TITLE: EFFICACY OF HEXANAL TREATMENT ON THE PHYSICAL AND BIOCHEMICAL ATTRIBUTES AND SHELF LIFE OF MANGO (*Mangifera indica L.*) FRUITS.

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DECLARATION

This thesis is my original work and has not been submitted for award of a degree in another university.

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LIST OF ABBREVIATIONS

ACC – Amino cyclopropane carboxylic acid

AEZ – Agro ecological zone

ANOVA – Analysis of variance

EFF – Enhanced Freshness Formulation

GDP- Gross domestic product

GRAS- Generally recognized as safe

HCD- Horticultural crops directorate

HPLC – High performance liquid chromatograph

LSD – Least significant difference

MAP – Modified atmosphere packaging

MCP – Methyl cyclopropene

MOA – Ministry of Agriculture

RID – Refractive index detector

SSC – Soluble solids content

TTA – Total titratable acidity

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ABSTRACT

Mango is a perishable fruit with a limited shelf life once ripe, resulting in significant postharvest losses. The objectives of this study are to determine the efficacy of hexanal treatment in prolonging the shelf life of mango fruits and to determine the effects of hexanal treatment on the physical and biochemical attributes of mango. A laboratory experiment was performed to determine the efficacy of hexanal treatment in different concentrations as a postharvest dip on the physical quality and extension of storage life of mangoes and its interaction with variety and agro ecological zone of production. Further studies were carried out to determine the effects of hexanal treatment on the biochemical attributes of mango. The study was done at two agro ecological zones namely Machakos (AEZ IV) and Meru (AEZ II) and on two varieties namely Apple and Tommy atkins. Fruits were harvested at mature green stage, cleaned, sorted and divided into several batches. A formulation of hexanal also known as Enhanced Freshness Formulation (EFF) was used at two concentrations 2% and 3% as a postharvest dip to treat the mangoes in the laboratory and observed under ambient room temperatures throughout the ripening process. The study also included untreated fruits which were only dipped in plain water to act as control. Various ripening parameters were evaluated at 3 day intervals to determine the effects of the EFF treatments. These parameters include: physiological loss of weight, colour, firmness, ethylene evolution rate and respiration rate. Additionally, Samples from all treatments were taken and refrigerated and later evaluated for several ripening biochemical parameters measured namely, brix, ascorbic acid content, total titratable acidity, Beta carotene and simple sugars (glucose, fructose and sucrose).The results showed that fruits treated with the EFF exhibited slowed ripening rate as compared to untreated fruits by 3 days in the Apple variety and 5 days in the Tommy Atkins variety. Hexanal treatment slowed down the rate of

cumulative weight loss by 5% -6%. It also delayed the drop in hue angle by 3-6 days as well as delayed drop in both peel and flesh firmness. This is indicative of slowed down ripening process resulting to prolonged shelf life. It was also observed that mangoes treated with 3% EFF had a longer shelf life by 3 days than those treated with 2% EFF indicating that 3% EFF was highly effective in prolonging the shelf life of the fruits. Hexanal formulation applied as a postharvest dip can therefore be adopted as a solution to reduce postharvest losses and prolong the shelf life of mango fruits for both domestic and commercial use. In all the ripening parameters measured in this study, hexanal treatment was observed to slow down the rate at which the ripening process progressed but did not significantly change the quality of the fruits compared to the untreated fruits. Hexanal treated fruits exhibited a slower ripening rate as well as a higher retention for sugars, vitamin C, Beta carotene and acidity. In some parameters such as TSS, Beta carotene, glucose and fructose content there was no significant difference between fruits from different varieties or even harvested from different zones. However, in parameters such as TTA differences were noted between varieties with Tommy atkins variety recording a higher TTA than apple mangoes. The varietal difference was also noted in sucrose content where apple mangoes had a higher level of sucrose content as compared to the Tommy atkins variety. It was concluded that hexanal treatment indeed prolonged the shelf life of mango fruits without altering the quality and biochemical attributes. Hexanal has been recommended for further studies and later commercialization.

CHAPTER ONE: Introduction

1. Introduction

1.1 Background information

The Agriculture sector contributes 30% of the Gross Domestic Product (HCD, 2018) and is among the major economic activities in the country. It contributes 60% of total export earnings (HCD, 2013) and is ranked the second main foreign exchange earner to the country. Agriculture is important in alleviating hunger and poverty to the poor communities in the society (FAO, 2012).

The horticulture sub-sector is an essential source of income, employment for farmers, traders and investors, government revenue as well as foreign exchange earnings (FAO, 2014). Horticulture has established itself over time as a main sub-sector in the agriculture sector. It has recorded an annual growth rate of 19% and has contributed 33% of the agricultural GDP (HCD, 2018). The most common fruits produced in Kenya in order of value are; bananas (36%), pineapple (21%), mangoes (18%), avocados (5%), pawpaw (5%), passion fruit (3%) oranges (2%), water melon (2%) and tangerines (2%) (HCD, 2018).

Mango (*Mangifera indica L.*) is among the most valued fruits in the subtropics as well as the tropics. Mango is produced commercially in over 90 countries in the world and is consumed in different forms. Mangoes can either be consumed fresh or in processed form (Mathooko *et al.*, 2013; Mujuka *et al.*, 2020). Additionally, the mango fruit has been placed in a valuable position as an income earner for farmers, international market and traders by its high nutritional attributes and its attractive flavour (Rodriguez *et al*., 2012; Bundi *et al*., 2020).

It is estimated that the world produces about 21.5% metric tonnes of mango fruits annually and it increases at an estimated rate of 2.6% annually (Okoth *et al*., 2014). Asia leads in the production of Mango fruits worldwide with 76.8% of total production, while America comes in second with 13.37%. Africa produces 9% while Europe and the Oceanic countries produce 1% (Jahurul *et al*., 2015). Globally, the mango value chain has gained popularity following its ability to offer jobs and its immense contribution to rural economies. This has been a response to the increase in demand for the fruit due to its ability to be value added to make jam, chutneys, pickles, jelly and natural juices (Korir *et al*., 2013; Chappalwar *et al*., 2020). Processing of mango fruits into these products is considered as an improvement of shelf life or enhancing the value of unprocessed mangoes thus minimizing postharvest losses.

Kenya is one of the major producers of mango in Africa (Galán, 2010). Additionally, mango is ranked second after banana in terms of quantity produced as well as total area of production (FAOSTAT, 2015). Statistically, mango production contributes about 5% of the agricultural GDP in Kenya and approximately 2% of the national GDP and employs a significant number of the seasonal work force (Ministry of Agriculture, Livestock and Fisheries, 2018). In 2015, the total area under mango cultivation was 46,363 hectares (ha) and the harvest was about 806,574 metric tonnes (MT), while in 2016 the area under mango production rose to 49,097 ha while the output dropped to 779,146 MT (HCD, 2016). In 2018, the area under mangoes decreased by 4% from 50,550ha in 2017 to 48,541ha (HCD, 2018). However, the value and volume in 2018 increased by 5.8% and 9.7%, respectively, compared to 2017. The leading counties in mango production as ranked by value were Makueni, Machakos, Kilifi, and Kwale, whose contribution to the total value was 24.9, 17.7, 14.6, and 4.7 percent respectively (HCD, 2018). Data from the HCD shows that the volumes of mango fruits exported in the year 2020 reduced by up to 2.2 million kilograms.

In 2019, Kenya exported approximately 9.3 million kilograms of mangoes to different destinations valued at KSh.1. 4 billion compared to 7.1 million kilograms exported in the year 2020 valued at KSh.1.1 billion.

The most common local varieties grown in Kenya include; Dodo, Kasukari, Ndoto, Sikio la punda, Katili, Kitui, Mombasa, while exotic varieties include; Apple, Batawi, sabine, Sensation, Tommy Atkins, Haden, Keitt, Kent, Ngowe, Nimrod, Maya and Van dyke (Toili *et al*., 2013). The fruit is considered a great source of antioxidants, phenolic compounds, ascorbic acid, carotenoids as well phenolic compounds (Talcott *et al*., 2005; Djioua, 2008).

1.1.1 Challenges in mango production

It is a fact that Mango production is a promising and profitable enterprise. However, this high potential is threatened by various challenges with the major one being high postharvest losses which are estimated at 40% (FAO, 2012). The great potential of the mango value chain for expansion and growth remains unexploited. This has been caused by constraints experienced at various stages of the value chain. The main stages in the value chain include; farm level, the marketing stage, the processing stage and the export stage (MOA, 2010). The main constraints faced at the farm level include: low yielding seedlings; poor quality of planting materials, low technological knowledge; lack of proper crop management practices, expensive inputs, oversupply at harvest time which leads to high postharvest losses and bad prices (FAO, 2013). At the marketing stage, farmers face constraints such as: inadequate market information, poor infrastructure and lack of financing to support their activities (MOA, 2010). At this marketing stage, lack of adequate knowledge on the uses and applications of postharvest technologies is in itself a big challenge. In Kenya, only about 7% of the mangoes harvested end up in processing (Marc-Peter, 2015). This is attributed to the low demand locally for dried mangoes and other processed products as well as high competition of mango puree. These challenges have been addressed in the Vision 2030

strategy, second medium term plan (2013 – 2017) which addresses issues surrounding agricultural market access and value addition aimed at enhancing agricultural product development and marketing systems.

Mango fruits are known to be highly perishable and ripen quickly once harvested or being transported. The storage life of mango fruits varies based on varieties and storage environment (Abbasi *et al*., 2009). Mango shelf life usually takes approximately 2 to3 weeks in cold storage and approximately 4 to 8 days at room temperature. (Herianus *et al.,* 2003). Postharvest losses in mangoes can however be reduced by coming up with other ways and measures to enhance the fruits storage life.

1.2 Problem statement

The mango fruit being a living tissue and a climacteric fruit is vulnerable to constant changes during ripening until it totally deteriorates. Postharvest losses in mango in Kenya are estimated at more than 40% (FAO, 2012). Postharvest technologies therefore need to be developed and applied in the mango value chain to reduce these losses. Over time, various post-harvest technologies have been studied and 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 mango include Modified Atmosphere Packaging (Githiga *et al.,* 2014), low temperature storage (Ambuko *et al.,* 2008), 1-Methylcyclopropene (Ricardo *et al.,* 2004; Ambuko *et al.,* 2013), hot water treatments (Mirshekari *et al.,* 2015) among others. However, the adoption rate of these technologies especially by small scale farmers is quite low due to high costs, application difficulties and non-availability (Lorevice *et al*., 2014). While storage in low temperatures is considered one of the most effective methods for extending the shelf life of most perishable commodities after harvest, majority of the small-scale farmers cannot afford the cold storage facilities. The application of 1-Methylcyclopropene (1-MCP) which is a known inhibitor of the action

of ethylene, has been used to prolong the storage life of several fruits especially 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), strawberry (Ku *et al*., 1999), oranges (Porat *et al*., 1999), and apples (Watkins *et al*., 2006). According to Sisler and Serek (1996), the mode of action of 1-MCP is by irreversibly and permanently binding to ethylene receptors which are present in the particular plant tissue. This in turn leads to slow ripening and softening of fruits, thus enabling distribution and prolonging shelf life while maintaining high quality of the fruits for longer (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 some fruits (Fan and Matthesis, 1999).

Modified atmosphere and controlled atmosphere storage have been reported to be effective in enhancing 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, Modified Atmosphere Packaging (MAP) is a simple technology that uses bags made of polythene to keep the fruits fresh for longer. 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). Several types of dip treatments have been tested and adopted in some countries for different fruits.

Various chemicals, such as combined solutions of ascorbic acid, calcium chloride and cysteine (Bico *et al*., 2009), soy lecithin along with natural lysophospholipid (Ahmed and Palta, 2016), phenylurea [CPPU] and gibberellins [GA_3] (Huang *et al*., 2014), salicylic acid (Srivastava and Dwivedi, 2000), potassium permanganate ($KMnO_4$) (Hassan, 2000), 1-MCP (Blankenship, 2001), oxalic acid (Huang *et al*., 2013) and nitrous oxide (N_2O) (Palomer *et al*., 2005) were reported to be effective in reducing the postharvest losses of

fruits during harvesting, transport and storage. In most cases, the chemicals have been found to act as inhibitors of ethylene production thus enhancing the storage life of the fruits. Regardless of the number of technologies and chemicals available for fruit preservation, their adoption is very minimal due to prohibitive costs, application difficulties and non-availability. Limitations of the applicable technologies described above calls for further research in other commercially viable and affordable technologies and innovations to prolong the storage life of mango fruits while maintaining its quality.

1.3 Justification

This study bridges the existing postharvest technologies gap by introducing a naturally occurring organic compound extracted from plants to help in overcoming the constraints that come with fast ripening and senescence in mangoes.

In the recent years, hexanal, which is a naturally occurring six carbon aldehyde resulting from the lipoxygenase pathway following tissue damage (Hildebrand, 1989), has been revealed to improve the quality and storage life of various temperate fruits, such as peaches, cherries, strawberries and nectarines (Sharma *et al*., 2010) and some tropical fruits such as tomato (Cheema *et al*., 2014), banana (Yumbya *et al*., 2020), mango (Anusuya *et al*., 2016), papaya (Hutchinson *et al*., 2018) and limequats (Debysingh *et al*., 2018). Hexanal works by hindering the activity of the phospholipase D enzyme which catalyzes membrane phospholipids hydrolysis and causes deterioration of membranes and thus initiates fruit softening (Paliyath *et al*., 2008). Treatment by Hexanal has been reported to result in keeping cell membranes stable and intact, enabling fruits to remain fresh looking and firm for longer periods of time. The mechanism of hexanal is achieved by hindering the work of the enzyme phospholipase D which catalyzes the membrane phospholipids hydrolysis and triggers break down of membranes causing fruit softening (Paliyath *et al*., 2008). Quantitative PCR focusing on genes associated with ethylene biosynthesis and softening

indicated that treating banana fruits with hexanal delayed the manifestation of four genes coding for different cell wall degrading enzymes which are xyloglucan endotransglucosylase, Pectin Methylesterase, Pectin Lyase and Polygalacturonase (Yumbya *et al*., 2020).

Hexanal has been approved by FDA in the US as a general food additive (US Patent 6,514,914;7,198,81) for use in processed plant-based foods. It is not retained in the treated tissues beyond 48hours of treatment (http:/www.accessdata.fda.gov/). In the human body, hexanal is readily oxidized to hexanoic acid. Like all other alcohols, hexanoic acid is further converted to carbon dioxide and water by the tricarboxylic acid cycle(TCA) during the process of respiration (Kruse *et al*., 2016)

Enhanced Freshness Formulation(EFF) is a biochemical formulation of an artificially synthesized version of hexanal. It is known to slow down the process of ripening in temperate fruits (Sharma *et al*., 2010). There have been successful studies on the use of Hexanal treatment in tomato (Cheema *et al*., 2014), papaya (Hutchinson *et al*., 2018) and Banana (Yumbya *et al*., 2020). Additionally, there has been studies on the efficacy of hexanal on enhancing the storage life of mango as a pre harvest spray in India (Anusuya *et al*., 2016). 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 grown in differing agro-ecological conditions, varietal differences, and mode of action among other factors. Kenya being a major producer of mango, there is a gap in information on the postharvest application of EFF as a dip, the best concentration of the EFF to give optimal results as well as the interaction of hexanal treatments with different varieties and agro-ecological zones of production of mangoes in Kenya. This study therefore focusses on the

use of the EFF as a postharvest dip in mangoes cv. Apple and Tommy Atkins grown at two different agro ecological zones (AEZ II and AEZ IV) in Kenya aimed at prolonging shelf life. Hexanal is easy to use and farmers can start using it with minimal training. It also does not require specialized equipment for application and is used in small quantities making the technology easily applicable to small scale farmers. The adoption and commercialization of hexanal application as a post- harvest technology will reduce losses and increase income to the farmers as well as all the players in the mango value chain.

1.4 Objectives

1.4.1 General objective

The broad objective of this study is to reduce the postharvest losses of mango fruits in Kenya by extension of shelf life through treatment with Hexanal**.**

1.4.2 Specific objectives

The specific objectives of this study include:

1. To determine the efficacy of hexanal, applied at different concentrations as a post-harvest dip, in improving the shelf life of mango fruits of different varieties harvested from two agro ecological zones in Kenya.
2. To determine the effects of Hexanal treatment on the physical and biochemical attributes of Mango fruits of different varieties grown in two different agro-ecological zones in Kenya.

1.5 Hypotheses

1. Hexanal has zero effect on the storage life of mango fruits of diverse varieties grown in contrasting climatic zones in Kenya.

2. Hexanal treatment has zero effect on the physical and biochemical attributes of diverse varieties grown in contrasting climatic zones in Kenya.

CHAPTER TWO: Literature Review

2.0 Literature Review

2.1 Mango Production Statistics

2.1.1 Global mango productions statistics

Reports show that commercial mango production has been reported in about 86 countries worldwide (Sivakumar *et al*., 2011) with China, India, Mexico, Philippines, Pakistan, Thailand and Indonesia, being the main mango producing countries. (Tharanathan *et al*., 2006). In mango export, however, the highest 10 countries are Brazil, Mexico, India, Netherlands, Ecuador, Pakistan, Peru, the Ivory Coast, Thailand and The Philippines. (FAOSTAT, 2016). Mango farming is growing away from the ancient geographical areas of mango production such as South and Central America, Australia, Southeast Asia, Egypt, Israel, Hawaii and South Africa, particularly for export. (Tharanathan, 2006).

2.1.2 Kenya production statistics

The favorite export varieties in Kenya are Apple, Ngowe, Boribo and Kent (ITC, 2015). In 2013, the area under mango production was 44,017 ha where 644, 828 tonnes of fruits were harvested valued at Kshs. 7.67 billion (HCD, 2013). The area under mango was 49,098 ha with a production of 779,147 million tons, valued at Kshs. 11.9 billion in 2016 (HCD, 2018).

In 2020, the area under mangoes increased from 56,090Ha to 63,437ha which was 13 percent increase while production dropped by 91,006 tones a 10 percent drop as compared to 2019. There was a marginal increase in value of 118.9 million which was only 1 percent increase compared to 2019 that could only be attributed to increase in production as the average farm gate prices dropped (Agriculture and Food Authority, 2020).

The leading counties in mango production by value are Makueni (30.4%), Machakos (23.2%), Kilifi (15.5%), Kwale (7.9%), Meru (4.5%), Embu (2.8%), Bungoma (2.1%), Tana

River (1.8%), Elgeyo Marakwet (1.1%), Muranga (1.1%), Tharaka Nithi (1%), Kitui (1%), Siaya (0.9%), Taita Taveta (0.8%), Busia (0.7%) and others (5%) (KARLO, 2019)

Kenya's export market for mango has grown significantly over the years. This export market is mostly around three regions namely: Asia, Latin America and some countries in Africa (Unctad, 2014). Despite the growing export market, Kenya has remained a minor player in the world mango trade, with around only 3% of total national production or 2% of the fresh fruits sold in the global market (ITC, 2012). In 2012, Kenya earned Kshs. 1 Billion ($11.2 Million) from export of mango fruits internationally. Mango fruits exported to the United Kingdom from Kenya increased in value by 154% between the years 2011 and 2012. During that same time, volumes of Kenyan mango exported to Germany improved by 92 % while mango exports to Qatar rose by 68 % (ITC, 2014).

2.2 The Botany of Mango

The mango fruit is a succulent stone fruit belonging to the genus *Mangifera*. There are two documented groups of mango cultivars, namely; Indian and Indian-Chinese. The Indian Chinese cultivars possess polyembryonic seeds and are typically green to light green to yellow when mature while the Indian types possess monoembryonic seeds which are commonly more coloured (Crane *et al*., 1997). Mangoes are now produced in most subtropical and tropical countries.

The mango tree is an evergreen tree, deep-rooted, which grows into big trees, particularly on good soils. The height and canopy shape varies significantly among cultivars. Under

optimum weather conditions, mango trees are erect and grow quite fast. The tree canopy can be either rounded and wide or vertical.

The developed leaves are dark green, glossy, simple and, leathery. The young leaves are usually red or pale green. They are pointed and short regularly measuring more than 30cm in length and about 13cm in width (Salim *et al*., 2002). Fresh leaves are formed in cyclic flushes up to two to three times annually.

Mostly, flowering in Kenya has been seen to last from late July to early November, based generally on climatic circumstances. At the coastal area, it is ordinary to find particular trees flowering from late February to March. Pollinators are typically flies, hardly bees or bats.

Flowers on mango trees are borne on greenish, yellow or pink coloured big terminal or axillary panicles up to 60cm long. Each individual panicle holds about 300-6000 flowers (Lyer *et al*., 1997). Based on the variety, the mango fruits may take three to four months from setting of fruits to maturity (Kader, 2003).

Mangoes from the numerous varieties differ significantly in shape, size, external and internal features. The fruit is a plump drupe, varying in dimension from 25 to 30cm long, may be kidney-shaped, ovate or round and weigh from roughly 200g to around 2000g.

2.3 Mango Varieties

2.3.1 Apple

This variety first originated from the Kenyan coastal area, possibly around the Malindi. The size of fruits varies from medium to large, and have a round shape. They also have a dense yellow orange to red color when fully ripe.

Usually, the skin is quite smooth and thin, and the succulent flesh is of exceptional flavor and of smooth texture free from any fiber. This is a monoembryonic variety whose trees if propagated by seed are extremely heterogeneous in color, shape and quality.

Based on locality, harvesting seasons of mangoes are from late December to March. The yields are high to medium (ICRAF, 2003).

2.3.2 Van dyke

This cultivar originated from Florida in the 1960s. It is known to have resistance to diseases such as anthracnose and has great color as well as a longer shelf life making it easy to transport. The fruits of this cultivar have a thick skin speckled with yellow lenticels. Its flesh possesses an attractive orange yellow color with a pleasant aroma. The seed which is approximately 7% of the total fruit weight is mono- embryonic. The trees are average producers and grow into a big and open canopy (ICRAF, 2003).

