EFFICACY OF HEXANAL AS A POTENTIAL PRESERVATIVE OF PAPAYA (*CARICA PAPAYA* L.) FRUIT

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Declaration

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

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Dedication

This work is dedicated to the Almighty God the source of wisdom, understanding, knowledge and life, my brothers (John Juma and Paul R. Obora) and sisters (Pauline Adhiambo and Seline Atieno), my best friend Edith Kevore who were always available to support me morally, emotionally and sacrificially to see me complete my studies.

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EFF	Enhanced Freshness Formulation.
HCDA	Horticultural Crop development Authority
KALRO	Kenya Agricultural and Livestock Research Organisations
MT	Metric Tonnes
SHEP-UP	Small Holder Horticulture Empowerment Programme Promotion Unit Project
ANOVA	Analysis of variance
AEZ	Agro ecological zones
IDRC	International development research centre
HPLC	High performance liquid chromatography
TSS	Total soluble solids
TTA	Total titratable acidity
AAS	Atomic absorption spectrophotometer
GC	Gas Chromatography
SF	Skin freckles
SOP	Standard Operation Protocol
TPC	Total Phenol Content
USFDA	Food and Drug Administration of the U.S

Abstract

Fruits are rich in vitamins and antioxidants that are required in our daily diets. Papaya fruit for instance, is rich in pro-vitamin A and vitamin C that provide humans with a protective effect against cancer and other health challenges associated with the scavenge harmful oxygen-free radicals. The preservation of papaya is therefore very important to ensure a sustained health benefit supplied by this fruit.

However, huge post-harvest losses, estimated above 40% have been reported due to quick ripening and softening of Papaya. The objective of this study was to determine the effects of hexanal as a potential organic compound for use to enhance the postharvest shelf life of papaya in Kenya. Hexanal is a volatile component of many plant tissues that occurs in traces in plants like cucumber, beans and grasses. Experiments were done using a liquid formulation of hexanal (hereinafter referred to as Enhanced Freshness Formulation, EFF) applied as a pre-harvest spray or postharvest dip on mature papaya fruits. Experiments were conducted using two concentrations of hexanal on volume by volume (v/v) basis at 1% and 2% v/v with a plain water treatment as a control and, applied to papaya fruits at two timings of 2.5 and 5 minutes in two Agro-ecological zone II (Meru) and IV (Machakos). Laboratory analyses we conducted at Jomo Kenyatta University of Agriculture and Technology (JKUAT). The parameters tested include Papaya firmness, peel and pulp colour, % cumulative weight loss, respiration rates, amounts of ethylene evolved, Total Titratable Acidity (TTA), Total Soluble Solids (TSS), Vitamin C and Beta-carotene.

The results of the study revealed that the application of EFF as a pre-harvest spray and a post-harvest dip on papaya fruits greatly improved fruits firmness by at least 37%, extended the shelf life of treated fruits by three days and enhanced the general appearances of papaya fruit. EFF treatment also significantly reduced the rate of Vitamin C degradation without hindering the concentration of beta-carotene content in ripening papaya fruits. Sprayed fruits showed a three week extension time on trees over the control fruits while dipped fruits had a six days extension on their shelf life in storage. Respiratory and ethylene peaks were delayed by 3 days in hexanal treated fruits occurring on day 6 in storage as opposed to the 3rd day on storage in the control fruits under ambient room temperature (25˚C). Hexanal application showed no significant ($P \leq 0.05$) effect on total titratable acidity (TTA) and total soluble solids (TSS) of papaya fruits.

Hexanal could therefore be a viable, natural and novel option for potential use to reduce the high postharvest losses experienced in delicate tropical fruits like papaya in Africa especially among the numerous small holder farmers.

Keywords: Postharvest losses, Kenya, Hexanal, Papaya.

CHAPTER 1

GENERAL INTRODUCTION

1.1. Background information

The Horticulture sector is a key component of the agriculture industry that feeds the Kenyan population and provides a third of the country's gross domestic production. This sector is further sub-divided into the flower, fruits and vegetables subsectors in order of revenue contribution to the Horticultural sector. Production of fruits is dominated by smallholder farmers. However, few medium and large scale growers also share a good percentage of the revenues from fruits production. Fruits are very essential in our daily diet providing nutrients, vitamins, irons, minerals and energy needed to fight diseases and provide a balanced state in the functioning of various organs (Aravind *et al*, 2013).

Papaya (*Carica papaya* L.), is key fruit crops in Kenya. The country's fruit industry is primarily dominated by seven (7) fruits. Banana tops at 37.6%, mangoes (19.6%), pineapples (12.1%), avocado (9.8%), papaya (5.4%), oranges (4.6%), water melon (4.2%), passion fruit (3.7%) and others (3%) (HCDA, 2014). Papaya fruit is thus the fifth most valued fruit in Kenya and has a potential of making it up to 3rd position of most utilised fruit after mango and banana nationally (HCDA, 2014).

The fruit has a thin skin which is easily damaged leading to massive postharvest losses approximated above 50% compared to the other priority fruits in Kenya during peak seasons in February and October (Asudi et al, 2010). The fruits take about 3 days to ripen at ambient room temperature (25˚C) in tropical countries like Kenya. Drastic ripening occurs when ethylene is triggered endogenously from wounds caused on the skin during handling or under exogenous ethylene trigger from ethylene evolved from other ripening fruits or the other source in the environment for instance burning coal.

According to HCDA (2013), fruits revenue amounted to Kshs. 48 billion an equivalent of 32% domestic value of horticultural produce in Kenya with the area under production being 160,000 Ha. Papaya alone contributed Kshs. 3.8 billion commanding 7.92% by value of the fruit subsector by the end of 2013. The contribution of papaya has been on the rise due to support from other projects that have been tailored towards empowering the small holder papaya producers. The SHEP-UP for instance, has reported a boost in papaya production in Bungoma and Kakamega Counties where papaya production volumes rose by 26% and 13% respectively.

1.2. **Major challenges to papaya production**

Papaya production is greatly undermined by the changing climatic and weather patterns and over reliance of rain fed production that limits the potential yields. Most farmers also have no access to reliable seeds and are usually not aware of the varieties they produce. The varieties also keep changing due to genetic erosion arising from open pollination. Other challenges include pathogen destructions especially spider mites and powdery mildew, birds' damage of ripe fruit on farm, lack of technical information on production, limited land devoted to papaya production, lack of extension support to the few growers and poor post-harvest handling technologies.

Marketing challenges include many market broker-agents that benefit at the expense of farmers; lack of farmers' groups to address their challenges with a common voice; subsistence level of production, lack of farm records for planned production and poor market targeting. Beyond the farmers' ability is the looming challenge of the lack of priority from the County government to papaya farmers as compared to other farming enterprises like dairy, mango and banana farming; declining local market demand due to challenges of papaya softening and limited information on external market.

1.3. **Problem statement**

A typical papaya fruit has a thin and a delicate exocarp which is less waxy and is easily wounded even in careful handling. The wounds also induce autocatalytic production of endogenous ethylene which triggers ripening and rapid softening of the fruit pulp. These wounds further provide avenues for pathogen invasion especially triggering germination of latent fungal spore on the peel. Moreover, the areas surrounding these wounds are prone to a lot of solute leakages which have been reported to provide a rich media for phyto-pathogens growth (Prasanna *et al.,* 2010) further shortening the fruit's post-harvest life.

Papaya is a climacteric fruit that ripens after harvesting. During this phase the pulp undergoes a quick softening (usually 3 days) during the climacteric and respiratory rise to give it its characteristic fruity flavour. The desired tastes, aroma and colour of papaya also develop during this biochemical changes to develop the desired sensory and nutritional properties in papaya. Softening, if left uncontrolled, predisposes papaya fruits to more physical damages, phyto-pathogens invasions and increases the post-harvest loss reported to be above 40% in Kenya and other developing countries (FAO, 2012).

1.4. **Justification**

Natural mechanisms to reduce the rate of fruits softening have not been fully exploited. Many technologies that have been tested in fruits, especially in papaya, to manage ripening and to reduce the rate of fruit softening mostly involve the use of chemical compounds that poses great threat to the environment and human health. Many natural mechanisms tried have been reported to only provide a limited relief to this challenge therefore, making the farmer opt for the chemical options. However, the current study bridges this gap by utilizing a natural organic compound extracted from plants to help alleviate the challenges associate with quick ripening and rapid softening of papaya fruit. Hexanal, a six carbon aldehyde occurs naturally in fruits like cucumber where it contributes to the characteristic green flavour (Misran, 2013) and in grasses commonly perceived as the smell in mowed grass. Hexanal is also produced by ripening fruits as a component of the flavour and in wounded tissues of fruits and vegetables as a degradative enzyme of fatty acids via lipoxygenase pathway (Hildebrand, 1989). When applied externally to mature fruits, Hexanal has been reported to work by initiating changes at the cell level where it works by inhibiting enzyme phospholipase D which is responsible for membrane deterioration (Paliyath and Murr, 2007). In mature fruits, the cell membrane is completely intact at maturity and only begins to degrade and become loosely held together as the fruit ripens (Tiwari and Paliyath, 2011). Sharma *et al* (2010) reported that Hexanal is capable of reducing the rate of cell wall degradation in a study conducted for sweet cherry fruits in Canada. Other studies have also reported the effectiveness of Hexanal in temperate fruits including apples (Fan *et al*, 2006), pear (Spotts *et al*, 2007) and in mango (Anusuya *et* al., 2016) and banana in India. However papaya is physiologically different from these fruits due to its relatively much thinner peel. Yet, it’s highly in demand by the growing population and therefore finding mechanism to preserve these fruits would contribute to the scientific solutions to our daily dietary challenges in a safe and environmentally sound approach.

1.5. **Objectives**

The overall objective of the study was to evaluate the efficacy of Hexanal as a potential pre-harvest and a postharvest preservative of papaya fruits.

Specific Objectives

1. To establish the appropriate method of application, optimum duration of exposure and the concentration of Hexanal in improving the post-harvest life of selected papaya cultivars grown in Kenya
2. To determine the effects of Hexanal on biochemical attributes of the selected papaya cultivars.

1.6. **Hypotheses**

1. Mode of application, duration of exposure and concentration of Hexanal has no negative effect on the physical and physiological attributes of papaya fruits grown in different agro-ecological zones in Kenya.
2. Different Hexanal treatments have no effect on the biochemical attributes of papaya fruits.

CHAPTER 2

LITERATURE REVIEW

2.1. **Description and origin of papaya**

It is believed that papaya (*Carica papaya* L) originated from tropical America around Mexico and the neighbouring Central America. Early Cultivation of Papaya is believed to be native to Andean regions where productive farming was done at altitudes between 1,800- 3,000 m (Morton, 1987). In early 16th Century cultivation was further spread to warm zones in South and Central America and Southern Mexico. By 1616, production was spread to West Indies, Bahamas and Bermuda. The centre of papaya diversification is believed to be in the Caribbean (around West Indies), Southern México and the lowlands of Central America (Crane, 2005). He reported a route through the Caribbean and South-east Asia as the initial centres of cultivation and diversification from where cultivation spread to India, Oceania, and Africa.

Asudi *et al* (2010) traces the papaya route to Africa from India. He reported that the first instance of papaya cultivation was in Zanzibar in the 18th century and then in Uganda in 1874 and later to Kenya possibly through the regional trade. Currently, the production of papaya is so wide spread in almost all the tropical and Sub-tropical countries, including Kenya, Ethiopia, South Africa, Democratic Republic of Congo, Cameroon, Morocco, and Tunisia among others.

Papaya is a semi-woody fruit tree propagated by seeds and is open pollinated. It is the most important and the most utilized plant of the *Caricaceae* family (Nakasone and Paull, 1998).Papaya plants grow in a wide range of altitude ranging from sea level to 1500 m above sea level (Griesbach, 1992) but optimum yields are realized from 0 – 800 m above sea level (masl.). The male and female papaya plants are quite distinct from each other occurring as separate plants with the former bearing mostly flowers only. The female plant readily sets fruits all year round under warm temperatures with good water supply.

2.1.1. Papaya tree and flowers

Classification based on the inflorescence types, distinguishes papaya trees into three kinds as either Pistillate/female, Staminate/male and hermaphrodite fruit trees. Female fruits have short stalks arising from the where flowers grow on. The flowers develop into almost round-shaped fruits. By contrast, male papaya trees produce in most cases, flowers that grow on long hanging stalks which usually do not set fruits. For good fruit set, female plants need pollen from nearby male tree. A ratio of one to ten male to female papaya trees is ideal for good yields.

Hermaphrodite papaya trees have both male and female flowers. The male flowers supply the pollen to the female flowers which produces fruits. However, a single hermaphrodite tree can bear fruits pear/oval shaped fruits without pollen from the separate male plants. (Agriculture Library, South Pacific Commission- New Caledonia, retrieved on 17th July, 2017, http://opac.spc.int/cgi-bin/koha/opac-search.pl?q=an:54761).

2.1.2. The fruits

Depending on the variety characteristics, fruits can be round, semi round or pear/oval shaped ranging in sizes from 200 g to 6 kgs for different cultivars and different growing zones. The huge fruits are of the local races adapted to particular locality overtime. The interior flesh of the fruit goes through colour changes from cream/dull white when immature to salmon/yellow in their pulp when ripe or pink or red depending on the variety (McGrath and Karahadian, 1994). According to Ali and Lazan (1998), Papaya skin is green when immature and turns to yellow when ripe and to orange when overripe while the flesh turns orange/red, and softens as it ripens with a distinct sweet flavour and a slight musk tang.

During ripening papaya fruits exhibit a climacteric and respiratory rise with a sudden burst of ethylene and CO_2 that drastically quickens fruit ripening within a few days (Zhu and Zhou, 2007) usually just three days in ambient room temperature (25^0C) storage.

2.2. **Cultivars grown in Kenya**

There are many landraces in Kenya which have not been characterized and therefore are commonly known under different names between the 42 different communities in Kenya. According to Asudi *et al* (2010), there are at least 66 strains of local papaya cultivars in Kenya with other various cultivars that have been selected from other parts of the world and are now being cultivated in many agro-ecological zones of Kenya. These include "Solo" varieties which are of Hawaiian origin that bears small pear shaped fruits of uniform size. 'Solo' types of papaya are hermaphrodites having both male and female flowers in one tree and thus are highly in-bred. 'Solo' group can be divided into three races including i) 'Solo sunrise', which is a high yielding plant with quality fruits bearing reddish orange flesh. 'Solo sunrise' fruits weigh between 400 – 650 g and are pear oblong shaped. This type is preferred for both local and export market; ii) Solo sunset and iii) Solo Kapoho that have yellow – orange flesh colour but with smaller fruits compared to those of sunrise types. Other cultivars of Hawaiian origin include 'Waimanalo' types which have smooth, shiny and round fruits with short necks. Their flesh is orange-yellow and usually thick and firm.

There are yet more cultivars from India that are grown both in Kenya and Tanzania. These include the 'Honey dew' which is a medium height plant producing juicy oval fruit of medium size; 'Kiru,' a Tanzanian variety bearing large fruits with high papain content suitable for table use and; the common 'Mountain' varieties that bear small fruits mostly used for Jam making and a good source of food preservatives.

2.3. **Socio-economic, medicinal/health and nutritional Benefits of papaya**

Papaya fruit is consumed fresh as a fruit salad/desert (Ali and Lazan, 1998) and can be blended into soft drinks, used in processing companies in candy slices and for making confections like "tutti-fruity." Papaya pulp can also be value added to prepare fruit jam, or be made into flavour ingredient. Green fruits, leaves and flowers are can be cooked and consumed as vegetable (www.infonet-biovision.org). Papaya fruits have also found many applications throughout the world in various food products and industries (Milind and Gurditta, 2012).

The fruit is rich in papain extracted in the latex of green and unripe fruits and is commonly used as a meat tenderizer and for clarification of beverages. Papaya fruits are rich in calcium, have high antioxidant content especially beta-carotene and vitamin A that counter the scavenge harmful oxygen-free radical in the human body. Peterson *et al*., (1982) reported that 100g of ripe papaya fruit contains 2500 I.U. of vitamin A and 70 mg of vitamin C (ascorbic acid). In the medicine industry, the family of *Caricaceae* have been found to have several uses as remedy against a several diseases (Munoz *et al*., 2000; Mello *et al*., 2008; Vij and Prashar, 2015). Other traditional and medicinal uses of papaya are have been reported by Aravind *et al*, 2013.

2.4. **Agro-ecological requirements**

According to FAO (1996), AEZ is defined as a division of an area lend into smaller units, which have similar characteristics related to land suitability, potential production and environmental impact. Profitable production of papaya occurs in warm tropical climate with mean temperature of 25^0C at altitudes below 1000 masl. in tropical and sub-tropical regions. However, the plant can be cultivated in a wide range of climates with the main challenge being chilling injuries at low temperatures (Yadava *et al*., 1990) and quality and yield decline in higher altitudes. Papaya is a semi woody tree and cannot withstand periods of prolonged drought. Therefore, supplemental irrigation is needed to avoid plant lodging and facilitate vigorous growth and extended production (Srinivas, 1996; de Lima *et al*., 2015). In Kenya, production of papaya is widespread throughout the entire country and is dominated by the numerous small holder farmers. Good yield in Kenya occurs in regions that receive rainfall in the range of 1000 mm to 1500 mm that is well distributed throughout the year.

Papaya grows best in warm areas below 1000masl. and at higher altitude their quality and yield declines. High temperature (25-30^{0}C) enhance sweetness and uniform ripening whereas cold (<20^{0}C) or freezing temperature result into poor tree growth (Protrade, 1993: Export manual for tropical fruits).

Production can be sustainably done for four to five years in fields with deep and well drained soils capable of retaining moisture without getting waterlogged at a pH of 6-7. Plant spacing in the farm mainly depends on soil fertility and vigour (varietal characteristic) of the plants. However, a standard spacing of 3 by 3m with 1,100 plants per hectare or 3 by 2m with 1,600 plants per hectare is recommended.

2.5. **Physiological disorders of papaya**

Many physiological disorders in papaya have been reported in soils with pH below 4 especially in dry seasons. Acidic soils are characterized with deficiency of boron, magnesium and calcium that are essential for papaya growth and yield (Campostrini *et al.*, 2008). Most physiological disorders associated with mineral deficiencies affect mainly fruits, leaves and roots (Bangerth, 1979). The most common papaya disorders in Kenya include pulp jellification and skin freckles common in the hotter agro-ecological zone (AEZ) IV that experience high solar intensity.

