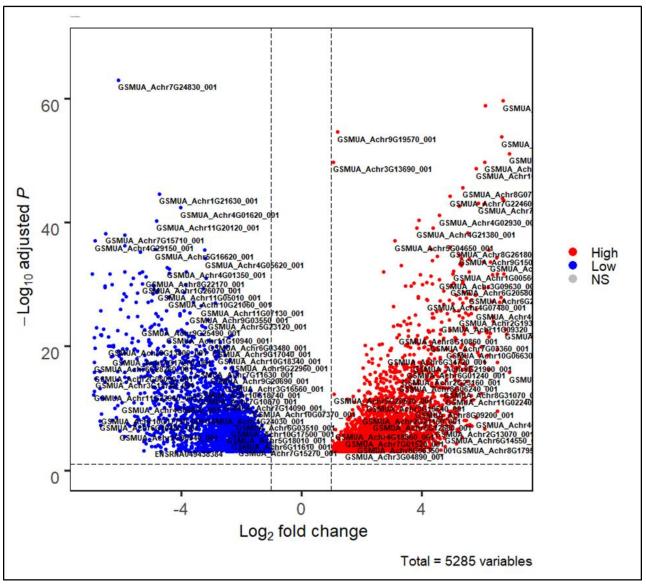


Figure 44: Enhanced volcano plot showing differentially expressed genes (DEGs) at p < 0.05 and a log2fold ratio  $\ge 1$  at day one of storage in the ethylene treated fruits

#### 5.4.3.2.2 Differentially Expressed Genes (DEGs) at day 4 of storage

A total of 5285 genes were differentially expressed at day 4 of storage after hexanal treatment, in which 2423 genes were up-regulated as compared to 2862 which were down-regulated (Figure 45). A similar trend to that of day 1 was observed were phospholipase D gene (GSMUA\_Achr4G16280\_001) which is involved in the cell membrane degradation pathway was down-regulated by 4.8 fold (figure 45). GSMUA\_Achr1G18250\_001 which codes for 1-aminocyclopropane-1-carboxylate oxidase and GSMUA\_Achr4G29150\_001 coding for 1-Aminocyclopropane-1-carboxylate synthase, key enzymes in the ethylene biosynthesis pathway were significantly down-regulated by 3.3 and 6.7 folds respectively (figure 45). Additionally, key genes involved in the cell wall degradation pathway such as Xyloglucan endotransglucosylase (GSMUA\_Achr3G10480\_001), Polygalacturonase At1g48100 (GSMUA\_Achr6G28260\_001), Expansin-A2 (GSMUA\_Achr5G07480\_001) and Pectate lyase 22 (GSMUA\_Achr6G28260\_001) were significantly down-regulated. Genes involved in respiration process such as Beta-amylase 3 (GSMUA\_Achr4G17840\_001) and synthesis of aroma volatiles such as 4-Coumarate-CoA ligase-like 6, and O-methyltransferase zrp4 were also down-regulated (figure 45).

In the ethylene treated fruits, 4820 genes were up-regulated as compared to 5395 genes which were down-regulated (figure 46) at day 4 of storage. Contrary to the hexanal treated fruits, most of the upregulated genes were those involved in various ripening related pathways such as ethylene biosynthesis, softening, synthesis of secondary metabolites as well as aroma volatiles. Two genes; GSMUA\_Achr1G18250\_001 and GSMUA\_Achr4G29150\_001 coding for 1-aminocyclopropane-1carboxylate oxidase 3 and 1-aminocyclopropane-1-carboxylate synthase respectively, were upregulated by 4.5 and 7.3 folds respectively (Figure 46). Several genes involved in cell wall degradation such as xyloglucan endotransglucosylase, Pectate lyase, expansins, Polygalacturonase and endo-1, 4-beta-mannosidase were also up-regulated. GSMUA\_Achr4G16280\_001 coding for phospholipase D enzyme involved in cell membrane degradation was up-regulated by 6.4 folds. Several genes involved in the aroma volatiles synthesis pathway in banana such as 4-coumarate-CoA ligase-like 3 (GSMUA\_Achr11G14670\_001) and Beta-fructofuranosidase 1 (GSMUA\_Achr5G19880\_001) were up-regulated. Most of the down-regulated genes identified are involved in growth and development processes such as cell division and elongation (GSMUA\_Achr7G18190\_001), light signalling (GSMUA\_Achr3G14630\_001) and regulation of stomata formation (GSMUA\_Achr2G06030\_001). Genes involved in respiration process such as Pyruvate decarboxylase isozyme 2 were also up-regulated (figure 46).



Hexanal treated fruits day 4

Figure 45: Enhanced volcano plot showing differentially expressed genes (DEGs) at p <0.05 and a log2fold ratio  $\ge 1$  at day 4 of storage in the hexanal treated fruits



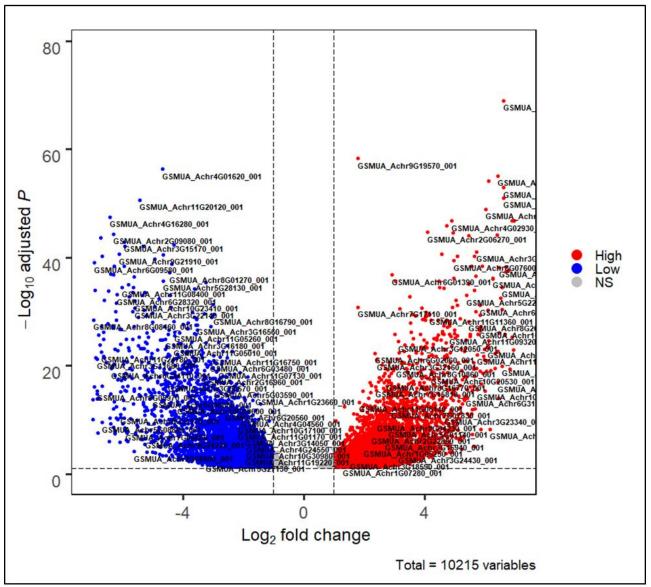


Figure 46: Enhanced volcano plot showing differentially expressed genes (DEGs) at p <0.05 and a log2fold ratio  $\ge 1$  at day 4 of storage in the ethylene treated fruits