2.3.3 Tommy atkins

The Tommy atkins cultivar tree is known to have developed from a Haden seed which was grown in the year 1922 on the orchard of one Thomas H. Atkins from Florida (Campbell, 1992). This tree grows into a huge and round canopy. It is also known for its resistance to diseases such as powdery mildew as well as anthracnose. Its production is high to medium

(Griesbach, 2003). The ripe fruits possess thick and tough skin which is orange to yellow in color. The flesh is firm and quite juicy, possesses a yellow to intense yellow color and a pleasant aroma.

2.3.4Kent

This cultivar whose origin is in Miami; Florida was first grown in the 1940s. The tree is known to have a dense and straight canopy with vigorous growth. The fruits which weigh around 500g are large in size (ICRAF, 2003). The fruit possesses a tough and thick skin which is green yellow in colour with yellow lenticels. The flesh is a little fibrous with a deep yellow color. The seed fixed in a woody, thick stone is also mono-embryonic (ICRAF, 2003).

2.3.5Ngowe

Ngowe variety was first planted in Lamu around 100 years ago and is known to have its origins in Zanzibar (Griesbach, 2003). The trees have a round canopy and are of small size. This variety produces good quality fruits which are easy to transport. However, the variety is prone to powdery mildew and is known to have alternate bearing (Griesbach, 2003). As the fruits ripen, the skin color changes from green to light yellow to orange. The flesh is known to have minimal fibre and is typically deep yellow in color with the seed being polyembryonic (ICRAF, 2003).

2.4 Mango Nutritional Quality

Mango fruit is a great source of vitamin A which is essential for maintenance of a healthy skin. The intake of mango fruits which are known to contain high levels of carotenes is well- known to protect the body from lung and oral cavity cancers. There are diverse phytochemicals categories found in mango fruits such as ascorbic acid, polyphenols and carotenoids (Talcott *et al*., 2004). The Mango fruit flesh has m-coumaric acid, mangiferin,

gallic acid, kaempfero acid among others (Schieber *et al*., 2003). Mango fruits from different varieties have different levels of nutrients and vitamins. Tommy Atkins and Kent varieties are known to contain lower β-carotene levels as compared to other mango types (Ornelas-Paz *et al*., 2006). Fresh mangoes contain high levels of Potassium which helps regulate the rate of heart beat as well as blood pressure in humans. 100g of fresh mango fruits contain around 155mg Potassium (Mervyn, 2000).

Mango fruits also contain about 27.6mg/100g or 46% of the prescribed daily intake level of vitamin C. It has been established that the intake of foods and fruits rich in ascorbic acid helps the human body build up strong resistance against dangerous and communicable diseases. Mango fruits also contain 0.133mg/100g of fruit or 10% of prescribed daily intake levels of Vitamin B6, which is important for Gamma-Aminobutyric acid hormone, produced within the brain (USDA, 2006). Additionally, mango fruits contain copper, which is 0.111mg/100g of fruit. Copper is essential in the production of red blood cells. The mango peel contains phytonutrients, such as carotenoids and polyphenols. Mango fruits are also a great source of thiamine, iron, niacin and calcium (Mervyn, 2000).

2.5 Ecological Requirements for Mango

2.5.1 Temperature

Mango fruits thrive in both tropical and subtropical climate conditions. The trees perform well at the optimum temperatures of between 21°C to 27°C although they can also survive at low temperatures of between 10°C to 65°C (Chako, 1986). The trees do well during a cool dry season with high heat levels throughout flowering and development of fruit. Fruit growth, development and the final quality is significantly affected by growing temperature with the most superior quality occurring in fruits produced in areas with cool non-freezing periods accompanied by an extended dry period just before flowering with quite hot temperature during fruit development. Low and freezing temperatures during the period

when the trees are in full bloom causes the fruit to grow into golf ball size, change color to yellow and then get aborted resulting to reduction in yield (Marsh *et al*., 1999). Some less tolerant cultivars to high temperature and low humidity show symptoms of sun burn when exposed to high temperatures. Lëchaudel *et al.* (2005), established that when exposed to high humidity and high temperature conditions photosynthetic efficiency in mango is significantly reduced. The rate of respiration also increases causing low Carbon accumulation which in turn leads to low yields.

2.5.2 Light environment

Light distribution inside and among the tree canopies has been reported to have a significant effect on the growth and development of fruits (Litz, 2009). Light exposure affects photosynthesis both directly and indirectly (Schaffer *et al*., 2009). When light falls below the thresh hold needed for light saturation during the process of photosynthesis, the resulting drop in available photo assimilates will significantly affect growth and development of the tree (Litz, 2009). A low light level within the canopy reduces the induction of flower buds, the fruit size and development of fruit colour due to crowding within the canopy (Flore, 1994). Mango fruits development and quality are favored when the fruit is exposed to longer period and full sunlight. Mendoza and Wills (1984) reported that soluble solids content and total sugars were low in mango fruits from the low portions of the tree canopy in Kensington mango. Simmons *et al*. (1998) also suggested that light exposure influences fruit colour intensity on the skin and flesh.

2.5.3 Water availability

Mango trees are known to tolerate quite a wide range of climate conditions. Mango trees can also thrive in swampy conditions for long periods of time (Villiers, 2008; KARI, 2009). The crop requires a distinct dry season of 3 months for flower induction and for best bearing. Low rainfall (<500mm/year) will restrict fruit yields while high rainfall

(>2000mm/year) can impact vigorous vegetative growth leading to biennial bearing. The preferred Mean annual rainfall is between 400 mm-2000mm per annum. High humidity during the flower development and fruit setting period, predisposes the tree to fungal diseases and eventually causes abortion of flowers and fruits (Davenport, 1997).

2.5.4 Altitude

Mango trees grow and produce within a wide range of altitudes from sea level up to an altitude of 1200 meters in the tropics. Mango trees can be grown up to elevations of 1300 meters above mean sea level. However, it has been established that commercial production is common in regions below the altitude of 600m above sea level (Mukherjee and Litz, 2009). Varieties like Apple and Ngowe grow well in areas below 1000 m above sea level (Griesbach, 1997).

2.5.5 Soils

Mango requires fertile, well drained sandy or loamy soil with less water logging that drain out easily after the rainy season, allowing the trees into a dormant period which is necessary for heavy flowering (Litz, 1997). A pH between 5.5 and 7.5 is preferred although mango have some tolerant of alkalinity.

2.6 Mango Fruit Growth and Maturation

2.6.1 Fruit growth

For mango trees to flower well, they require mature stems(terminals) as well as a quiescent and resting period of time which can be induced by either dry conditions or cool and non-freezing temperatures (Jules, 2008). The rate of growth and the fruit size is primarily determined by cell enlargements and cell division. Most fruits such as banana, strawberry,

mango and avocado have been found to show a single sigmoid pattern of growth throughout the season. This means that if the fruit growth rate is plotted in a graph against time, the rate of growth is slow at the beginning, increases in a rapid linear version but then drops while approaching maturity (Tadesse, 1997).

2.7 Factors Affecting Maturation of Fruits

2.7.1 Climatic conditions

Kenya is categorized into seven agricultural zones based on moisture index which is calculated by expressing annual rainfall as a % of evaporation potential (Sombroek *et al*., 1982). Zones whose moisture index is greater than 50 percent are categorized as zones I, II and III. These zones cover 12% of Kenya's total area and possess high cropping potential. On the other hand, zones with moisture indices of less than 50% are referred to as Kenya rangelands and are categorized into zones IV, V, VI, and VII. These semi-humid to arid areas have a mean annual rainfall of less that 1100mm and cover 88% of the total land area.

Each of Kenya's agricultural zones is sub-divided based on the mean annual temperature to determine suitability to produce major food and cash crops. The majority of high production areas are situated above 1200m above sea level with mean annual temperatures of less than 18°C, whereas about 90% of the semi-arid and arid areas are located below 1260 m with mean annual temperatures ranging from 23° C to 40° C.

Mango can be grown in a variety of conditions throughout Kenya, ranging from sub-humid to semi-arid (Kehlenbeck *et al*., 2010). The ideal growing temperature for mango is 24°C–27°C. Extreme temperatures have an impact on the mango tree's and fruit's growth rates. The trees can also flourish in a variety of rainfall conditions. The preferred annual rainfall range is 400 mm-2000 mm (Griesbach, 1997). Mango trees do not require nutrient-rich soils, but they do need to be deep and well-drained. The tree can thrive in pH levels ranging from 5.5 to 7.5.

Too much acidity is detrimental to growth. Mangoes mature at different times depending on the variety and the weather conditions (ICRAF, 2003).

Temperature plays a role in a variety of processes during fruit development, especially at the sink level (Léchaudel *et al*., 2005). In apples and pears, high temperatures are thought to produce early maturity compared to low temperatures (Lasko *et al*., 1995). Mangoes of the Apple and Ngowe varieties produced in two locations with varying agro ecological zones (AEZs) (high and low temperatures), were found to ripen at different times. The AEZ conditions were also found to influence the final attributes of the mangoes (Ouma, 2015). According to studies, high temperatures shorten fruit development time in other fruits (Marsh *et al*., 1999).

The amount of light that reaches the fruit-bearing branch depends on its location within the tree's canopy. Farquhar *et al*. (1980) found that light had a direct effect on the rate of electron flow and a less direct effect on leaf photosynthetic capacity. Carbon assimilation is lower in lower leaves. If the carbon supply is low, the pace of fruit growth slows (Hofman *et al*., 1995).

The amount of water deficit, the duration of the lack, and the stage of growth in which the stress occurred can all be used to explain water stress in mango trees. The size of mango fruits has been found to be affected by this (Simmons *et al*., 1995). Water stress reduced fruit growth rate and final fruit size if irrigation was discontinued between flowering and mid-growing time, according to Simmons *et al*. (1995). After maturity, water stress has little effect on the pace of growth or the final size of the fruit.

2.7.2 Cultural practices

Nutrient management, watering, pest and disease control, thinning and pruning are some of the cultural practices in mango production. Thinning and pruning are critical for reduction of

fruit load, increase of the fruit size and allowing water and air circulation (Kader, 2003). The pruning should be done as soon as possible after harvest.

Reduced water availability on mango growing between flower development and the mid-growing period, according to Simmons *et al*. (1995), causes water stress, which reduces cell number. Irrigation is essential to mango trees four years and older during extended dry seasons, as it promotes growth and high yields.

Since majority of the essential cultural activities are not implemented, the crop is reliant on the soil nutrients contained naturally in the soil. Most of the mango fruit orchards are usually neglected, which as a result allows pests and diseases to thrive. Mango fruit is also attacked by fruit flies, which degrades the quality (Kader, 2003). Although fertilizer addition is necessary for vegetative development, too much fertilizer might impair flowering and fruit set (Griesbach, 1997).

2.7.3 Varietal differences

Mango varieties differ primarily due to mutation of genes. They are sensitive to various climatic factors and thus adaptable over a large area (Chakrabarti, 2011). Mangoes can either be propagated vegetatively or by seed. Seedlings are planted to create new cultivars, serve as rootstocks, or reproduce existing polyembryonic cultivars. Mono-embryonic cultivars, on the other hand, require vegetative propagation in order to retain all of the desired traits (Griesbach, 1997).

2.8 Maturity indices for fruits

Changes in fruit parameters can be used to predict mango maturity; however, these factors differ with variety, region of cultivation, market and consumer. A range of physical, physiological, biochemical and chronological maturity indices have been used to determine commodity maturity. Physical characteristics of fruits and vegetables, such as form, size, and

surface attributes are widely utilized as ripeness indices.

In bananas, maturity is measured by measuring the diameter of the fingers whereas in some melons, such as honeydew, variations in the surface gloss or feel (waxiness) are utilized as a practical tool in harvesting.

The color shift that happens when many fruits mature is also often utilized as a maturity indicator. Through the human eye, color can be utilized to subjectively measure mango maturity. However, because the human eye is incapable of evaluating a single color, color comparison techniques are widely employed to determine fruit maturity.

Fruit softens as it matures, whereas over-mature vegetables become fibrous and rough. These textural characteristics can be used to assess ripeness. The chemical composition of the fruits changes during maturation and they can also serve as indices of maturity. In most fruits, the soluble solid content (SSC) increases as the total titratable acidity (TTA) decreases. The SSC/TTA ratio affects taste that characterizes some cultivars and their maturity. To be utilized as a commercial maturity measure for mangoes, these chemical criteria are supplemented by other indices such as shoulder formation and flesh color (Mizrach *et al*., 1999).

Furthermore, as determined by changing patterns of ethylene evolution and respiration rate, maturation of commodities is linked to changes in their physiology. These qualities are also used to determine maturity. However, because of the wide variation in absolute rates of respiration and ethylene production across similar individuals of the same commodity, its use as a maturity gauge has been limited. On a commercial scale, the procedures are also complicated and costly to apply. Nonetheless, some growers use the rate of ethylene generation in a sample of apples to determine the apples' maturity. For several fruits, such as bananas (Ahmed, 1998), guava (El-Buluk et al., 1995), melons (Ahmed, 2009), and strawberries, the number of days from blooming and fruit set to harvest has been found to be

a suitable non-destructive maturity metric (ElMasry *et al*., 2007). Days after bloom, on the other hand, vary by variety and geographical region.

2.9 Maturity indices for mango

Mango maturity indices are challenging to develop due to the variety of cultivars and growing circumstances (Ouma, 2015). Mango is traditionally collected by producers based on the appearance of the fruit (Yahia, 1994). These observational maturity indexes, however, are subjective because they differ between persons and locations. The choice of appropriate ripeness indices for harvest is critical since it impacts mango fruit quality and postharvest life (Ouma, 2015). According to Jha *et al*. (2006), physical characteristics such as color, size and firmness can be used to predict mango ripeness. Chemical standards have also been employed to determine mango harvest maturity. Total titratable acidity, TSS, pH, reducing sugars, acid/sugar ratio, volatile compounds, tannins, ascorbic acid, internal flesh color and oil content are among them (Abbasi *et al*., 2009). Late-harvested fruits were sweeter and had a distinct volatile profile than earlier-harvested fruits, according to solids and acids data (Lebrun *et al*., 2008). Mangoes harvested 100 days after flowering developed greater organoleptic qualities than those collected earlier, according to Abbasi *et al*. (2009). In mango harvesting (Abbasi *et al*., 2009), skin color was used as a maturity index as well as in citrus (Ahmed, 2009). In mango, the number of days from flowering to fruit set to harvest was discovered to be a suitable non-destructive maturity index (Lebrun *et al*., 2008; Slaughter, 2009).

Harvest maturity and ripening ability of climacteric fruit, as well as maturity indices, are affected by climatic changes between cropping seasons and production localities. Fruit flesh firmness decreases more quickly in high temperatures (Lotze and Bergh, 2005). Total soluble

solid levels in mango are increased by high accumulated heat units before harvest due to high photosynthesis rates as well as increased carbohydrate reserves (Lotze and Bergh, 2005). The acid and sugar content of mangos during long cold storage periods was found to be influenced by daily hourly average temperatures occurring over the last six weeks prior to harvest (Ferguson *et al*., 1999). Increased light exposure enhances fruit size, total soluble solids, and flesh firmness (Tahir *et al*., 2007).

2.10 Changes in Quality Attributes Associated with Mango Ripening

Ripening is the process of a fruit's biochemical and physiological changes to achieve the desired eating characteristics of color, taste, and flavour. Ripening occurs when a fruit reaches full maturity, and a completely matured mango fruit will ripen even after harvest (Bender *et al*., 2000). The ripening of mango fruit involves a number of chemical and physiological changes.

2.10.1 Changes in color

Depending on the variety, the skin color of mango fruit can vary from green to yellow to orange as it ripens (Jha *et al*., 2007). In ripe fruit, most types lose their green color, while some keep it. In most cultivars, the flesh color varies from green to yellow to orange (Yahia, 2009). Some mango types, such as Tommy Atkins, develop a yellow color during ripening due to a loss of chlorophyll and an increase in carotenoids (Medlicott *et al*., 1988). Chloroplasts experience considerable disorganization, which is associated to the production of huge osmiophilic globules, according to Medlicott *et al*. (1988).

2.10.2 Changes in firmness

Fruit firmness decreases during ripening as the structure of the cell wall's pectin polymers changes, eventually stabilizing to indicate the conclusion of the ripening process (Kalra *et al*., 1995). According to Hosakote *et al*. (2006), mango ripening is followed by a series of

metabolic changes that cause slow softening. Mango fruit softens due to increased cell wall solubility (Nasrijal, 1993). Mango ripening begins in the inner mesocarp tissue and progresses outwards, resulting in decreased tissue stiffness (Lazan *et al*., 1993).

2.10.3 Changes in flavour

The fruit flavour is created by the balance of sugar content and organic acids (Medlicott *et al*., 1985). Flavor is a crucial qualitative feature that determines the fruit's customer acceptability (Dharini *et al*., 2010). Flavor, according to Baldwin, 2010, is made up of taste and odour, and is primarily made up of sweetness, fragrance, and sourness, which correspond to sugars, volatile chemicals and acids. The aroma of mango fruit is attributed to changes in fatty acid profile during ripening (Dharini *et al*., 2010). Mango fruit aroma emerges during ripening and is caused by the development of numerous volatile chemicals.

2.10.4 Ethylene production

As the fruit matures, ethylene production decreases and increases as the fruit ripens (Akamine *et al*., 1973). Mango is a climacteric fruit which means that the rate of ethylene evolution increases drastically during the ripening process, reaches a peak level then drops significantly. Ethylene causes mango fruit to ripen, which is an irreversible process that improves the eating quality.

2.10.5 Changes in soluble sugars

The main compositional alteration associated to mango flavour is sweetness. As the starch content of the mango fruit is hydrolyzed to simple sugars, soluble sugars rise (Ito *et al*., 1997). On a flesh-weight basis, mature mango contains 10–20 percent sugars, but this varies depending on mango type and ripening stage. At the start of ripening, reducing sugars have the maximum sugar content. In ripe fruit, non-reducing sugars account for 18%, whereas reducing sugars account for only 4%. During ripening, starch that accumulates in the

chloroplast as a fruit matures is entirely hydrolyzed to simple sugars (Kumar *et al*., 1994). After ripening, starch is nearly undetectable in mangoes, but sucrose increases dramatically while fructose increases just slightly (Ouma, 2015).

2.10.6 Changes in vitamins

Vitamins like ascorbic acid, K, folic acid, BI and B2 have been found in several ripe mango cultivars (Tharanathan *et al*., 2006). Ascorbic acid is the most important vitamin for human nutrition. Because of its antioxidant and curative effects, it is an important food component (Okiei *et al*., 2009). Vitamin C level decreases when mango fruit ripens (Mamiro *et al*., 2007). This is due to vitamin C's sensitivity to oxidative degradation during ripening, according to Aina (1990). Unripe fruits have more ascorbic acid than ripe fruits, but this increases with temperature, ripening, and exposure time.

2.10.7 Changes in β-carotenes

As mango fruits get closer to maturity and ripening, their total carotenoids concentration rises (Joao *et al*., 2010). In most mango types, β -carotene is said to account for 60% of the total carotenoid (Tharanathan *et al*., 2006). β -carotene build-up in mango fruits has been previously highlighted in terms of interrelationships with flesh color and vitamin A throughout the subsequent ripening process. Carotenoids accumulate in the inner tissue of most mango cultivars as their color develops from yellow to orange during post-harvest ripening (Vasquez *et al*., 2005).

2.10.8 Changes in mineral nutrients

As the ripening process advances, minerals including calcium, phosphorus, and sodium decrease (Appiah *et al*., 2011). The magnesium concentration in Keitt mango fruits increased with maturity, according to Appiah *et al*. (2011). The essential elements sodium and potassium were found in immature mango fruits (Mujahid *et al*., 2013). Mineral levels differ

between cultivars. The Dusheri and Langra mango types were found to have the greatest potassium contents (Akhtar *et al*., 2010). Othman *et al*. (2009) reported that mango fruits harvested at maturity stage had higher potassium levels. Mango fruit calcium content varies depending on type and maturation stage (Akhtar *et al*., 2010).

2.10.9 Changes in acidity

During the maturing and ripening process, mango fruits lose a significant amount of organic acids. Citric, malic, succinic and tartaric acids are the most important acids in mango fruit (Medlicott *et al*., 1985). Citric acid has the highest concentration and climbs steadily during fruit growth until the endocarp begins to harden, after which it gradually decreases (Ito *et al*., 1997). Succinic and citric acids decrease throughout ripening, but malic acid differs depending on the cultivar (Lizada, 1993). The decrease in acidity that occurs during ripening is caused by starch hydrolysis, which results in an increase in total sugars and a decrease in acidity (Fuchs, 1980).

2.11 Applicable Post Harvest Technologies in Mango

Examples of the postharvest technologies that have been developed and tested in mango include Modified Atmosphere Packaging (Githiga *et al*., 2014)) low temperature storage (Ambuko *et al*., 2018), 1-Methylcyclopropene (Ricardo *et al*., 2004; Ambuko *et al*., 2013), hot water treatments (Mirshekari *et al*., 2015) among others. However, the adoption rate of these technologies especially by small scale farmers is quite minimal due to prohibitive costs, practical difficulties and non-availability (Lorevice *et al*., 2014).

2.11.1 The use of 1-methylcycopropene (1-MCP)

One of the strategies used to stop the adverse effects of ethylene in horticultural produce is the use of the ethylene action inhibitor 1-methylcyclopropene (1-MCP) (Blankenship and Dole, 2003). 1-MCP works by preventing or delaying the metabolic processes that are

ordinarily caused by ethylene by inhibiting the binding of ethylene to its receptors (Serek *et al*., 1994). 1-MCP has a tenfold higher affinity for ethylene receptors than ethylene, making it a viable rival. 1-MCP is colourless, odourless and nontoxic and it can be used at extremely low doses with minimum quantifiable residues in food (Sisler and Serek, 1997).