2.5.1. Pulp Jellification in papaya

Papaya pulp jellification is caused by limiting environmental factors and is frequent in papaya fruits grown on soils with nutrient imbalances and/or deficiencies of calcium and magnesium. According Campostrini *et al*, (2010), affected papaya shows an intense red colouring with visible translucence due to extracellular accumulation of water on the fruit pulp. According to Oliveira *et al*, (2010) water is trapped in the fruit tissues as a result of changes in proton gradient needed to drive water through the affected cell membranes. A review by Saure (2005) on calcium translocation in fruits indicated that calcium imbalance is likely related to pulp jellification in fruits. The cell wall structures of affected fruits are further weakened. In addition, Maathius, (2009) related this phenomenon to plants susceptibility to fungal and bacterial invasion during ripening of fruits.

2.5.2. Skin Freckles (SF) of papaya

This is a visual disorder common in papaya fruits exposed to high intensity of direct sunlight. SF was first reported in the 1950s by Le Roux (Cited by Campostrini, 2008), and later in 1963 and 1965 in papaya plantations in Hawaii (Eloisa *et al*, 1994) and the specific cause of SF was not yet determined. Reyes and Paull (1994) later reported that SF is not caused by bacterial or fungal pathogens and Kaiser *et al*., (1996) suggested that probable cause is genetically and/or environmentally related with greater incidences reported in the driest months of the production years. SF is easily noticeable in the side of the fruits directly exposed to sun appearing as patches of dry and cracked skin that in some cases have been referred to as ‘frog skin’. Heat from the sun induces latex exudation from the fruit surface that dehydrates as they evaporate off the skin leaving behind cracked spots on the fruit pulp (Campostrini *et al*, 2008). Skin freckled papaya tend to be harder unlike papaya with early soften disorder (Jacomino *et al*, 2010).

2.6. **Pests and postharvest disease of papaya**

2.6.1. Field pest and diseases of Papaya

The most stubborn pests are the spider mites (*Tetranychus spp.*) belonging to tetranychidae family. These mites occur in large colonies usually on leaf underside and on active growing points on the plant. Spider mites pierce the leaves which then begin to turn yellow and reduce the photosynthetic ability of the affected leaves. Spider mites infestation is critical in dry and dusty seasons during which papaya plants turns as one of the major favourite vegetation for spider mites. Infested fruits show yellowish discolorations that turn brown upon drying and become roughened with greyish colour on their skin. The common distinction of spider mites attack from normal nutrient deficiencies is the characteristic webbings on the leaf underside with several distinct shot holes that turn yellow. Spider mites are managed by regular hosing using irrigation water or by spaying with Kelthane 27g/10L or Morocide 40g/20L. (http://www.kalro.org/alris/index.php/home/emimilist). Other pests range from birds; insects like fruit flies and Systates bugs weevil (Campostrini *et al*, 2010).

2.6.2. Postharvest pests and diseases of Papaya

Fungal infections are the most prevalent causes of papaya diseases both before and after harvest. The most important fungal pathogens on papaya fruit include *Glomerella cingulaae* (anthracnose), *Mycophaerella caricae* (black rot) and *Asperisporium caricarae* (black spot). These fungal infections are latent and only grow as the fruit tissues soften during ripening resulting into enhanced postharvest damages. A comprehensive list of the most important pathogens in fruit and vegetables can be accessed from publications from Beattie *et al*., 1989 and Snowdon, 1990; 1991.

Papaya skin and flesh has been shown to have an antimicrobial compounds sufficient to offer protection against the potential most fungal pathogens (Aked, 2002). Therefore, many successful invasions are facilitated by damaged tissues through which fungal pathogens obtain entry into fruit. For example, the *Penicillium* species are typical wound pathogens, incapable of invading an undamaged fruit but readily colonise a ripe fruit (Swinburne, 1983). Hexanal has been reported to have a mild antifungal effect in fruits against *Penicillium* by Fan *et al*, (2006) and significant effect against various microbial pathogens by Lanciotti *et al,* (2003).

Black rots are of two types, i) *Mycophaerella caricae* which is a major post-harvest fungal pathogen and ii) *Aschochyta caricarae* which is common on leaves. These pathogens can be managed using chemical sprays like Zineb, maneb, Captain or Copper Sulphate. Black spot affects both the leaves and fruits appearing as circular lesions that results to plant die-back under severe infections. Black rot is controlled by spraying with Dithiocarbonate (like Zineb or Dithane M45) or with a systemic fungicide like Benlate (www.kalro.org).

Viral infections are not very common except when the planting material is contaminated by a viral pathogen or when a vector transmits viral matter from nearby farms. Some of the common symptoms of viral infestation include leaf distortion, stunted growth associated with apical death, small brittle and rolled leaves, reduced leaf number due to suppressed growth of plant and a bright yellow discrete spotting/mottle. Infected fruits shrivel and abscess when 3 – 5cm long with some showing ring spots signs. There is no chemical control against viral diseases therefore beginning from clean planting material is key to avoiding viral infection in the farm. Affected plants may also be removed and destroyed through burning or by burying deep in the soil to arrest potential of spread. (Medina *et al*., 2003)

2.7. **Yield, maturity, harvesting and ripening of papaya**

The life span of a papaya plantation can be extended to 10 years but the productive and economical period is within the first 3 – 4 years (Campostrini *et al*., 2008). It's therefore advisable to renew the plantation after 4 years to maintain a high yield. Yields per tree vary but on average one tree can produce 90 fruits yearly which translates to approximately 38 tonnes of marketable fruit per hectare (Campostrini and Glenn, 2007). For best quality, harvesting should be based on days after anthesis and not on colour as a visual harvest index (Addai *et al*, 2013). Papaya takes approximately 10 months from seed to 1st harvest in the tropical climate. Flowering begins in the 5th month after transplanting and fruits then take 3-4 months to fully mature from time of flower set depending on the variety and the prevailing weather condition. Warmer conditions shorten the harvest time unlike cool conditions.

Mature fruits begin to break colour from the blossom end of the fruit with yellow strips along the fruit edges. Depending on the market and /or the use of the fruit, harvesting may begin after a third of the colour has broken from the base. It’s recommended to harvest the fruit manually and to leave a short fruit stalk about 1cm attached then transport the fruits in wooden/plastic crate carefully to avoid injuries caused through mechanical damages and to limit wounds that may serve as entry points of pathogens.

Colour is the most commonly used maturity index although it’s sometimes confounding to use especially when colour change is due to environmental stress like high temperatures which causes sunscald. Other indices include size which largely depends on variety (refer to varietal description in sub-section 2.2 above); skin characteristics like gloss; texture and tone (Aked, 2002; Santamaría *et al.*, 2009).

2.8. **Post-harvest handling of papaya**

The major cause of post-harvest deterioration in fruit is temperature and humidity. It’s therefore always necessary to remove field heat immediately after harvesting by precooling papaya through water spray or by dipping for a short time in water with a biocide to manage pathogens as well. The fruit stalk should be left at harvesting and only trimmed back to

0.6mm during packaging using a pair of secateurs and packed into crates or intended fibre board boxes to reduce chances of pathogen invasion through the point of stalk attachment. The fruits can be stored at 14^0C and relative humidity between 85 – 90% (http://ucanr.edu/sites/Postharvest_Tech-nology_Center_/files/231952.pdf).

Some of the readily available measures to control fungal pathogens and control some pests include use of hot water treatment. This will kill latent fungal infections and control fruit fly. Duration of exposure depends on the water temperature and fruits sensitivity to heat which decreases with the degree of ripeness. According to export manual by Protrade (1993), immersion time of 20 minutes is recommended for papaya at a temperature of 49^0C or 30 minutes exposure at 42^0C. However, fruits from areas where stem rot is prevalent are dipped at a higher temperature at 60^0C for a shorter duration of 30 seconds. Fungicides with thiabendazole (TBZ) as the main active ingredient have been reported to be most effective in controlling fungal pathogens (Protrade, 1993).

Careful handling of papaya fruit is highly necessary to avoid injuries to the highly sensitive peel. Fruits can always be sorted by size, weight, and colour and packaged in clusters not exceeding 8 fruits, each put without resting on other fruits in standard boxes (400 by 300 by 140 mm) to avoid skin abrasion. Management of ethylene can be done using ethylene scrubbers like Potassium permanganate and/or ethylene synthesis inhibitors like 1-MCP or by avoiding mixing of papaya with other fruits like mango and avocado or avoiding smoke conditions in store.

2.9. **Hexanal**

Hexanal is a volatile six carbon aldehyde and a natural component of many plant tissues that occurs in traces in plants like cucumber, beans and grasses. In grasses for instance, the volatile hexanal odour gives grass its characteristic smell when mowed (Misran, 2013). The chemical formula of Hexanal is $C_6H_{12}O$ with a molar mass of 100.16 g/mol and a boiling point of 130 to 131°C (https://en.wikipedia.org/wiki/Hexanal).

Studies on temperate fruits like peaches, cherries, nectarines and apple have reported that Hexanal has the potential to enhance the shelf life of these temperate fruits (Sharma *et al*, 2010). The use of Hexanal in pears has also been reported to have a mild antifungal effect in delaying the emergence of latent infections of post-harvest diseases associated with Penicillium (Fan *et al*., 2006) species. Hexanal has also been approved by FDA in the United States as a generally safe food additive (US Patent #6,514,914; 7, 198, 81) for use in processed plant based foods as a safe green flavour which does not remain in the treated tissues after 48 hours of treatment (http://www.accessdata.fda.gov/). In the human body, Hexanal is readily oxidized to hexanoic acid within 48 hours. Similar to other alcohols, hexanoic acid is further oxidized to carbon (IV) oxide and water through the tricarboxylic acid cycle during respiration (Kruse *et al*., 2006).

Hexanal has been proposed to work by inhibiting phospholipase D (PLD) enzyme which is responsible for the degradation of cellular membrane. It has been reported that Hexanal tightly binds with PLD enzyme preventing the degradation of the cellular membrane (Paliyath and Subramanian, 2008). Hexanal structure has a double bond and thus is unstable as shown below:

Source: http://www.chemspider.com/Chemical-Structure.5949.html

Hexanal is immiscible in water and to increase its solubility, a formulation containing Tween 20, ethanol and distilled water in ratio by volume basis was made and referred to as

‘Enhanced Freshness Formulation’ (EFF). Addition of Tween 20 increases the formulations solubility in water. The formulation was then tested on two varieties of papaya fruit grown in Kenya.

CHAPTER 3

Determination of the concentration, time of exposure and the appropriate mode of Hexanal application as a potential preservative of papaya fruit

Abstract

Papaya (*Carica papaya* L) is the fifth priority fruits in Kenya that is available all year round. However, huge postharvest losses estimated at approximately above 40%, is one of the major challenges to the production of thin-skinned fruit. Under ambient conditions, (25±2°C and 85±5% relative humidity), papaya readily ripens and softens usually in just 3 days, predisposing the fruit to more physical damages and phyto-pathogens invasions even in careful handling. Therefore, the objective of this study was to establish the appropriate mode, optimal concentration and time of Hexanal exposure in managing the postharvest shelf life of papaya in two agro-ecological zones, Machakos (AEZ IV) and Meru (AEZ II) in Kenya. Hexanal is an organic volatile compound that has been tested in various temperate fruits. Preliminary trials were conducted at various timings (1, 2, 3, 4, 5, 6 minutes) and dose ranges (1%, 2%, 3% and 4%) to fine tune different treatment combinations for the main experiment, which later informed the concentration adopted for the main experiments. A formulation of Hexanal was applied as either spray or dip treatment at 1 and 2% in 'Solo sunrise' and 'Mountain' papaya cultivars.

The results revealed that Hexanal applied as a spray extended fruit retention on tree by at least 13 days longer compared to the control fruits. Hexanal treatment at 2% revealed improved effect on managing papayas postharvest shelf life regardless of the mode of application. Fruit firmness was improved by up to 37.4% at 2% Hexanal exposure for 5 minutes when compared to the control fruits across the 15 days storage period in ambient room conditions. All the fruits treated with Hexanal significantly showed reduced incidences of skin damages, reduced rate of colour break and softening and enhanced extension of fruit shelf life by at least 6 days. Hexanal treatment also delayed ethylene and respiratory peaks by three days and showed no significant (*P*≤0.05) difference in the levels of total titratable acidity and total soluble solids in papaya fruit.

These results indicate that Hexanal could be a viable organic option in papaya fruit preservation especially when applied as a pre-harvest spray on mature green ‘Solo sunrise’ and ‘Mountain’ papaya cultivars.

Key word: Postharvest losses, Papaya, Hexanal, Fruit preservation

3.1. **Introduction**

Papaya is a fleshy and highly perishable fruit that contains 89.7% and has a very thin skin that easily wounds. As a climacteric fruit, papaya continues to ripen under ethylene trigger causing softening and senescence (Payasi and Sanwal, 2010). Perception of ethylene by papaya fruit tissues, either from an exogenous source like other ripening fruits or those induced by wounding during handling causes an increase in the rate of softening especially during the late stage of ripening (Kubo, 2015) thus limiting papaya shelf life to less than a week. Ripening and softening process in papaya involves disassembly of cell wall, breakdown of starch into sugars, increase in ion leakage due to increased membrane permeability and loss of cell integrity, peel and pulp colour changes (Seymour, 1993), development of flavour and taste, and synthesis of antioxidants like beta-carotene and degradation of vitamin C (Abu-Goukh *et al*., 2010).

Several technologies have been applied to manage the postharvest life of papaya including use of Methyl Jasmonate and modified atmosphere packaging, MAP (Gonzalez-Aguilar *et al*., 2003), low temperature storage (Kays and Paull, 2004) which is constrained by fruit sensitivity and chilling injuries (Paull, 1990), use of calcium (Mahmud *et al*., 2008), use of gum Arabic and essential oils (Maqbool *et al*., 2011), modified atmosphere packaging (MAP)

(Waghmare and Annapure, 2013), technologies tailored to regulate and/or inhibit ethylene in papaya (Bayogan *et al*., 2012; Ahmed *et al*., 2013) and those intended to preserve taste and biochemical constituents of papaya like the use of calcium chloride and calcium lactate in dried papaya (Udomkun *et al*., 2014). However, these technologies are specific to preserve single attribute of a fruit and in most case have limited other quality characteristics like causing abnormal colouring of fruits while others inhibit the production of essential volatiles and esters (Fan and Mathias, 1999). The use of 1-MCP has produced commendable results but is not affordable to the numerous peasant farmers and many users are still sceptical to use chemicals in the fruits.

The current study involved the use of Hexanal as a potential alternative organic compound to manage the pre-harvest and post-harvest life of papaya fruits in Kenya. Hexanal is a volatiles six carbon aldehyde found in plants where it's associated with the characteristics green flavour (Misran, 2013) perceived when a plant tissue is wounded for instance, the smell in mowed grass. Hexanal is generally regarded as a safe product and it's been approved as a safe food additive by FDA (US Patent # 6,514,914; 7, 198, 81) since it readily dissipates in 48 hours leaving no traces (Cheema *et al*, 2014) in plant tissues. In the human body, Hexanal is oxidized to hexanoic acid which is further broken down to carbon IV oxide, CO_2 and water through the tricarboxylic acid (TCA) cycle (Kruse *et al.*, 2006). Hexanal has been tested in temperate fruits including apples (Fan *et al*, 2006), pear (Spotts *et al*, 2007), strawberry, peach, nectarines and cherries (Sharma *et al*., 2010; Tiwari and Paliyath, 2011) where it has been reported to enhance the shelf life of these temperate fruits. Hexanal is proposed to work by inhibition of phospholipase D enzyme which initiates membrane degradation (Paliyath and Subramanian, 2008). However, to the best of my knowledge, no study has been conducted in Kenya or in any other part of Africa especially on papaya as a tropical fruit.

3.2. **Materials and Methods**

3.2.1. Study Site

Papaya fruits were obtained from Meru and Machakos representing agro-ecological zones (AEZs) II and IV in Kenya. AEZ II receives rainfall in the range of 1000-1600 mm per annum with average temperature of 21^0C and AEZ IV receives annual rainfall ranging 600-1100 mm with an average temperature of 28^0C (FAO, 1996). The two counties were selected because they are the leading in papaya production in the country. Within each County, farm/farmer selection was based on the ease of accessibility, farmer's willingness to participate in the study, the farmers' ability to carry-out good agricultural practice (GAP) and the farmers' information on the different varieties he/she grows. Only farmers' growing 'Solo sunrise' and 'Mountain' papaya cultivars were selected for the study. The two cultivars are the most commonly grown by farmers and are also the most preferred varieties both for local consumption and export. 'Solo sunrise' varieties are preferred for the export market whereas 'Mountain' types are least affected by papaya disease. A total of 192 papaya plants were used for the whole study; 96 papaya fruit trees in season I (January to end of March, 2016) and the other for season II (July to end of September, 2016).

3.2.2. Experimental design and treatments

A factorial complete randomized design was used for fruits brought to the laboratory. Two concentrations of Hexanal (1 and 2% v/v) were applied in papaya as a pre-harvest spray or as a post-harvest dip in the selected farms for each site. Hexanal was sprayed on mature green papaya at three timings for 30 days, 30+15 days and 15 days to harvest time on site. Dip treatment was applied for 2.5 minutes and 5 minutes in papaya brought to the laboratory at Jomo Kenyatta University of Agriculture and Technology (JKUAT) in Juja, Kenya. A total of 10 dip treatment combinations were evaluated (control (plain water dip), and 1% Hexanal at 2.5 minutes, 1% at 5 minutes, 2% at 2.5 minutes and 2% at 5 minutes Hexanal dip per variety. A sum of 14 treatment combinations for sprayed samples included a control (plain water spray), 1% Hexanal spray at 30 days, 1% at 30+15 days, 1% at 15 days, 2% at 30 days, 2% at 30+15 days and 2% Hexanal spray at 15 days to harvesting per variety.

In every farm in Machakos and Meru, papaya plants with substantial fruits were tagged using strings with unique colour codes for ease of identification. ‘Mountain’ papayas were tagged using a blue string and ‘Solo sunrise’ varieties tagged with a green string in a randomised complete block design (RCBD), blocking was done by variety. The fruits were monitored twice every week (on Mondays and Thursdays) for changes in colour and fruit retention on tree. Samples for dip experiment were hand harvested from unsprayed trees to minimise damage to skin early in the morning and packed into plastic crates lined with used newspapers. A small amount of water was then sprinkled over the newspaper covers to remove field heat and temporarily manage storage temperatures during transportation to JKUAT laboratories. In the lab, fruits were washed and the 1cm stalk left attached to all the harvested fruits removed. The fruits were allowed to drip completely their sap from the point of stalk attachment overnight before dipping in Hexanal formulation the following morning. A factorial complete randomised design was used in the Lab.