#### 5.4.3.2.3 Differentially Expressed Genes (DEGs) at day 18 of storage

Most of the genes (4516) were up-regulated by day 18 of storage in the hexanal treated fruits as compared to 3121 genes which were down-regulated (figure 47). Key to note was the up-regulation of most of the ripening-related genes which were previsiouly down-regulated following hexanal treatment in day one and four post treatment. Genes involved in the ethylene biosynthensis pathway such as 1-aminocyclopropane-1-carboxylate synthase 3 gene (GSMUA\_Achr2G08600\_001) and 1aminocyclopropane-1-carboxylate oxidase (GSMUA\_Achr1G18250\_001) were highly up-regulated by 5.5 and 2.8 folds respectively (figure 47). Phospholipase D alpha 1 (GSMUA Achr1G14590 001) and Phospholipase D delta (GSMUA\_Achr9G21120\_001) genes controlling the cell membrane degradation pathway were also up-regulated at the 18<sup>th</sup> day of storage post hexanal treatment by 3.8 and 6.2 folds respectively. Most of the genes involved in cell wall degradation pathway such as Expansin-A1 (GSMUA\_Achr1G02650\_001), Pectate lyase (GSMUA\_Achr2G16830\_001) and Polygalacturonase At1q48100 (GSMUA Achr11G04870 001) were also up-regulated (Figure 47). Some of the gene families involved in the synthensis of aroma volatiles such as Anthocyanin 5aromatic acyltransferase, Glutathione S-transferase, Zeatin O-glucosyltransferase and 3hydroxybenzoate 6-hydroxylase 1 were also up-regulated (figure 47). However, some genes such as endotransqlucosylase (GSMUA Achr3G10480 001) Xyloqlucan and Expansin-A2 (GSMUA\_Achr5G07480\_001) were still down-regulated.

In the ethylene treated fruit, 5038 genes were up-regulated while 4835 genes were down-regulated (Figure 48). At this point, the fruits were completely over-ripe. In addition, to the ripening related genes, some of the genes involved in biotic and abiotic stress in plants were up-regulated. Some of this genes are GSMUA\_Achr7G23680\_001 which codes for peroxidase 47 regulates plant cell response to stress. Genes involved in protein synthensis, RNA biosynthesis as well as transmembrane transporters genes were among those highly down-regulated (Figure 48).

Hexanal treated fruits day 18

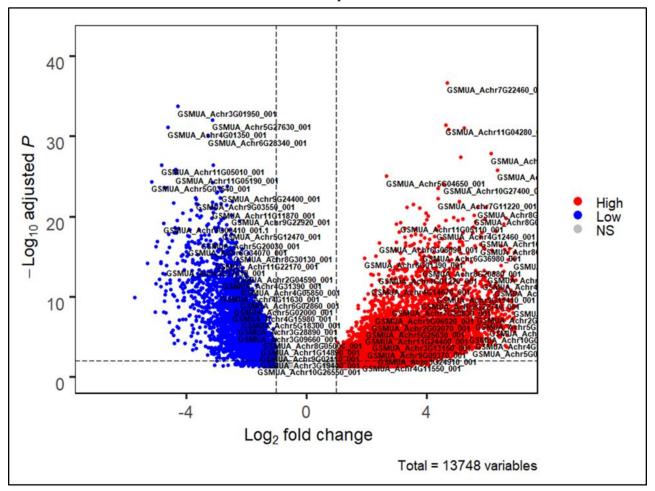


Figure 47: Enhanced volcano plot showing differentially expressed genes (DEGs) at p <0.05 and a log2fold ratio  $\ge 1$  at day 18 of storage in the hexanal treated fruits



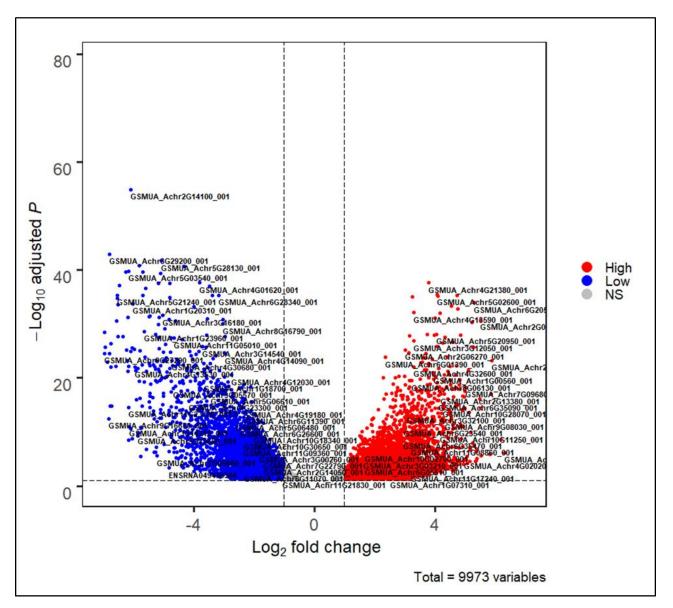


Figure 48: Enhanced volcano plot showing differentially expressed genes (DEGs) at p <0.05 and a log2fold ratio  $\geq$  1 at day of 18 storage in the ethylene treated fruits

# **5.4.3.2.4 Differentially Expressed Genes (DEGs) at day 24 of storage in the Hexanal treated fruits**

By the 24<sup>th</sup> day of storage, 568 genes were up-regulated while 41 genes were down-regulated in the hexanal treated fruits (Figure 49). A similar trend to that observed in day 18 of storage was noted, were most of the ripening related genes were up-regulated. Some genes involved in fruit softening such endoglucanase 9 (GSMUA\_Achr5G07510\_001) and expasin-A15 as (GSMUA\_Achr2G21970\_001) as well as phospholipase D alpha 1 gene (GSMUA Achr1G14590 001) involved in cell membrane degradation were up-regulated (Figure 49). Some gene families involved in starch metabolism such as sucrose-phosphatase 2 as well as those involved in aroma synthesis; Flavonoid 3', 5'-hydroxylase, Caffeic acid 3-O-methyltransferase and galacturonosyltransferase 7 were up-regulated.