1-MCP treatment has been shown to postpone or slow ethylene development, respiratory activity, acidity loss, softening, color changes and other ripening and senescence-related changes (Blankenship and Dole, 2003).

The beneficial attributes of 1-MCP therapy are determined by factors in pre harvest production, storage temperature, maturity stage and treatment period (Blankenship and Dole, 2003; Watkins, 2006). In apricots (Fan *et al*., 1999), bananas (Harris *et al*., 2000) and mangoes (Ricardo *et al*., 2004; Ambuko *et al.,* 2013), the efficacy of 1-MCP therapy declined as fruit development progressed. According to Guillén *et al*. (2006), tomatoes harvested at later stages of maturity responded better to 1-MCP treatment than tomatoes gathered earlier. Passion fruits obtained from two different agro-ecological situations responded differentially to 1-MCP treatment (Baraza *et al*., 2013).

However, after treating fruits with 1-MCP, several issues were discovered (Yumbya *et al*., 2020). It was discovered that bananas treated with 1-MCP remained green or ripened unevenly (Harris *et al*., 2000). Furthermore, total volatile generation, particularly esters, was decreased in banana fruits treated with 1-MCP (Golding *et al*., 1998).

2.11.2 Cold chain management

Low-temperature storage is one of the most successful methods for preserving quality and extending the shelf life of fruits after harvest. This is feasible because low temperatures slow down fruit metabolism, which slows the expression of ripening characteristics (Yumbya *et al*., 2020). Maintaining a suitable cold chain for fresh horticulture products is critical for

maintaining quality and avoiding postharvest losses in perishable commodities (M. Aung, 2014). Simple harvest procedures combined with low-cost cold storage can be used to generate a desirable cold chain for smallholder farmers with limited resources (Ambuko *et al.*, 2018).

In mango, efficient cold chain management dramatically reduced the rate of changes associated with ripening such as peel and flesh color, physiological weight loss, peel and flesh stiffness, and increase in TSS, resulting in an additional 18 days of shelf life (Amwoka *et.al*., 2021).

2.11.3 Modified Atmosphere Packaging(MAP)

Modified atmosphere packaging (MAP) entails packaging actively respiring horticultural produce in polymeric film packages in order to change the levels of oxygen and carbon dioxide in the package atmosphere (Githiga *et al*., 2014). Delayed ripening by decreasing ethylene production, reduced transpiration water loss, delayed biochemical activity associated with ripening, and greater resistance to postharvest pathogen attack are some of the benefits of MAP (Valero and Serrano, 2010).

Polymeric films employed in MAP obstruct water vapour diffusion, causing the internal atmosphere package to become saturated with water vapour pressure, limiting tissue transpiration and weight loss. Physiological activities such as respiration and ethylene biosynthesis and activity are affected by the low oxygen environment induced in MAP. Reduced respiration rates caused by a low oxygen environment impede starch decomposition and sugar consumption in packaged goods (Githiga *et al.*, 2014). Apart from lowering respiration rates, the use of MAP in climacteric fruits like mango delays climacteric respiration (Singh and Rao, 2005; Yahia, 2006).

Furthermore, the low oxygen and high carbon dioxide conditions established in MAP inhibit ethylene production by inhibiting the activity of 1-Aminocyclopropane-1- carboxylic acid

(ACC) oxidase, the enzyme that catalyzes the conversion of ACC to ethylene. Carbon dioxide has also been proven to be an ethylene antagonist, preventing its autocatalytic production (Yang and Hoffman, 1984, Githiga *et al*., 2014).

2.11.4 Hexanal

Hexanal is a six-carbon aldehyde produced spontaneously in plants by the lipoxygenase route from linoleic acid (Hildebrand, 1989). It is very volatile and has been shown to have antifungal activities against *Botrytis cinerea*, *Alternaria alternative* and *Penicillium expansum* infections (Hamilton-Kemp *et al*., 1992; Song *et al*., 1998). Externally applied hexanal as a post-harvest dip, pre-harvest spray or vapour extends the storage life of fruit. It has been found to be a phospholipase D inhibitor and is generally recognized as safe (GRAS). As a result, technology is currently being developed to enable its application to improve the shelf life and quality of vegetables, fruits, and flowers (Paliyath *et al*., 2003).

Many fruits, including pears, strawberries, sweet cherries, apple, peach, mango (Paliyath *et al*., 1999, Anusuya *et al*., 2006) and tomato (Utto *et al*., 2008) have been observed to benefit from hexanal formulations used as vapour treatments, pre-harvest treatments and post-harvest dips.

Hexanal's antimicrobial effect is complemented by its fragrance volatiles, which enhance the sensory qualities of ripe fruit (Archbold *et al*., 2000). It has been observed that hexanal enhances aroma production in apple slices (Song *et al*., 1998). When administered under MAP, hexanal formulations also prevented browning reactions in apples for 16 days at 15°C (Lanciotti *et al*., 1999). Despite the fact that hexanal formulations have been extensively researched in temperate fruits and vegetables, there have been relatively few studies in tropical fruits. There have been successful experiments using Hexanal therapy in tomato (Cheema *et al*., 2014), papaya (Hutchinson *et al*., 2018), and banana (Yumbya *et al*., 2020).

In India, there have also been studies on the usefulness of hexanal as a pre harvest spray for extending mango storage life (Anusuya *et al*., 2016). It is thought that dipping mango fruit in a hexanal formulation inhibits the phospholipase D enzyme in the fruit's epidermis and suppresses ethylene production; these two processes allow the fruit to last longer in storage.

The mechanism of hexanal is achieved by hindering the work of the enzyme phospholipase D which catalyzes the membrane phospholipids hydrolysis and triggers break down of membranes causing fruit softening (Paliyath *et al*., 2008). Quantitative PCR focusing on genes associated with ethylene biosynthesis and softening indicated that treating banana fruits with hexanal delayed the manifestation of four genes coding for different cell wall degrading enzymes which are xyloglucan endotransglucosylase, Pectin Methylesterase, Pectin Lyase and Polygalacturonase (Yumbya *et al*., 2020).

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 horticultural commodities grown under different agricultural zones with different climatic conditions, varietal differences and mode of action among other factors.

Hexanal has been formulated to form an ‘Enhanced Freshness Formulation’ (EFF) having geraniol, ascorbate, ethanol and calcium chloride (Sharma *et al*., 2010). Since hexanal is normally insoluble in water, Tween 20 is added to help it dissolve (Tiwari and Paliyath, 2011).

CHAPTER 3: Efficacy of Hexanal Treatment In Improving The Shelf Life Of Mango (*Mangifera Indica L.*) Fruits Of Different Varieties Harvested From Different Agro Ecological Zones In Kenya.

3.1 Abstract.

Mango is a perishable fruit with a limited shelf life once ripe, resulting in significant postharvest losses. The objective of this study is to determine the efficacy of hexanal treatment in prolonging the shelf life of mango fruits of different varieties harvested from different agro ecological zones in Kenya. A laboratory experiment was performed to determine the efficacy of hexanal treatment in different concentrations as a postharvest dip on the physical quality and extension of storage life of mangoes and its interaction with variety and agro ecological zone of production. The study was done at two agro ecological zones namely Machakos (AEZ IV) and Meru (AEZ II) and on two varieties namely Apple and Tommy atkins. Fruits were harvested at mature green stage, cleaned, sorted and divided into several batches. A formulation of hexanal also known as Enhanced Freshness Formulation (EFF) was used at two concentrations 2% and 3% as a postharvest dip to treat the mangoes in the laboratory and observed under ambient room temperatures throughout the ripening process. The study also included untreated fruits which were only dipped in plain water to act as control. Various ripening parameters were evaluated at 3 day intervals to determine the effects of the EFF treatments. These parameters include: physiological loss of weight, colour, firmness, ethylene evolution rate and respiration rate. The results showed that fruits treated with the EFF exhibited slowed ripening rate as compared to untreated fruits by 3 days in the Apple variety and 5 days in the Tommy Atkins variety. Hexanal treatment slowed down the rate of

cumulative weight loss by 5% -6%. It also delayed the drop in hue angle by 3-6 days as well as delayed drop in both peel and flesh firmness. This is indicative of slowed down ripening process resulting to prolonged shelf life. It was also observed that mangoes treated with 3% EFF had a longer shelf life by 3 days than those treated with 2% EFF indicating that 3% EFF was highly effective in prolonging the shelf life of the fruits. Hexanal formulation applied as a postharvest dip has been found to extend the shelf life of mango fruits and is therefore recommended for adoption as a solution to reduce postharvest losses and prolong the shelf life of mango fruits for both domestic and commercial use.

3.2 Introduction

Mango (*Mangifera indica* L.) is considered one of the high value fruits in Kenya, cultivated in various agro-ecological zones in the country (Griesbach, 2003). It flourishes from 0m-1500m altitude (Nakasone and Paul, 1998) but can also flourish in high elevations. Mango is currently considered third in value among fruit crops after banana and pineapple (HCD, 2011). The current demand for the mango fruit is attributed to its rich content of Mineral, Vitamin and fibre level as well as the products made from it following value addition. In Kenya, the overall area under mango cultivation is projected at 14,386 Hectares having a production of 281,880 Metric tonnes (MoA, 2010).

Mango fruits are consumed locally while the rest exported or processed into numerous products such as pulp, pickles, dried mango chips and chutney. Processing of mango fruits into these products is considered as an improvement of shelf life or enhancing the value of unprocessed mangoes thus minimizing postharvest losses. Mango fruits are potential source of raw material for the industry, household income for the farmer and foreign exchange.

During post-harvest management of mangoes, 40-45% of mango fruits are lost (HCD, 2003). Mango is considered to be quite perishable due to its fast ripening after harvest or

upon transport. Mango being climacteric produces high levels of ethylene triggering senescence. Ripening in mango is accompanied by rapid softening which makes them less marketable. Moreover, harvested mangoes are vulnerable to several postharvest pests and diseases, which cause a drop in quality of the fruits as well as consumer acceptability. Postharvest losses in mangoes can however be reduced by coming up with other ways and measures to prolong the fruits shelf life.

The use of 1-MCP to enhance storage life has already been studied in avocado (Meyers *et al*., 2011) and in mango (Ambuko *et al*., 2013). Its use though has not been commercialized yet. The use of low temperature storage has also been proven effective in slowing down the ripening of mango. Its high cost has however discouraged many small- scale farmers from applying the technology in their production. Hexanal, a naturally occurring six carbon aldehyde produced by the lipoxygenase pathway in response to tissue injury (Hildebrand,1989), has been shown to extend the storage life and improve the quality of several fruits, including strawberries, cherries, nectarines and peaches (Sharma *et al*., 2010).

The mechanism of hexanal is achieved by hindering the work of the enzyme phospholipase D which catalyzes the membrane phospholipids hydrolysis and triggers break down of membranes causing fruit softening (Paliyath *et al*., 2008). Quantitative PCR focussing on genes associated with ethylene biosynthesis and softening indicated that treating fruits with hexanal delayed the manifestation of four genes coding for different cell wall degrading enzymes which are xyloglucan endotransglucosylase, Pectin Methyl esterase, Pectin Lyase and Polygalacturonase (Yumbya *et al*., 2020). Hexanal treatment has been reported to result in increased stability of cell membranes resulting to fruits remaining intact, firm and fresh looking for long.

Hexanal has been approved by FDA in the US as a general food additive (US Patent 6,514,914;7,198,81) for use in processed plant-based foods. It is not retained in the treated tissues beyond 48hours of treatment (http:/www.accessdata.fda.gov/). In the human tissues, hexanal is easily converted to hexanoic acid by oxidation. Like all other alcohols, hexanoic acid is further broken down to water and carbon dioxide during respiration through the tricarboxylic acid cycle (Kruse *et.al*., 2006).

An artificially created biochemical version of hexanal also known as the Enhanced Freshness Formulation (EFF) which slows down the process of ripening in both temperate and tropical fruits has been formulated (Sharma *et al*., 2010). It has been applied both as a preharvest spray and a postharvest dip. It has indicated great results in several countries. There have been successful studies on the use of Hexanal treatment in tomato (Cheema *et al*., 2014), papaya (Hutchinson *et al*., 2018) and Banana (Yumbya *et al*., 2020). Being a relatively new technology, several studies are currently underway in different fruits to establish the effects of hexanal on storage 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 treatment with hexanal suppressed expression of genes responsible for ethylene evolution, 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 flavour compounds such as terpenes and alcohols. In tomato fruits, the color intensity was slightly high in hexanal treated fruits compared to the control ones as reported by Cheema *et al*. (2014). In Cherries, Sharma *et al*. (2010), reported a deepening in the intensity of the red color 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 polygalacturonase and β-galactosidase 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. An experiment 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). In addition to increasing fruits shelf-life, hexanal treatment has been shown to increase fruits retention on the tree in Mango fruits (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.

There has been studies on the efficacy of hexanal on enhancing the storage life of mango as a pre-harvest spray in India (Anusuya *et al*., 2016). However, there is a gap in information on the postharvest application of EFF as a dip, the best concentration of the EFF to give optimal results as well as the interaction of hexanal treatments with different varieties and agro ecological zones of production in Kenya. This study therefore focusses on the use of the EFF as a postharvest dip in mangoes cv. Apple and Tommy Atkins grown at two different agro ecological zones in Kenya aimed at prolonging shelf life.

3.3 Materials and Methods

3.3.1 Site description

Mango fruits from two varieties Apple and Tommy Atkins were harvested at two agro

ecological zones with contrasting climatic conditions. Meru County (AEZ II) with an altitude of 1980m-2700m above sea level and with a mean annual rainfall of 1500mm and Machakos County (AEZ IV) which is a semi- arid region with an altitude of 1000m-1600m above sea level and with a mean annual rainfall of 600mm. The orchards selected for this study were 10-15 years old to ensure uniformity of the samples. Selection was also based on the application of good agricultural practices by the farmer as well as willingness to participate in the study. The mango varieties selected, Apple and Tommy atkins are the most commonly grown varieties in the two areas of study.

3.3.2 Samples and treatments

The fruits were picked from orchards consisting of trees aged between 10 to 15years in which good agricultural practices are applied. The mangoes were harvested at the optimal maturity stage (mature green) from healthy trees based on fruit size and shape and packed in crates, cushioned with old newspapers and sprinkled with a little water to remove field heat. The fruits were immediately transported to the lab where they were washed, dried, and randomly selected based on uniformity and freedom from visible injuries. This experiment was carried out at Jomo Kenyatta University of Agriculture and Technology (JKUAT) Food Science lab.

Once the fruits had been washed and dried, they were divided into three batches and EFF was applied. The protocol developed by (Tiwari and Paliyath, 2010) was applied in the preparation of the formulation.

Fruits were dipped in hexanal concentrations of 2% EFF and 3% EFF or plain water (control). All the fruits were stored in crates and left to undergo the typical ripening process under normal room temperature.

The experimental design used was Complete Randomized Design(CRD) with factorial arrangements. The factors included: variety, agro ecological zones and hexanal concentration.3

Three mango fruits from each treatment i.e. control, 2% and 3% EFF were sampled randomly after in 3-day intervals to assess ripening parameters namely: percentage cumulative weight loss, firmness, colour, ethylene evolution rate and rate of respiration.

3.3.3 Percentage cumulative loss of weight

Three fruits from each treatment were weighed throughout the ripening process and their weight recorded in grams. % CWL was calculated as follows:

$$CWL\ (\%) = Initial\ weight\ (g) - Final\ weight\ (g) / Initial\ weight\ (g) \times 100.$$

3.3.4 Peel and flesh colour

Three mango fruits from each treatment were tested every 3 days for peel and flesh color using a color spectrophotometer (NF-333-Color spectrophotometer (Nippon Denshoku Industries, Japan) and the recorded a* and b* values changed to Hue angle (H^o) whereby,

$$(H^o) = tan\text{-}1(b^*/a^*) *s$$

3.3.5 Fruit firmness

A penetrometer (CR-100D, Sun Scientific Co. Ltd, Japan) fitted with a 10mm probe was used to measure the firmness of three fruits from each batch. For all treatments, peel firmness was measured using three separate sections of the fruit's peel, whereas flesh firmness was determined utilizing peeled pieces of the sampled fruits. Fruit and peel firmness is expressed in newton's (N).

3.3.6 Rate of ethylene evolution and respiration

A gas chromatograph was used to determine the rate of ethylene generation and respiration (Model GC-8A, Shimadzu Corp., Kyoto, Japan) Three mango fruits were placed in plastic jars, one for each treatment. A self-sealing rubber septum had been installed on the jar lids. The fruits were incubated at room temperature for 2-3 hours. Gas chromatographs were

used to extract samples from the headspace gas. At standard atmospheric pressure, carbon dioxide production was measured in ml/kg/hr while ethylene evolution was measured in µl/kg/hr.

3.4 Statistical Analysis

The data was analyzed using the GENSTAT statistical software 13th edition (ANOVA) and the means were compared using the Least Significance Difference (LSD) at $P<0.05$.

3.5 Results

3.5.1 Percent Cumulative weight loss

Throughout the storage period, all fruits gradually lost weight as the ripening process progressed irrespective of AEZ of production, variety and concentration of hexanal treatment (Figure 1). The percentage weight loss was significantly different ($P<0.05$) between fruits from the two varieties. It was observed that Apple mangoes lost more weigh recording a percentage weight loss of up to 20% by the expiration of the experimental period as compared to Tommy atkins mangoes which registered a percentage cumulative weight loss of up to 15% by the end of the experiment period. The percentage cumulative loss of weight between mangoes harvested from different agro ecological zones was significantly different ($P<0.05$) whereby fruits from the hotter region of Machakos seemed to lose weight faster during the ripening process as compared to fruits harvested from colder regions.

It was also noted that the levels of concentration of hexanal significantly ($p<0.05$) reduced the rate at which the fruits lost weight with fruits treated with 3% EFF having the slowest rate of weight loss (Figure 1). The different concentrations of hexanal gave significantly different results ($p<0.05$) with 3% EFF proving to be more effective in reducing the rate at which the fruits lost weight.

In Apple mangoes from Machakos, hexanal treatment slowed down the rate at which the

fruits lost weight. Control fruits lost 15.5% of their weight by day 12 as compared to 10.5% and 9.16% for fruits treated with 2% and 3% hexanal concentrations respectively (1A). In Tommy Atkins variety, control fruits lost 12.2% of their weight while fruits treated with 2% and 3% hexanal had lost 8.6% and 7.26% of their weight respectively by day 12 for fruits harvested in Machakos (1A).

The same trend was observed in mangoes harvested from Meru. For Apple mangoes, untreated mangoes had lost 12.8% of their weight as opposed to 11.3% and 9.9% for fruits treated with 2% and 3% hexanal concentrations by day 12(1B). In Tommy Atkins variety untreated fruits had lost 9.7% of their weight as compared to 8.5% and 6.9% for fruits treated with 2% and 3% concentrations of hexanal respectively within the same period (1B).

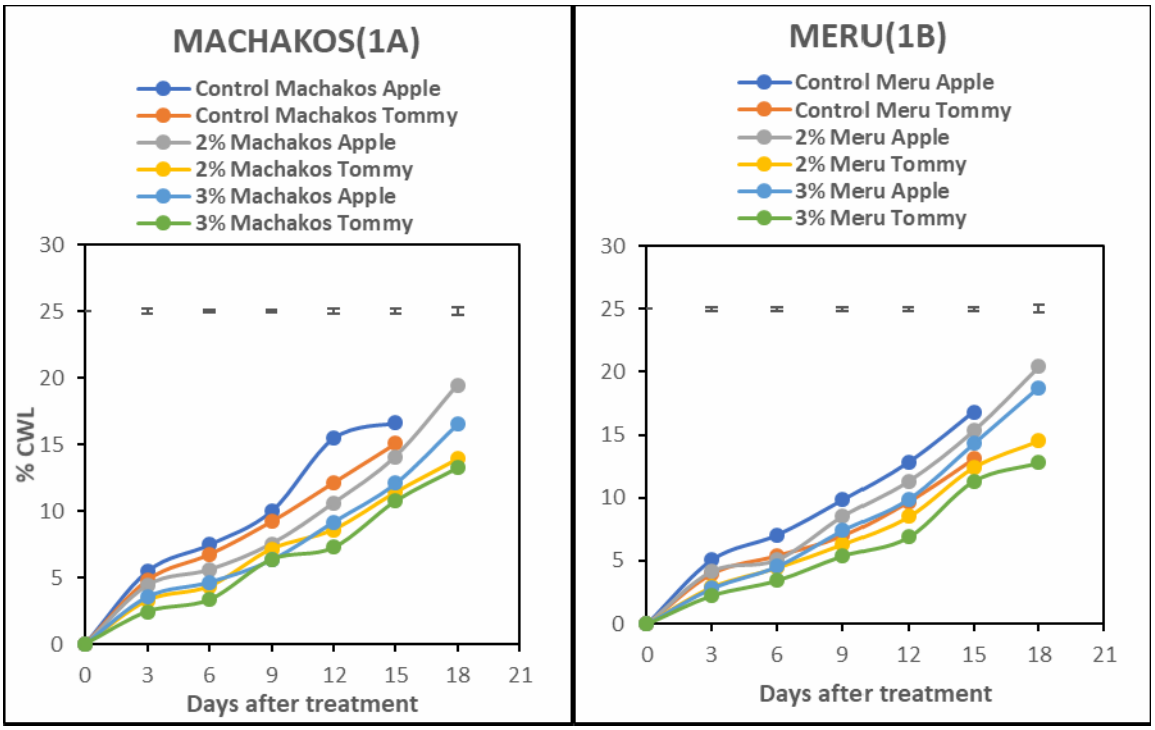

Figure 1: Effects of hexanal treatment on the percentage Cumulative weight loss of Apple and Tommy Atkins varieties of Mango harvested in Machakos and Meru. The vertical bars are representative of the LSD at ($p<0.05$)

3.5.2 Flesh colour

The intensity of yellow colour increased steadily as the fruits ripened with a hue angle drop from 110º (unripe green) to 60º (ripe yellow) (Figure 2). There was a significant difference ($p<0.05$) between varieties with Apple mangoes observed to reach the 60º hue angle 3 days earlier as compared to the Tommy Atkins variety (Figure 2). Different agro ecological zones of production did not show any significant difference in the flesh colour of the fruits.