3.2.3. Experimental set up and data analysis

About 160 kgs (480 fruits) of fruits were harvested from 48 papaya plants, (24 plants from each variety) and only 120 Kgs (360 fruits) utilized. More fruits were harvested to compensate for any injury and allow for proper sorting of fruits used for the analysis in the laboratory. The fruits were then grouped by variety, and sorted to remove injured fruits. Weight basis was used ensure uniformity of fruit samples. The 360 fruit samples for dip treatment were then divided into 10 equal lots of 36 fruits for every treatment combination as above (3.2.2). The sprayed samples were harvested when most of the fruits revealed 2 – 3 yellow stripes from the lower end and a total of 504 fruits utilised for analysis of sprayed samples brought to the laboratory. Similar replicates as for dip treated fruits was adopted for the sprayed papaya samples to allow for destructive sampling of fruits during firmness measurement.

Data were recorded at intervals of 3 days for respiration rate, ethylene production rate, firmness, peel and pulp colour, physiological weight loss, total soluble solids (TTS), total titratable acidity (TTA), beta-carotene content and vitamin C content. All the data were subjected to analysis of Variance (ANOVA) in Genstat software version 15 and the means separated using Fishers Protected Least Significant Differences.

3.2.4. Measurement of physical parameter

I. Fruit firmness

A destructive sampling method was used where two fruits were randomly picked from each treatment lot for Hexanal sprayed and dip fruits, and examined for firmness using a penetrometer (Model CR-100D, Sun Scientific Co. Ltd, Japan) fitted with an 8 mm probe. The probe was allowed to penetrate the fruit to a depth of 10mm and fruit firmness expressed in Newton (N) according to Jiang *et al.*, 1999.

II. Peel and Flesh colour

Colour determination was done for the two fruits examined for firmness (as above) using Minolta colour difference meter (Model CR-200, Osaka, Japan) calibrated with a clean white and black standard tile. The L*, a* and b* coordinates were recorded and a* and b* values converted to hue angle (H^0) according to McClellan *et al.*, (1995) as indicated in equation 1.

Equation 1: Hue angle formulae

$$Hue\ angle\ (\mathrm{H}^{\circ}) = \arctan\left(\frac{b}{a}\right) for + a\ and + b\ values$$

$$= \arctan\left(\frac{b}{a}\right) + 180\ for - a\ and + b\ values$$

$$= \arctan\left(\frac{b}{a}\right) + 180\ for - a\ and - b\ values$$

3.2.5. Analysis of changes in physiological parameters

III. Percentage weight loss (% PLW)

Five fruits were marked and monitored throughout the storage period for changes in weight using a digital weighing balance (Model Libror AEG-220, Shimadzu Corp. Kyoto, Japan). The initial weight of each fruit was recorded and then the subsequent weight measured at three day interval. Percent PLW was then calculated using equation 2.

Equation 2: Percentage weight loss formula (% PLW)

$$\frac{OW - NW}{OW} * 100$$

Where OW= original weight and NW= new weight

IV. Ethylene production and respiration rates

Papaya fruits from each lot (treated and control) were incubated in an air tight transparent lockable containers 1450 ml, 4500 ml and 6300 ml capacity with a third headspace, and tightly sealed under room conditions (25±2°C, 80±5% RH) . Papaya fruits were incubated for at least one hour and headspace gas collected using 1 ml hypodermic syringe and then injected into a Gas chromatograph (Model GC-8A and GC-9A, Shimadzu Corp., Kyoto, Japan) for quantifications of respiration and ethylene production, respectively. The GC for carbon dioxide determination was fitted with a thermal conductivity detector (TDC) and a Poropak N column while that of ethylene determination was fitted with an activated alumina column and a flame ionization detector (FID). The rate of CO_2 production (used to estimate respiration rate) was expressed as millilitres per kilogram per hour (ml/kg/hr.) at standard atmospheric pressure. Ethylene levels were expressed as microliters per kilogram per hour (µl/kg/hr.)

3.3. **Results**

3.3.1. Firmness

Papaya firmness continued to drop as the fruit ripened from 58.5 N consistently with no significant difference in the mode of application. However, Hexanal treatment by day and papaya variety by day revealed a significant ($P<0.05$) effect on papaya firmness (Figure 1). Hexanal at 2% v/v dip for 5 minutes and Hexanal at 2% v/v sprays at 30+15 days caused significant drop in fruit firmness between the 3rd and 9th day after treatment. By the 3rd day after treatment, papaya fruit firmness had dropped to 84.6% (from 58.5 to 49.5 N) for 'Solo sunrise' treated at 2% v/v for 30+15 days and to 15.2% (from 58.8 N to 8.9 N) for the control (Figure 1, 1A) fruits from Machakos County. 'Solo sunrise' variety responded better to Hexanal treatment compared to 'Mountain' cultivars at all the levels of treatment with firmness advantage of 69.4%. Location differences were also noticed to have impact on Hexanal effects on papaya firmness where dip treatments at 2% for 5 minutes on the 3rd day showed better response at 88.8% (58.5 – 52N) for papaya obtained from Machakos County (Figure 1, 2A) compared to 70.2% (52.6 – 36.9N) for papaya fruits from Meru County (Figure 1, 3A)

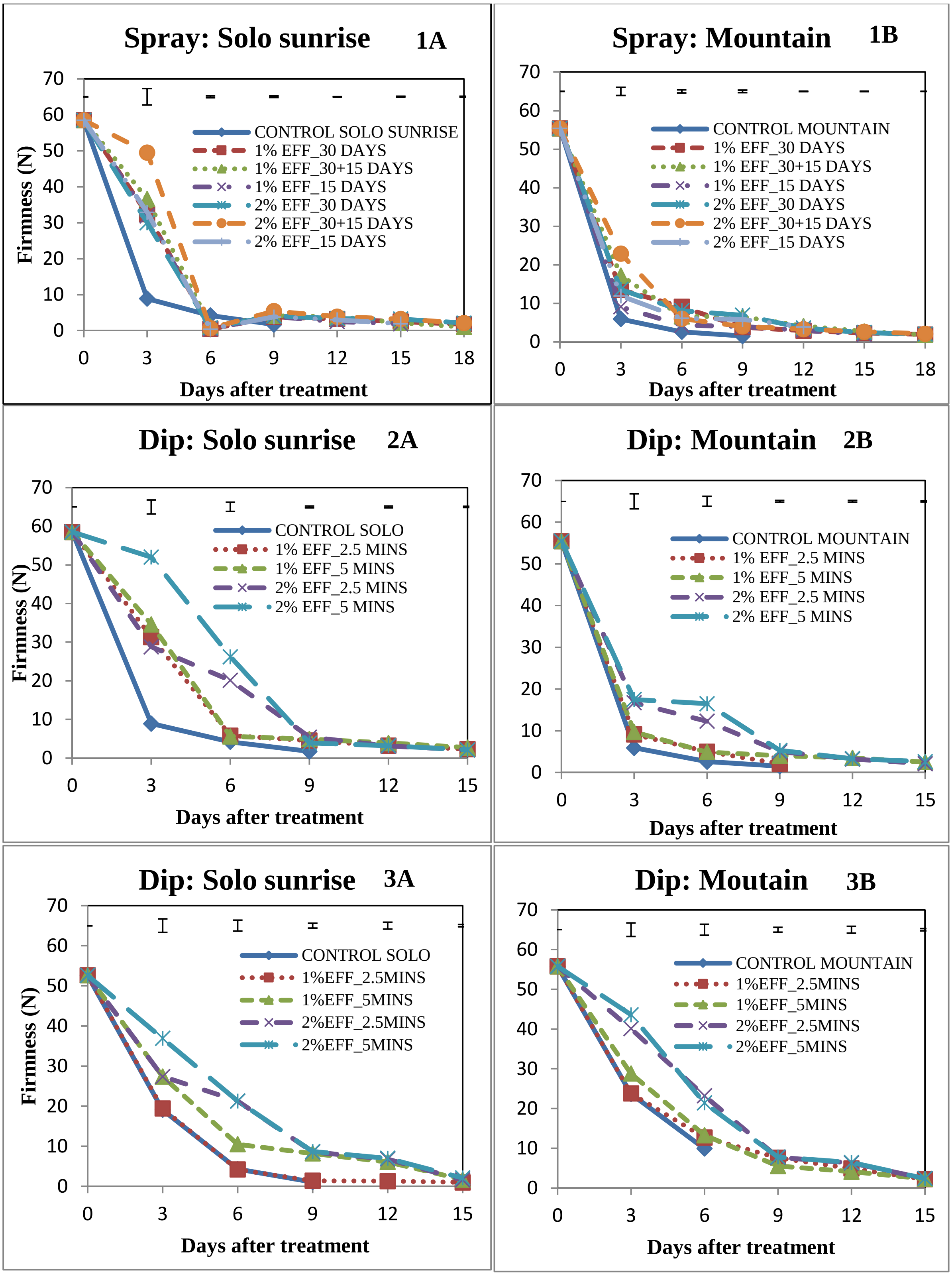

Figure 1: Firmness (N) of papaya fruits treated in Hexanal applied as a spray (1A and 1B) and dip (2A and 2B) for fruits obtained from Machakos County and dipped fruits from Meru County (3A and 3B) for experiment 1. Top bars represent LSD at 0.05.

3.3.2. Peel Colour

The mode of application and Hexanal treatment revealed a significant ($P<0.05$) difference in Papaya (Figure 2). Papaya peel hue gradually and uniformly dropped under ambient (25˚C) room conditions as the skin colour changed from green (120˚) to lime(90˚) to yellow (60˚) and then amber (50˚) across the storage days. The average rate of colour break for papaya fruits sprayed with Hexanal was more gradual and changed from a green colour hue of 127^0 to a yellow hue at 65^0. However, fruits dipped in Hexanal rapidly changed their peel colour from 121^0 to 56^0 with no difference between the means of papaya treated by dipping or spraying. All the papaya fruits treated at 2% v/v Hexanal dip/spay revealed a reduced rate of hue angle decline preserving the fruits peel colour at lime appearance for at least three days. The control fruits depicted a faster rate of colour break compared to papaya treated at 2% Hexanal in sprayed and dipped fruits. By the end of the 4th day in storage, 90% of the control batch, comprising 18 fruits, had completely turned yellow with a hue angle below 90˚ for all the varieties and for all the mode of treatment. 'Mountain' papaya revealed a linear transition compared to 'Solo sunrise' papaya.

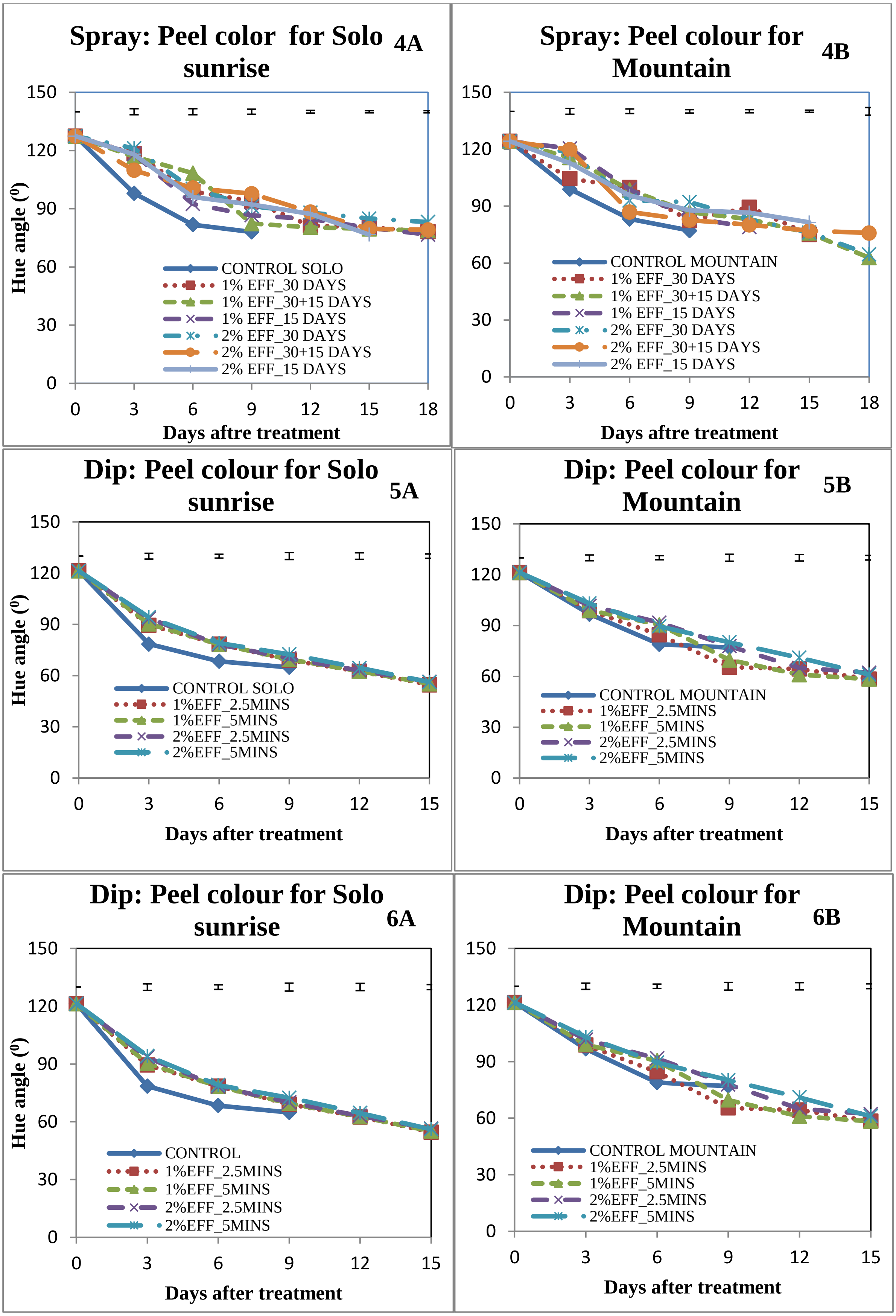

Figure 2: Peel colour of papaya fruits treated in Hexanal applied as a spray (4A and 4B) and dip (5A and 5B) for fruits obtained from Machakos County and dipped fruits from Meru County (6A and 6B) for experiment 1. Top bars represent LSD at 0.05.

3.3.3. Pulp colour

Hexanal treatment and the mode of application showed significant ($P<0.05$) difference in pulp colour break in the two varieties. (Figure 3). The intensity of red colour continued to increase in papaya pulp as the fruit ripened with a steady drop in hue angle over a narrow range from 85^0(unripe) to 48^0(fully ripe) across the storage period. Hexanal treatment significantly delayed coloured changes within the first 6 days under ambient room conditions. However, no clear trend was observed for effect of the various concentrations of Hexanal in the two papaya varieties. The mode of applications also did not differ within the first experiment. A combined analysis of variance between the means of the two experiments in all the zones revealed a high significant difference in the mode of applications with high averages for dip treatment. The rate of pulp colour change was more stable for pulp compared to the peel. It was observed that the control fruits developed a watery pulp by day 9 in storage. This lowered the pulp hue reading to 47.7˚ compared to less watery pulp in Hexanal treated fruit at 53.6˚ (Figure 2, 7A). Beyond day 12, the pulp firmness was below 1N for all the fruits with a more solid pulp and a higher hue angle above 53˚ for all the treatment combinations of Hexanal in sprayed and dipped fruits.

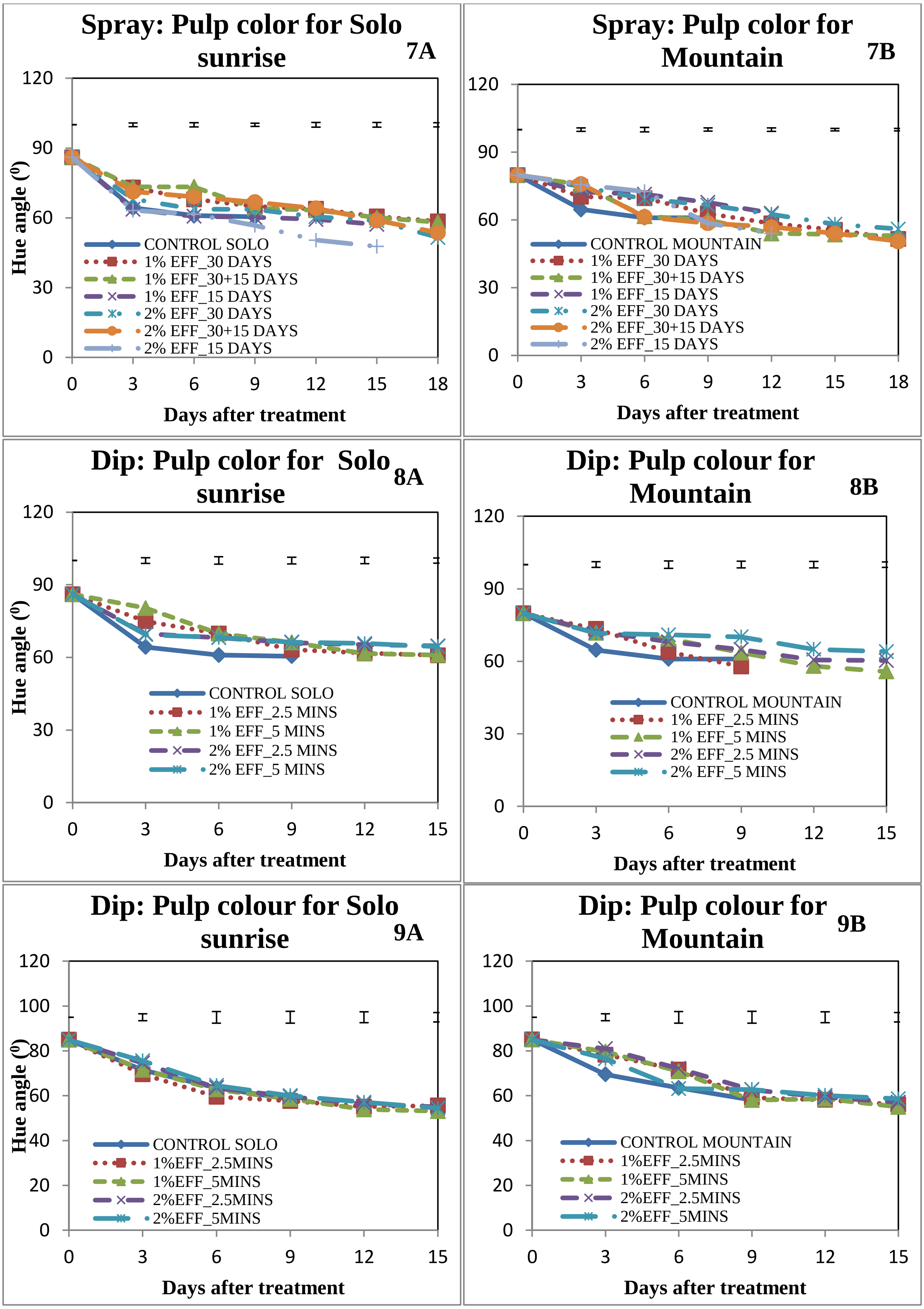

Figure 3: Pulp colour (H^0) of papaya fruits treated in Hexanal applied as a spray (7A and 7B) and dip (8A and 8B) for fruits obtained from Machakos County and dipped fruits from Meru County (9A and 9B) for experiment 1. Top bars represent LSD at 0.05.