60 GSMUA\_Achr7G16790\_001 40 -Log<sub>10</sub> adjusted P GSMUA\_Achr7G17110\_001 GSMUA Achr4G14920\_001 High Low NS 20 GSMUA\_Achr4G24180\_001 GSMUA\_Achr6G02240\_001 GSMUA\_Achr11G07220\_001 GSMUA\_Achr1G09880\_001 GSMUA\_Achr10G30480\_001 GSMUA\_Achr2G15930\_001 GSMUA\_Achr7G11880\_001 GSMUA\_Achr6G33140\_001 GSMUA\_Achr6G01600\_0 001 Achr6G12420 001 GSMUA GSMUA Achr1G24422 GSMUA Ach: 11G12620 GSMUA Achr3G06 GSMUA\_Adm8G31460\_001 GSMUA\_Achr6G21690\_001 ACHITTG05110 001 GSMI GSMUA\_Achr5G06200\_001 0 -4 0 4 Log<sub>2</sub> fold change Total = 609 variables

Hexanal treated fruits day 24

Figure 49: Enhanced volcano plot showing differentially expressed genes (DEGs) at p < 0.05 and a log2fold ratio  $\ge 1$  at day 24 of storage in the hexanal treated fruits

## 5.5. Discussion

Several biochemical and physiological changes take place during fruit ripening process in which several genes are recruited in various metabolic pathways to produce a ripe fruit (Asif *et al.*, 2014). Softening is one of the significant physical changes associated with banana ripening that increases the susceptibility of the fruit to mechanical injuries and pathogen invasions. Therefore, there is need to use effective post-harvest technologies and innovations to delay ripening and the softening process. Use of hexanal and its formulations have shown great potential to delay banana softening and ripening in general without compromising on quality. Previous studies by Brummel (2006), showed that softening in banana fruit is largely due to disassembly of the cell wall, deterioration of the cell membrane and breakdown of starch into sugars. This study sought to understand the molecular basis of hexanal mode of action in delaying banana ripening and the softening process.

Results of this study showed that, most of the identified DEGs either in the hexanal or ethylene treated fruits were involved in different ripening processes such as ethylene biosynthesis, respiration, softening, breakdown of starch, cell membrane degradation, synthesis of secondary metabolites as well as aroma volatiles. At day one of storage, most of the DEGs were suppressed, an indication that ripening process had not been initiated in both ethylene and hexanal treated fruits. Of interest was the suppression of GSMUA\_Achr1G14590\_001 which codes for phospholipase D gene, the key enzyme in the cell membrane degradation pathway following hexanal treatment. According to Paliyath et al. (2008), cell membrane degradation involves the activity of several enzymes, in which one enzyme acts on the product released by the previous enzyme in the sequence. However, phospholipase D (PLD) is the key enzyme which initiates the cell membrane degradation action. It is believed that PLD enzyme is bound to the membrane resulting in a cascade of catabolic reactions leading to the generation of several neutral lipids, the accumulation of which leads to membrane destabilization (Paliyath et al., 2008). Additionally, low expression of two genes coding for expansin enzyme and one coding for mannan endo-1,4-beta-mannosidase were detected in the hexanal treated fruits. According to Brummel (2006), expansin enzyme is one of the cell-wall modifying enzymes engaged in loosening of hydrogen bonds between cellulose microfibrils and matrix glycans. On the other hand mannan endo-1,4-beta-mannosidase enzyme is involved in cleavage of the mannan backbone in hemicellulose polysaccharides found in the cell wall (Schroder et al., 2009). The softening process is known to begin at the onset of ripening according to Asif *et al.* (2014), possibly explaining the low expression of these genes on day one of storage in the hexanal treated fruits. Endoglucanase and xyloglucan endotransglucosylase (XET) genes were down-regulated in day one of storage in both ethylene and hexanal treated fruits, an indication fruit softening had not begun. This observations correlates well with the physical and physiological data collected were the fruits had high peel and pulp firmness at day one of storage. Various genes involved in the synthesis of aroma volatile were also suppressed in the hexanal treated fruits. A previous study by Asif *et al.* (2014), showed that genes involved in aroma synthesis pathways such as phenylpropanoid pathway, fatty acid biosynthesis pathway and iso- leucine biosynthesis pathway were down-regulated in unripe banana fruits.

At day four of storage, genes coding for 1-aminocyclopropane-1-carboxylate oxidase (ACO) and 1-Aminocyclopropane-1-carboxylate synthase (ACS), key enzymes in the ethylene biosynthesis pathway were significantly suppressed in the hexanal treated fruits. However, these two enzymes were highly induced in the ethylene treated fruits. Both ACS and ACO, are rate-limiting enzyme in ethylene biosynthesis pathway. Ethylene initiates ripening process in climacteric fruits such as banana. A study by Ahmad *et al.* (2001), reported that harvested bananas fruits pass through four physiological developmental stages which include pre-climacteric, climacteric, ripening and senescence stage. The pre climacteric stage also known as the "green stage" represents the period from harvest until the onset of respiratory climacteric (Gowen, 1995). This probably explains why genes involved in ethylene biosynthesis pathway such ACS and ACO were suppressed in the hexanal treated fruits, an indication ripening had not been initiated contrary to the induction of the same genes in the ethylene treated fruits.

Transcriptomic and qPCR data showed that genes involved in cell wall degradation (XET, PG, PL) were suppressed in the hexanal treated fruits at day four of storage. A previous study by Valero and Serrano (2010), reported that pectin degradation by polygalacturonase (PG) enzymes is a major contributor to fruit softening in banana fruits and this might explain the observed suppression of the PG gene in the present study following hexanal treatment. This is in contrast with a study by Tiwari and Paliyath, (2011) in tomatoes which showed that hexanal down-regulated expression of only beta-

galactosidase gene coding for  $\beta$ -galactosidase enzymes which is a cell wall modifying enzyme.  $\beta$ galactan occurs primarily on the side chains of rhamnogalacturon-I polysaccharide as reported by Schols *et al.* (1995). According to Zykwinska *et al.* (2007), these chains are intertwined with cellulose glucan chains creating a thick network that adds to the cell wall's extensibility, strength and porosity (Ulvskov *et al.*, 2005; Larsen *et al.*, 2011). In addition, hexanal treatment suppressed the expression of Phospholipase D by 4.8. These results supports hexanal role in inhibiting the activities of PLD enzyme as earlier reported by Paliyath and Padmanabhan, (2019).