Hexanal treatment delayed the drop in hue angle. There was a significant difference ($p<0.05$) between means for fruits treated with different levels of hexanal with 3% EFF showing better results in slowing down the change of flesh colour from green to yellow (Figure 2).

In apple mango, untreated fruits reached the 60º hue angle (ripe yellow) on day 12, while fruits treated with 2% EFF reached the same on day 15. Fruits treated with 3% EFF reached the 60º hue angle on day18 for fruits harvested in both Machakos and Meru (2A and 2B).

In Tommy atkins mango, the control fruits reached the 60º hue angle on day 15 while fruits treated with 2% EFF and 3% EFF reached the same on day 18 for fruits harvested from both Machakos and Meru (2A and 2B).

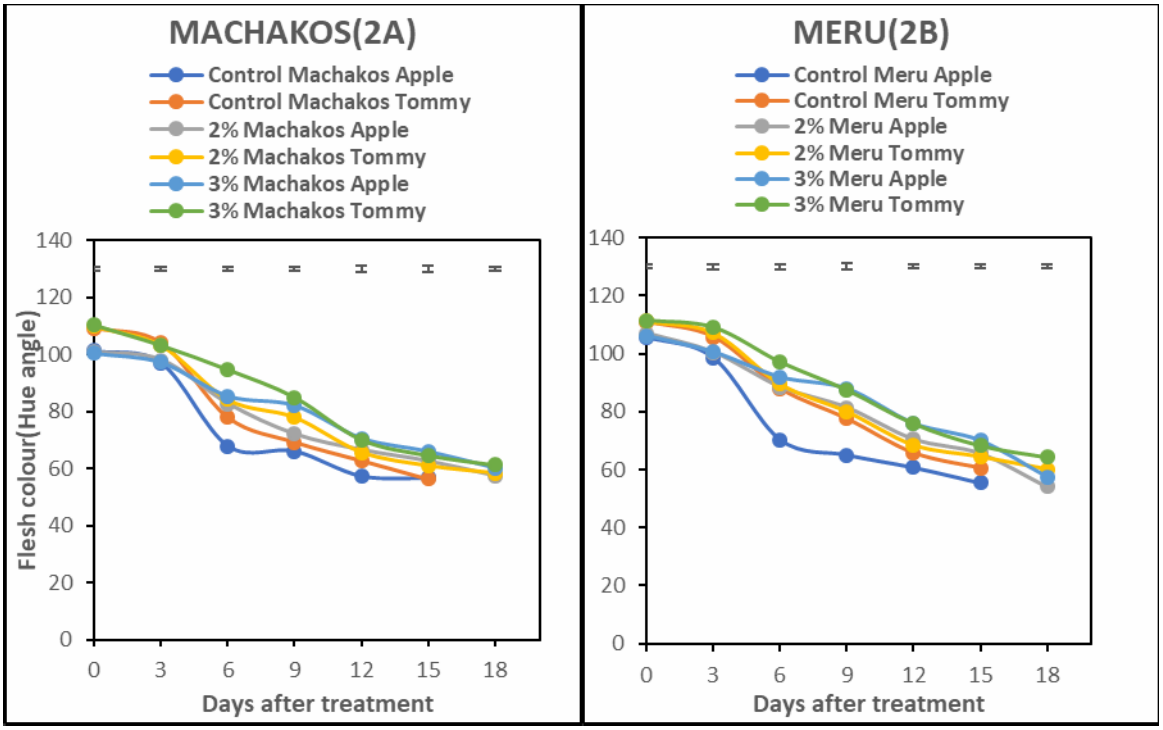

Figure 2: Effects of hexanal treatment on the flesh colour of Apple and Tommy Atkins varieties of Mango harvested in Machakos and Meru. The vertical bars are representative of the LSD at ($p<0.05$)

3.5.3 Peel colour

The mango peels steadily changed from colour green to colour yellow during the ripening process. There was a drop of hue angle from 120º (unripe) to 60º (ripe) (Figure 3). There was a significant difference ($P<0.05$) between means in peel colour for fruits obtained from different agro ecological zones with fruits from hotter regions of Machakos showing a bigger drop in hue angle as compared to fruits from the colder areas of Meru. There was also a significant difference ($P<0.05$) between fruits of different varieties with Tommy atkins mangoes showing a slower rate in change of colour as compared to the Apple variety.

The different levels of hexanal treatments caused a significant difference ($P<0.05$) in the changes in peel colour as the fruits ripened with 3%EFF proving to be more effective in slowing down the rate at which the hue angle dropped (Figure 3).

In apple mango obtained from Machakos, the untreated mangoes reached hue angle of 60º on day 9, the fruits treated with 2% EFF reached 60º on day 15 while the fruits treated with 3%

EFF reached on day 18. The hexanal treatment in apple mango obtained from Machakos delayed the drop in hue angle for 6 days in 2% EFF concentration and 9 days in fruits treated with 3% EFF. In Tommy atkins mango, the change in colour was more gradual as compared to apple mangoes. In Machakos, the untreated tommy mango fruits reached the 60º on day 12 and on day 18 for both 2% EFF and 3%EFF concentrations. This was a 6-day delay due to hexanal treatment (3A).

For apple mangoes obtained from Meru, untreated mangoes reached the 60º hue angle on day 12, 2% EFF on day 15 and 3% EFF on day18. This showed a 3-day delay in mangoes treated with 2% EFF concentration and 6-day delay in mangoes treated with 3% EFF concentration (3C). In Tommy mangoes from Meru the untreated mangoes reached 60º on day 12 and day 18 for both 2% and 3% concentrations (3B).

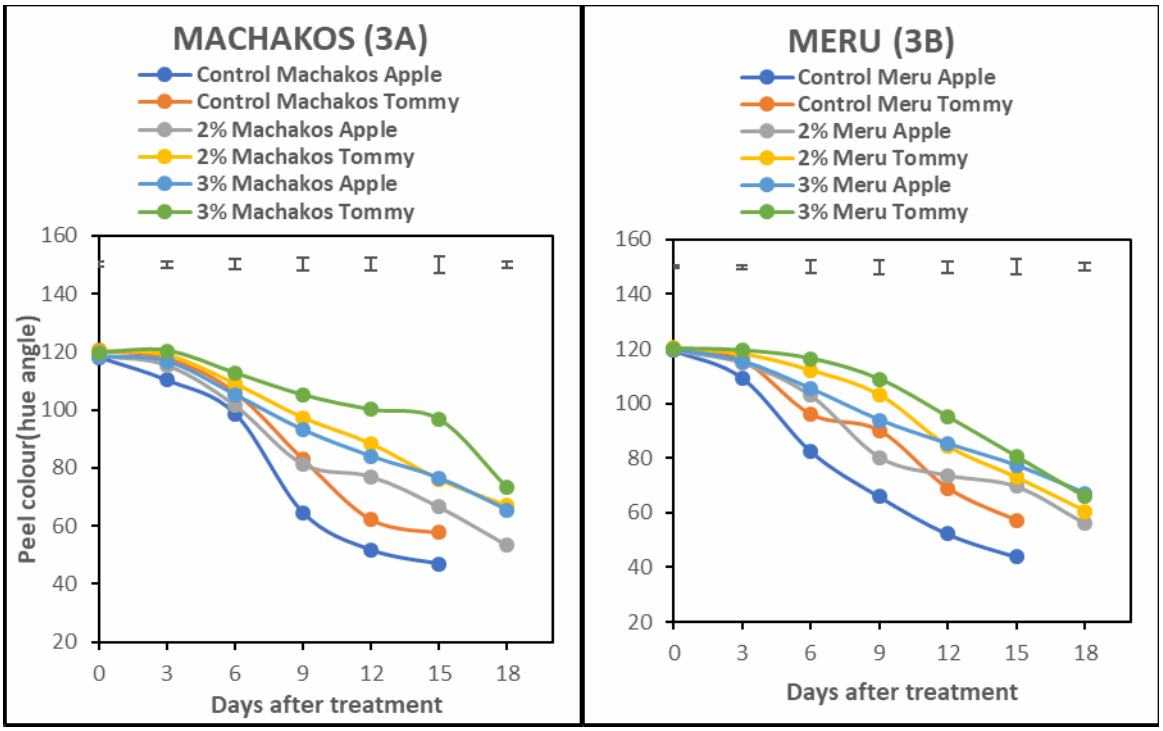

Figure 3: Effects of hexanal treatment on the peel colour of Apple and Tommy Atkins varieties of Mango harvested in Machakos and Meru. The vertical bars are representative of the LSD at (p<0.05)

3.5.4 Flesh firmness

The flesh firmness declined continuously throughout the ripening process across all treatments (Figure 4). The rate at which flesh firmness declined between fruits from different varieties differed significantly (P<0.05), with apple mangoes showing a faster decline than the Tommy Atkins variety. There was also a significant difference(P<0.05) between means for fruits obtained from different locations with mangoes from Machakos showing a faster decline in flesh firmness as compared to fruits from Meru.

Application of hexanal at different concentrations showed a significant (P<0.05) effect on the rate of which the flesh firmness declined. It was observed that treatment with hexanal slowed the rate at which the flesh firmness declined with fruits treated with 3%EFF having the slowest rate (Figure 4).

In apple mangoes harvested at Machakos, flesh firmness reduced drastically for untreated mangoes as compared to the treated ones. By day 3 after treatment, the flesh firmness of untreated mangoes had declined by 56% (24.3N-10.6N) and by 77% (24.3N-5.5N) by day 6. Fruits treated with 2% EFF, firmness had a decline by 38% (24.2N-15.1N) on day 3 and 68% (24.2N-7.6N) on day 6 as compared to 32% (24.3N-16.5N) on day 3 and 62% (24.3N- 9.2N) on day 6 in fruits treated with 3% concentration of hexanal (Figure 4A). In Tommy mangoes harvested in Machakos, the decrease in firmness was observed on day 6. On day 3, the decrease was only by 10% across all three treatments. On day 6, untreated fruits had a decline in flesh firmness of 65% (29.1N-10.0N) as compared to 55% (28.7N-12.9N) in fruits treated with 2% concentration of hexanal and 40% (28.9N-17.3N) in mangoes treated with 3% concentration of hexanal (4A).

In apple mangoes harvested in Meru, the flesh firmness in untreated fruits had declined by 47% (26.7N-14.1N) by day 3 and up to 79% (26.7N-5.3N) by day 6. In fruits treated with 2% concentration of hexanal, the flesh firmness decreased by 39% (25.0N-15.2N) on day 3 and up to 69% (25.0N-7.5N) on day 6 while fruits treated with 3% concentration of hexanal had a decline of 37%(24.9N-15.6N) on day 3 and 61% (24.9N-9.5N) on day 6 (4B). In Tommy mangoes obtained from Meru, untreated fruits had a flesh firmness decrease of 14% (28.3N-24.2N) on day 3 and 64%(28.3N-9.9N) on day 6. In fruits treated with 2% concentration, the flesh firmness declined by 14% (29.0N-24.7N) on day 3 and 49% (29.0N- 14.7N) on day 6 while in fruits treated with 3% concentration there was 12% (29.2N-25.6N) decline on day 3 and 47% (29.2N-15.4N) decline by day 6 (4B) (Figure 4). In all the groups it was observed that fruits treated with 3% concentration of hexanal had the slowest decline in flesh firmness over the storage period.

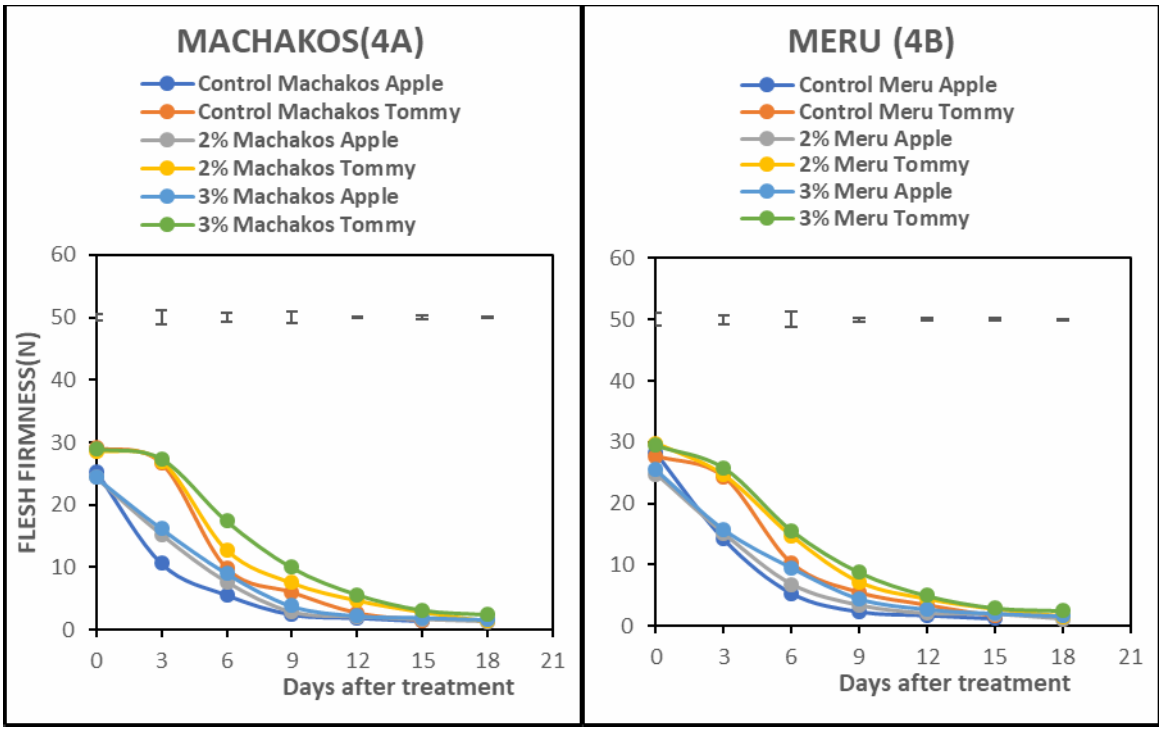

Figure 4: Effects of hexanal treatment on the flesh firmness of Apple and Tommy Atkins varieties of Mango harvested in Machakos and Meru. The vertical bars are representative of the LSD at ($p<0.05$)

3.5.5 Peel firmness

Peel firmness expressed in Newtons (N), declined steadily throughout the ripening period in all fruits irrespective of variety, agro ecological zone of production or treatment (Figure 5). There was no significant difference ($P<0.05$) in peel firmness between fruits harvested from different agro ecological zones. Variety had a significant effect ($P<0.05$) on the peel firmness of the fruits with Tommy atkins mangoes showing a higher peel firmness as compared to apple mangoes throughout the treatment period.

Hexanal treatment at different concentrations caused a significant difference ($P<0.05$) in the rate at which peel firmness declined (Figure 5). Fruits treated with 3% EFF recorded the slowest rate of decline in peel firmness as compared to the other treatments. This indicates that 3% EFF was more effective.

In apple mango obtained from Machakos, the peel firmness decreased faster in untreated fruits (control) as compared to the fruits treated with hexanal. In untreated fruits, firmness declined by 53% (61.6N-28.9N) on day 3 and 71%(61.6N-17.6N) by day 6. In fruits treated with 2% concentration of hexanal, peel firmness dropped by 40% (59.8N-35.5N) on day 3 and 56% (59.8-26.1N) by day 6. In apple mangoes treated with 3% the drop in peel firmness was at 31% (59.7N-41.0N) by day 3 and 53% (59.7N- 27.7N) by day 6 (5A). In Tommy Atkins mangoes harvested from Machakos the drop in in peel firmness was observed to be more gradual within the first 3 days as compared to apple mangoes harvested from the same location. In untreated fruits(control), peel firmness dropped by 21% (59.4N-47.2N) by day 3 and 66% (59.4N- 20.1N) by day 6. Mangoes treated with 2% concentration of hexanal had a drop in peel firmness of 22% (61.3N- 48.1N) on day 3 and 54% (61.3N-28.0N) by day 6 as compared to fruits treated with 3% concentration whose drop was at 12%(59.5N-52.4N) by day 3 and 49%(59.5N-30.5N) by day 6 (5A).

In apple mangoes harvested from Meru, untreated fruits had a drop of 47% (60.2N-31.7N) by day 3 and 72% (60.2N- 16.9N) by day 6. Fruits treated with 2% concentration had a peel firmness drop of 38% (59.5N-37.1N) by day 3 and 57% (59.4N-25.5N) by day 6 while mangoes treated with 3% concentration of hexanal had a drop of 24%(60.1N-45.9N) by day 3 and 52% (60.1N-28.5N) by day 6 (5B). In Tommy Atkins mangoes harvested from Meru, the decline in peel firmness was observed to be slow in the first 3 days after treatment. In untreated fruits, peel firmness declined by 35%(58.2N-37.4N) by day 3 and 70% (58.2N-17.1N) by day 6. Fruits treated with 2% concentration of hexanal had a decline of 17%(58.6N-48.6N) by day 3 and 56%(58.6N-25.9N) by day 6 as compared to 12%(59.3N-52.3N) on day 3 and 51% (59.3N-28.8N) on day 6 for fruits treated with 3% concentration of hexanal (5B) (Figure 5).

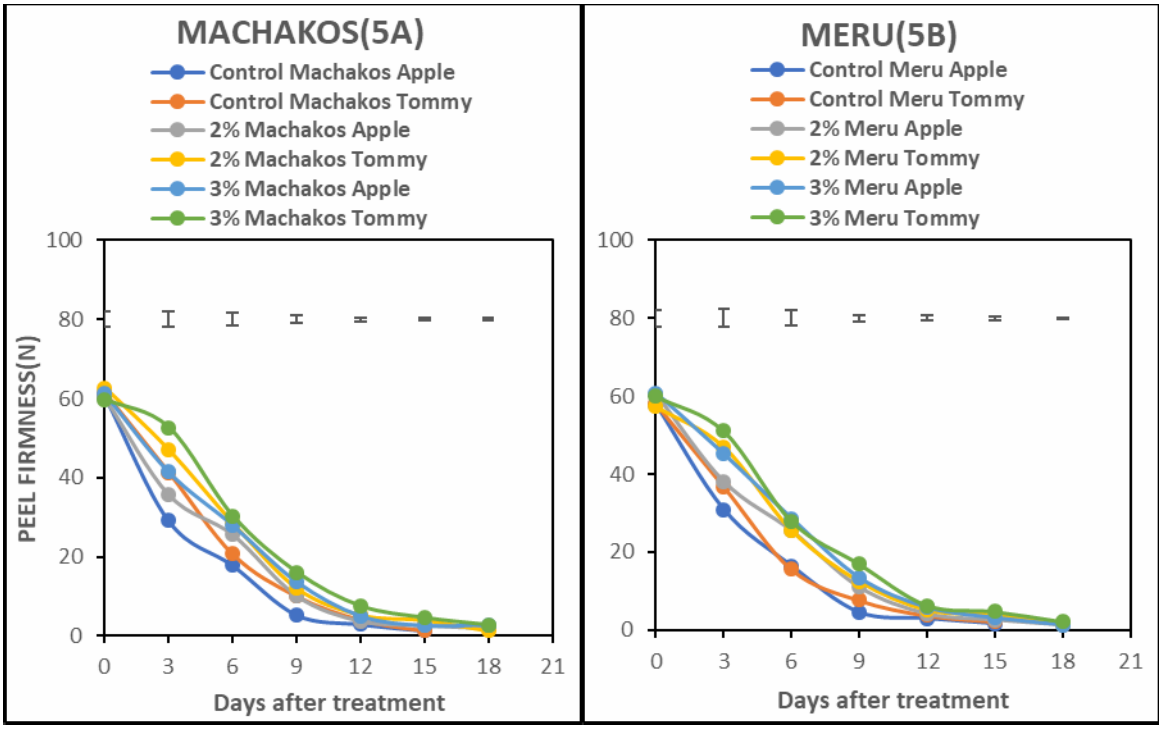

Figure 5: Effects of hexanal treatment on the peel firmness of Apple and Tommy Atkins varieties of Mango harvested in Machakos and Meru. The vertical bars are representative of the LSD at ($p<0.05$)

3.5.6 Rate of respiration

The respiration rate/ CO_2 evolution in mango fruits increased steadily reached a peak level and dropped fast in all fruits irrespective of variety and AEZ exhibiting a clear climacteric behaviour (Figure 6). Respiration rates of fruits from different varieties were significantly different ($P<0.05$) where Tommy Atkins variety had a lower respiration rate compared to the Apple variety, irrespective of treatment. There was also a significant difference($P<0.05$) in rates of respiration between fruits harvested from different zones of production with fruits from Machakos having a higher respiration rate as compared to fruits harvested from Meru.

Hexanal treatment at different concentrations caused a significant difference ($P<0.05$) in the rate of respiration. It was observed that hexanal treatment delayed the peak period by 3 days in both varieties. Untreated fruits reached peak levels on day 3 while fruits treated with 2%

and 3% concentrations of hexanal reached peak levels on day 6. It was observed that fruits treated with 3% EFF had the slowest rate of respiration while untreated fruits had the highest (Figure 6).