3.3.4. Percent Weight Loss (% PLW)

A significant (*P*≤0.05) difference was revealed in papaya treated with different concentrations of Hexanal and between the two modes of applications (Figure 4). The total Percent cumulative weight loss consistently increased in all the fruits as the fruits ripened. Papaya fruits from the cooler AEZ-II lost more weight compared to fruits from the hotter AEZ-IV (Figure 4). Sprayed samples depicted a reduced rate of total weight loss compared to their controls (Figure 4, 10A and 10B). Dip exposure for 2.5 minutes did not show any difference in their weight loss trend from the control fruits both at 1% and 2% v/v dip. Hexanal dipping at 2 for 5 minutes reduced the total weight loss by 8.4% compared to the control fruits by the end of day 9 in storage. Hexanal treatment was more effective in reducing physiological weight loss in ‘Mountain’ papaya (Figure 4, 11B) compared to ‘Solo sunrise (Figure 4, 11A).

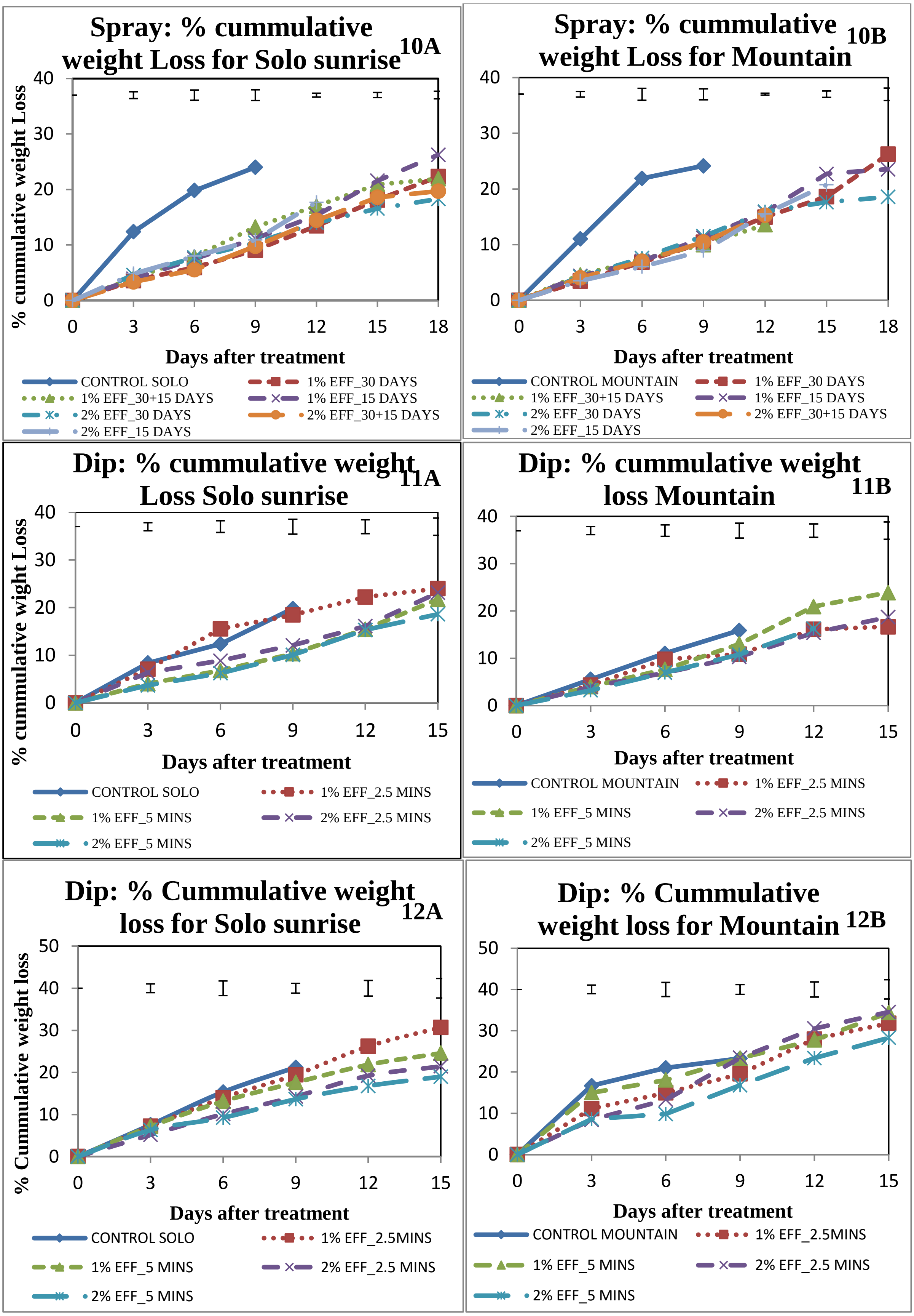

Figure 4: Percent cumulative weight of papaya fruits treated in Hexanal applied as a spray (10A and 10B) and dip (11A and 11B) for fruits obtained from Machakos County and dipped fruits from Meru County (12A and 12B) for experiment 1. Top bars represent LSD at 0.05.

3.3.5. Respiration rate/ CO_2evolution rate

Hexanal treatment and the mode of application had a significant ($P \leq 0.05$) effect in the rate of Co_2 evolution (Figure 5). Hexanal treatment delayed the respiratory peak by 3 days. However dipping at 1% for 2.5 minutes and spraying at 1% Hexanal at 30 days to harvesting did not differ from the control fruits. The rate of Co_2 evolution was high in 'Solo sunrise' papaya compared to 'Mountain' cultivars across the storage period. 'Solo sunrise' cultivars evolved more Co_2 at a peak of 23.33ml/kg/hour (Figure 2, 14A) compared to 'Mountain' varieties that peaked at 21.55ml/kg/hour (Figure 2, 14B). The respiratory peaks occurred at full yellow peel colour ($H^0 < 90^0$) in both two varieties.

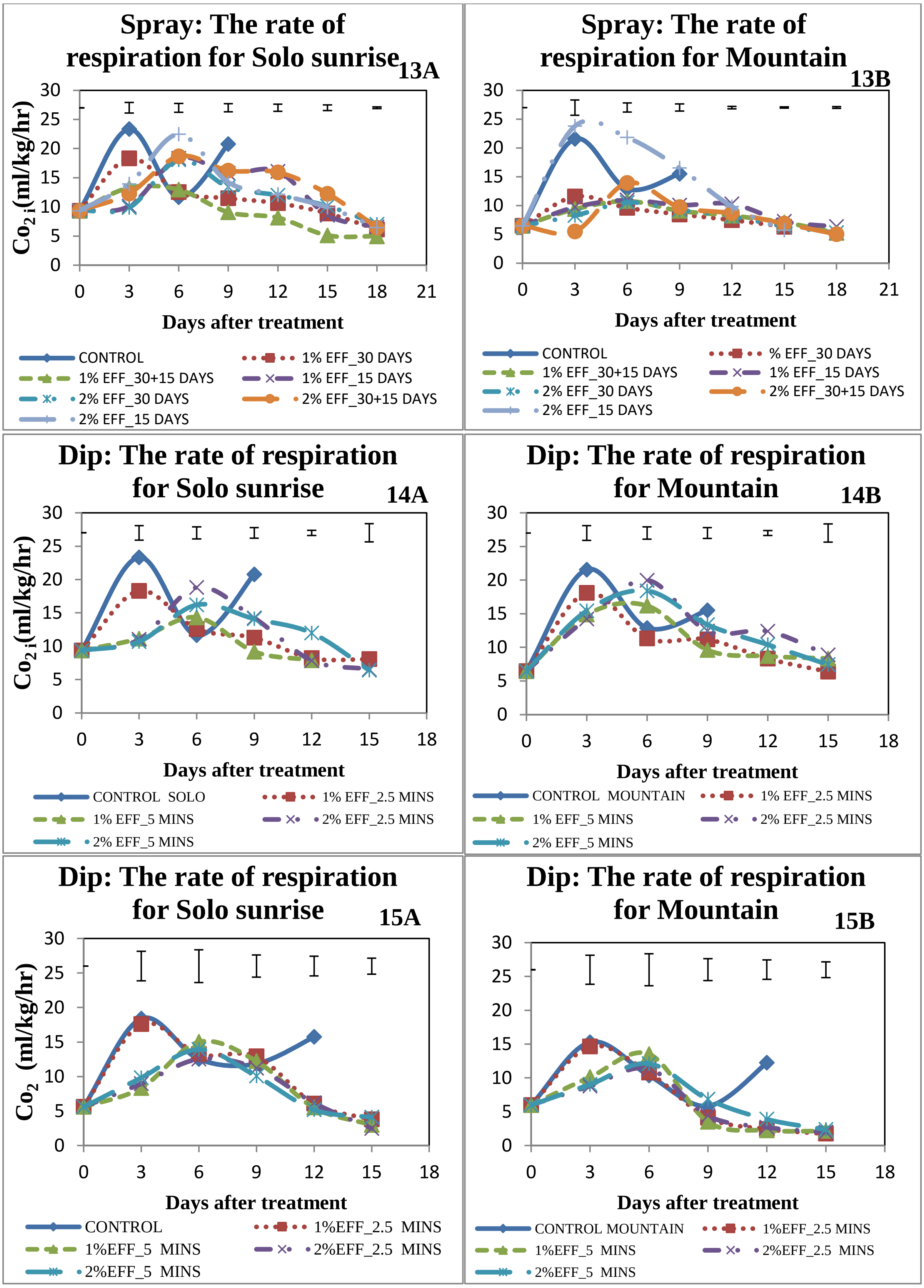

Figure 5: The rate of respiration/Co$_2$ evolved from ripening papaya fruits treated with Hexanal spray (13A and 13B) and dip (14A and 14B) from Machakos County and dipped fruits from Meru (15A and 15B) for experiment 1. Top bars represent LSD at 0.05

3.3.6. Ethylene evolution rates

The mode of application and different concentrations of Hexanal showed a significant ($P \leq 0.05$) effect on the rate of ethylene evolution on both the sprayed and dipped fruits (Figure 6). Papaya fruits revealed a climacteric pattern as the fruits ripened. Ethylene peaks followed a similar behaviour as respiratory peaks with treatment at 2% Hexanal yielding lower volumes of ethylene. Dipping papaya fruits at 2% for 5 minutes and spraying at 30 days to harvest both at 1% and 2% were significantly different from the control fruits with lower amounts of ethylene evolved from the treated papaya. However, application of Hexanal at 1% for 5 minutes and all dip exposure at 2% were not significantly different from each other in the rate of ethylene evolved as the fruits ripened. Control fruits and treatment at 1% for 2.5 minutes showed a similar behaviour. Highest ethylene peak was noticed in control fruits at3.53µl/kg/hour compared to a high of 2.63µl/kg/hour in treated fruits (Figure 6, 17A) in fruits from the hotter AEZ-IV.

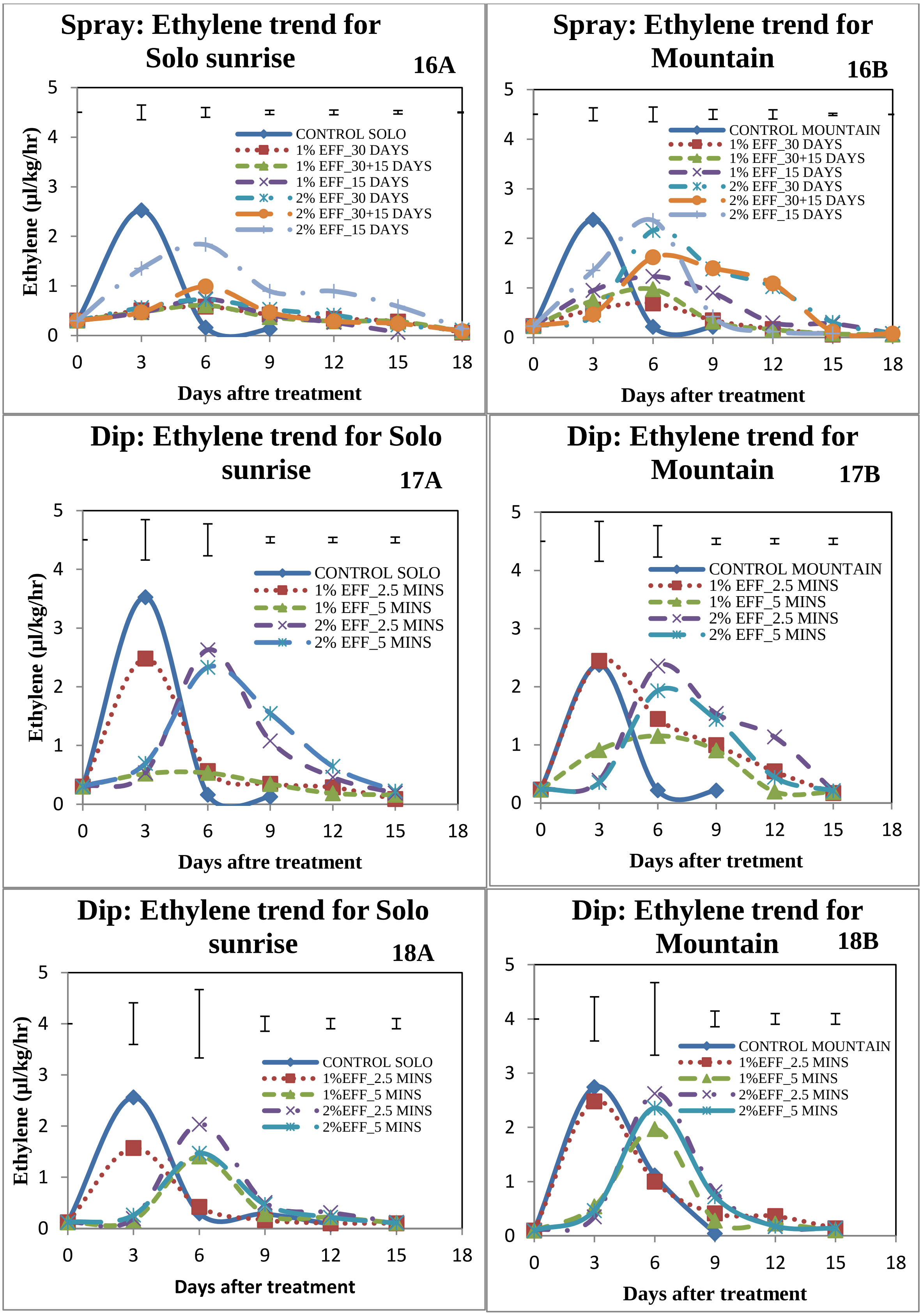

Figure 6: The rate of ethylene evolved from ripening papaya fruits treated with Hexanal spray (16A and 16B) and dip (17A and 17B) from Machakos County and dipped fruits from Meru (18A and 18B) for experiment 1. Top bars represent LSD at 0.05

The overall summary of means for various parameters tested is shown in the table 1 below:

Table 1: Effects of Hexanal dip/spray in various quality attributes of papaya fruit.

Effects of Hexanal on Papaya

Parameter	**Spray**	**Dip**	**LSD**
Colour	102.49*	94.88	2.9140
CO_2	10.97*	14.12	2.3900
C_2H_4	0.61	0.85	0.2780
Firmness	7.93	4.74	4.3750
% PLW	7.44	7.87	0.8264
TSS	11.00*	10.00	0.9311
TTA	0.08	0.10	0.0161
Vitamin C	52.18	48.25	7.7840
Beta-carotene	154.10*	142.70	3.3320

These results revealed that Hexanal applied as spray was significantly better than dip at 2% v/v. Spray application of Hexanal significantly reduced the rate of colour break, reduced the amount of CO2 evolution without limiting total soluble sugar and beta-carotene accumulation. However, no significant differences of Hexanal mode of application were noticed for fruits firmness, % cumulative weight loss, Ethylene evolution, total titratable acidity and vitamin C. The best effects for Hexanal dip treatment were realized at 2% dip exposure for 5 minutes and at 2% Hexanal double spray at 30+15 days in the two cultivars studied.

3.4. **Discussion**

Use of Hexanal extended the shelf life of papaya by six days. Papaya left to ripen without Hexanal application were completely ripe in 9 that marked their end use stage unlike 15 days realized for Hexanal dipped fruits. Shelf life is inversely related to the rate of respiration and the lower the rate the longer shelf-life (Day, 1993). The low respiratory activity observed in the treated fruits could partially contribute to the six days extension of Hexanal treated papaya fruits in ambient storage (25 ±2°C and 80±5% relative humidity). The use of Hexanal in papaya has shown a potential to enhance the pre- and postharvest life of papaya fruits in

Kenya. ‘Solo sunrise’ and ‘Mountain’ papaya cultivars revealed different response rates of Hexanal applied as a pre-harvest spray and a postharvest dip to mature green papaya fruits grown in cool and hot zones in Kenya. It was observed that 2% Hexanal dip for 5 minutes and 2% Hexanal spray at 30+15 days enhanced papaya fruit firmness by up to 38%. Softening in fruits is caused by disassembly of cell wall that contribute to changes in the internal pressure and loss of cell integrity Luza *et al*., (1992). In the current study, the rate of softening in papaya treated with Hexanal was delayed as the fruits continued to ripen. The slow rate of softening may be associated with the inhibition of phospholipase D (PLD) enzyme that is responsible for the degradation of cell wall (Tiwari and Paliyath, 2011). Fruit softening has also been associated with several enzymatic activities that catalyses the breakdown of the pectin chains, hemicellulosic and cellulosic fractions of the cell (Krongyut *et al*., 2011a; 2011b). Pectin methylesterase (PME) enzymes demethylates the pectin chain in the cell wall and then Polygalacturonase (PG) enzymes catalyses the depolymerising of the pectin chains that hold the cell membranes and the cell wall intact (de Souz *et al*., 2014). The mechanism of enzymatic catalysis on softening has also been described by Hayama *et al*., (2006; 2008) in peach fruit. In the current study, it was observed that by day 6 in storage, papaya fruit firmness had dropped below 5N in control fruits and above 8N in Hexanal treated at 2% for 5 minutes dip and 2% Hexanal spray at 30 days and 30+15days.However, papaya fruits firmness had dropped below 2N when all the fruits were 100% yellow beyond day 9. The possible cause for this loss of papaya firmness may be a result of increased rate of skin injury after respiratory and climacteric peak (Quintana and Paull, 1993).