On the other hand, fruits treated with exogenous ethylene had most of the cell wall softening genes induced. The qPCR data showed that xyloglucan endotransglucosylase, Pectate lyase and polygalacturonase genes were all up-regulated at day four following exogenous ethylene treatment in both peel and pulp. However, the PG gene was highly up-regulated in the peel as compared to the pulp. Additionally, genes involved in cell membrane degradation such as Phospholipase D alpha 1 and Phospholipase D delta as well as calmodulin-binding protein genes were induced. Phospholipase D is the key enzyme in the cell membrane deterioration and its activity is stimulated by high calcium levels. Calmodulin are calcium sensing proteins and high calcium levels have been previously reported to activate phospholipase D enzymes (Kayal et al., 2017). Some of the induced genes are involved in production of various aroma volatiles such as 4-coumarate-CoA ligase-like 3 and Betafructofuranosidase 1 which were all induced, an indication of ripening process. In banana fruit, aroma is mostly as a result of different volatiles such as isoamyl acetate, isoamyl alcohol, elemecine as well as butyl acetate among others (Boudhrioua et al., 2003). Genes involved in respiration were also induced, an indication of the concomitant increase in respiration in banana fruits during the climacteric phase as indicated by the physiological data on respiration (Zhu et al., 2015). This coincides with physicochemical and biochemical modifications that turn the fruit into an edible state with the most favourable characteristics of quality (Mbeguie *et al.*, 2009).

The qPCR results showed that hexanal transiently suppressed expression of 1-Aminocyclopropane-1-Carboxylic Acid Oxidase gene (ACO gene), a rate limiting enzyme in ethylene biosynthesis pathway up to day 8 of storage in both the peel and pulp tissues. Interestingly, in 1-MCP treated fruits, ACO gene was suppressed upto day 20 and 22 of storage in both the peel and pulp tissues. This is because 1-MCP mode of action is by competitively binding to ethylene receptors and autocompeting ethylene action as reported by Blankenship, (2003). Once the ethylene has been locked out by 1-MCP, the ethylene signal cannot proceed and all the ethylene dependent ripening process are inhibited. However, after some time new ethylene receptors are generated by the cells and hence ethylene binds to the new receptors to initiate ripening at later days of treatment. This is shown by induction of the expression of ACO gene, a key enzyme in the ethylene biosynthensis pathway. The fact that ACO recovers earlier in hexanal treated fruits in the present study indicates that the suppression is weaker as compared to that 1-MCP. This results concur with the observation of Tiwari and Paliyath (2011), who reported that hexanal treatment caused temporal down regulation of 1-aminocyclopropane-1-carboxylate synthase 6 (ACS6) and 1-aminocyclopropane-1-carboxylate oxidase (ACO) genes in tomato fruits. On the other hand, most of the suppressed genes are those that are involved in plant growth and development such as cell division and elongation, light signalling and regulation of stomata formation. This is probably because, as the fruit is fully mature and undergoing the ripening process, growth and development genes are no longer required. This concurs with results of Asif et al. (2014), who found out that genes involved in growth and development are down-regulated at ripe stage of banana fruit.

By the 18<sup>th</sup> day of storage, fruits treated with ethylene were completely over-ripe and unmarketable while those treated with hexanal had started to ripen as indicated by the physical data on peel color and firmness. A previous study by John and Marchal, (1995), showed that fruits treated with exogenous ethylene ripen within 4-5 days then if not marketed immediately they become unsuitable for sale within 1-3 days after turning yellow. On the other hand, in fruits treated with hexanal, ripening had been initiated as indicated by peel color changes and softening from the physical data. Most of the ripening related gene were induced including those involved in cell wall degradation such as expansin, pectate lyase and polygalacturonase as well as phospholipase D alpha 1 involved in cell membrane degradation. This shows that hexanal doesn't irreversibly inhibit the activities of phospholipase D enzyme hence the fruit will finally undergo normal ripening and softening. This coincudes with results of a study by Tiwari and Paliyath, (2011) who reported that despite hexanal ability to delay softening and other physical attributes such as peel color, the fruit will finally undergo normal ripening. This coincides with the qPCR data were XET and PL genes were highly up-

regulated at day 18 of storage in both the peel and pulp of banana fruits. The qPCR results however, showed that PG gene was highly up-regulated between days 10 to 12 of storage. Pectin methylesterase (PME) enzyme activity is a pre-requisite for PG activity during cell wall degradation process. The qPCR results in this study revealed that the expression of PME gene was induced between days 6 -8 and 10-12 in the ethylene and hexanal treated fruits respectively.

Previous studies by Paliyath *et al.* (2008) and results of chapter 4 of this study have shown that hexanal has the potential to enhance quality attributes of the treated fruits. This may be as a result of hexanal treatment influencing the expression profile of genes involved in various pathways such as synthesis of aroma volatiles, starch degradation among others. In this study, some gene families involved in the synthensis of aroma volatiles such as Anthocyanin 5-aromatic acyltransferase, glutathione S-transferase, Zeatin O-glucosyltransferase and putative 3-hydroxybenzoate 6-hydroxylase 1 were down-regulated by 4<sup>th</sup> day of storage after hexanal treatment. Interestingly, by the 18<sup>th</sup> - 24<sup>th</sup> day of storage most of the gene families involved in starch metabolism as well as aroma synthesis were up-regulated. This strongly shows that hexanal treated fruits once ripe will attain the normal or even better quality in terms of flavour and aroma. This agrees with results of Tiwari and Paliyath (2011), who reported that hexanal treatment caused up-regulation of various genes involved in the aroma synthesis pathway.