Untreated Apple mangoes harvested from Machakos peaked at 25.03ml/kg/hr on day 3 while the ones treated with 2% EFF peaked at 20.36 ml/kg/hr on day 6. Fruits treated with 3% EFF in this group peaked at 15.23 ml/kg/hr on day 6 (6A). Untreated Tommy Atkins mangoes from Machakos peaked at 21.86 ml/kg/hr on day 3, 2% EFF treated ones peaked at 22.1 ml/kg/hr while those treated with 3% EFF peaked at 18.1 ml/kg/hr on day 6 (6A).

Untreated Apple mangoes from Meru peaked at 15.26 ml/kg/hr on day 3, 2% EFF treated fruits peaked at 16.6 ml/kg/hr while those treated with 3% EFF peaked at 13.6 ml/kg/hr on day 6(6B). The same trend was observed from fruits harvested in Meru whereby control Tommy Atkins fruits peaked at 16.7kg/ml/hr on day 3 while fruits treated with 2% EFF peaked at

15.26 ml/kg/hr on day 6. Fruits treated with 3% EFF peaked at 13.63 ml/kg/hr on day 6 too (6B).

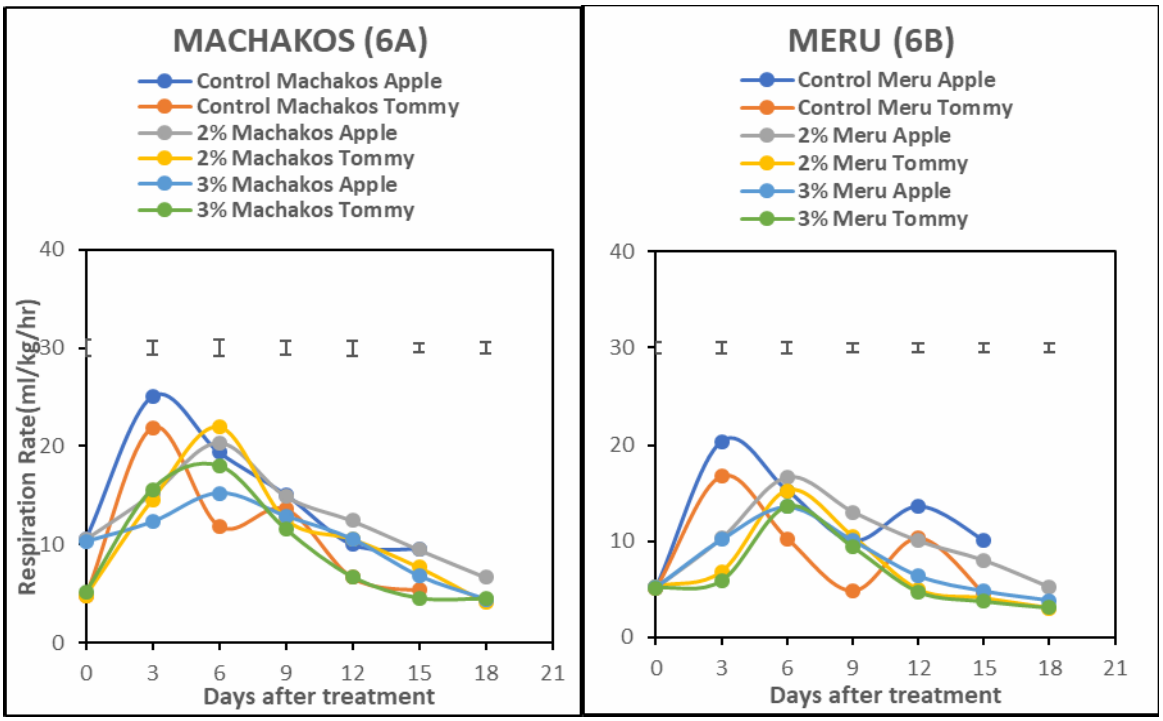

Figure 6: Effects of hexanal treatments on the respiration rate(ml/kg/hr) of Apple and Tommy Atkins varieties of Mango harvested in Machakos and Meru. The vertical bars are representative of the LSD at ($p<0.05$)

3.5.7 Ethylene rate

Ethylene evolution rate increased drastically reaching peak levels then dropped gradually across all treatments with fruits exhibiting clear climacteric behavior (Figure 7). There was no significant difference in ethylene rates between fruits from different varieties. However, ethylene rates were significantly different($P<0.05$) in mangoes harvested from different agro ecological zones with fruits from hotter regions (Machakos) producing higher levels of ethylene as compared to fruits from colder regions (Meru).

The different concentrations of hexanal treatments significantly ($P<0.05$) affected the level of ethylene evolution rate (Figure 7). Hexanal treatment was observed to delay reaching of peak levels by 3 days across all treatments irrespective of agro ecological zone of origin and variety. Hexanal treated fruits had lower rates of ethylene evolution as compared to the control fruits.

In apple mangoes harvested from Machakos, untreated fruits reached peak level on day 3 with 3.9µL/kg/hr ethylene rate while fruits treated with 2% concentration reached peak levels on day 6 with 2.9µL/kg/hr evolution rate. Fruits treated with 3% concentration of hexanal reached peak levels on day 6 with a rate of 2.3µL/kg/hr (7A). In Tommy Atkins mangoes harvested in Machakos, untreated fruits reached peak levels on day 3 with a rate of 2.5µL/kg/hr while fruits treated with 2% and 3% reached peak levels on day 6 with a rate of 2.3µL/kg/hr and 1.6µL/kg/hr respectively (7A).

In Apple mangoes harvested from Meru, peak level for untreated mangoes was reached on day 3 at 2.9µL/kg/hr while fruits treated with 2% and 3% reached peak levels on day 6 with 2.3µL/kg/hr and 1.7 µL/kg/hr respectively (7B). Tommy Atkins mangoes from Meru reached peak levels on day 3 for untreated fruits at 3.1µL/kg/hr as compared to day 6 for fruits treated with 2% and 3% hexanal at 2.5µL/kg/hr and 2.0µL/kg/hr respectively (7B).

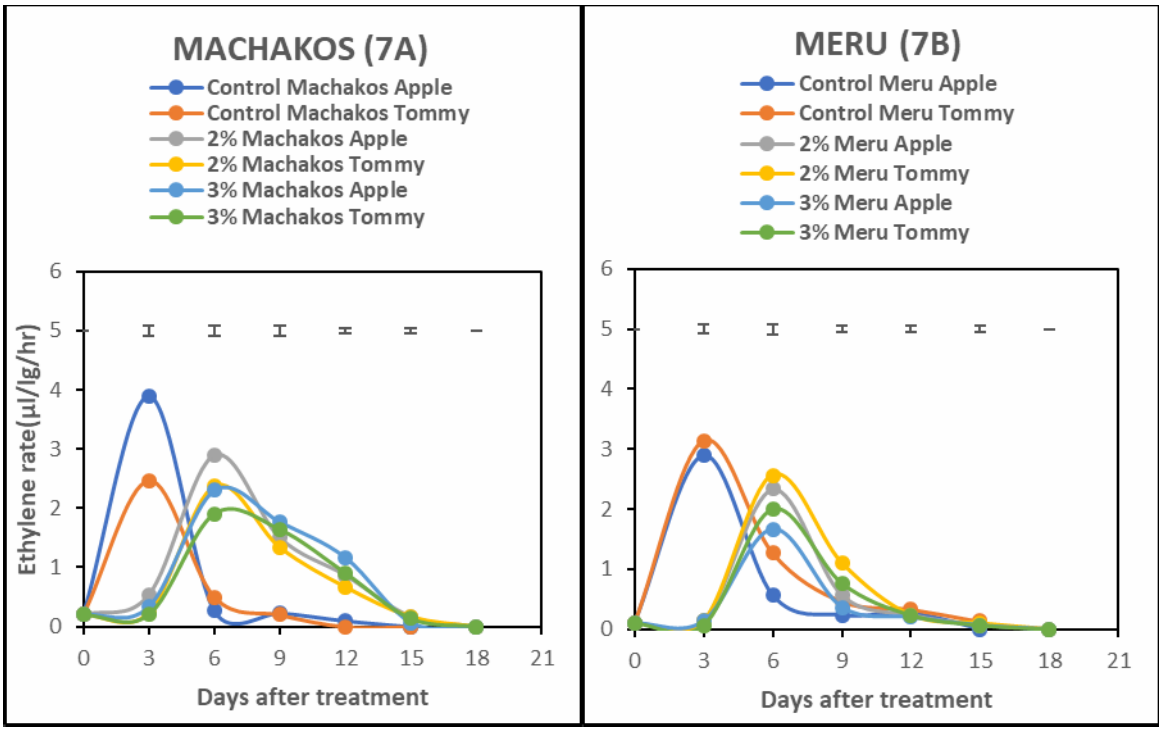

Figure 7: Effects of hexanal treatments on the ethylene rate(µl/kg/hr) of Apple and Tommy Atkins varieties of Mango harvested in Machakos and Meru. The vertical bars are representative of the LSD at ($p<0.05$)

3.6 Discussion

It is important to note that hexanal treatment has been tested in other fruits in Kenya. Studies have shown promising results on the use of hexanal in Papaya (Hutchinson *et al*., 2018) and Banana (Yumbya *et al*., 2020). Additionally, there has been successful studies on the use of Hexanal treatment in tomato (Cheema *et al*., 2014). On mango, there has been research on the efficiency of hexanal as a pre harvest treatment applied as a spray in prolonging the shelf life of mango in India (Anusuya *et al*., 2016).

Hexanal is a plant-derived compound that has been shown to inhibit the action of the phospholipase D enzyme on the peel of fruits, thereby extending the shelf life of fruits in temperate (Paliyath and Subramanian, 2008; Sharma *et al*., 2010) as well as tropical climates (Anusuya *et al*., 2016; Jincy *et al*., 2017). In this study, the mango fruits treated with EFF as a postharvest dip exhibited lower cumulative weight loss during the storage period irrespective of the concentration used. This is as a result of thickening of the cell wall caused by hexanal inhibiting the action of the lipoxygenase enzyme. The results from this study suggest that hexanal causes thickening of cell walls hence reducing the rate at which fruits lose weight during storage.

The EFF-treated mangoes exhibited higher firmness as compared to untreated fruits during the experiment period. This is attributed to the action of hexanal, which inhibits the enzymes associated with the breakdown of hemicellulose and pectin. The delayed softening could also be attributed to decrease of the biosynthesis of the hydrolysis of the cell wall as well as the inhibition in ethylene evolution. Peel and pulp softening in fruits could be caused by these

three mechanisms: degradation of starch, loss of turgor or the breakdown of cell walls which are all inhibited by the action of Hexanal.

The change in colour in Mango is a clear indication of ripening and is usually used by consumers to determine the ripeness of the fruits. It is as a result of loss in chlorophyll. This study has shown that hexanal treatments slow done the progression of change in colour as indicated by the slower progression of hue angle values in treated fruits. Treated fruits showed a slower change of colour as compared to control fruits indicating a slowed down ripening process.

Mangoes being climacteric seem to continue respiration even after harvesting leading to a shortened shelf life. Fruits treated with EFF seemed to have a lower respiration rate as indicated by lower CO^2 evolution. The respiration rate increases to peak levels during ripening (Albert, 1926). The increase in respiration rate is caused by the conversion of starch to sugar (Clendennen and May, 1997; Chen and Ramaswamy, 2002). The rate then drops after the climacteric phase (Cordenunsi and Lajolo, 1995; Waliszewski *et al*., 2003). Hexanal has been associated with preserving starch granules hence reducing the rate of respiration (Saltveit, 2004). Ethylene production also causes hastened senescence. Hexanal seemed to act as an ethylene blocker hence reducing its production rate. Studies carried out by Tiwari and Paliyath, (2011) confirmed that in tomatoes, hexanal treatments slowed down the evolution of ethylene.

In terms of varieties, the Tommy Atkins variety seemed to respond better to the hexanal treatments as compared to the Apple variety. This was indicated by the difference in days after treatment that the two varieties took to reach a certain level of ripeness. In most parameters, the Tommy Atkins variety was observed to delay by 3-5 days as compared to the Apple variety. This could be attributed to difference in genotypes in the two varieties.

Mangoes from hotter regions (AEZ IV) were observed to ripen faster as compared to the fruits from colder regions (AEZ II). This could be caused by the difference in temperatures and other climatic conditions associated with different agro ecological zones. Machakos county is known to be quite hot recording a mean temperature of 30ºC while Meru is relatively colder with temperatures ranging between 18ºC and 21ºC. Such cool temperatures in Meru could be the cause of the slower ripening. Similar observations have been made in apples (Paliyath *et al*., 2008), sweet cherries (Sharma *et al*., 2010) and tomato (Cheema *et al*., 2014).

This study indicated that 3% EFF was more effective in delaying the ripening parameters as compared to 2% EFF. This means that the optimal concentration of Hexanal treatments in mango as a postharvest dip is 3% EFF. This could be due to higher levels of hexanal getting into contact with the fruits. The same was observed in a study by Yumbya *et al*. (2020), which showed that bananas ripened slower when exposed to hexanal for longer and at higher concentrations.

3.7 Conclusion

This study indicates that indeed hexanal used as a dip applied after harvest prolongs the shelf life of mango fruits and preserves the physical attributes of fruits as they ripen. Fruits treated with the EFF retained a higher firmness, slower change in colour and low rates of respiration and ethylene production. Treated fruits also had a low rate of weight loss throughout the storage period indicative of slowed down ripening and senescence.

CHAPTER 4: The Effects of Hexanal Treatment on the Biochemical Quality Characteristics of Mango Fruits

4.1 Abstract

This research was conducted to evaluate the effects of hexanal treatment on the postharvest quality attributes of mango fruits from two varieties namely Apple and Tommy Atkins harvested from two agro ecological zones with contrasting climatic conditions, Machakos (AEZ IV) and Meru (AEZ II). The objective was to determine the effects of hexanal treatment on the biochemical and quality attributes of mango fruits. Samples from all treatments were taken and refrigerated and later evaluated for several ripening biochemical parameters measured namely, brix, ascorbic acid content, total titratable acidity, Beta carotene and simple sugars (glucose, fructose and sucrose). In all the ripening parameters measured in this study, hexanal treatment was observed to slow down the rate at which the ripening process progressed but did not significantly change the quality of the fruits compared to the untreated fruits. Hexanal treated fruits exhibited a slower ripening rate as well as a higher retention for sugars, vitamin C, Beta carotene and acidity. In some parameters such as TSS, Beta carotene, glucose and fructose content there was no significant difference between fruits from different varieties or even harvested from different zones. However, in parameters such as TTA differences were noted between varieties with Tommy atkins variety recording a higher TTA than apple mangoes. The varietal difference was also noted in sucrose content where apple mangoes had a higher level of sucrose content as compared to the Tommy atkins variety.

In some parameters such as ascorbic acid level, differences in the content was noted in fruits harvested from different agro ecological zones. Fruits harvested in Machakos recorded higher vitamin C level as compared to fruits harvested from Machakos. This study therefore finds hexanal treatment an effective postharvest technology since it does not alter the quality attributes of fruits and recommends wider research and subsequent commercializing.

4.1 Introduction

Mango is an essential crop in Kenya and its cultivation has increased over the years as a result of increased demand. It is also known to be a great source of vitamin C as well as other important anti-oxidants. The nutritional value and health benefits of fruit has become a major attribute in consumer preference (Michael, 2002). The optimum quality, flavour and taste of mango develops when the fruits are picked after they reach physiological maturity **(**Reid, 2002; Slaughter, 2009). These attributes are also affected by climatic conditions, varietal differences, cultural practices during production as well as postharvest handling.

Mango is classified as a climacteric fruit which is harvested raw and ripened for the market. It undergoes various physiological and biochemical changes that transform the fruit from raw to ripe and edible state. The changes include changes in both peel and pulp colour, softening, increase in sugar content as well as accumulation of aroma volatiles and changes in acid concentrations. These changes are what leads to ripening and appeal of the fruits to the consumers. However, after ripening, senescence sets in where reactions of anabolic nature are inhibited by degradative changes leading to decay and death of the fruit tissue (Valero and Serrano, 2010) which in turn leads to decline in quality of the fruits. If not checked this may lead to huge losses to farmers and traders since the fruits become less appealing to consumers and begin to rot. Post-harvest losses in mango have been reported to be more than 40% in Kenya (FAO, 2012).

It is therefore important that postharvest technologies to prolong shelf life are applied to reduce the losses. Several technologies have been developed and Hexanal is one of them. Treating fruits with hexanal slows down fruits metabolic activities hence keeping them in better quality for longer. A good postharvest technology should be able to prolong shelf life without affecting the quality of the produce. In mango, hexanal has been used as a field spray with promising results (Anusuya *et al*., 2016). Being a new technology there is need to investigate its effectiveness in prolonging shelf life and how it affects the postharvest quality of fruits. There have been successful studies on the use of Hexanal treatment in tomato (Cheema *et al*., 2014), papaya (Hutchinson *et al*., 2018) and Banana (Yumbya *et al*., 2020).

4.2 Materials and methods

4.3.1 Sample preparation

Fruit samples from each treatment (control, 2% EFF and 3% EFF) classified in varieties and AEZ of production were analyzed for various biochemical attributes namely total titratable acidity, total soluble solids, ascorbic acid content, Beta carotene content and major soluble sugars content (glucose, sucrose and fructose). Portions of fruits from all treatments were chopped and stored in zip lock bags which were marked and stored in a freezer at -20°C. The quality attributes analysis was done at the end when all samples had been collected. The factors considered were agro ecological zones (AEZ II and AEZ IV), varieties (Apple and Tommy atkins) and treatments (Control, 2% EFF and 3% EFF).

4.4 Measurements of bio chemical attributes of mango

4.4.1 Total soluble solids (TSS)

Juice squeezed from three selected fruits sampled from each treatment was used. 5ml of mango fruit extract obtained from 3 fruits in each treatment were placed in a prism of a digital refractometer (Model 500, Atago, and Tokyo, Japan) and recorded °Brix as per the instrument readings.

4.4.2 Total titratable acidity (TTA)

Titration was used to determine the (TTA), in which 5g of the fruit pulp was crushed and diluted with 20ml of distilled water. 10ml of the diluted solution was set aside and mixed with 2-3 drops of phenolphthalein indicator before being titrated with 0.1N Sodium hydroxide until the solution turned a faint pink color. The titre volume was measured and recorded, and the TTA was calculated as a percentage of citric acid equivalent using the formula

%Citric acid equivalent. = Sample reading (ml)*Dilution factor (0.0064) *100/sample weight (g)

4.4.3 Ascorbic Acid content

The HPLC method was used to determine the ascorbic acid content (Mamun *et al,* .2012). 5g of sample was weighed and extracted with 0.8 percent metaphosphoric acid under low light conditions. The extract was created by centrifuging 20 mL of juice for 10 minutes at 10000 rpm at 4°C. The supernatant was filtered and diluted in 10 mL with 0.8 percent meta-phosphoric acid. The filtrate was then passed through 0.45 micro filters.

After that, the samples were programmed as a post-run into an HPLC machine (Model LC-10AS, Shimadzu Corp., Kyoto, Japan), where 20L of the micro-filtered sample was automatically injected on the same day of extraction. An ODS C-18 column measuring 250 mm x 4.6mm x 0.5l was used for the analysis. By preparing various concentrations of ascorbic acid standards at 10, 20, 40, 60, 80, and 100 ppm, as well as a blank containing only degassed meta-phosphoric acid, a calibration curve was obtained. Shimadzu UV-VIS detector was used for HPLC analysis. The mobile phase was 0.8 percent metaphosphoric acid at 1.2 mL/min flow rate and 266.0 nm wavelength. The amount of ascorbic acid was calculated using the AOAC (2019) method, which yielded the standard vitamin C regression curve with freshly prepared Vitamin C standards as follows;

$$\boldsymbol{Ascorbic\ acid}\ (\boldsymbol{mg/100ml}) = (\boldsymbol{Peak\ area\ from\ graphs\ y}) * (\boldsymbol{Dilution\ volume}\ \boldsymbol{Sample\ weight}\ (\boldsymbol{g})) * (\mathbf{100})$$

Where y =Calibration coefficient obtained from standard regression curve when y-intercept is zero (AOAC, 2019).

4.4.4 Beta carotene content

Beta carotene content was determines using UV spectrophotometry as explained in the Harvestplus Handbook for Carotenoid Analysis by Rodriguez-Amaya and Kimura, 2004. 2g of mango pulp was crushed with acetone until the sample gave no colour. Portioning using 25ml of petroleum ether was done and a little distilled water added to facilitate separation. The lower mix of acetone and water was carefully separated from the upper layer of petroleum ether and carotenoids. To remove the remaining water, the mixture was poured through a funnel and filter paper containing anhydrous sodium sulphate. All of the extractions were carried out in low-light conditions. From freshly prepared Beta carotene standards, standards at 0, 2, 4, 6, 8, 10, 20, 40, 60, 80 and 100 ppm were made and used to plot a calibration curve used to calculate Beta carotene content in the samples. UV spectrophotometry was used to take absorbance readings at 440nm (Shimadzu model UV-1610 PC, Kyoto, Japan). Beta carotene content was calculated as follows:

Beta carotene content (mg/100g) = Absorbance *Volume of extract(ml)*10^4 /2592*Weight of sample (g)*100

Note - 2592 is the Beta carotene extinction coefficient in petroleum ether.