Antunes *et al*., (2006) reported that ethylene has a strong participation in modulating enzymes associated with ripening and softening of fruits. In this study, it was noticed that Hexanal treatment delayed the respiratory and climacteric peaks in ‘Solo sunrise’ and ‘Mountain’ papaya cultivars by 3 days. Ethylene plays a key role in fruit ripening and softening (Barry and Giovanoni, 2007) and therefore the suppressed amounts of ethylene and delayed ethylene peak observed in Hexanal treated papaya fruits the suggest that Hexanal has a potential mild impact to suppress ethylene evolution in papaya. Tiwari and Paliyath (2011) also reported that Hexanal has potential action in down-regulating ACC-synthase which limits the amount of ethylene produced from the fruits. However, Schobert and Elstner (1980) reported an antagonistic response between Hexanal and ethylene in *Phaeodactylum triconutum*. The external application of Hexanal may have triggered the suppression of ethylene in the treated papaya fruits. However, this requires investigations to ascertain the levels of ethylene beyond which Hexanal may present no antagonist influence. The overall ethylene rates were lower than those reported by Paull and Chen (1983) and Paull (1993).

The amount of Co_2 evolved continued to rise from 9.35ml/kg/hour in ‘Solo sunrise’ and 6.48 ml/kg/hour in ‘Mountain’ papaya as the fruits ripened and peaked on day 3 in control fruits and day 9 in Hexanal treated fruits. Respiration rate is a reflection of the metabolic activities in a given cell. Therefore the reduced rate of respiration observed in papaya fruits dipped/sprayed in Hexanal may point to a limiting factor in fruits metabolic activity and the extended storage life.

All the fruits tested in this study were harvested at two to three stripes yellow colour break to ensure taste and sweetness is not affected and to allow the fruit to ripen normally in ambient room conditions (Zhou and Paull, 2001). Peel colour is a very important visual parameter widely used in fruits used as a maturity index (Adil, 2002) and to subjectively judge the taste/preference of fruit by consumers. In papaya, colour change from green to yellow informs the purchase of this delicious fruit and must therefore colour development must not be hampered by any technologies tailored to preserve this fruit. Hexanal treatment did not significantly interfere with the peel colour change of papaya from green to yellow and the intensity of red colour development in the fruits pulp. Fruit colour changes is associated by the enzymatic degradation of chlorophyll in the peel (Ding *et al*., 2007) and the concentration of carotenoids in both peel and pulp which gives the fruit its characteristic colour. In this study, it was observed that the hue angles consistently dropped as a* and b* values increased during ripening ensuring a uniform colour break in the peel and an intense red colour concentration in the pulp. A complete description of the objective colour measurement in fruits is described by McGuire, (1992). In this study it was noted that the hue angle of Hexanal treated papaya changed from green (127˚ to 107˚) to lime (101˚ to 90˚)towards yellow (85˚ to 60˚) and amber (58˚ to 50˚) as chlorophyll continued to degrade. These findings agree with the guidelines given for colour measurements using Minolta instruments describe by McGuire (1992). See colour wheel in appendix 4a.

Papaya is a fleshy fruit which is 89.7% composed of water (Mia *et al*, 2010) that is lost as moisture from fruits and is therefore the main component of physiological weight loss especially during ripening and softening. The amount of water capable of being lost is also directly dependent on the physical status of the fruit. For instance, presence of skin freckles and other skin injuries, presence of fungal pathogens enhance the rate of water loss from the skin the size and surface area/volume ratio correlations of the produce. In this study, the Percent cumulative weight loss reached a high of 34.5% without affecting the fruits appearance in ambient room temperature (25˚C) surpassing the 8% reported by Paull and Chen (2000). Both the control and the papaya fruits treated in Hexanal were still good looking and saleable even after exceeding a loss moisture loss of 8% of their initial weight. The huge difference may be attributed to the genetic and size differences of ‘Solo sunrise’ and ‘Mountain’ papaya over papaya cultivars studied by Paull and Chen (2000), environmental differences and the size of the papaya fruits investigated in the studies.

The use of Hexanal could therefore be a viable organic option to manage papaya fruit, contribute to reduction of postharvest losses and extend nutritional benefits in our daily diet.

CHAPTER 4

Effects of Hexanal treatment on the postharvest quality attributes in two varieties of papaya (*Carica papaya* L.) fruits in Kenya

Abstract

Papaya fruits are a rich source of vital vitamins and mineral ion required in our daily diet. Therefore, finding natural mechanisms to preserve these nutrients is important in research. This study involved the use of Hexanal applied in two modes as a pre-harvest spray or a postharvest dip and evaluated to determine the best mode and the optimal concentration of Hexanal that can be used to enhance the postharvest shelf life of papaya fruits. Hexanal concentration of 1% and 2% v/v exposed as dip for 5 minutes or spray for 30+15days was adopted from a previous study. The objective was to determine the effect of the optimal concentration of Hexanal on the quality attributes of two varieties of papaya fruit. A factorial complete randomized design was used to analyses the effects of Hexanal on total soluble solids, total titratable acidity, and beta-carotene content and vitamin C contents in papaya.

The results revealed that Hexanal spray/dip significantly reduced the rate of vitamin C degradation in ripening papaya fruit, without negatively impacting on the concentration on beta-carotene. Exposure to Hexanal did not show any significant effect in the level of total titratable acidity and total soluble solids. Therefore, Hexanal is a potential alternative organic volatile compound for use to preserve the quality attributes of papaya for an extended period which when adopted can aid in reducing the postharvest losses in papaya value chain.

Key word: Vitamin C, Beta-carotene, Total soluble solids, Total titratable acidity, Hexanal, Papaya.

4.1. **Introduction**

Papaya is a good source of Vitamin C and carotenoids especially pro-vitamin A (Bari *et al*, 2006) that is vital in human diet. The availability of this antioxidant widely referred to as beta-carotene is affected by the maturity stage of a fruit, genetic composition of the cultivar and cultural practices during the production period. Papaya reaches physiological maturity after 120 days after anthesis (DAA) and ripening phase 125 – 145 DAA (Abu-Goukh *et al*, 2010). During this phase all the desired fruits characteristics are fully developed including total soluble solids (TSS) which is a good indicator of fruit sweetness and ripeness.

In papaya, human preference for taste increases with the advancement in papaya maturity stage. Some of the desired changes in papaya fruit as it ripens include development of aroma with every rise in climacteric, decline in acidity as maturity advances, increase of flavour and sweetness (Bron and Jacomino, 2006) and slight acidity in pH (5.72). These attributes are fully developed when the fruit has accumulated the highest antioxidants activity and has the capacity to scavenge free radicals (Addai *et al*., 2013).

The quality of fruit both physical, physiological and biochemical is influenced by the agro-ecological (AEZ) factors where the fruit is grown. Papaya fruits quality is therefore expected to vary from AEZs due to difference of the environmental impact. However, several factors including cultural practises like application of plant nutrients and irrigation among other practices influence the quality of fruits even within a specific zone. For instance, the biochemical attributes of papaya including total titratable acidity, total soluble solids, vitamin C, beta-carotene and sugars are influence by both pre-harvest and post-harvest factors.

This study was conducted in Machakos and Meru Counties in Kenya on ‘Solo sunrise’ and ‘Mountain’ papaya cultivars. Machakos is classified as AEZ-IV based on its natural potential. The area receives rainfall below 1100mm with average temperature of 28˚C occurring in a bimodal rainfall pattern with two dry seasons. Meru, AEZ-II, receives rainfall between 1100 – 1600 mm annually with average temperature of 21˚C with only one or two dry months. Papaya fruits from these two zones therefore may vary in the composition of the biochemical attributes despite the similarity in variety and genotypes.

The objective of this study was to evaluate the effects of Hexanal treatment in the biochemical attribute of papaya including total soluble solids, total titratable acidity, and antioxidants including beta-carotene and ascorbic acid and major sugars (fructose, glucose and sucrose) in papaya fruit.

4.2. **Material and Methods**

4.2.1. Fruit sampling

After analysis of the physical and physiological attributes of the treated samples, only samples from the best level of treatment were analysed for biochemical parameters. All the parameters were evaluated once all the samples had been obtained at the end of season I and II for the two zones.

4.2.2. Measurement of Biochemical attributes of Papaya

V. Total Soluble Solids (TSS/%Brix)

Papaya samples from destructive sampling which were used to evaluate fruits firmness were peeled and the seeds removed. The pulp was then placed in zip lock bags of size 6 by 4 and stored in a freezer at -20˚C. At the end of the sampling seasons, 5 grams of juice was squeezed from the papaya pulp using a clean muslin cloth for ripe fruits while unripe papaya pulp was crushed using a pestle and a mortar. A Hanna digital hand held refractometer 0-85% Brix (Model HI 96801, USA) was used to determine the TSS and expressed as % brix.

VI. Total Titratable Acidity (TTA)

Total titratable acidity was determined through titration where 5 g of fruit pulp was macerated and diluted with 20ml of distilled water. Ten millilitres of the diluted solution was obtained, mixed with 3 drops phenolphthalein indicator (colourless in acid medium) for titration using 0.1N sodium hydroxide with constant shaking. The reaction end point was the appearance of faint pink colour that persisted for about 30 seconds. The titre volume was then recorded and the results expressed as Percent citric acid content (titratable acidity) of fruit juice according to the method of Ranganna (1991).

VII. Vitamin C /Ascorbic acid content

Vitamin C was determined as described by Mamun *et al*., (2012) with a few modifications where; about 2 – 3g of papaya pulp from the stored samples (as described for TSS) were weighed and extracted with 0.8 % meta-phosphoric (MPA) acid under subdued light conditions. The extract was made to 25 ml of juice and centrifuged at 100 revolutions per minute (rpm) (Kokusan H-200 Tokyo Japan), at 4°C for 10 minutes. The supernatant was using a syringe and filtered into vials through 0.45µ micro-filters. The samples were then set as a post-run in HPLC machine (Model LC-10AS, Shimadzu Corp., Kyoto, Japan) where 20 µL of the filtered sample was automatically injected into the HPLC machine on the same day of extraction. High Performance Liquid Chromatogram analysis was done using C18-4D column and Shimadzu UV-VIS detector. Various concentrations of ascorbic acid standards were prepared at 10, 20, 40, 60, 80 and 100ppm and a blank containing only degassed MPA and used to obtain a standard calibration curve. The mobile phase was 0.8 % meta-phosphoric acid, at 1.2 mL/min flow rate and wavelength of 266.0 nm. The quantity of ascorbic acid was calculated using methods described by AOAC (1998) where standard vitamin C concentration regression curve was obtained with the freshly prepared vitamin C standards and calculated as shown in equation 3.

Equation 3: Ascorbic acid formula

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

Where y = calibration coefficient obtained from standard regression curve when y-intercept is zero (AOAC, 1998).

VIII. Beta- Carotene

Beta- carotene was analysed using UV spectrophotometry using the method described by Rodriguez-Amaya and Kimura, (2004). About 2g of the stored papaya pulp was quantitatively transferred to a pestle and a mortar and ground with acetone and the extract transferred to 100ml volumetric flask. This was repeated until the sample gave no colour in acetone. Partitioning was done using 25 ml of petroleum ether in a separating funnel. Small amount of distilled water was added to the mixture of acetone, extract and petroleum ether to facilitate separation. The lower elute mixture of water and acetone was carefully channelled out to leave the upper layer mixture of carotenoids and petroleum ether. This was then transferred to 25 ml volumetric flask through a funnel and filter paper with anhydrous Sodium sulphate to remove water from the petroleum carotene mixture. All extractions were done under subdued light conditions. Standards at 0, 2, 4, 8, 10, 20, 40, 60, 80 and 100 ppm were also made from a freshly prepared Beta-carotene standard and used to plot a calibration curve used to calculate beta-carotene amounts in the samples. Absorbance readings were done at 440nm in a UV-spectrophotometry (Shimadzu model UV-1610 PC, Kyoto, Japan). Beat-carotene content was determined using equation 4

Equation 4: Carotenoid content (μg/g)

$$Carotenoid\ contents\ \left(\frac{\mu g}{g}\right) = \frac{A \times V(ml) \times 10^4}{A_{1cm}^{1\%} \times P(g)}$$

Where: A= absorbance

V= Total extract volume

P= Sample weight in grams

$A_{1cm}^{1\%}$= 2592 (β-carotene extinction coefficient in petroleum ether)

The result is multiplied by 100 to convert µg/g to µg/100g.

(Source: HarvestPlus Handbook for Carotenoid Analysis, Page 36. By Rodriguez-Amaya and Kimura, 2004)

4.2.3. Data analysis

Analysis of variance was done using Genstat software version 15 and the means separated using LSDs at 5% level of significance to determine the effects of the optimal treatment of Hexanal on papaya fruits.

4.3. Results

4.3.1. Total soluble solids (TSS)

Total soluble solids in papaya fruits increased with ripening across the storage period of 15 days for dip sample and 18 days for sprayed (Figure 7). The rate of TSS increase gradually reduced as the fruit advanced in ripening. Optimal Hexanal treatment did not show any significant ($P<0.05$) effect in papaya levels of total soluble solids between the initial day and the 9th day in storage. Varietal differences were realized were 'Solo sunrise' papaya had relatively higher initial TSS level (0.6% higher) at 9.3% against 8.7% for 'Mountain' papaya (Figure 7, 20A and 20B). The highest level of % TSS was observed in control fruits.

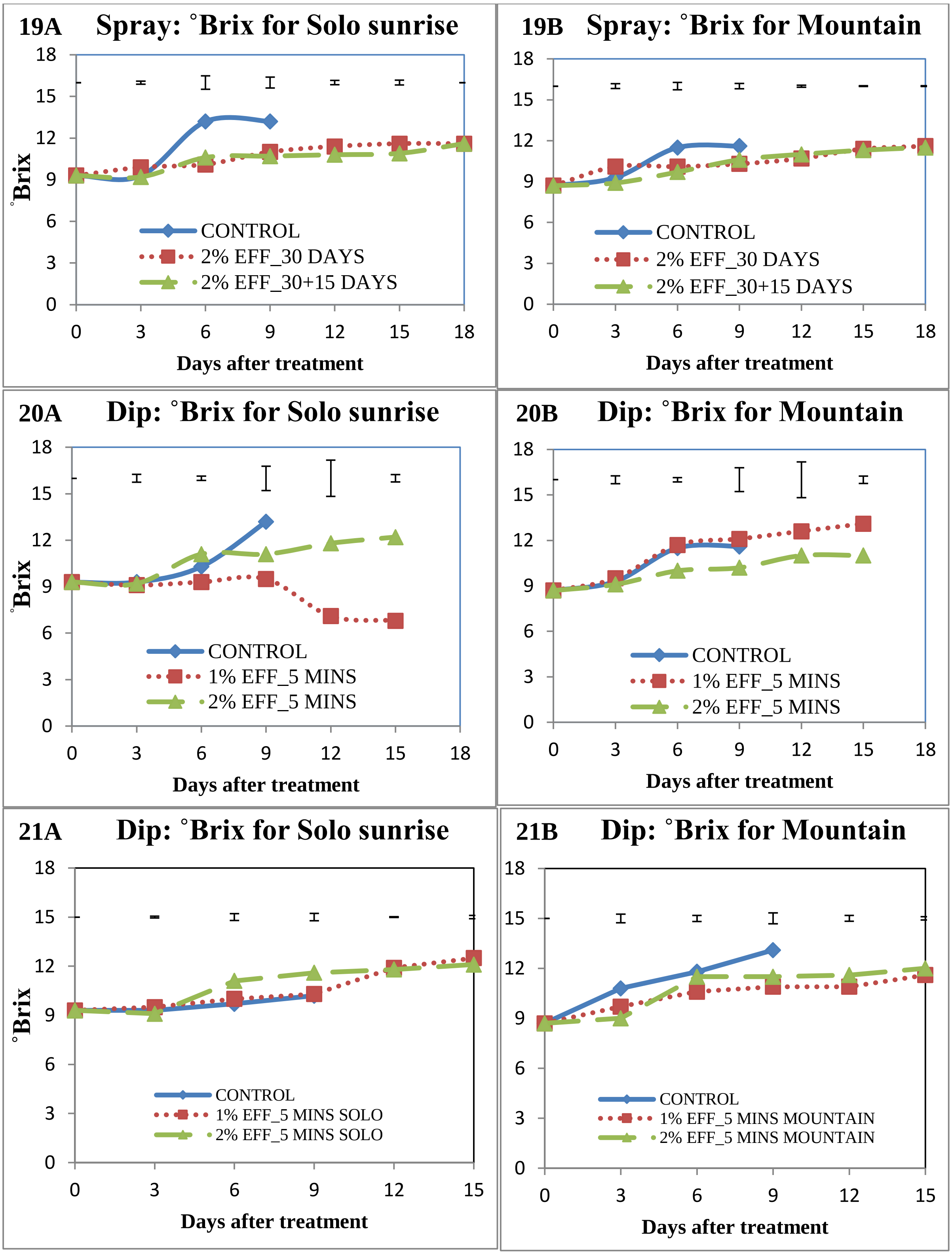

Figure 7: Percent total soluble solids present in papaya fruits treated with Hexanal spray (19A and 19B) and dip (20A and 20B) from Machakos County and dipped fruits from Meru (21A and 21B) for experiment 1. Top bars represent LSD at 0.05

4.3.2. Total Titratable Acidity (% TTA)

Percent TTA varied inconsistently in unstable fashion in both the treated and the control fruit. However the overall trend showed a slight Percent drop across the storage period in the treated and control papaya fruits (Figure 8). A reverse trend was observed for dip samples from Machakos County in season I: where the % TTA levels dropped in control fruits but increased in treated samples significantly (Figure 8, 23A and 23B). ‘Solo sunrise’ cultivars had higher levels of % TTA compared to ‘Mountain’ papaya across the storage period. Optimal Hexanal treatment at 2% dip exposure for 5 minutes and 2% spray at 30+15 days did not have any significant ($P<0.05$) effect on the levels % TTA in treated papaya fruits. Percent TTA trend seem to mimic the climacteric and respiratory peaks in both the dip and spray treatments.