#### **5.6.** Conclusion

The results of this study have demonstrated the important role of hexanal in temporarily delaying fruit softening and ripening process in general. The physical and physiological data showed that fruits treated with exogenous ethylene ripened faster followed by the controls then those treated with either hexanal or 1-MCP with no significant differences between the two. Quantitative PCR which focused on genes involved in ethylene biosynthesis and softening showed that hexanal treatment delayed the expression of four genes coding for different cell wall degrading enzymes which are xyloglucan endotransglucosylase (XET), Pectin Lyase (PL), Pectin Methylesterase (PME) and Polygalacturonase (PG) as compared to fruits treated with ethylene and the controls. However, expression of these four cell wall degrading genes occurred 2- 4 days earlier in the hexanal treated fruits as compared to those treated with 1-MCP in both the peel and pulp. Transcriptomic data which focused on hexanal and ethylene treated fruits as well as the control showed that hexanal treatment

transiently suppressed the expression of various ripening related genes at day one and four of storage. Phospholipase D gene involved in cell membrane degradation as well as genes coding for key enzymes in cell wall degradation including xyloglucan endotransglucosylase, Expansin, Pectate Lyase and Polygalacturonase were suppressed by hexanal treatment. Results of qPCR showed that expression of 1-Aminocyclopropane-1-Carboxylic Acid Oxidase gene (ACO gene), a key enzyme in ethylene biosynthesis was suppressed till day 8 and 22 of storage in the hexanal and 1-MCP treated fruits, respectively. This shows that the suppression of ACO gene by hexanal is temporal and weaker compared to that of 1-MCP. Later on, at days 18-24 of storage genes involved in ethylene biosynthesis, cell wall and cell membrane degradation, aroma synthesis and glucose synthesis were induced, an indication that hexanal treatment does not impair ripening process as well as quality attributes at the end of storage

## CHAPTER SIX

#### 6.0 GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

#### **6.1 General Discussion**

Banana crop is widely grown in the country and plays a key role in Kenya's economy and food security that is in tandem with 2030 vision of the country. However, bananas' full potential remains unexploited despite various initiatives to support fruit production and value addition in the country (FAO, 2014). This is attributed to various constraints along the value chain such as disease and pest attack, poor crop husbandry, low soil fertility, high post-harvest losses and poor marketing infrastructure (Acharya and Mackey, 2008). High post-harvest losses has been highlighted as one of the factors contributing to unsustainability of food systems. In addition, high post-harvest losses contributes to food insecurity as it affects availability and access to food for the smallholder farmers involved. To address this challenge of high post-harvest losses, various post-harvest technologies have been developed. These technologies are used to preserve the postharvest quality by slowing down deteriorative processes that lead to loss of quality after harvest. Hexanal, is one of the applicable technologies that has shown great potential to enhance fruits shelf-life without compromising on quality. This study was therefore, conducted to evaluate the effectiveness of hexanal in enhancing the shelf-life and post-harvest quality of banana fruits in Kenya.

The first objective of this study sought to establish the effective concentration, treatment duration and best application method of hexanal in banana fruits. Application of 2% or 3% hexanal as a preharvest spray significantly (p<0.05) delayed time to fruit harvesting by 12-18 days depending on the variety and agro ecological zone of production. The observed effect of production zone in hexanal response could be attributed to the impact of hexanal treatment coupled with the cool temperatures in AEZ II which might have led to the longer time (18 days) taken by the fruits to reach harvest maturity as compared to 12 days in the hotter AEZ IV. Duration of hexanal application significantly (p<0.05) affected hexanal efficacy in the two varieties studied. Applying hexanal as a post-harvest dip for 5-minutes was more effective compared to a 2.5 minutes dip in both varieties. This could be attributed to banana fruit, thick and fibrous peel which might have affected the penetration of hexanal solution. Consequently, the longer period of exposure to hexanal for 5 minutes probably permitted sufficient penetration of the treatment solution compared to 2.5 minutes resulting to better effectiveness. This concurs with observation of Venkatachalam *et al.* (2018) in banana and Hutchinson *et al.* (2018) in papaya fruits.

The second objective sought to establish the effect of hexanal treatment on the post-harvest quality of banana fruits. For commercialization of any post-harvest technology including hexanal, it should enhance the fruits' post-harvest shelf-life without compromising the quality. Findings of this study shows that, hexanal treatment significantly ((*p*<0.05) slowed down the rate of increase of the various quality parameters analysed. However, at the end of storage period, the hexanal-treated fruits ultimately attained the desirable quality characteristics comparable to the untreated control fruits. Additionally, hexanal-treated fruits retained high levels of vitamin C which is associated with good nutritional quality as compared to the untreated controls. This could be as a result of reduced enzymatic oxidation by hexanal as reported by Paliyath and Padmanabhan, (2019). Further, sensory analysis results showed no significant differences in the general acceptability, aroma, peel color, texture and mouth feel between the hexanal-treated and untreated (control) fruits.

The third objective, sought to elucidate the molecular basis of hexanal's mode of action in delaying ripening in banana fruits. Most post-harvest technologies applied to preserve postharvest quality of fruits works by slowing down ethylene synthesis and/or action. However, the current research explored other mechanisms such as membrane degradation and cell wall softening that occur during fruit ripening. Results of this study show that hexanal treatment transiently suppressed Phospholipase D (PLD) genes. However, later on in storage as the treated fruits commenced ripening, PLD genes were induced. This is a unique characteristic of hexanal which unlike other treatments used to preserve quality does not completely stop the ripening process with no possibility of reversing the arrested reaction. Reversing the action of post-harvest treatments that slow down or inhibit ripening is necessary for quality and general consumer acceptability. Further, genes coding for various cell wall softening enzymes such as Polygalacturonase (PG), xyloglucan endotransglucosylase (XET), Pectin Lyase (PL) and Pectin Methylesterase (PME) were also transiently suppressed following hexanal treatment. Additionally, hexanal treatment caused transient suppression of 1-Aminocyclopropane-1-Carboxylic Acid Oxidase (ACO) and 1-Aminocyclopropane-1-Carboxylic

Acid Synthase (ACS) genes involved in ethylene biosynthesis pathway. This concurs with a previous study by Tiwari and Paliyath, (2011), who reported that hexanal is a weak inhibitor of ethylene.

## **6.2 General Conclusion**

From this study, it can be concluded that, efficacy of hexanal is affected by variety, mode of application used and duration of application. Best results were obtained in 'Grand nain' variety when using post harvest dip mode of application which enhanced fruits' shelf life by 9 days. Best results when using hexanal as a pre-harvest spray, were obtained when applied twice at 30 days and 30 + 15 days before harvest.

Overall, this study revealed that the use of hexanal did not have any negative effect on the postharvest quality parameters analyzed. Infact, better results were observed in the hexanal-treated fruits when compared to the controls in some parameters such as vitamin C. This is further supported by transcriptome analysis results, which showed up-regulation of genes involved in various quality biosynthetic pathways in hexanal treated fruits later on in storage (day 18-24).