4.4.5 Major sugars (fructose, glucose and sucrose)

The AOAC 2019 standards were used to analyze sugars. 10g mango pulp was blended, and 96 percent ethanol was added. Refluxing was carried out for one hour at 100°C before being cooled under running water. After that, the solution was filtered through 42mm Whatman filter paper. 5ml of 96 percent ethanol was used to rinse. At 60°C, the solution was rotary evaporated to dryness. Following that, 5ml of 50% acetonitrile was added and micro-filtered (0.45). A high performance liquid chromatography (HPLC) system equipped with a refractive index detector (RID) was used to analyze the sugars (Model LC-20AS, Shimadzu Corp., Kyoto, Japan). The sugars present (glucose, fructose, and sucrose) were identified and their concentrations calculated. A graph was drawn showing the concentration of the standard (X-axis) versus absorbance (Y-axis) and sugar concentration as follows:

$$\boldsymbol{Amount\ of}\ \textbf{sugar}\ \boldsymbol{in\ sample}\ (\boldsymbol{mg}/\textbf{100g}) = \boldsymbol{Sugar\ value\ from\ graph}\ (\boldsymbol{mg})/\ \boldsymbol{Aliquot\ sample\ used} * \boldsymbol{Total\ volume\ of\ extract}\ (\boldsymbol{ml})\ /\boldsymbol{Weight\ of\ sample}\ (\boldsymbol{mg})\ *\textbf{10}$$

4.5 Data analysis

The collected data was analyzed using the GENSTAT 13th edition statistical software, and the means were compared using the Least Significance Difference (LSD) at $P<0.05$.

4.6 Results

4.6.1 Total soluble solids (°Brix)

The TSS or brix content increased gradually as the fruits continued to ripen across all treatments. The highest level was realized on day 9 and then the increase slowed down towards the end of the storage period (Figure 8). There was significant difference in the TSS level for fruits the two agro ecological zones with mango fruits from Machakos recording higher TSS as compared to fruits from Meru at maturity. There was also a significant difference ($p<0.05$) between mangoes from the two varieties with Apple mangoes having higher TSS level as compared to Tommy Atkins variety.

Hexanal treatment slowed down the rate at which the TSS level changed. The 2% and 3% EFF treated mangoes had a slower rate in the increase and eventual decline of TSS level as compared to the untreated fruits which indicated slowed down ripening process. The treatment did not however alter the level of TSS in the treated fruits at the end of the ripening period (Figure 8).

For Apple mangoes harvested from Machakos, the TSS increased steadily for untreated mangoes from 10.06°Brix at the beginning of the storage period, reached a peak of 21.63°Brix on day 9 and dropped to 12.1°Brix on day 15. The same trend was observed from the treated mangoes though the rate of increase and eventual decline was slower. The apple mangoes treated with 2% EFF had a TSS increase from 10.03°Brix on day 0 to 19.1°Brix on day 9 and dropped to 12.2°Brix on day 15 while those treated with 3% EFF increased from 9.9°Brix on day 0 to 17°Brix on day 9 then dropped to 11.8°Brix by day 15 (8A). In Tommy mangoes harvested in Machakos, the same trend was observed with TSS in untreated fruits increasing from 10.53°Brix at the beginning of the ripening process to 15.73°Brix on day 9 and declining to 14.7°Brix on day 15. The TSS in 2% EFF treated fruits increased from

10.4°Brix to 14.23°Brix on day 9 and declined to 12.1°Brix on day 15. While TSS in 3% EFF treated fruits rose from 10.4°Brix on day 0 to 12.9°Brix on day 9 and dropped to 11.1°Brix on day 15(8A).

The same trend was observed in Apple mangoes harvested in Meru. The TSS in untreated fruits increased from 9.6°Brix at the beginning of the storage period to 20.13°Brix on day 9 then dropped to 15.5°Brix on day 15. For the 2% EFF treated fruits, TSS increased from 9.7°Brix to 18.76°Brix on day 9 then dropped to 13.43°Brix on day 15 while for the 3% EFF treated fruits TSS increased from 9.5°Brix to 15.5°Brix on day 9 and declined to 8.93°Brix on day 15(8B). For Tommy mangoes harvested from Meru the TSS in untreated fruits increased from 9.03°Brix at the beginning of the storage period to 14.46°Brix on day 9 then dropped to 11.7°Brix on day 15. The TSS in the 2% EFF treated fruits rose from 9.33°Brix to 13.33°Brix on day 9 and then declined to 10.53°Brix on day 15 while TSS in the 3% EFF treated fruits rose from 8.96°Brix on day 0 to 12.35°Brix on day 9 then dropped to 10.16°Brix on day 15(8B).

The slower rate of changes in TSS in treated mangoes could be attributed to slowed down ripening rate caused by hexanal treatment. 3% EFF treated mangoes had the slowest increase in TSS. The treatment of the fruits with hexanal did not affect the level of TSS content but only slowed the rate of increase by 3 days (day 15 – day 18).

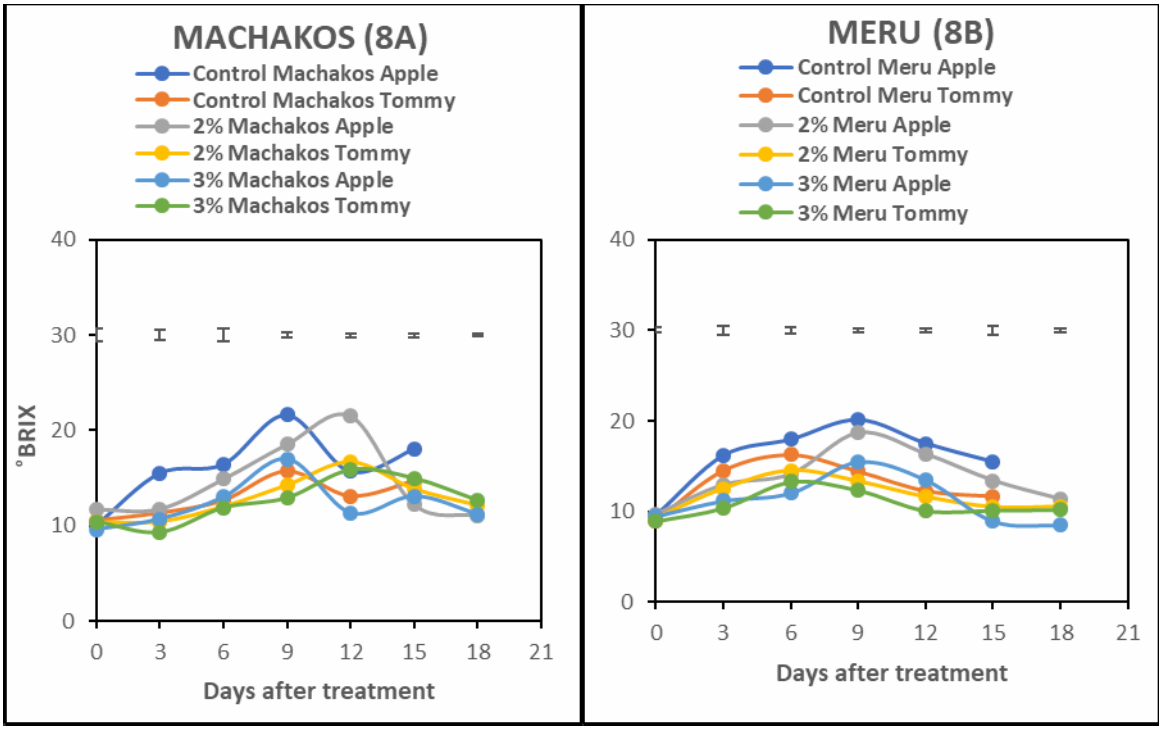

Figure 8: The Effects of hexanal treatment on the TSS/ Brix of Apple and Tommy Atkins varieties of Mango harvested in Machakos and Meru. The vertical bars are representative of the LSD at ($p<0.05$)

4.6.2 Ascorbic Acid (Vitamin C)

Ascorbic Acid drops drastically at the beginning of the ripening process and slows down from day 9 onwards in all fruits regardless of the variety, treatment or agro ecological zone of production (Figure 9). The ascorbic acid content of fruits harvested from different locations differed significantly ($P<0.05$) with fruits harvested from Machakos (AEZ IV) having higher levels of ascorbic acid as compared to the fruits harvested from Meru (AEZ II). Difference in variety significantly ($P<0.05$) affected the level of ascorbic acid with apple mangoes showing slightly higher levels than Tommy atkins variety.

This study also revealed that the fruits treated with hexanal had a slower rate of the decline of ascorbic acid levels in comparison with the untreated fruits (Figure 9). Fruits treated with 3% EFF recorded the slowest rate of decline in ascorbic acid. In the apple variety harvested from Machakos untreated fruits had a drop of 91.8% by day 9 as compared to 87.4% in fruits treated with 2% EFF and 73.3% for fruits treated with 3% EFF (Figure 9A). Tommy variety demonstrated the same trend where Machakos untreated fruits had a drop of 93.7% while 2%

and 3% EFF treated fruits had a drop of 90.5% and 86.7% respectively by day 9 of the storage period (Figure 9A).

Apple mangoes harvested from Meru had a similar trend where control fruits had an ascorbic acid drop of 94.2% while 2% and 3% EFF had a drop of 90.1% and 84.5% respectively by day 9 (Figure 9B). In Meru, control Tommy mangoes had a decline of 95% while 2% and 3% EFF treated fruits had a decline of 92.8% and 89.04% respectively by day 9 (Figure 9D). The hexanal treatment had no effect on Vitamin C levels in the fruits but caused a three-day delay in the treated fruits as compared to the control fruits. However, the drastic drop of Vitamin C at the end of the ripening period seems inconsistent with earlier studies on mango and therefore calls for further investigation.

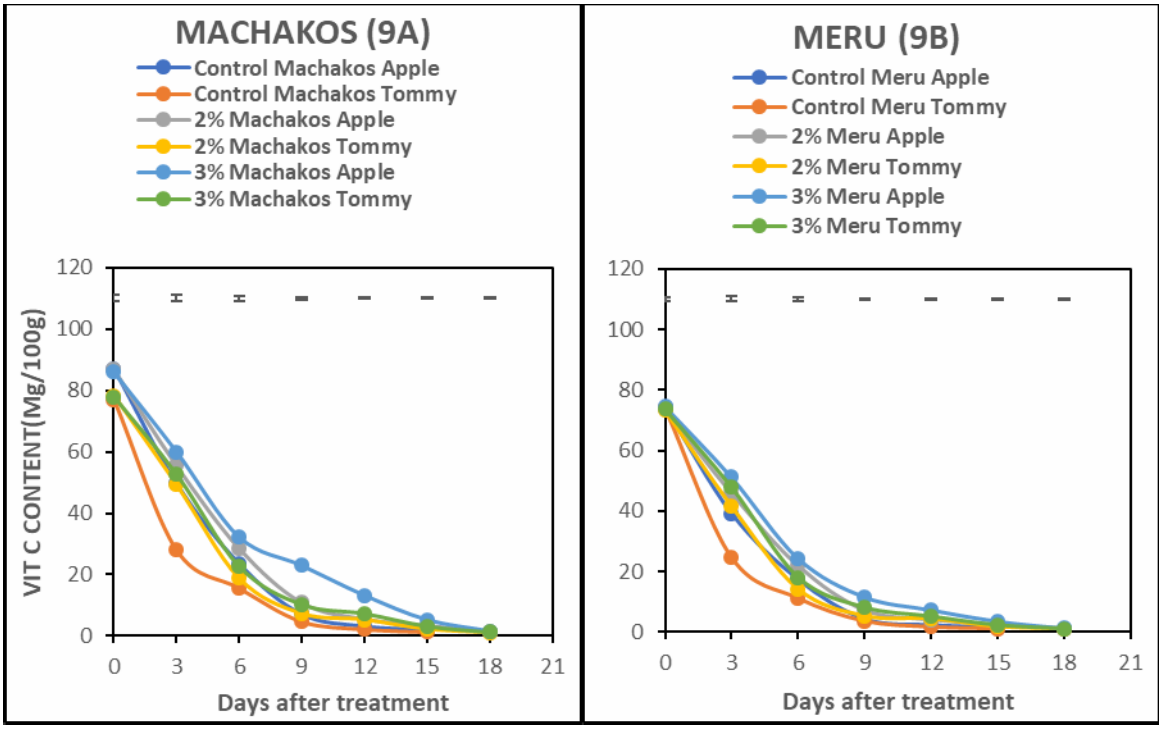

Figure 9: Effects of hexanal treatments on the Ascorbic acid/ Vitamin C of Apple and Tommy Atkins varieties of Mango harvested in Machakos and Meru. The vertical bars are representative of the LSD at ($p<0.05$)

4.6.3 Total Titratable Acidity (TTA)

The percent TTA declined rapidly and then slowed down in mangoes throughout the storage period across all varieties, treatments as well as agro ecological zones of production (Figure 10). The apple mango variety from both agro ecological zones had a higher %TTA at harvest as compared to the Tommy Atkins variety. Hexanal treatment did not affect the %TTA in the treated fruits but only slowed down the rate of decline. The untreated fruits had a more drastic drop in % TTA as compared to the 2% and 3% EFF treated fruits (Figure 10).

In Apple mangoes harvested from Machakos, the %TTA in untreated fruits drastically dropped from 83.94% on day 0 to 8.6% on day 15. The % TTA in 2% EFF treated fruits dropped from 83.42% at the beginning of the storage period to 10.66% by day 15 while the TTA in 3% EFF treated fruits dropped drastically from 83.2% on day 0 to 10.85% on day 15 (Figure 10A). For the Tommy atkins mangoes harvested from Machakos, the TTA in untreated fruits dropped drastically from 73.68% at the beginning of the storage period to 9.17% by day 15. The TTA in the 2% EFF treated fruits dropped from 74.29% on day 0 to 10.45% on day 15 while in the 3% EFF treated fruits it declined from 73.7% on day 0 to 12.37% on day 15 (Figure 10A).

For apple mangoes harvested in Meru, the TTA in untreated fruits dropped from 72.96% on day 0 to 8.74% on day 15. The TTA in the 2% EFF treated fruits dropped from 73.38% on day 0 to 8.32% on day 15 while in the 3% EFF treated fruits it declined from 75.09% on day 0 to 9.6% on day 15 (Figure 10B). The same trend was observed in Tommy atkins mangoes harvested in Meru. The TTA in untreated fruits declined from 59.7% at the beginning of the storage period to 7.65% on day 15. In the 2% EFF treated fruits it dropped from 60.37% on day 0 to 10.02% on day 15 while in the 3% EFF treated fruits it dropped from 61.22% on day 0 to 10.45% on day 15 (Figure 10B).

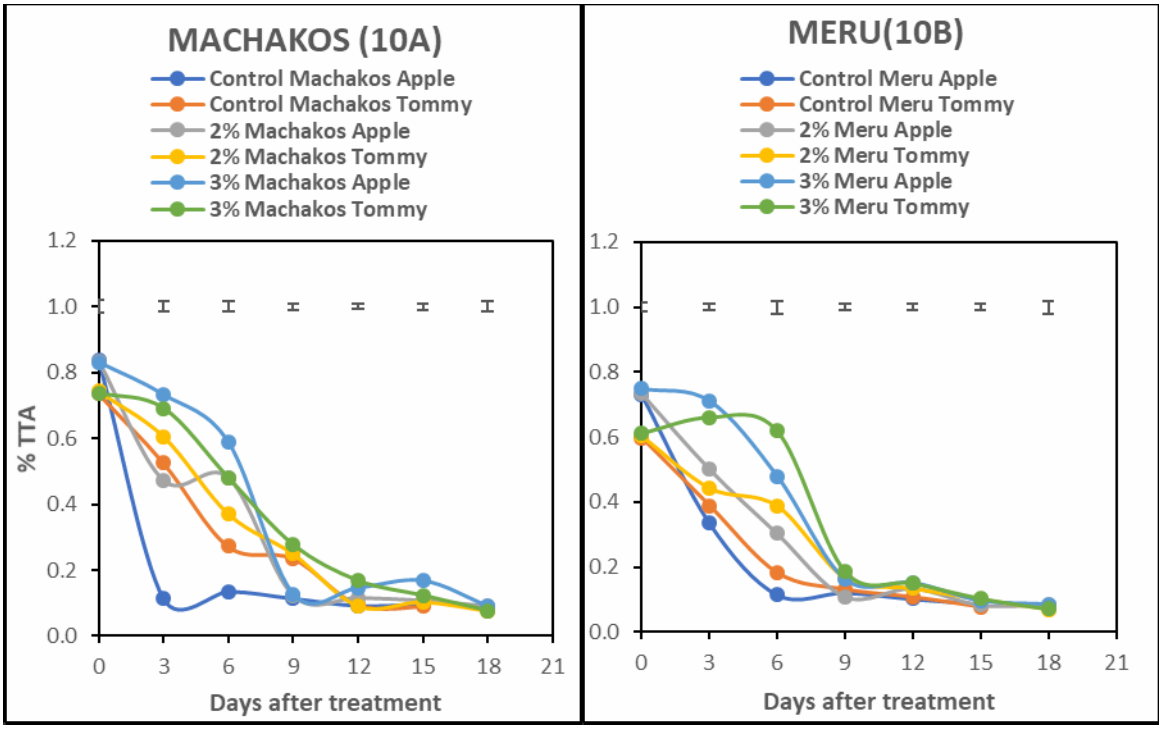

Figure 10: Effects of hexanal treatments on the TTA of Apple and Tommy Atkins varieties of Mango harvested in Machakos and Meru. The vertical bars are representative of the LSD at ($p<0.05$)

4.6.4 Beta Carotene

The beta carotene content in mangoes gradually increased throughout the ripening process in all treatments regardless of variety and agro ecological zone of production (Figure 11). There was observed a significant difference ($P<0.05$) in Beta carotene content between fruits harvested from different agro ecological zones with fruits from the hotter region of Machakos recording a higher level of Beta carotene in both Apple and Tommy varieties. Variety did not have a significant difference in the content level of Beta carotene in the fruits. Treatment by hexanal did not affect the level of beta carotene in the treated fruits but only slowed down the rate at which the rate increased (Figure 11).

By day 15 of storage, the beta carotene content in untreated apple mangoes harvested from Machakos increased from 1.33mg/100g to 14.33mg/100g while the fruits treated with 2% EFF had an increase from 1.33mg/100g to 12.46mg/100g. Mangoes treated with 3% EFF had an increase from1.36mg/100g to 12.03mg/100g (Figure 11A). The same trend was

observed in Tommy atkins mangoes. Control tommy fruits harvested from Machakos had an increase of 1.83mg/100g to 11.36mg/100g between day 1 and day 15. Within the same storage period the beta carotene level of mangoes treated with 2% increased from 1.76mg/100g to 10.43mg/100g while those treated with 3% EFF had an increase from 1.8mg/100g to 9.93mg/100g (Figure 11A).

The increase rate in the beta carotene content in mangoes harvested from Meru followed a similar trend. For the apple mangoes, the untreated mangoes increased from 0.3mg/100g on day 0 to 11.2mg/100g by day 15. For the treated mangoes, 2% EFF increased from 0.36mg/100g to 10.33mg/100g while 3% EFF increased from 0.33 to 9.56 between day 0 and day 15 (Figure 11B). In the Tommy Atkins variety harvested from Meru, the beta carotene content in untreated mangoes increased from 0.8mg/100g to 11.33 mg/100g between day 0 and day 15 of the storage period. Within the same period fruits treated with 2% EFF increased from 0.8mg/100g to 10.33mg/100g while fruits treated with 3%EFF increased from 0.76mg/100g to 9.8mg/100g (Figure 11B).

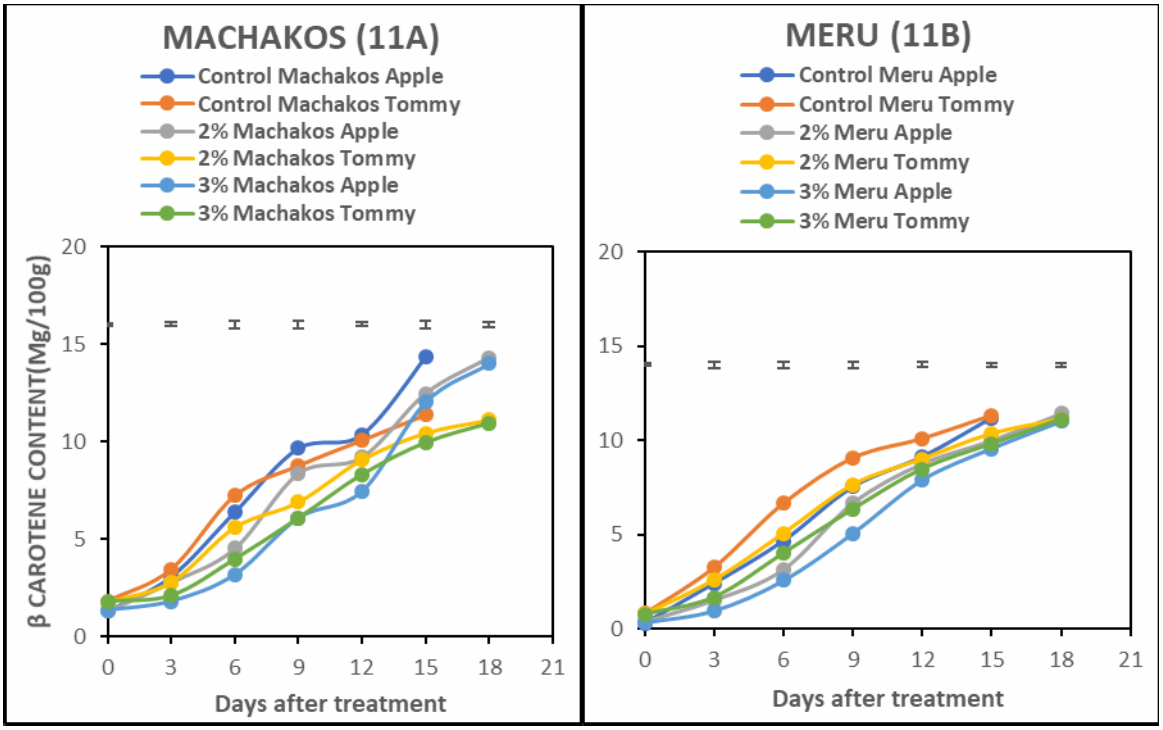

Figure 11: Effects of hexanal treatments on the Beta Carotene content in (mg/100g) of Apple and Tommy Atkins varieties of Mango harvested in Machakos and Meru. The vertical bars are representative of the LSD at ($p<0.05$)

4.6.5 Simple sugars

4.6.5.1 Glucose

Glucose content in mango increased gradually throughout the ripening process regardless of the variety, treatment and zone of production. In this study the increase was more drastic for untreated mangoes as compared to the hexanal treated ones. Hexanal treatment seemed to slow down the ripening process hence the slower increase of glucose in the treated fruits (Figure 12).