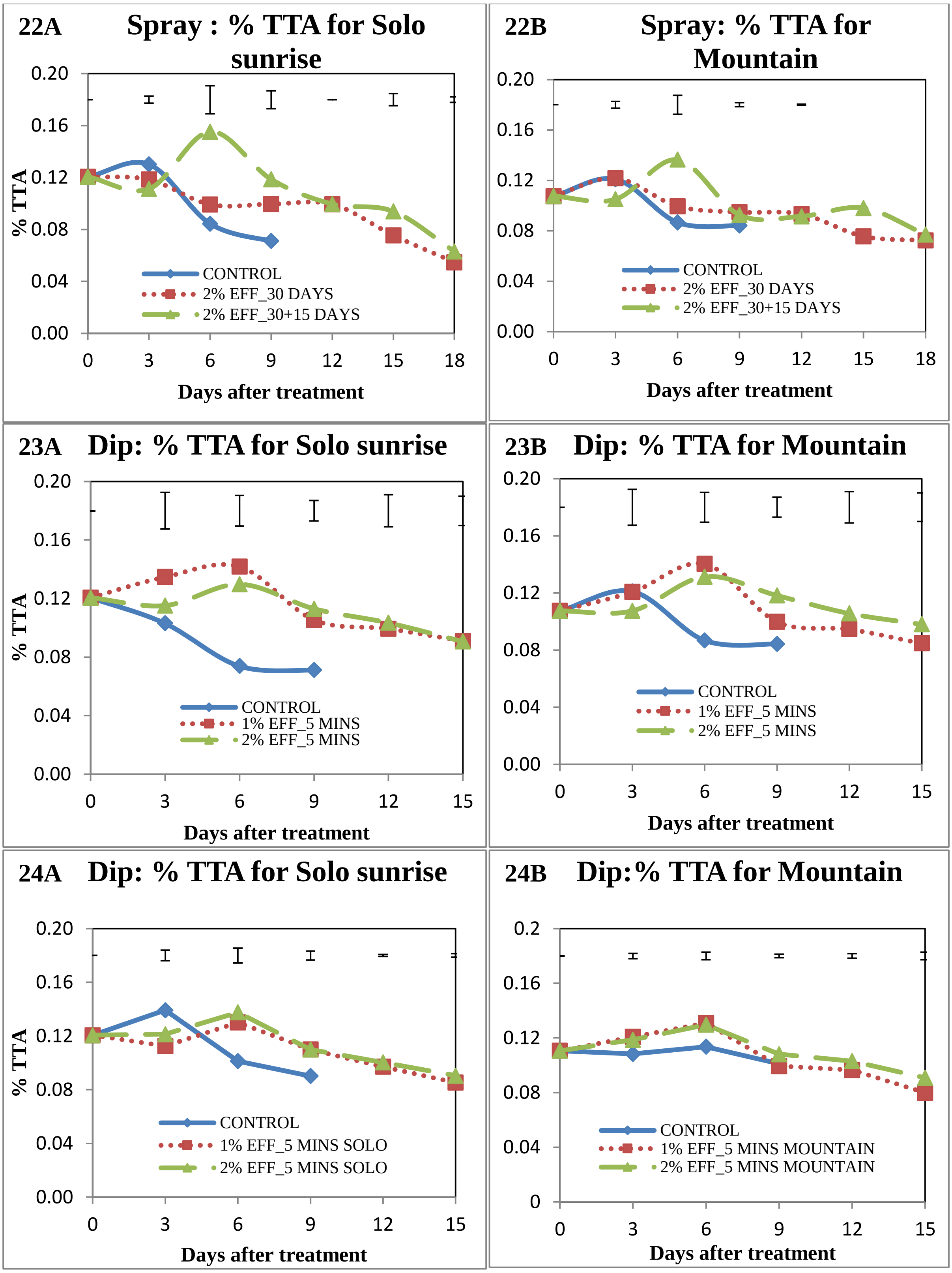

Figure 8: Percent Total titratable acidity present in papaya fruits treated with Hexanal spray (22A and 22B) and dip (23A and 23B) from Machakos County and dipped fruits from Meru (24A and 24B) for experiment 1. Top bars represent LSD at 0.05

4.3.3. Beta-carotene

The amounts of beta-carotene in papaya pulp increased with ripening gradually and consistently throughout the storage period (Figure 9 and 10). Optimal Hexanal treatment did not show any significant (*P*<0.05) impact on the concentration of beta-carotene within each mode of application. However, the overall effect of Hexanal revealed a significant (*P*<0.05) difference between the modes of application and treatments.

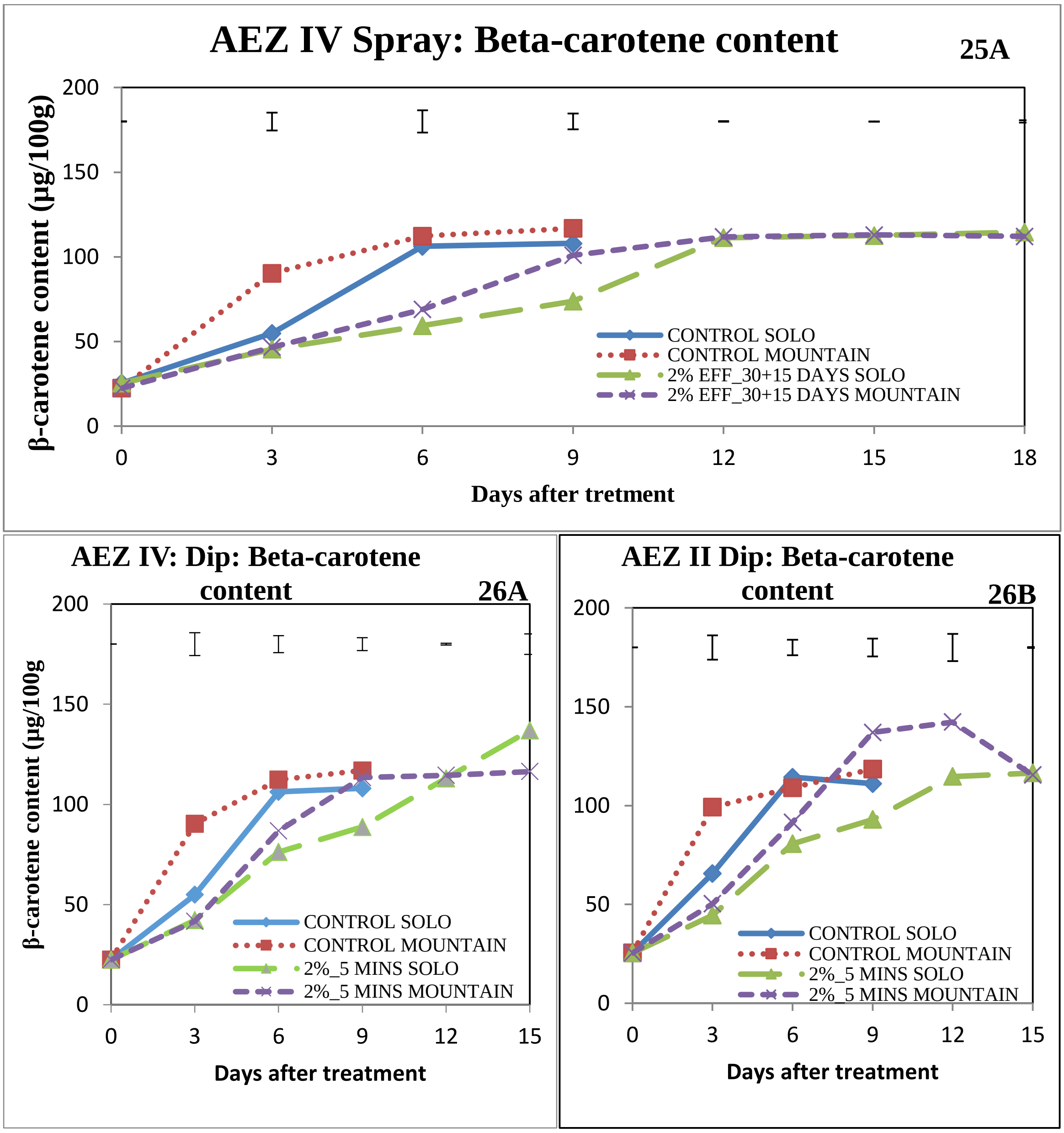

Figure 9: Beta-carotene content of papaya fruits treated with Hexanal spray (25A) and dip (26A) from Machakos County and dipped fruits from Meru (26B) for experiment 2. Top bars represent standard error of mean (*P*≤0.05)

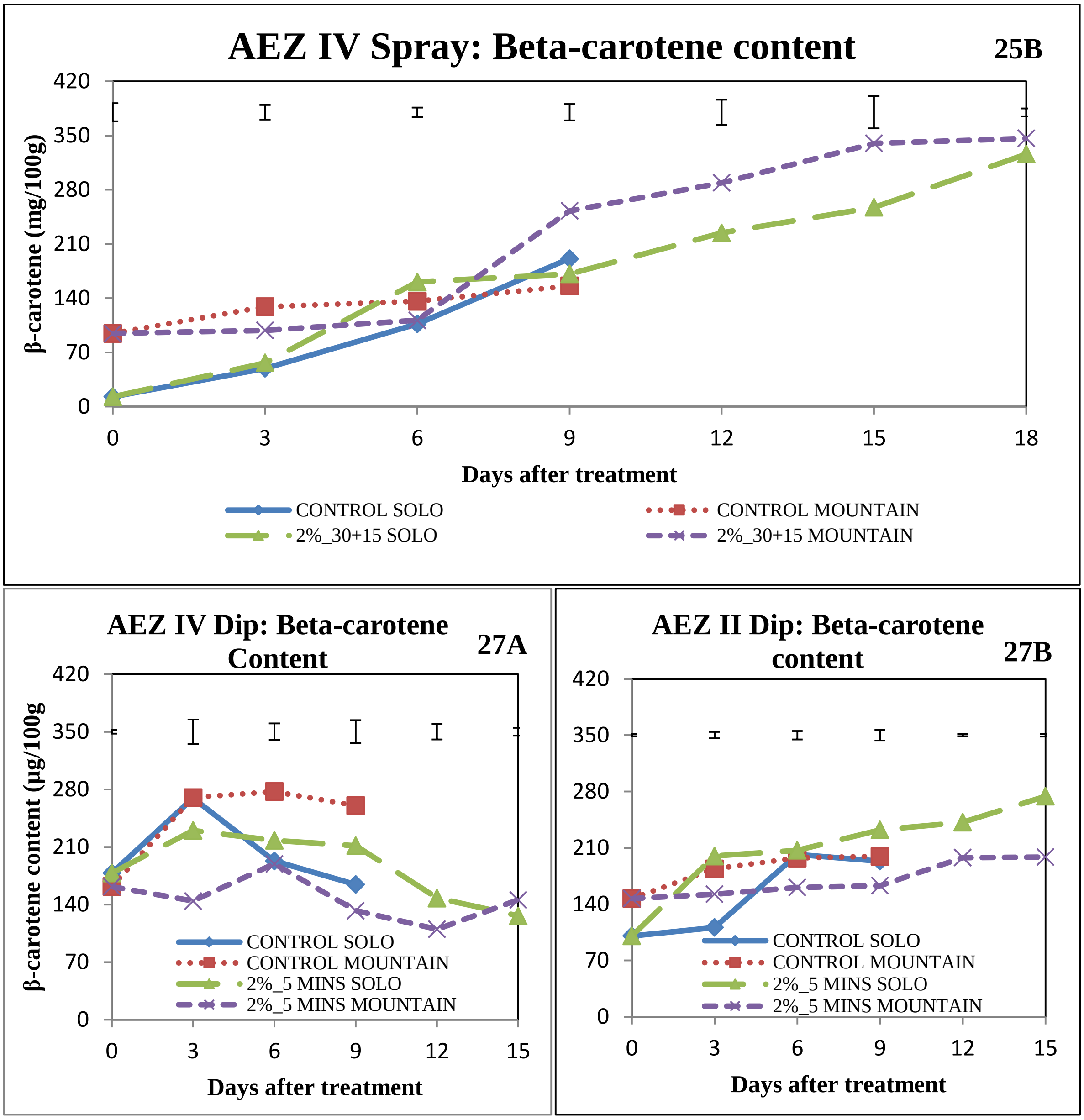

Figure 10: Beta-carotene content of papaya fruits treated with Hexanal spray (25B) and dip (27A) from Machakos County and dipped fruits from Meru (27B) for experiment 2. Top bars represent standard error of mean (*P*≤0.05)

4.3.4. Vitamin C / Ascorbic acid

Hexanal treatment significantly (*P*<0.05) reduced the rate of vitamin C decline in papaya fruits dipped in 2% Hexanal for 5 minutes and analysed for 15 days and in fruits sprayed at 2% Hexanal for 30+15 and analysed for 18 days during ripening (Figure 11 and 12). Vitamin C declined gradually for every extension in storage in control and Hexanal treated papaya fruits. Vitamin C ranged from 78.7 to 28.0 mg/100g in 'Mountain' papaya (Figure 11, 28A) and from 73.1 to 33.1 mg/100g in ‘Solo sunrise’ with higher means in fruits from the hotter AEZ-IV.

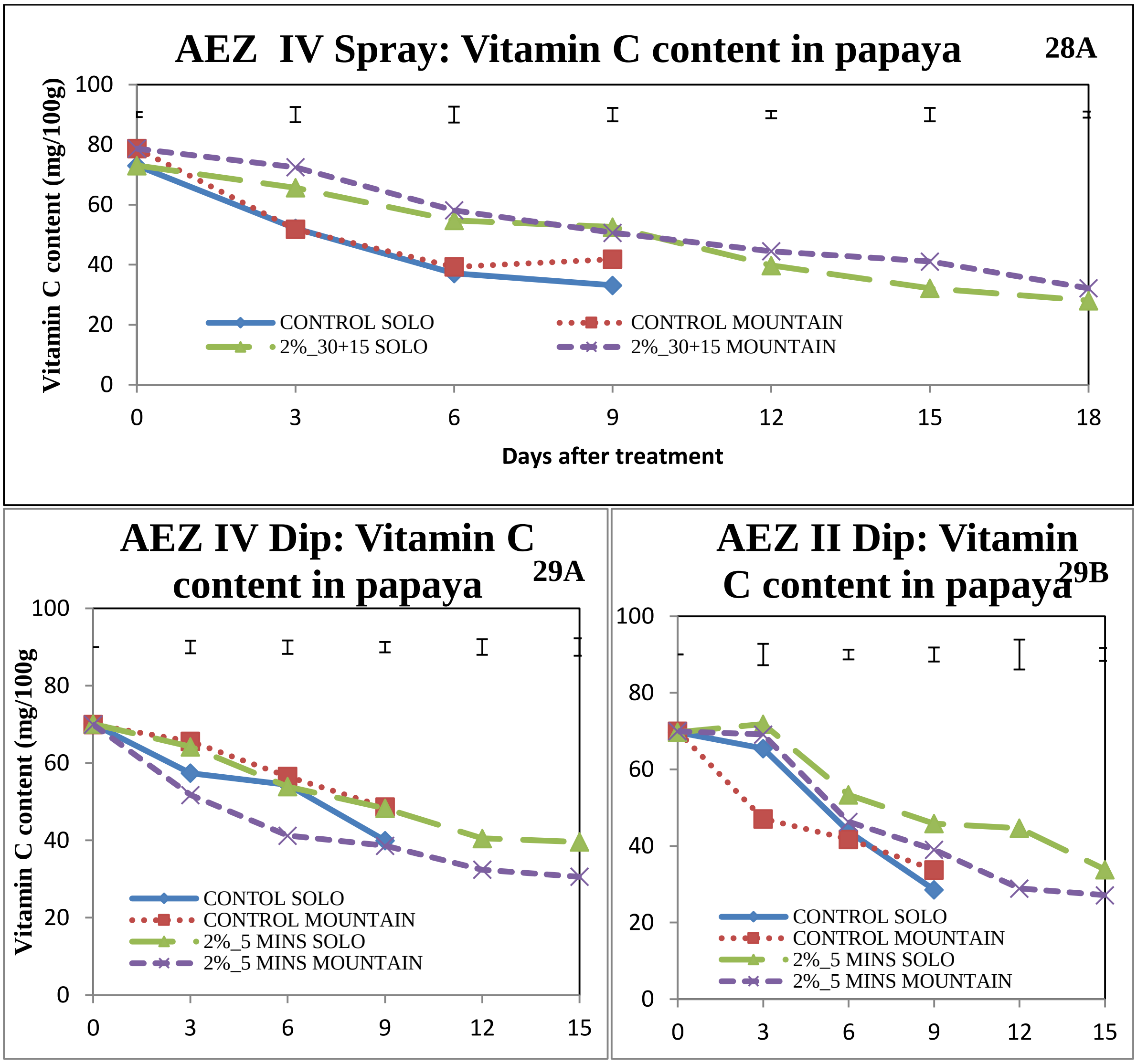

Figure 11: Vitamin C content of papaya fruits treated with Hexanal spray (28A) and dip (29A) from Machakos County and dipped fruits from Meru (29B) for experiment 1. Top bars represent standard error of mean ($P \leq 0.05$)

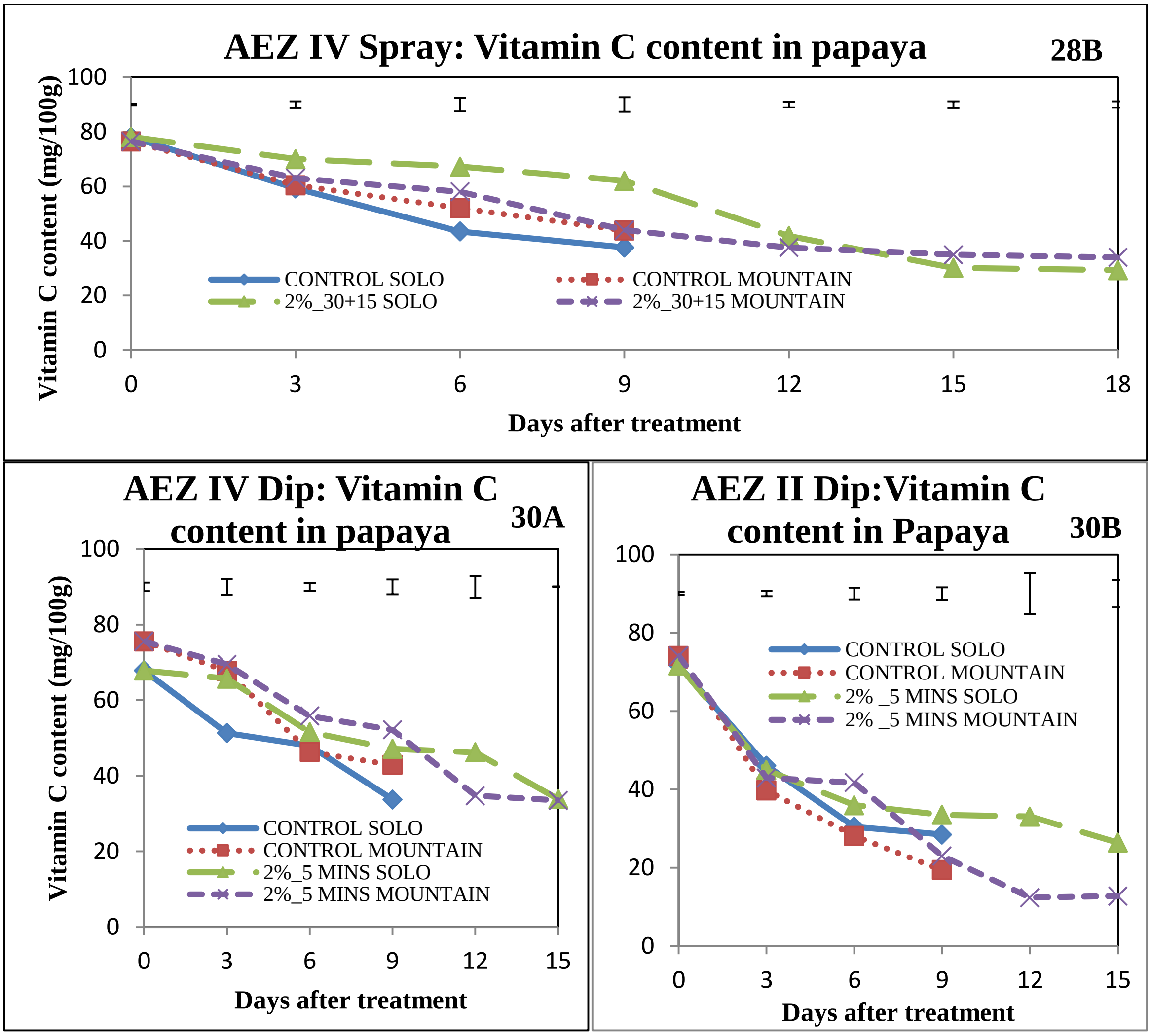

Figure 12: Vitamin C content of papaya fruits treated with Hexanal spray (28B) and dip (30A) from Machakos County and dipped fruits from Meru (30B) for experiment 2. Top bars represent standard error of mean (*P*≤0.05).