Results from this study have shown that hexanal's mode of action in banana fruits is by temporal suppression of genes involved in ethylene biosynthesis, cell membrane degradation and cell wall softening. The specific genes are; ACS and ACO genes involved in ethylene biosynthesis pathway, phospholipase D genes (PLD) involved cell membrane deterioration, xyloglucan endotransglucosylase (XET), Expansin, Pectate Lyase (PL) and Polygalacturonase (PG) genes involved in cell wall degradation.

## **6.3. Recommendations**

The following are specific recommendations to farmers, extension officers and retailers;

1. Hexanal can be applied as either a pre-harvest spray or post-harvest dip at 2% or 3% depending on the user's convenience. A double pre-harvest spray at 30 and 15 days before harvest is recommended for both small scale and large scale farmers to delay time to fruit harvesting and hence extend the marketing period for their fruits. This is because its more convenient, and easy to apply hexanal as a spray while the crops are still in the field. This will

save farmers on labor cost which is incurred incase of post-harvest dip which involves dehanding the fruits before dipping owing to the large volumes of fruits involved. However, for banana traders, a post-harvest dip for 5-minutes is recommended. Most of the traders handle manageable volumes of banana fruits, which can be dehanded before dipping them in hexanal. This will significantly delayed the rate of fruit ripening and the traders will have ample time to market their fruits without any losses from fast ripening and rotting.

The following are recommendations for further research;

- This study tested only two methods of hexanal application namely; post-harvest dip and preharvest spray. However, hexanal is a volatile compound which can also be applied as a vapor. To this extent, a recent formulation of hexanal in vaporizable satchets has been developed. It is therefore, necessary to test the efficacy of hexanal when used as a vapor which is a more convenient option during long distance transportation of bananas and during storage by traders.
- 2. Hexanal efficacy varied between the two varieties tested with shelf-life being extended by 9 days in 'Grand nain' variety as compared to 6 days in 'Sweet banana'. It is therefore necessary to conduct wide-scale trials in the various local banana varieties and other fruits inorder to commercialize its use in Kenya.
- 3. Banana fruits have a short shelf life once they ripen. Hence, low temperature storage has been incorporated into the post-harvest storage systems for enhanced shelf-life. However, best results have been obtained when banana fruits are stored at temperatures above 13°C due to its sensitivity to chilling injuries. The current study was conducted at ambient room temperature and hexanal extended banana shelf-life by approximately 6-9 days depending on the variety. However, it is anticipated that the effect would have been greater under cold storage. It is therefore, recommended that further studies be conducted to compare hexanal effectiveness under cold storage and ambient room temperatures.
- 4. The current study focused majorly on the expression profiles of genes involved in the ethylene biosynthesis, membrane degradation and cell wall softening pathways. Extensive research is recommended to study the different metabolic pathways of all the other genes identified

through RNA sequencing. This will help in fully understanding the molecular mechanism underlying hexanal mode of action in delaying banana ripening.

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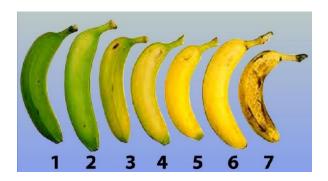
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## **APPENDICES**

## Appendix 1. Banana ripening chart



## Appendix 2. Sensory evaluation questionnaire for banana fruits

Name......Gender...... Age group.....

(A= 19-24 years, B= 25-29 years, C= 30-35 years, D= Above 35 years)

You are provided with coded samples of banana fruits. Please score the samples from 1-5 according to the scale provided below by filling in the table against each sample attributes.

## **STATION 1**

- 1. Dislike (worst)
- 2. Dislike moderately
- 3. Neither like nor dislike
- 4. Like moderately
- 5. Like extremely (Best)

## STATION 1

SAMPLE	COLOR	TEXTURE	TASTE/FLAVOR	AROMA	MOUTHFEEL	GENERAL
CODE						ACCEPTABILITY
Ksb1						
Ksb2						

## **STATION 2**

- 1. Dislike (worst)
- 2. Dislike moderately
- 3. Neither like nor dislike
- 4. Like moderately
- 5. Like extremely (Best)

SAMPLE	COLOR	TEXTURE	TASTE/FLAVOR	AROMA	MOUTHFEEL	GENERAL
CODE						ACCEPTABILITY
Rsb 1						
Rsb 2						

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Treatments	4	5316.56	1329.14	49.95	<.001
AEZ	1	5767.99	5767.99	216.77	<.001
Variety	1	1545.17	1545.17	58.07	<.001
Days	10	39421.68	3942.17	148.15	<.001
Treatments.AEZ	4	2 <b>37</b> .6	59.4	2.23	0.065
Treatments.variety	4	62.77	15.69	0.59	0.67
AEZ.variety	1	538	538	20.22	<.001
Treatments.Days	<b>3</b> 6	4085.64	113.49	4.27	<.001
AEZ.Days	9	5714.13	634.9	2 <b>3.8</b> 6	<.001
Variety.Days	8	2609.82	326.23	12.26	<.001
Treatments.AEZ.Variety	4	6 <b>9.9</b> 6	17.49	0.66	0.622
Treatments.AEZ.Days	34	900.42	26.48	1	0.48
Treatments.Variety.Days	30	1606.4	53.55	2.01	0.002
AEZ.variety.Days	7	389.59	55.66	2.09	0.044
Treatments.AEZ.variety.Days	24	1125.17	46.88	1.76	0.016
Residual	354	9419.64	26.61		
Total	533	47965.63			

Appendix 3. ANOVA for peel color field

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Application_mode	1	41.24	41.2367	148.82	<.001
Treatments	9	85.18	9.4647	<b>34.1</b> 6	<.001
Variety	1	1.57	1.573	5.68	0.018
AEZ	1	13.54	13.5398	<b>48.8</b> 6	<.001
Days	5	1336.58	267.3161	964.7	<.001
Application mode.variety	1	162.61	162.6098	586.83	<.001
Treatments.variety	9	105.6	11.7335	42.34	<.001
Application mode.AEZ	1	149.31	149.297	538.79	<.001
Treatments.AEZ	9	15.03	1.6702	6.03	<.001
Variety.AEZ	1	116.4	116.4045	420.09	<.001
Application mode.variety.AEZ	1	33.67	33.6725	121.52	<.001
Treatments.variety.AEZ	9	17.74	1.9706	7.11	<.001
Application mode.variety.Days	4	108.35	27.0867	97.75	<.001
Treatments.variety.Days	38	427.77	11.2571	40.63	<.001
Application mode.AEZ.Days	5	36.38	7.2762	26.26	<.001
Treatments.AEZ.Days	41	171.29	4.178	15.08	<.001
variety.AEZ.Days	5	<b>49.7</b> 6	9.9521	35.92	<.001
Mode.treatment.variety.AEZ	4	37.29	9.3237	33.65	<.001
Treatments.variety.AEZ.Days	37	80.38	2.1723	7.84	<.001
Residual	410	113.61	0.2771		
Total	650	5159.97			