For apple mangoes produced in Machakos, untreated fruits recorded an increase of 0.23mg/100g on day 3 to 1.31mg/100g on day 15. Fruits treated with 2%EFF had an increase from 0.14mg/100g on day 3 to 0.96mg/100g on day 15 while the fruits treated with 3% EFF recorded an increase from 0.1mg/100g on day 3 to 0.89mg/100g on day 15(12A). For Tommy atkins mangoes from the same region, untreated mangoes had an increase from0.21mg/100g on day 3 to 1.11mg/100g on day 1. Mangoes treated with 2%EFF had an

increase of from 0.13mg/100g to 0.84mg/100g between day 3 and day 15 while fruits treated with 3% EFF recorded an increase of 0.1mg/100g to 0.81mg/100g within the same period (Figure 12A).

The same pattern was observed in mangoes harvested from Meru (AEZ II). Untreated apple mangoes had an increase from 0.22mg/100g to 1.29mg/100g between day 3 and day 15. Fruits treated with 2%EFF had an increase from 0.12mg/100g to 0.94mg/100g between day 3 and day 15, while mangoes treated with 3% EFF had an increase from 0.1mg/100g to 0.9mg/100g within the same period (12B). For the Tommy atkins variety the same trend was observed. Control mangoes recorded an increase from 0.2mg/100g on day 3 to 1.2mg/100g on day 15. Fruits treated with 2%EFF had an increase from 0.13mg/100g to 0.79mg/100g between day 3 and day 15 while fruits treated with 3% EFF had an increase from 0.11mg/100g to 0.77mg/100g within the same period (Figure 12B).

Hexanal treatment only slowed down the increase in glucose content but did not affect the level of glucose by the end of the storage period.

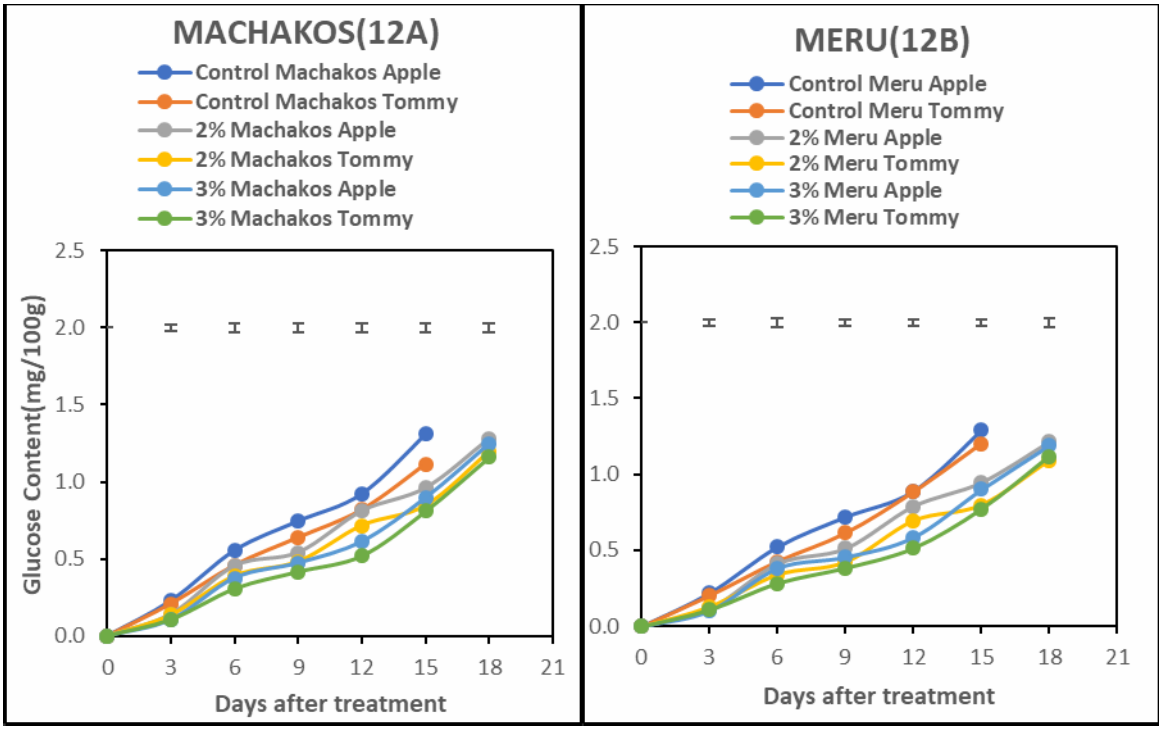

Figure 12: Effects of hexanal treatments on the Glucose content in(mg/100g) of Apple and Tommy Atkins varieties of Mango harvested in Machakos and Meru. The vertical bars are representative of the LSD at ($p<0.05$)

4.6.5.2 Fructose

In this study, the fructose content in mango increased gradually throughout the storage period irrespective of the variety, location of production as well as treatment. It was however observed that the rate of increase was slower in fruits treated with hexanal with the 3% EFF treated fruits recording the slowest rate (Figure 13).

In Machakos, untreated apple mangoes had a rise of fructose content from 1.39mg/100g at the beginning of the storage period to 6.01mg/100g by day 15. For 2% EFF treated fruits, the rise was from 1.43mg/100g at the beginning of the storage period to 5.97mg/100g by day 18, while 3% EFF treated fruits had an increase from 1.43mg/100g to 6.01mg/100g within the same period (13A). For the Tommy atkins variety, control mangoes had a rise of between 1.31mg/100g to 5.51mg/100g, 2%EFF treated mangoes between 1.3mg/100g and 5.26mg/100g and 3% EFF treated fruits between 1.31mg/100g to 5.35mg/100g from the beginning to the end of the ripening period (Figure 13A).

Mangoes harvested in Meru also followed the same trend with control apple mangoes recording a rise in fructose content from1.81mg/100g to 6.13mg/100g, 2% EFF treated mangoes from 1.82mg/100g to 5.99mg/100g and 3% EFF treated ones from 1.81mg/100g to 6.01mg/100g between the beginning to the end of the storage period (Figure 13B). Same pattern is observed in Tommy atkins variety where untreated mangoes had an increase in fructose content from 0.63mg/100g to 5.58mg/100g, 2% EFF treated mangoes from 0.67mg/100g to 5.53mg/100g while 3% EFF treated fruits from 0.65mg/100g to 5.68mg/100g between the beginning to the end of the storage period (Figure 13B).

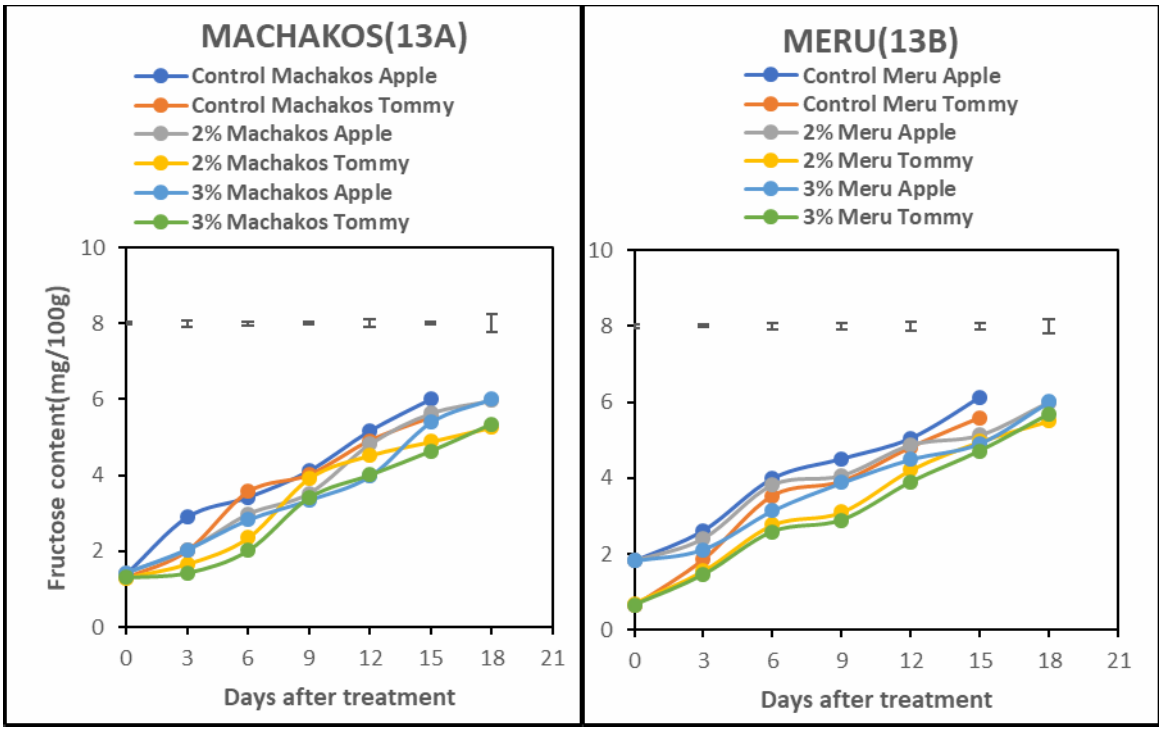

Figure 13: Effects of hexanal treatments on the Fructose content in(mg/100g) of Apple and Tommy Atkins varieties of Mango harvested in Machakos and Meru. The vertical bars are representative of the LSD at ($p<0.05$).

4.6.5.3 Sucrose

The sucrose content in mango rose steadily throughout the ripening period in all the treatments, zones of production as well as varieties. It was observed that fruits treated with hexanal had a slower rise in the sucrose content as compared to untreated ones (Figure 14). A slight difference in sucrose content was however noted in fruits from the two varieties with Apple mangoes recording slightly higher sucrose content that Tommy atkins variety. The hexanal treatment did not affect the level of the sucrose content in the fruits by the conclusion of the ripening period but only slowed down the rate of increase.

For fruits harvested in Machakos, the untreated apple mangoes had a rise from 2.12mg/100g to 8.17mg/100g, 2% EFF treated mangoes from 2.11mg/100g to 8.21mg/100g and 3% EFF treated fruits from 2.1mg/100g to 8.06mg/100g (Figure 14A). For Tommy atkins variety from the same region, control fruits had a rise in sucrose content from 1.72mg/100g to 7.83mg/100g, 2% EFF treated fruits from 1.71mg/100g to 7.52mg/100g and 3% EFF treated mangoes from 1.71mg/100g to 7.11mg/100g from the beginning to the end of the storage period (Figure 14A).

Mangoes from Meru exhibited the same pattern with untreated apple mangoes recording an increase in sucrose content from 2.1mg/100g to 8.21mg/100g, 2% EFF treated fruits from 2.11mg/100g to 8.2mg/100g and 3% EFF treated fruits from 2.1mg/100g to 8.012mg/100g (Figure 14B). In the Tommy atkins variety, untreated fruits had a rise from 1.71mg/100g to 7.43mg/100g, 2% EFF treated fruits from 1.71mg/100g to 7.09mg/100g and 3% EFF treated fruits from 1.71mg/100g to 7.12mg/100g between the start and the end of the ripening period (Figure 14B).

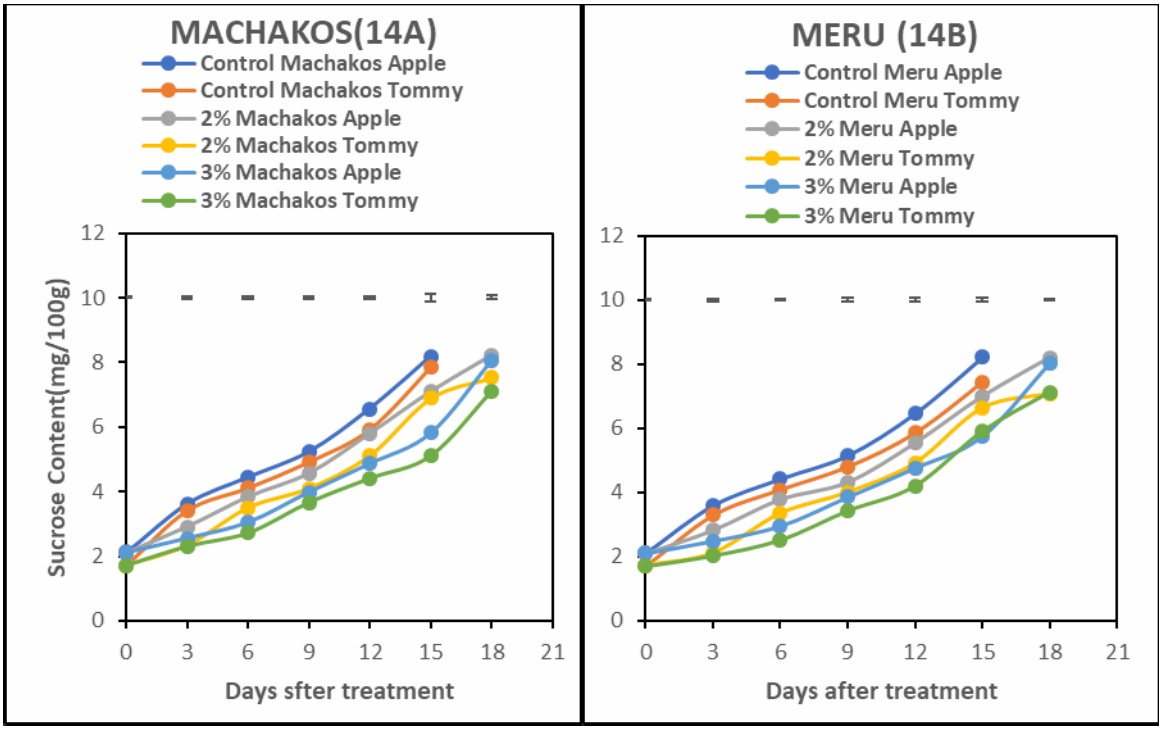

Figure 14: Effects of hexanal treatments on the Sucrose content in (mg/100g) of Apple and Tommy Atkins varieties of Mango harvested in Machakos and Meru. The vertical bars are representative of the LSD at ($p<0.05$)

4.7 Discussion

In this study, several biochemical attributes were measured across all treatments to determine the effect of hexanal on the mango fruits. The rise in TSS throughout the storage period of mangoes is mainly due to the breakdown of starch into simple sugars associated with the ripening process (Siddiqui and Dhua, 2010). The increase could also be due to the partial breakdown of cellulose and pectins (DeLima and Lima, 2001). Hexanal treatment did not affect the level of TSS content in the fruits both across agro ecological zones as well as varieties. The observation of this study was that hexanal only slowed down the rate at which TSS content increased and eventually stabilized by only 3 days (Day 15-day 18) for treated fruits. This could be attributed to the action of hexanal in slowing down the activity of enzymes such as amylase and invertase which are linked to the conversion of stored carbohydrates into soluble sugars (Kumar *et al*., 1994). The mangoes from the different agro ecological zones showed a slight difference in TSS level with mangoes from Machakos

recording slightly higher TSS level as compared to the ones from Meru. This could be attributed to the difference in climatic conditions between both regions. Machakos experiences a lower annual rainfall of 1100mm and higher temperatures of about 28°C. The region also has a higher solar density accompanied by longer durations of sunlight. This results to a higher photosynthetic activity as well as greater carbon accumulation in fruits (Lechaudel *et al*., 2005) as compared to fruits from Meru which has higher rainfalls and lower temperatures. Similar observations have been made in banana (Ambuko *et al*., 2006), passion fruit (Baraza *et al*., 2012), papaya (Hutchinson *et al*., 2018), avocado fruits (Ferguson *et al*., 1999) and Mango (Mendoza *et al*., 1972). A positive relationship between TSS content and light has been formed in mango fruits (Mendoza *et al.,* 1972). The varietal differences observed could be attributed to merely the genetic composition of the different varieties of mangoes under this study.

Vitamin C is an essential quality trait in mango as a fruit. In this study the ascorbic acid level seems to decline throughout the ripening process in both control and EFF treated mangoes. The drop was however slower in treated fruits compared to the untreated ones. This drop could be attributed to the oxidative degradation of ascorbic acid which occurs during respiration or its conversion to metabolites such as amino acids and sugars (Opara *et al*., 2012). Fruits treated with hexanal showed a higher retention of ascorbic acid which could be explained by the inhibition action of hexanal to the enzymatic oxidation of ascorbic acid. This trend had been reported in cherries (Sharma *et al*., 2010) and in tomatoes (Cheema *et al*., 2014). Similar behavior has been studied and noted in papaya where hexanal treatment delayed the decline of vitamin C significantly (Hutchinson *et al*., 2018). However, the drastic drop of Vitamin C at the end of the ripening period in this study seems inconsistent with earlier studies on mango and therefore calls for further investigation.

Studies have shown that vitamin C level could be affected by the climatic conditions, genotype variations, cultural practices as well as postharvest handling. This could be the

cause of the variations in vitamin C content between the varieties and the agro ecological zones of production.

The reduced rate in decline of TTA in the treated fruits could be due to the action of hexanal to slow down acid oxidation. Genetic variations played a role in the different levels of TTA observed between varieties at the beginning of the storage period.

One of the precursors of vitamin A is Beta carotene. It is an oxidant that is useful in the human body to prevent aging, protect against many cancers as well as enhancing vision by protecting the retina and macula. The mango fruit is one of the sources of beta carotene (Yahia, 2006). Studies have shown that the carotenoid content of mangoes increase with ripening in several varieties (Mercadante *et al*., 1998). The difference in beta carotene content in fruits harvested in different agro ecological zones could be attributed to the variations in climatic conditions. Machakos which is a drier region and has high solar intensity and limited water produced mangoes that had a higher beta carotene content. This could be because of the accumulation of dry matter content in the fruits caused by the hot and dry climatic conditions (Lechaudel and Joas, 2006).

All soluble sugars studied (glucose, fructose and sucrose) increased steadily throughout the ripening process. While chloroplast starch content increases during mango fruit development, it is hydrolyzed completely to simple sugars during the ripening process (Ito, 1997). This is because starch is hydrolyzed into simple sugars like fructose, glucose, and sucrose by the action of enzymes like sucrose synthase, invertase, and amylase (Kumar *et al*., 1992). The same trend was observed in banana (Yumbya *et al.*, 2010) where sugar levels rose from 2% to 20% during the ripening process. Hexanal seemed to slow down the rate at which all three soluble sugars rose during ripening. This could be due to the slowed down rate of respiration (where starch is catabolized into sugars used as respiratory substrates) in the treated fruits which is an action associated with hexanal. Venkatachalam *et al*. (2018)

also reported the same trend in banana where hexanal treated bananas had a slower rate of sugar accumulation from 10% to 19% in 27 days of storage.

Varietal variations were noted in some of the sugars as well as differences between fruits from AEZ II and AEZ IV. This could be associated with genotype differences and climatic conditions. The increase of sugars in the pulp of fruits has been reported before to be affected by genotypes as well as different climatic conditions (Mawaduwathi *et al*., 2017).

4.8 Conclusion

The use of hexanal as a postharvest treatment has been studied before in banana, sweet cherries, tomatoes, avocado and mango and has been found to be effective. For a postharvest technology to be considered effective and suitable for commercialization it must not only prolong storage life but also preserve the postharvest quality of the fruits

This study indicates that indeed hexanal used as a postharvest dip extends the shelf life of mango fruits and preserves the physical attributes of fruits as they ripen. Fruits treated with the EFF retained higher levels of TSS and acidity as compared to the untreated controls. The increase in sugars and Beta carotene was also slower in treated fruits as compared to the untreated ones suggesting that hexanal preserved the quality of the harvested fruits better throughout the storage period. It was also noted that 3% EFF was more effective than 2% EFF in preserving the postharvest quality of the mangoes. Varietal differences were also noted with hexanal treatment exhibiting better results with Tommy atkins variety as compared to Apple variety.

CHAPTER 5: General Discussion, Conclusion and Recommendations

5.1 General Discussion

Applying an effective postharvest technology in the handling of mango fruits is important considering the huge losses reported in the value chain. However, consumer needs and safety have to be considered when applying these technologies. It has been noted that consumers and other value chain actors prefer technologies that do not alter the postharvest quality of produce and one that is environmentally friendly, safe for consumption and easy to use.

Hexanal has been proven to extend the storage life of various fruits including tomatoes (Cheema *et al*., 2014), papaya (Hutchinson *et al.*, 2018), mango (Anusuya *et al*., 2016) and cherries (Sharma *et al*., 2010). For a postharvest technology to be considered effective and suitable for commercialization it must not only prolong shelf life but also preserve the postharvest quality of the fruits.

Hexanal treated fruits exhibited slowed down ripening as indicated by the results for both physical and biochemical parameters. Hexanal seemed to slow down the rate of physiological weight loss, loss of firmness, change of colour as well as rate in ethylene evolution and respiration. The rate at which biochemical ripening parameters changed was also slowed down by hexanal treatment.