4.4. **Discussion**

The quality of papaya fruits investigated in this study was not negatively affected by the Hexanal treatment. However, differences were realized between the fruits from the two locations even within same varieties under similar treatment. This could be attributed to the environmental impacts such as prevailing environmental weather condition/climate in production site, different cultural practices and the plant genetic composition.

Percent TSS level was consistently higher in papaya fruits from the hotter AEZ-IV (Machakos County) compared to fruits from the wetter AEZ-II (Meru County). Machakos experiences a longer duration of full sunlight with high solar intensity, lower rainfall below

1100 mm annually and higher average temperatures of 28˚C. This leads to more carbon accumulation and greater photosynthetic activity (Léchaudel *et al*., 2005) as compared to Meru. A positive relationship between light and TSS level has been reported in mango fruits by Mendoza *et al*., (1972) and this partially explains the differences in vitamin C contents in fruits from these zones.

Variations in total titratable acidity noticed in the Hexanal treated fruits compared to the control fruits in this study could have arisen from genetic variations of each genetic material that continues to change with environmental interactions, differences in cultural practice like use of fertilizer and spacing, as well as harvest date/ maturity stage of harvesting (Soares *et al*., 2009; de-Souza *et al*., 2015). The overall trend revealed slight rise in % TTA from the initial day in storage to around day 6 afterwards dropping slightly. This seemed to mimic the respiratory and climacteric rise in the ripening fruits. A similar phenomenon has been reported by Wills and Widjanarko (1995) who indicated that TTA increased with the maturity stage starting from 0.04 to 0.14 and reaches the maximum when the fruit colour becomes completely yellow. Results from the present study agrees with findings from Lazan *et al*. (2006) who reported that the TTA increases with fruit ripening to about 75% and decreases thereafter. The peel colour had dropped by 75% to a hue angle approximately 80˚around day 6 for treated fruits corresponding to the peaks of % TTA.

Seasonal variation of beta-carotene content of papaya observed could be attributed to variations and/or limiting environmental factors. Studies were conducted in two successive seasons with one being moderately wetter (February to early April) and another drier (July to early October, 2017). High solar intensity was a characteristic of the dry season with water being a limiting factor. Such factors that influence accumulation of water and fruit dry matter have been reported by Léchaudel and Joas (2006) to impact on the biochemical attributes of the fruits. The increase in solar intensity therefore allowed for good accumulation of antioxidants in the fruit since the fruit was the priority sink. The levels of vitamin C content followed a similar trend but with less frequency in fluctuation. A narrow difference of approximately 5 mg/100g could also be attributed to time in cold storage. Papaya fruits sprayed with Hexanal displayed higher means compared to dip samples due to extended time in the field that allowed for proper time of accumulation of this antioxidant. These findings suggest that Hexanal could be a viable organic option of papaya fruits preservation.

CHAPTER 5

GENERAL CONCLUSION AND RECOMMENDATIONS

5.1. **General conclusion**

Papaya fruit is one of the chief sources of vitamins in the tropical and sub-tropical regions where this fruit is grown. However, due to the huge postharvest losses that have been reported to be greater than 50%, Kenya continues to be deprived of these benefits. In Kenya, for instance, papaya fruit is the fifth priority fruit that is also available throughout the seasons. It is therefore the main fruit available that can be consumed as food and utilised in hospitals to supply its readily available nutrients and vitamins to patients. The major challenge has always been the short shelf life and the drastic softening of papaya that is not desirable to consumers. From this study, Hexanal has shown a potential as a viable and a novel option to reduce the rate of papaya softening, extend papaya's availability and maintain the main nutrients including antioxidants like pro-vitamin A and vitamin C in these delicate fruit. Hexanal is a natural compound extracted from plants and is also generally regarded as a safe compound for use in food additive and has been approved by FDA (US Patent # 6,514,914; 7, 198, 81). The results from this study have indicated that Hexanal has the potential to delay respiration and slightly reduced the amounts of ethylene that contributes to postharvest challenges with fruits. Hexanal also improved fruit firmness by at least 35% and was observed to preserve the biochemical attributes of papaya fruits. Application of Hexanal on papaya fruits especially as a spray was realized to possess a great potential in preserving Vitamin C. Spraying also allows for full accumulation of antioxidants during the extended retention time of at least three weeks in the field. The two modes of application of Hexanal are also easy and most of the numerous small holder farmers targeted by this technology can fully apply Hexanal using the locally available tools within their farmsteads.

5.2. **Recommendations**

Hexanal is currently not available for public use in Kenya and tests are still restricted to research institutions like the University of Nairobi by the Ethics Board of Kenya. From the two experiment conducted in Machakos and Meru, Hexanal application to papaya has indicated a good potential to enhance the postharvest life of this delicate and top among preferred fruits in Kenya and the tropical regions.

1. Hexanal could be promoted and licenced as a safe organic volatile compound for use to extend shelf life and preserve quality attribute of papaya. Hexanal can be easily applied as a pre-harvest spray or a postharvest dip in papaya and possibly other fruits by small holder farmers and/or traders.
2. Wide scale experiments be conducted for other fruits and vegetables along various points of the value chains in order to increase benefits for various commodities
3. A detailed socio-economic determination of Hexanal use be conducted in Kenya among different users.

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APPENDICES

Appendix 1: ANOVA for Experiment 1 Firmness

Experiment 1: Firmness (N) in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	2643.42	2643.42	38.18	<.001
Treatment	10	1509.07	150.91	2.18	0.018
Variety	1	379.92	379.92	5.49	0.02
Mode	1	53.87	53.87	0.78	0.378
Day	6	200591.3	33431.89	482.91	<.001
Treatment.Variety	10	481.98	48.2	0.7	0.728
Variety.Mode	1	31.55	31.55	0.46	0.5
Treatment.Day	53	8341.15	157.38	2.27	<.001
Variety.Day	6	5133.56	855.59	12.36	<.001
Mode.Day	3	369.11	123.04	1.78	0.151
Reatment.Variety.Day	46	3588.14	78	1.13	0.27
Variety.Mode.Day	3	17.66	5.89	0.09	0.968
Residual	446	30876.31	69.23		
Total	**587**	**254017**	**432.74**		

Appendix 2: ANOVA for Experiment 2 Firmness

Experiment 2: Firmness (N) in Papaya treated with Hexanal

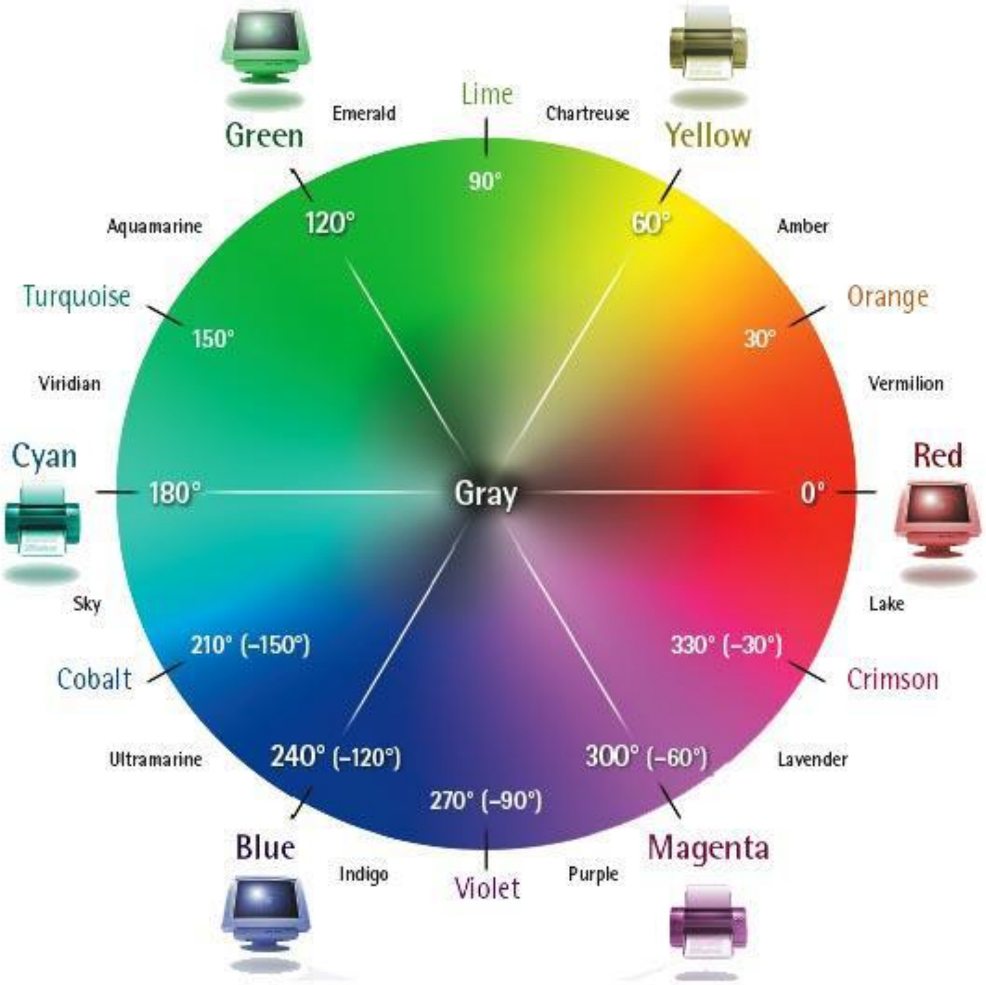
Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	5406.51	5406.51	278.2	<.001
Treatment	10	1885.29	188.53	9.7	<.001
Variety	1	151.26	151.26	7.78	0.006
Mode	1	21.05	21.05	1.08	0.299
Day	5	190833.2	38166.63	1963.9	<.001
Treatment.Variety	10	973.17	97.32	5.01	<.001
Variety.Mode	1	1.25	1.25	0.06	0.8
Treatment.Day	48	9672.15	201.5	10.37	<.001
Variety.Day	5	1602.52	320.5	16.49	<.001
Mode.Day	3	192.56	64.19	3.3	0.02
Treatment.Variety.Day	47	3894.31	82.86	4.26	<.001
Variety.Mode.Day	3	11.62	3.87	0.2	0.897
Residual	434	8434.42	19.43		
Total	**569**	**223079.3**	**392.05**		

Appendix 3: Combined ANOVA for Papaya Firmness

Experiment 1 and2: Firmness (N) in Papaya treated with Hexanal

Source of Variation	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	29.99	29.99	0.48	0.49
Mode	1	122.24	122.24	1.96	0.16
Treatment	10	2550.26	255.03	4.09	<.001
Day	6	388972.9	64828.81	1038.83	<.001
Variety.Mode	1	61.27	61.27	0.98	0.32
Variety.Treatment	10	602.51	60.25	0.97	0.47
Mode.Day	5	3141.6	628.32	10.07	<.001
Treatment.Day	51	9532.62	186.91	3	<.001
Variety.Day	6	5626.63	937.77	15.03	<.001
Variety.Mode.Day	5	253.72	50.74	0.81	0.54
Variety.Treatment.Day	47	3307.05	70.36	1.13	0.26
Residual	1014	63279.21	62.41		
Total	**1157**	**477480**	**412.69**		

Appendix 4a: Colour wheel



Appendix 4b: ANOVA for Experiment 1 Peel Colour

Experiment 1: Peel Colour Hue (H˚)in Papaya treated with Hexanal					
Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	48045.6	48045.6	1191.76	<.001
Treatment	10	2560.76	256.08	6.35	<.001
Variety	1	42.49	42.49	1.05	0.305
Mode	1	120.65	120.65	2.99	0.084
Day	6	192089.6	32014.94	794.13	<.001
Treatment.Variety	10	1066.01	106.6	2.64	0.004
Variety.Mode	1	49.28	49.28	1.22	0.269
Treatment.Day	53	7081.06	133.6	3.31	<.001
Variety.Day	6	791.09	131.85	3.27	0.004
Mode.Day	3	442.79	147.6	3.66	0.012
Treatment.Variety.Day	50	2400.3	48.01	1.19	0.183
Variety.Mode.Day	3	55.59	18.53	0.46	0.711
Residual	457	18423.83	40.31		
Total	**602**	**273169.1**	**453.77**		

Appendix 5:ANOVA for Experiment 2 Peel Colour

Experiment 2: Peel Colour Hue (H˚) in Papaya treated with Hexanal					
Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	21201.8	21201.8	1030.06	<.001
Variety	1	1147.3	1147.3	55.74	<.001
Treatment	10	984.38	98.44	4.78	<.001
Day	6	136032.3	22672.06	1101.49	<.001
Variety.Treatment	10	774.98	77.5	3.77	<.001
Variety.Day	6	770.75	128.46	6.24	<.001
Treatment.Day	53	5986.06	112.94	5.49	<.001
Variety.Treatment.Day	50	2883.79	57.68	2.8	<.001
Residual	464	9550.59	20.58		
Total	**601**	**179332**	**298.39**		

Appendix 6: Combined ANOVA for Papaya Peel Colour

Experiment 1 and 2: Peel Colour Hue (H˚) in Papaya treated with Hexanal					
Change	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	914.59	914.59	10.84	0.001
Mode	1	17163.08	17163.08	203.49	<.001
Treatment	10	2617.84	261.78	3.1	<.001
Day	6	325008.9	54168.15	642.22	<.001
Variety.Mode	1	136.18	136.18	1.61	0.204

Variety.Treatment	10	1103.51	110.35	1.31	0.221
Mode.Day	5	3549.53	709.91	8.42	<.001
Treatment.Day	51	7153.52	140.27	1.66	0.003
Variety.Day	6	1127.27	187.88	2.23	0.038
Variety.Mode.Day	5	142.32	28.46	0.34	0.89
Variety.Treatment.Day	49	4613.44	94.15	1.12	0.273
Residual	1059	89321.58	84.35		
Total	**1204**	**452851.8**	**376.12**		

Appendix 7: ANOVA for Experiment 1 Pulp Colour

Experiment 1: Pulp Colour Hue (H˚) in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	479.335	479.335	50.48	<.001
Treatment	10	2188.844	218.884	23.05	<.001
Variety	1	8.338	8.338	0.88	0.349
Mode	1	9.062	9.062	0.95	0.329
Day	6	44069.42	7344.903	773.46	<.001
Treatment.Variety	10	1381.235	138.123	14.55	<.001
Variety.Mode	1	1.246	1.246	0.13	0.717
Treatment.Day	53	2134.035	40.265	4.24	<.001
Variety.Day	6	875.002	145.834	15.36	<.001
Mode.Day	3	59.678	19.893	2.09	0.1
Treatment.Variety.Day	50	1319.156	26.383	2.78	<.001
Variety.Mode.Day	3	12.753	4.251	0.45	0.719
Residual	457	4339.765	9.496		
Total	**602**	**56877.86**	**94.482**		

Appendix 8: ANOVA for Experiment 2 Pulp Colour

Experiment 2: Pulp Colour Hue (H˚) in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	705.08	705.08	36.47	<.001
Variety	1	266.53	266.53	13.79	<.001
Treatment	10	2969.01	296.9	15.36	<.001
Day	6	35149.09	5858.18	303.01	<.001
Variety.Treatment	10	1345.7	134.57	6.96	<.001
Variety.Day	6	736.33	122.72	6.35	<.001
Treatment.Day	53	4742.1	89.47	4.63	<.001
Variety.Treatment.Day	50	3045	60.9	3.15	<.001
Residual	464	8970.71	19.33		
Total	**601**	**57929.55**	**96.39**		

Appendix 9: Combined ANOVA for Papaya Pulp Colour

Experiment 1 and 2: Pulp Colour Hue (H˚) in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	171.45	171.45	8.22	0.004
Mode	1	898.35	898.35	43.06	<.001
Treatment	10	1463.56	146.36	7.02	<.001
Day	6	78012.28	13002.05	623.28	<.001
Variety.Mode	1	2.82	2.82	0.14	0.713
Variety.Treatment	10	2542.07	254.21	12.19	<.001
Mode.Day	5	562.3	112.46	5.39	<.001
Treatment.Day	51	4653.53	91.25	4.37	<.001
Variety.Day	6	1325.97	220.99	10.59	<.001
Variety.Mode.Day	5	300.55	60.11	2.88	0.014
Variety.Treatment.Day	49	2929.25	59.78	2.87	<.001
Residual	1059	22091.43	20.86		
Total	**1204**	**114953.6**	**95.48**		