Appendix 4. ANOVA for ethylene production levels

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Application mode	1	5193.81	5193.81	1165.87	<.001
Treatments	9	2218.047	246.45	55 <b>.3</b> 2	<.001
Variety	1	5152.859	5152.859	1156.68	<.001
AEZ	1	920.588	920.588	206.65	<.001
Days	5	<b>37018.34</b> 6	7403.669	<b>1</b> 66 <b>1.9</b> 2	<.001
Application mode.variety	1	7.484	7.484	1.68	0.196
Treatments.variety	9	814.433	90.493	20.31	<.001
Application mode.AEZ	1	0.615	0.615	0.14	0.71
Treatments.AEZ	9	942.433	104.715	23.51	<.001
variety.AEZ	1	4.819	4.819	1.08	0.299
Application mode.variety.AEZ	1	130.499	130.499	29.29	<.001
Treatments.variety.zone	9	507.029	56.337	12.65	<.001
Application mode.variety.Days	4	11932.53	2983.132	669.63	<.001
Treatments.variety.Days	39	20970.907	537.716	120.7	<.001
Application mode.AEZ.Days	5	2623.673	524.735	117.79	<.001
Treatments.zone.Days	41	4783.817	116.678	26.19	<.001
variety.zone.Days	5	3616.854	723.371	162.38	<.001
Mode.variety.AEZ.Treatment	4	3037.669	759.417	170.47	<.001
Treatments.variety.zone.Days	37	2305.278	62.305	13.99	<.001
Residual	<b>41</b> 2	1835.412	4.455		
Total	653	155995.782			

Appendix 5. ANOVA for respiration rate

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Mode_of_application	1	99.93	99.93	2.73	0.099
Treatments	9	595.8	66.2	1.81	0.063
Variety	1	176.1	176.1	4.81	0.019
Zone	1	217.81	217.81	5.95	0.045
Mode_of_application.variety	1	492.01	492.01	13.43	<.001
Treatments.variety	9	199.53	22.17	0.61	0.793
Mode_of_application.zone	1	<b>93.1</b> 6	93.16	2.54	0.111
Treatments.zone	9	120.31	13.37	0.37	0.952
variety.zone	1	0	0	0	0.997
Mode_of_application.variety.zone	1	2.97	2.97	0.08	0.776
Treatments.variety.zone	9	163.59	18.18	0.5	0.878
Residual	1212	44385.28	<b>3</b> 6.62		
Total	1259	45766.03			

Appendix 6. ANOVA for cumulative weight loss

# Appendix 7. ANOVA for peel firmness

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Application mode	1	15835.925	15835.93	7805.77	<.001
Treatments	9	7256.211	806.246	397.41	<.001
Variety	1	<b>3</b> 55 <b>4.88</b> 6	<b>3</b> 55 <b>4.88</b> 6	1752.26	<.001
AEZ	1	9.194	9.194	4.53	0.034
Days	5	<b>88758.3</b> 2	17751.66	8750.07	<.001
Application mode.variety	1	1220.314	1220.314	601.51	<.001
Treatments.variety	9	152.829	16.981	8.37	<.001
Application mode.AEZ	1	268.206	268.206	132.2	<.001
Treatments.AEZ	9	48.767	5.419	2.67	0.005
Variety.AEZ	1	97.409	97.409	48.01	<.001
Application mode.variety.AEZ	1	114.857	114.857	56.61	<.001
Treatments.variety.AEZ	9	121.071	<b>13.4</b> 52	6.63	<.001
Application mode.variety.Days	5	2557.809	511.562	252.16	<.001
Treatments.variety.Days	40	<b>998.8</b> 62	2 <b>4.97</b> 2	12.31	<.001
Application mode.AEZ.Days	5	221.572	44.314	21.84	<.001
Treatments.AEZ.Days	41	395.855	9.655	<b>4.7</b> 6	<.001
Variety.AEZ.Days	5	816.645	163.329	80.51	<.001
Mode.variety.AEZ.Treatment	4	442.238	110.559	54.5	<.001

Total	665	111816.33			
Residual	419	850.044	2.029		
Treatments.variety.AEZ.Days	39	215.93	5.537	2.73	<.001

# Appendix 8. ANOVA for pulp firmness

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Application mode	1	10145.44	10145.44	6385.39	<.001
Treatments	9	3114.97	346.11	217.84	<.001
Variety	1	16.31	16.31	10.26	0.001
AEZ	1	154.97	154.97	97.54	0.006
Days	5	44271.29	8854.26	5572.73	<.001
Application mode.variety	1	87.61	87.61	55.14	<.001
Treatments.variety	9	156.41	17.38	10.94	<.001
Application mode.AEZ	1	130.7	130.7	82.26	<.001
Treatments.AEZ	9	105.95	11.77	7.41	<.001
variety.AEZ	1	5.79	5.79	3.64	0.057
Application mode.variety.AEZ	1	10.79	10.79	6.79	0.009
Treatments.variety.zone	9	26.51	2.95	1.85	0.057
Application mode.variety.Days	4	<b>1823.3</b> 6	455.84	286.9	<.001
Treatments.variety.Days	39	798.69	20.48	12.89	<.001
Application mode.AEZ.Days	5	504.31	100.86	63.48	<.001
Treatments.AEZ.Days	41	199.05	<b>4.8</b> 6	3.06	<.001
Variety.AEZ.Days	5	<b>38</b> 6.46	77.29	48.65	<.001
Mode.variety.AEZ.Treatment	4	52.09	13.02	8.2	<.001
Treatments.variety.AEZ.Days	38	162.65	4.28	2.69	<.001
Residual	410	651.43	1.59		
Total	652	56352.93			