In terms of varieties, the Tommy Atkins variety seemed to respond better to the hexanal treatments as compared to the Apple variety. This was indicated by the difference in days after treatment that the two varieties took to reach a certain level of ripeness. In most parameters, the Tommy Atkins variety was observed to delay by 3-5 days as compared to the Apple variety. This could be attributed to difference in genotypes in the two varieties.

Mangoes from hotter regions (AEZ IV) were observed to ripen faster as compared to the fruits from colder regions (AEZ II). This could be caused by the difference in temperatures

and other climatic conditions associated with different agro ecological zones. Machakos county is known to be quite hot recording a mean temperature of 30ºC while Meru is relatively colder with temperatures ranging between 18ºC and 21ºC. Such cool temperatures in Meru could be the cause of the slower ripening. Similar observations have been made in apples (Paliyath *et al*., 2008), sweet cherries (Sharma *et al*., 2010) and tomato (Cheema *et al*., 2014).

5.2 General Conclusion

The use of hexanal as a postharvest treatment has been studied before in banana, sweet cherries, tomatoes, avocado and mango and has been found to be effective. For a postharvest technology to be considered effective and suitable for commercialization it must not only prolong storage life but also preserve the postharvest quality of the fruits. This study indicates that hexanal used as a dip applied after harvest prolongs the shelf life of mango fruits and preserves the physical attributes of fruits as they ripen. Fruits treated with the EFF retained a higher firmness, slower change in colour and low rates of respiration and ethylene production. Treated fruits also had a low rate of weight loss throughout the storage period. It was also noted that 3% EFF was more effective than 2% EFF. Results from the study suggest and recommends the use of 3% EFF on mangoes as a postharvest dip to effectively extend shelf life.

This study further indicates that hexanal preserves the physical attributes of fruits as they ripen. Fruits treated with the EFF retained higher levels of TSS and acidity as compared to the untreated controls. The increase in sugars and Beta carotene was also slower in treated fruits as compared to the untreated ones suggesting that hexanal preserved the quality of the harvested fruits better throughout the storage period. It was also noted that 3% EFF was more effective than 2% EFF in preserving the postharvest quality of the mangoes. This study therefore recommends the use of 3% EFF on mangoes as a postharvest dip to effectively extend shelf life. Varietal differences were also noted with hexanal treatment

exhibiting better results with Tommy atkins variety as compared to Apple variety.

5.3 General Recommendations

Hexanal has shown encouraging results in the preservation of shelf life in mango as well as other fruits. This means that its use could help reduce the high postharvest losses recorded in various fruit value chains. In this study the following recommendations have been made:

1. Develop and implement a legal and policy framework towards licensing of Hexanal as a natural and safe postharvest treatment for fresh horticultural produce.
2. More studies and experiments could be commissioned to further evaluate efficacy of Hexanal as a post-harvest treatment in different fruits and vegetables as well as elucidate its mode of action
3. Create awareness among farmers on the use of various postharvest technologies already studied and recommended by researchers. This will bridge the knowledge gap between research and private sector players/ stakeholders along the entire value chain.

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Appendices

Appendix 1: Anova for cumulative weight loss for mango fruits treated with hexanal

% Cumulative weight loss

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AEZ	1	0.81420	0.81420	22.99	<.001
DAYS	6	7377.25332	1229.54222	34716.49	<.001
TREATMENT	2	275.40427	137.70213	3888.06	<.001
VARIETY	1	251.75067	251.75067	7108.25	<.001
AEZ.DAYS	6	11.32694	1.88782	53.30	<.001
AEZ.TREATMENT	2	20.47038	10.23519	288.99	<.001
DAYS.TREATMENT	11	66.59055	6.05369	170.93	<.001
AEZ.VARIETY	1	10.23946	10.23946	289.11	<.001
DAYS.VARIETY	6	162.88250	27.14708	766.51	<.001
TREATMENT.VARIETY	2	2.31644	1.15822	32.70	<.001
AEZ.DAYS.TREATMENT	11	12.33191	1.12108	31.65	<.001
AEZ.DAYS.VARIETY	6	9.86477	1.64413	46.42	<.001
AEZ.TREATMENT.VARIETY	2	1.22022	0.61011	17.23	<.001
DAYS.TREATMENT.VARIETY	11	3.52842	0.32077	9.06	<.001
AEZ.DAYS.TREATMENT.VARIETY	11	4.40605	0.40055	11.31	<.001
Residual	160	5.66667	0.03542		
Total	**239**	**6841.97583**			

Appendix 2: Anova for flesh colour for mango fruits treated with hexanal

FLESH COLOUR

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AEZ	1	418.2	418.2	3.29	0.071
DAYS	6	117238.9	19539.8	153.95	<.001
TREATMENT	2	3604.0	1802.0	14.20	<.001
VARIETY	1	753.0	753.0	5.93	0.016
AEZ.DAYS	6	301.0	50.2	0.40	0.881
AEZ.TREATMENT	2	6.1	3.1	0.02	0.976
DAYS.TREATMENT	12	8366.0	697.2	5.49	<.001
AEZ.VARIETY	1	53.5	53.5	0.42	0.517
DAYS.VARIETY	6	674.6	112.4	0.89	0.507
TREATMENT.VARIETY	2	11.0	5.5	0.04	0.958
AEZ.DAYS.TREATMENT	12	480.5	40.0	0.32	0.986
AEZ.DAYS.VARIETY	6	120.7	20.1	0.16	0.987
AEZ.TREATMENT.VARIETY	2	105.4	52.7	0.42	0.661
DAYS.TREATMENT.VARIETY	12	533.3	44.4	0.35	0.978
AEZ.DAYS.TREATMENT.VARIETY	12	183.9	15.3	0.12	1.000
Residual	168	21323.7	126.9		
Total	**251**	**154173.8**			

Appendix 3: Anova for peel colour for mango fruits treated with hexanal

PEEL COLOUR

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AEZ	1	33.617	33.617	7.33	0.007
DAYS	6	166388.805	27731.468	6045.93	<.001
TREATMENT	2	27835.012	13917.506	3034.25	<.001
VARIETY	1	4761.459	4761.459	1038.08	<.001
AEZ.DAYS	6	303.619	50.603	11.03	<.001
AEZ.TREATMENT	2	14.477	7.239	1.58	0.209
DAYS.TREATMENT	12	22173.161	1847.763	402.84	<.001
AEZ.VARIETY	1	5.821	5.821	1.27	0.262
DAYS.VARIETY	6	1935.402	322.567	70.33	<.001
TREATMENT.VARIETY	2	35.228	17.614	3.84	0.023
AEZ.DAYS.TREATMENT	12	689.537	57.461	12.53	<.001
AEZ.DAYS.VARIETY	6	318.403	53.067	11.57	<.001
AEZ.TREATMENT.VARIETY	2	116.564	58.282	12.71	<.001
DAYS.TREATMENT.VARIETY	12	348.770	29.064	6.34	<.001
AEZ.DAYS.TREATMENT.VARIETY	12	148.978	12.415	2.71	0.002
Residual	168	770.582	4.587		
Total	**251**	**225879.435**			

Appendix 4: Anova for flesh firmness for mango fruits treated with hexanal

FLESH FIRMNESS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AEZ	1	0.0159	0.0159	0.03	0.868
DAYS	6	21932.7754	3655.4626	6425.17	<.001
TREATMENT	2	172.1793	86.0896	151.32	<.001
VARIETY	1	1068.0692	1068.0692	1877.33	<.001
AEZ.DAYS	6	8.4486	1.4081	2.47	0.025
AEZ.TREATMENT	2	1.5634	0.7817	1.37	0.256
DAYS.TREATMENT	12	112.3913	9.3659	16.46	<.001
AEZ.VARIETY	1	8.6173	8.6173	15.15	<.001
DAYS.VARIETY	6	780.6197	130.1033	228.68	<.001
TREATMENT.VARIETY	2	14.0406	7.0203	12.34	<.001
AEZ.DAYS.TREATMENT	12	7.6671	0.6389	1.12	0.345
AEZ.DAYS.VARIETY	6	19.0249	3.1708	5.57	<.001
AEZ.TREATMENT.VARIETY	2	4.7529	2.3765	4.18	0.017
DAYS.TREATMENT.VARIETY	12	36.7056	3.0588	5.38	<.001
AEZ.DAYS.TREATMENT.VARIETY	12	28.6332	2.3861	4.19	<.001
Residual	168	95.5800	0.5689		
Total	**251**	**24291.0843**			

Appendix 5: Anova for peel firmness for mango fruits treated with hexanal

PEEL FIRMNESS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AEZ	1	19.948	19.948	7.31	0.008
DAYS	6	108795.914	18132.652	6644.89	<.001
TREATMENT	2	1429.807	714.903	261.98	<.001
VARIETY	1	304.260	304.260	111.50	<.001
AEZ.DAYS	6	47.492	7.915	2.90	0.010
AEZ.TREATMENT	2	16.349	8.174	3.00	0.053
DAYS.TREATMENT	12	922.692	76.891	28.18	<.001
AEZ.VARIETY	1	33.223	33.223	12.17	<.001
DAYS.VARIETY	6	582.455	97.076	35.57	<.001
TREATMENT.VARIETY	2	0.161	0.080	0.03	0.971
AEZ.DAYS.TREATMENT	12	31.539	2.628	0.96	0.486
AEZ.DAYS.VARIETY	6	48.450	8.075	2.96	0.009
AEZ.TREATMENT.VARIETY	2	0.850	0.425	0.16	0.856
DAYS.TREATMENT.VARIETY	12	19.905	1.659	0.61	0.834
AEZ.DAYS.TREATMENT.VARIETY	12	27.852	2.321	0.85	0.598
Residual	168	458.440	2.729		
Total	**251**	**112739.336**			

Appendix 6: Anova for Respiration Rate for mango fruits treated with hexanal

RESPIRATION RATE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AEZ	1	465.4744	465.4744	891.83	<.001
DAYS	6	3687.8693	614.6449	1177.63	<.001
TREATMENT	2	240.4865	120.2433	230.38	<.001
VARIETY	1	379.1889	379.1889	726.51	<.001
AEZ.DAYS	6	168.1471	28.0245	53.69	<.001
AEZ.TREATMENT	2	22.7613	11.3806	21.80	<.001
DAYS.TREATMENT	11	973.0070	88.4552	169.48	<.001
AEZ.VARIETY	1	0.5814	0.5814	1.11	0.293
DAYS.VARIETY	6	86.6313	14.4386	27.66	<.001
TREATMENT.VARIETY	2	91.5653	45.7826	87.72	<.001
AEZ.DAYS.TREATMENT	11	207.3240	18.8476	36.11	<.001
AEZ.DAYS.VARIETY	6	143.8661	23.9777	45.94	<.001
AEZ.TREATMENT.VARIETY	2	4.3597	2.1799	4.18	0.017
DAYS.TREATMENT.VARIETY	11	16.8108	1.5283	2.93	0.001
AEZ.DAYS.TREATMENT.VARIETY	11	56.8878	5.1716	9.91	<.001
Residual	160	83.5092	0.5219		
Total	**239**	**6382.1972**			

Appendix 7: Anova for ethylene evolution rate for mango fruits treated with hexanal

Ethylene Evolution Rate

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AEZ	1	1.322248	1.322248	280.83	<.001
DAYS	6	90.255504	15.042584	3194.89	<.001
TREATMENT	2	0.268526	0.134263	28.52	<.001
VARIETY	1	0.008181	0.008181	1.74	0.189
AEZ.DAYS	6	3.075508	0.512585	108.87	<.001
AEZ.TREATMENT	2	3.304839	1.652419	350.96	<.001
DAYS.TREATMENT	11	95.722219	8.702020	1848.22	<.001
AEZ.VARIETY	1	1.474334	1.474334	313.13	<.001
DAYS.VARIETY	6	1.037012	0.172835	36.71	<.001
TREATMENT.VARIETY	2	0.012768	0.006384	1.36	0.261
AEZ.DAYS.TREATMENT	11	2.639008	0.239910	50.95	<.001
AEZ.DAYS.VARIETY	6	1.208604	0.201434	42.78	<.001
AEZ.TREATMENT.VARIETY	2	0.092613	0.046306	9.83	<.001
DAYS.TREATMENT.VARIETY	11	1.119284	0.101753	21.61	<.001
AEZ.DAYS.TREATMENT.VARIETY	11	1.174931	0.106812	22.69	<.001
Residual	160	0.753333	0.004708		
Total 239	**197.647333**				

Appendix 8: Anova for % Brix for mango fruits treated with hexanal

% BRIX

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AEZ	1	38.0334	38.0334	188.44	<.001
DAYS	6	946.3877	157.7313	781.49	<.001
TREATMENT	2	292.1088	146.0544	723.64	<.001
VARIETY	1	165.8143	165.8143	821.54	<.001
AEZ.DAYS	6	137.9797	22.9966	113.94	<.001
AEZ.TREATMENT	2	16.6334	8.3167	41.21	<.001
DAYS.TREATMENT	11	137.4017	12.4911	61.89	<.001
AEZ.VARIETY	1	1.7970	1.7970	8.90	0.003
DAYS.VARIETY	6	151.8244	25.3041	125.37	<.001
TREATMENT.VARIETY	2	81.6703	40.8351	202.32	<.001
AEZ.DAYS.TREATMENT	11	68.9310	6.2665	31.05	<.001
AEZ.DAYS.VARIETY	6	54.0149	9.0025	44.60	<.001
AEZ.TREATMENT.VARIETY	2	2.1323	1.0661	5.28	0.006
DAYS.TREATMENT.VARIETY	11	47.1516	4.2865	21.24	<.001
AEZ.DAYS.TREATMENT.VARIETY	11	38.8121	3.5284	17.48	<.001
Residual	160	32.2933	0.2018		
Total	**239**	**2207.2940**			

Appendix 9: Anova for TTA for mango fruits treated with hexanal

TTA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AEZ	1	0.0655981	0.0655981	284.55	<.001
DAYS	6	13.7122606	2.2853768	9913.56	<.001
TREATMENT	2	0.7298983	0.3649492	1583.08	<.001
VARIETY	1	0.0029609	0.0029609	12.84	<.001
AEZ.DAYS	6	0.0851499	0.0141916	61.56	<.001
AEZ.TREATMENT	2	0.0018128	0.0009064	3.93	0.021
DAYS.TREATMENT	12	0.9456744	0.0788062	341.85	<.001
AEZ.VARIETY	1	0.0073851	0.0073851	32.04	<.001

DAYS.VARIETY	6	0.2740182	0.0456697	198.11	<.001
TREATMENT.VARIETY	2	0.0375448	0.0187724	81.43	<.001
AEZ.DAYS.TREATMENT	12	0.0429669	0.0035806	15.53	<.001
AEZ.DAYS.VARIETY	6	0.1304627	0.0217438	94.32	<.001
AEZ.TREATMENT.VARIETY	2	0.0327971	0.0163986	71.13	<.001
DAYS.TREATMENT.VARIETY	12	0.1136552	0.0094713	41.08	<.001
AEZ.DAYS.TREATMENT.VARIETY	12	0.0639066	0.0053255	23.10	<.001
Residual	168	0.0387291	0.0002305		
Total	**251**	**16.2848208**			

Appendix 10: Anova for Vitamin C for mango fruits treated with hexanal

VITAMIN C

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AEZ	1	1.040	1.040	2599.06	<.001
DAYS	6	1.792	2.987	74650.71	<.001
TREATMENT	2	1.531	7.655	1913.14	<.001
VARIETY	1	1.081	1.081	2701.59	<.001
AEZ.DAYS	6	5.489	9.148	228.63	<.001
AEZ.TREATMENT	2	2.213	1.107	27.66	<.001
DAYS.TREATMENT	12	1.423	1.186	296.32	<.001
AEZ.VARIETY	1	1.444	1.444	360.78	<.001
DAYS.VARIETY	6	6.872	1.145	286.24	<.001
TREATMENT.VARIETY	2	2.942	1.471	36.77	<.001
AEZ.DAYS.TREATMENT	12	4.785	3.987	9.97	<.001
AEZ.DAYS.VARIETY	6	1.100	1.834	45.83	<.001
AEZ.TREATMENT.VARIETY	2	9.111	4.556	11.39	<.001
DAYS.TREATMENT.VARIETY	12	4.023	3.352	83.78	<.001
AEZ.DAYS.TREATMENT.VARIETY	12	3.705	3.088	7.72	<.001
Residual	168	6.722	4.001		
Total	**251**	**1.864**			

Appendix 11: Anova for Beta Carotene for mango fruits treated with hexanal

BETA CAROTENE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AEZ	1	45.34766	45.34766	1459.46	<.001
DAYS	6	3777.95913	629.65985	20264.91	<.001
TREATMENT	2	94.97770	47.48885	1528.38	<.001
VARIETY	1	0.18893	0.18893	6.08	0.015
AEZ.DAYS	6	10.31595	1.71933	55.33	<.001
AEZ.TREATMENT	2	0.98437	0.49218	15.84	<.001
DAYS.TREATMENT	12	45.71897	3.80991	122.62	<.001
AEZ.VARIETY	1	29.82893	29.82893	960.01	<.001
DAYS.VARIETY	6	52.85357	8.80893	283.51	<.001
TREATMENT.VARIETY	2	0.35643	0.17821	5.74	0.004
AEZ.DAYS.TREATMENT	12	2.16452	0.18038	5.81	<.001
AEZ.DAYS.VARIETY	6	17.91802	2.98634	96.11	<.001
AEZ.TREATMENT.VARIETY	2	0.72881	0.36440	11.73	<.001
DAYS.TREATMENT.VARIETY	12	2.15357	0.17946	5.78	<.001
AEZ.DAYS.TREATMENT.VARIETY	12	1.24008	0.10334	3.33	<.001
Residual	168	5.22000	0.03107		
Total	**251**	**4087.95663**			

Appendix 12: Anova for Fructose content for mango fruits treated with hexanal

FRUCTOSE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AEZ	1	0.134107	0.134107	49.46	<.001
DAYS	6	618.934192	103.155699	38041.44	<.001
TREATMENT	2	19.312382	9.656191	3560.98	<.001
VARIETY	1	16.519997	16.519997	6092.19	<.001
AEZ.DAYS	6	2.053219	0.342203	126.20	<.001
AEZ.TREATMENT	2	0.025081	0.012540	4.62	0.011
DAYS.TREATMENT	11	5.085176	0.462289	170.48	<.001
AEZ.VARIETY	1	1.351124	1.351124	498.26	<.001
DAYS.VARIETY	6	0.935542	0.155924	57.50	<.001
TREATMENT.VARIETY	2	0.106926	0.053463	19.72	<.001
AEZ.DAYS.TREATMENT	11	0.887809	0.080710	29.76	<.001
AEZ.DAYS.VARIETY	6	4.199422	0.699904	258.11	<.001
AEZ.TREATMENT.VARIETY	2	0.097838	0.048919	18.04	<.001
DAYS.TREATMENT.VARIETY	11	0.912214	0.082929	30.58	<.001
AEZ.DAYS.TREATMENT.VARIETY	11	1.008162	0.091651	33.80	<.001
Residual	160	0.433867	0.002712		
Total	**239**	**586.505730**			

Appendix 13: Anova for Glusose for mango fruits treated with hexanal

GLUCOSE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AEZ	1	0.0290880	0.0290880	143.64	<.001
DAYS	6	42.4965894	7.0827649	34976.62	<.001
TREATMENT	2	1.7705117	0.8852559	4371.63	<.001
VARIETY	1	0.2901798	0.2901798	1432.99	<.001
AEZ.DAYS	6	0.0282186	0.0047031	23.23	<.001
AEZ.TREATMENT	2	0.0071315	0.0035658	17.61	<.001
DAYS.TREATMENT	11	0.7815463	0.0710497	350.86	<.001
AEZ.VARIETY	1	0.0003647	0.0003647	1.80	0.182
DAYS.VARIETY	6	0.1244494	0.0207416	102.43	<.001
TREATMENT.VARIETY	2	0.0026843	0.0013421	6.63	0.002
AEZ.DAYS.TREATMENT	11	0.0082207	0.0007473	3.69	<.001
AEZ.DAYS.VARIETY	6	0.0043520	0.0007253	3.58	0.002
AEZ.TREATMENT.VARIETY	2	0.0067624	0.0033812	16.70	<.001
DAYS.TREATMENT.VARIETY	11	0.0083773	0.0007616	3.76	<.001
AEZ.DAYS.TREATMENT.VARIETY	11	0.0100409	0.0009128	4.51	<.001
Residual	160	0.0324000	0.0002025		
Total	**239**	**37.9218933**			

Appendix 14: Anova for Sucrose for mango fruits treated with hexanal

SUCROSE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AEZ	1	4.646	4.646	565.57	<.001
DAYS	6	5.996	9.993	1.217E+05	<.001
TREATMENT	2	1.514	7.571	9216.74	<.001
VARIETY	1	1.251	1.251	15225.60	<.001
AEZ.DAYS	6	2.263	3.772	45.92	<.001
AEZ.TREATMENT	2	1.431	7.155	87.11	<.001
DAYS.TREATMENT	12	5.342	4.451	54190.77	<.001
AEZ.VARIETY	1	1.717	1.717	20.90	<.001
DAYS.VARIETY	6	6.334	1.056	128.51	<.001
TREATMENT.VARIETY	2	2.896	1.448	176.27	<.001
AEZ.DAYS.TREATMENT	12	6.201	5.168	62.91	<.001
AEZ.DAYS.VARIETY	6	9.240	1.540	18.75	<.001
AEZ.TREATMENT.VARIETY	2	6.864	3.432	41.78	<.001
DAYS.TREATMENT.VARIETY	12	1.826	1.522	185.27	<.001
AEZ.DAYS.TREATMENT.VARIETY	12	7.721	6.434	78.33	<.001
Residual	168	1.380	8.214		
Total	**251**	**1.167**			

Appendix 15: Colour wheel