Appendix 10: ANOVA for Experiment 1 % Cumulative Weight Loss

Experiment 1: % Cumulative Weight Loss in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	1278.171	1278.171	328.68	<.001
Treatment	10	502.696	50.27	12.93	<.001
Variety	1	0.983	0.983	0.25	0.615
Mode	1	156.819	156.819	40.33	<.001
Day	6	26421.73	4403.621	1132.37	<.001
Treatment.Variety	10	137.26	13.726	3.53	<.001
Variety.Mode	1	2.462	2.462	0.63	0.427
Treatment.Day	53	1088.633	20.54	5.28	<.001
Variety.Day	6	33.799	5.633	1.45	0.194
Mode.Day	3	79.578	26.526	6.82	<.001
Treatment.Variety.Day	53	204.075	3.85	0.99	0.498
Variety.Mode.Day	3	2.164	0.721	0.19	0.906
Residual	463	1800.534	3.889		
Total	**611**	**31708.9**	**51.897**		

Appendix 11: ANOVA for Experiment 2 % Cumulative Weight Loss

Experiment 2: % Cumulative Weight Loss in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	361.51	361.51	109.49	<.001
Treatment	10	967.524	96.752	29.3	<.001
Variety	1	30.836	30.836	9.34	0.002
Mode	1	10.659	10.659	3.23	0.073

Day	6	16266.66	2711.11	821.11	<.001
Treatment.Variety	10	91.858	9.186	2.78	0.002
Variety.Mode	1	0.011	0.011	0	0.955
Treatment.Day	52	508.591	9.781	2.96	<.001
Variety.Day	6	28.18	4.697	1.42	0.204
Mode.Day	3	27.029	9.01	2.73	0.044
Treatment.Variety.Day	44	139.831	3.178	0.96	0.543
Variety.Mode.Day	3	0.647	0.216	0.07	0.978
Residual	440	1452.777	3.302		
Total	**578**	**19886.11**	**34.405**		

Appendix 12: Combined ANOVA for Papaya % Cumulative Weight Loss

Experiment 1 and 2: % Cumulative Weight Loss in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	14.748	14.748	2.23	0.136
Mode	1	54.878	54.878	8.29	0.004
Treatment	10	1046.13	104.613	15.81	<.001
Day	6	42807.68	7134.613	1078.24	<.001
Variety.Mode	1	3.225	3.225	0.49	0.485
Variety.Treatment	10	137.67	13.767	2.08	0.023
Mode.Day	5	299.822	59.964	9.06	<.001
Treatment.Day	51	882.849	17.311	2.62	<.001
Variety.Day	6	40.429	6.738	1.02	0.412
Variety.Mode.Day	5	13.537	2.707	0.41	0.843
Variety.Treatment.Day	51	247.518	4.853	0.73	0.919
Residual	1043	6901.413	6.617		
Total	**1190**	**52449.9**	**44.076**		

Appendix 13: ANOVA for experiment 1 respiration rate

Experiment 1: Respiration rate in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	9035.83	9035.83	581.98	<.001
Treatment	10	2209.37	220.94	14.23	<.001
Variety	1	193.21	193.21	12.44	<.001
Day	6	11809.24	1968.21	126.77	<.001
Treatment.Variety	10	355.03	35.5	2.29	0.013
Variety.Mode	1	15.96	15.96	1.03	0.311
Treatment.Day	53	5259.72	99.24	6.39	<.001
Variety.Day	6	153.26	25.54	1.65	0.133
Mode.Day	3	609.81	203.27	13.09	<.001
Treatment.Variety.Day	46	512.71	11.15	0.72	0.917
Variety.Mode.Day	3	27.98	9.33	0.6	0.615

Residual	446	6924.64	15.53		
Total	**587**	**37106.75**	**63.21**		

Appendix 14: ANOVA for experiment 2 respiration rate

Experiment 2: Respiration rate in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	2463.315	2463.315	378.62	<.001
Variety	1	144.02	144.02	22.14	<.001
Treatment	10	806.626	80.663	12.4	<.001
Mode	1	3.668	3.668	0.56	0.453
Day	5	3676.224	735.245	113.01	<.001
Variety.Treatment	10	692.812	69.281	10.65	<.001
Variety.Mode	1	24.937	24.937	3.83	0.051
Variety.Day	5	256.207	51.241	7.88	<.001
Treatment.Day	48	1838.653	38.305	5.89	<.001
Mode.Day	3	215.852	71.951	11.06	<.001
Variety.Treatment.Day	44	1044.357	23.735	3.65	<.001
Variety.Mode.Day	3	56.251	18.75	2.88	0.036
Residual	428	2784.599	6.506		
Total	**560**	**14007.52**	**25.013**		

Appendix 15: Combined ANOVA for Papaya respiration rate

Experiment 1 and2: Respiration rate in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	289.56	289.56	10.69	0.001
Mode	1	2817.68	2817.68	104.03	<.001
Treatment	10	2019.53	201.95	7.46	<.001
Day	6	11749.94	1958.32	72.3	<.001
Variety.Mode	1	165.48	165.48	6.11	0.014
Variety.Treatment	10	661.56	66.16	2.44	0.007
Mode.Day	5	2296.99	459.4	16.96	<.001
Treatment.Day	51	3452.81	67.7	2.5	<.001
Variety.Day	6	310.39	51.73	1.91	0.076
Variety.Mode.Day	5	175.93	35.19	1.3	0.262
Variety.Treatment.Day	44	869.75	19.77	0.73	0.905
Residual	1008	27301.94	27.09		
Total	**1148**	**52111.54**	**45.39**		

Appendix 16: ANOVA for experiment 1 ethylene rate

Experiment 1: Ethylene evolution rate in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	88.8394	88.8394	172.07	<.001
Treatment	10	19.3394	1.9339	3.75	<.001
Variety	1	6.4911	6.4911	12.57	<.001
Day	6	337.8255	56.3042	109.05	<.001
Treatment.Variety	10	4.7902	0.479	0.93	0.507
Treatment.Day	53	128.4893	2.4243	4.7	<.001
Variety.Day	6	5.4227	0.9038	1.75	0.108
Treatment.Variety.Day	46	12.6907	0.2759	0.53	0.995
Residual	454	234.4018	0.5163		
Total	**587**	**838.2902**	**1.4281**		

Appendix 17: ANOVA for experiment 2 ethylene rate

Experiment 2: Ethylene evolution rate in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	49.7557	49.7557	172.95	<.001
Treatment	10	61.2966	6.1297	21.31	<.001
Variety	1	6.6552	6.6552	23.13	<.001
Mode	1	6.9052	6.9052	24	<.001
Day	5	69.6873	13.9375	48.45	<.001
Treatment.Variety	10	24.4899	2.449	8.51	<.001
Variety.Mode	1	0.2729	0.2729	0.95	0.331
Treatment.Day	48	149.2917	3.1102	10.81	<.001
Variety.Day	5	4.6672	0.9334	3.24	0.007
Mode.Day	3	1.2543	0.4181	1.45	0.227
Treatment.Variety.Day	44	42.7213	0.9709	3.37	<.001
Variety.Mode.Day	3	0.2953	0.0984	0.34	0.795
Residual	428	123.131	0.2877		
Total	**560**	**540.4235**	**0.965**		

Appendix 18: Combined ANOVA for Papaya ethylene rate

Experiment 1 and 2Ethylene evolution rate in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	13.4741	13.4741	18.46	<.001
Mode	1	15.3327	15.3327	21.01	<.001
Treatment	10	64.8497	6.485	8.88	<.001
Day	6	314.6737	52.4456	71.85	<.001
Variety.Mode	1	0.2404	0.2404	0.33	0.566
Variety.Treatment	10	17.4372	1.7437	2.39	0.008
Mode.Day	5	80.1662	16.0332	21.97	<.001

Treatment.Day	51	118.8127	2.3297	3.19	<.001
Variety.Day	6	4.6196	0.7699	1.05	0.388
Variety.Mode.Day	5	2.9594	0.5919	0.81	0.542
Variety.Treatment.Day	44	26.0283	0.5916	0.81	0.807
Residual	1008	735.771	0.7299		
Total	**1148**	**1394.365**	**1.2146**		

Appendix 19: ANOVA for experiment 1 ˚Brix

Experiment 1: ˚Brix in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	5.494	5.494	6.28	0.013
Treatment	4	31.0587	7.7647	8.88	<.001
Variety	1	3.7339	3.7339	4.27	0.04
Mode	1	3.7627	3.7627	4.3	0.039
Day	6	267.7709	44.6285	51.04	<.001
Treatment.Variety	4	6.0686	1.5171	1.74	0.143
Variety.Mode	1	4.0669	4.0669	4.65	0.032
Treatment.Day	19	65.6318	3.4543	3.95	<.001
Variety.Day	6	3.0186	0.5031	0.58	0.75
Mode.Day	3	2.0081	0.6694	0.77	0.514
Treatment.Variety.Day	19	16.2358	0.8545	0.98	0.489
Variety.Mode.Day	3	4.2969	1.4323	1.64	0.181
Residual	228	199.3526	0.8744		
Total	**296**	**612.4995**	**2.0693**		

Appendix 20: ANOVA for experiment 2 % Brix

Experiment 2: ˚Brix in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	31.1026	31.1026	79.72	<.001
Treatment	3	115.1465	38.3822	98.37	<.001
Variety	1	23.5901	23.5901	60.46	<.001
Mode	1	113.7671	113.7671	291.58	<.001
Day	6	204.1653	34.0275	87.21	<.001
Treatment.Variety	3	23.5949	7.865	20.16	<.001
Variety.Mode	1	11.3906	11.3906	29.19	<.001
Treatment.Day	8	24.9615	3.1202	8	<.001
Variety.Day	6	12.5543	2.0924	5.36	<.001
Mode.Day	3	20.4591	6.8197	17.48	<.001
Treatment.Variety.Day	8	10.7283	1.341	3.44	0.001
Variety.Mode.Day	3	5.7752	1.9251	4.93	0.003
Residual	147	57.3553	0.3902		
Total	**191**	**654.5908**	**3.4272**		

Appendix 21: Combined ANOVA for Papaya % Brix

Experiment 1 and 2: ˚Brix in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	2.362	2.362	1.77	0.184
Mode	1	115.321	115.321	86.65	<.001
Treatment	4	22	5.5	4.13	0.003
Day	6	447.189	74.532	56	<.001
Variety.Mode	1	5.04	5.04	3.79	0.052
Variety.Treatment	4	6.044	1.511	1.14	0.339
Mode.Day	5	9.436	1.887	1.42	0.217
Treatment.Day	17	59.02	3.472	2.61	<.001
Variety.Day	6	5.551	0.925	0.7	0.654
Variety.Mode.Day	5	15.656	3.131	2.35	0.04
Variety.Treatment.Day	17	19.676	1.157	0.87	0.611
Residual	421	560.307	1.331		
Total	**488**	**1267.602**	**2.598**		

Appendix 22: ANOVA for experiment 1 % TTA

Experiment 1: % TTA in Papaya treated with Hexanal

Change	d.f	s.s.	m.s.	v.r.	F pr.
Location	1	0.008345	0.008345	53.7	<.001
Treatment	4	0.004859	0.001215	7.82	<.001
Variety	1	0.000121	0.000121	0.78	0.378
Mode	1	0.000149	0.000149	0.96	0.329
Day	6	0.024228	0.004038	25.98	<.001
Treatment.Variety	4	0.00099	0.000247	1.59	0.177
Variety.Mode	1	0.000228	0.000228	1.47	0.227
Treatment.Day	19	0.014056	0.00074	4.76	<.001
Variety.Day	6	0.00418	0.000697	4.48	<.001
Mode.Day	3	0.001131	0.000377	2.43	0.066
Treatment.Variety.Day	17	0.005787	0.00034	2.19	0.005
Variety.Mode.Day	3	0.000388	0.000129	0.83	0.478
Residual	224	0.03481	0.000155		
Total	**290**	**0.099272**	**0.000342**		

Appendix 23: ANOVA for experiment 2 % TTA

Experiment 2: % TTA in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	0.012955	0.012955	33.33	<.001
Treatment	3	0.0221	0.007367	18.95	<.001
Variety	1	0.00152	0.00152	3.91	0.051
Mode	1	0.00688	0.00688	17.7	<.001

Day	6	0.008822	0.00147	3.78	0.002
Treatment.Variety	3	0.00204	0.00068	1.75	0.162
Variety.Mode	1	0.000226	0.000226	0.58	0.448
Treatment.Day	8	0.004755	0.000594	1.53	0.156
Variety.Day	6	0.004886	0.000814	2.09	0.06
Mode.Day	3	0.000907	0.000302	0.78	0.509
Treatment.Variety.Day	8	0.003496	0.000437	1.12	0.353
Variety.Mode.Day	3	0.001038	0.000346	0.89	0.449
Residual	103	0.040039	0.000389		
Total	**147**	**0.109664**	**0.000746**		

Appendix 24: Combined ANOVA for Papaya % TTA

Experiment 1 and 2: % TTA in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	1	0.000296	0.000296	0.45	0.501
Mode	1	0.013596	0.013596	20.85	<.001
Treatment	4	0.011126	0.002782	4.26	0.002
Day	6	0.018903	0.00315	4.83	<.001
Variety.Mode	1	0.000546	0.000546	0.84	0.361
Variety.Treatment	4	0.002727	0.000682	1.05	0.384
Mode.Day	5	0.003417	0.000683	1.05	0.389
Variety.Day	6	0.007351	0.001225	1.88	0.083
Treatment.Day	17	0.011508	0.000677	1.04	0.416
Variety.Mode.Day	5	0.0013	0.00026	0.4	0.85
Variety.Treatment.Day	17	0.008181	0.000481	0.74	0.764
Residual	372	0.242618	0.000652		
Total	**439**	**0.321568**	**0.000733**		

Appendix 25: ANOVA for experiment 1 Beta-carotene

Experiment 1: Beta-carotene content in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	144.2	144.2	1.26	0.263
Treatment	2	146.2	73.1	0.64	0.529
Mode	1	282.1	282.1	2.47	0.118
Day	6	203253.7	33875.6	296.83	<.001
Treatment.Day	8	5088.6	636.1	5.57	<.001
Mode.Day	3	844.9	281.6	2.47	0.065
Residual	142	16205.8	114.1		
Total	**163**	**225965.6**	**1386.3**		

Appendix 26: ANOVA for experiment 2 Beta-carotene

Experiment 2: Beta-carotene content in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	4643	4643	2.59	0.11
Treatment	2	79283	39641	22.11	<.001
Variety	2	72611	36306	20.25	<.001
Mode	1	15159	15159	8.46	0.004
Day	6	455860	75977	42.38	<.001
Treatment.Variety	2	68511	34255	19.11	<.001
Treatment.Day	8	312656	39082	21.8	<.001
Variety.Day	11	44706	4064	2.27	0.014
Mode.Day	3	35465	11822	6.59	<.001
Treatment.Variety.Day	6	98721	16453	9.18	<.001
Residual	143	256377	1793		
Total	**185**	**1443993**	**7805**		

Appendix 27: Combined ANOVA for Papaya Beta-carotene

Experiment 1 and 2: Beta-carotene content in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	9	289748	32194	7.18	<.001
Mode	1	151361	151361	33.74	<.001
Treatment	3	133563	44521	9.92	<.001
Day	7	584310	83473	18.61	<.001
Variety.Treatment	8	35281	4410	0.98	0.449
Mode.Day	5	316409	63282	14.11	<.001
Treatment.Day	7	32378	4625	1.03	0.409
Variety.Day	14	33246	2375	0.53	0.915
Variety.Treatment.Day	9	85196	9466	2.11	0.029
Residual	286	1283034	4486		
Total	**349**	**2944525**	**8437**		

Appendix 28: ANOVA for experiment 1 Vitamin C

Experiment 1: Vitamin C content in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	47.31	47.31	2.41	0.125
Treatment	2	277.87	138.94	7.07	0.001
Variety	1	106.88	106.88	5.44	0.022
Mode	1	176.37	176.37	8.98	0.004
Day	6	23169.92	3861.65	196.54	<.001
Treatment.Variety	2	838.92	419.46	21.35	<.001
Variety.Mode	1	28.23	28.23	1.44	0.234
Treatment.Day	8	334.8	41.85	2.13	0.042

Variety.Day	6	200.02	33.34	1.7	0.132
Mode.Day	3	381.61	127.2	6.47	<.001
Treatment.Variety.Day	8	213.71	26.71	1.36	0.227
Variety.Mode.Day	3	12.44	4.15	0.21	0.888
Residual	81	1591.54	19.65		
Total	**123**	**27379.63**	**222.6**		

Appendix 29: ANOVA for experiment 2 Vitamin C

Experiment 2: Vitamin C content in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Location	1	5011.36	5011.36	213.96	<.001
Treatment	2	285.18	142.59	6.09	0.003
Variety	1	20.76	20.76	0.89	0.349
Mode	1	101.25	101.25	4.32	0.041
Day	6	29248.01	4874.67	208.13	<.001
Treatment.Variety	2	223.79	111.89	4.78	0.011
Variety.Mode	1	0.27	0.27	0.01	0.915
Treatment.Day	8	711.22	88.9	3.8	<.001
Variety.Day	6	301.42	50.24	2.14	0.057
Mode.Day	3	72.57	24.19	1.03	0.383
Treatment.Variety.Day	8	404.26	50.53	2.16	0.039
Variety.Mode.Day	3	114.79	38.26	1.63	0.188
Residual	81	1897.15	23.42		
Total	**123**	**38392.03**	**312.13**		

Appendix 30: Combine ANOVA for Papaya Vitamin C

Experiment 1 and 2: Vitamin C content in Papaya treated with Hexanal

Change	d.f.	s.s.	m.s.	v.r.	F pr.
Variety	3	2045.76	681.92	16.13	<.001
Mode	1	85.66	85.66	2.03	0.156
Treatment	9	20321.72	2257.97	53.43	<.001
Day	7	33346.11	4763.73	112.71	<.001
Variety.Treatment	4	269.31	67.33	1.59	0.178
Mode.Day	5	319.54	63.91	1.51	0.188
Treatment.Day	19	1494.63	78.66	1.86	0.019
Variety.Day	7	80.22	11.46	0.27	0.964
Variety.Treatment.Day	6	246.72	41.12	0.97	0.445
Residual	186	7861.04	42.26		
Total	**247**	**66070.72**	**267.49**		