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Application mode	1	41842.71	41842.71	1873.99	<.001
Treatments	9	9131.82	1014.65	45.44	<.001
Variety	1	5536.56	55 <b>3</b> 6.56	247.96	<.001
AEZ	1	771.76	771.76	<b>34</b> .56	<.001
Days	5	102185.53	20437.11	915.31	<.001
Application mode.variety	1	1313.79	1313.79	58.84	<.001
Treatments.variety	9	421.84	46.87	2.1	0.029
Application mode.AEZ	1	800.15	800.15	35.84	<.001
Treatments.AEZ	9	209.37	23.26	1.04	0.406
variety.AEZ	1	357.53	357.53	16.01	<.001
Application mode.variety.AEZ	1	73.93	73.93	3.31	0.07
Treatments.variety.AEZ	9	118.78	13.2	0.59	0.805
Application mode.variety.Days	4	2086.03	521.51	23.36	<.001
Treatments.variety.Days	39	1580.26	40.52	1.81	0.003
Application mode.AEZ.Days	5	579.77	115.95	5.19	<.001
Treatments.AEZ.Days	41	1316.3	32.1	1.44	0.044
variety.AEZ.Days	5	523.37	104.67	4.69	<.001
Mode.variety.AEZ.Treatment	4	628.14	157.03	7.03	<.001
Treatments.variety.AEZ.Days	38	576.13	15.16	0.68	0.928
Residual	<b>41</b> 6	9288.5	22.33		
Total	659	143291.74			

# Appendix 9. ANOVA for peel color

# Appendix 10. ANOVA for Total soluble solids

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Application_mode	1	7208.35	7208.35	82.78	<.001
Treatments	5	1648.57	329.71	3.79	0.002
Variety	1	3391.57	3391.57	38.95	<.001
AEZ	1	210.78	210.78	2.42	0.121
Application_mode.variety	1	17.67	17.67	0.2	0.653
Treatments.variety	5	102.06	20.41	0.23	0.947
Application_mode.AEZ	1	9.61	9.61	0.11	0.74
Treatments.AEZ	5	40.82	<b>8.1</b> 6	0.09	0.993
variety.AEZ	1	0.36	0.36	0	0.948
Application_mode.variety.AEZ	1	17.87	17.87	0.21	0.651
Treatments.variety.zone	5	96.94	19.39	0.22	0.953
Residual	<b>3</b> 66	31868.95	87.07		
Total	395	43078.86			

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Application_mode	1	19.8523	19.8523	2191.85	<.001
Treatments	5	1.4717	0.2943	32.5	<.001
Variety	1	<b>3.427</b> 5	3.4275	378.42	<.001
AEZ	1	0.5306	0.5306	58.58	<.001
Application_mode.variety	1	0.2492	0.2492	27.51	<.001
Treatments.variety	5	0.1015	0.0203	2.24	0.05
Application_mode.AEZ	1	0.4992	0.4992	55.12	<.001
Treatments.AEZ	5	0.177	0.0354	3.91	0.002
Variety.AEZ	1	1.8766	1.8766	207.2	<.001
Application_mode.variety.AEZ	1	0.0573	0.0573	6.32	0.012
Treatments.variety.zone	5	0.0825	0.0165	1.82	0.108
Residual	399	3.6139	0.0091		
Total	434	25.3513			

# Appendix 11. ANOVA for Total titratable acidity

# Appendix 12. ANOVA for Vitamin C

Source of variation	d.f.	S.S.	m.s.	<b>v.r.</b>	F pr.
Application_mode	1	0.18	0.18	0.07	0.798
Treatments	5	337.21	67.44	24.39	<.001
Variety	1	46.42	46.42	16.79	<.001
AEZ	1	35.42	35.42	12.81	<.001
Application_mode.AEZ	1	19.08	19.08	6.9	0.009
Treatments.variety	5	12.79	2.56	0.93	0.464
Application_mode.AEZ	1	29.69	29.69	10.74	0.001
Treatments.AEZ	5	32.54	6.51	2.35	0.04
Variety.AEZ	1	2.43	2.43	0.88	0.349
Application_mode.variety.AEZ	1	8.82	8.82	3.19	0.075
Treatments.variety.AEZ	5	22.78	4.56	1.65	0.146
Residual	405	1119.85	2.77		
Total	440	<b>3043</b> .26			

Source of variation	d.f.	<b>S.S.</b>	m.s.	v.r.	F pr.
Application_mode	1	24799.9	24799.9	341.1	<.001
Treatments	5	26190.7	5238.1	72.1	<.001
Variety	1	4971.1	4971.1	68.4	<.001
AEZ	1	2883.2	2883.2	39.7	<.001
Application_mode.variety	1	366.8	366.8	5.1	0.025
Treatments.variety	5	1010.2	202.1	2.8	0.018
Application_mode.AEZ	1	753.8	753.8	10.4	0.001
Treatments.AEZ	5	936.7	187.3	2.6	0.026
Variety.AEZ	1	55.8	55.8	0.8	0.381
Application_mode.variety.AEZ	1	548.8	548.8	7.6	0.006
Treatments.variety.AEZ	5	1314.3	262.9	<b>3</b> .6	0.003
Residual	399	29010.9	72.7		
Total	434	131733.7			

# Appendix 13. ANOVA for fructose

# Appendix 14. ANOVA for glucose

Source of variation	d.f.	<b>S.S.</b>	m.s.	v.r.	F pr.
Application_mode	1	12695	12695	7.32	0.007
Treatments	5	<b>4398</b> 6	8797	5.07	<.001
AEZ	1	<b>178</b> 6	<b>178</b> 6	1.03	0.311
Variety	1	9863	9863	5.69	0.018
Application_mode.AEZ	1	235	235	0.14	0.713
Treatments.AEZ	5	2076	415	0.24	0.945
Application_mode.variety	1	608	608	0.35	0.554
Treatments.variety	5	7207	1441	0.83	0.528
AEZ.variety	1	528	528	0.3	0.581
Application_mode.AEZ.variety	1	65	65	0.04	0.847
Treatments.AEZ.variety	5	1643	329	0.19	0.967
Residual	<b>40</b> 2	697274	1735		
Total	431	762042			