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Effect of Hexanal as a Post-harvest Treatment to Extend the Shelf-life of Banana Fruits (*Musa acuminata* var. Sweet Banana) in Kenya

Peninah Yumbya[1*], Margaret Hutchinson[1], Jane Ambuko[1] and Willis Owino[2]

[1]Department of Plant Science and Crop Protection, University of Nairobi, P.O.Box 29053-00625, Nairobi, Kenya.
[2]Department of Food Science and Technology, Jomo Kenyatta University of Agriculture and Technology, P.O.Box 62000, Code 00200, Nairobi, Kenya.

Authors' contributions

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ABSTRACT

The short shelf-life of fruits in the tropics continues to be a pressing problem for farmers and other value chain actors. Hexanal is a naturally occurring compound that has received attention as a novel postharvest compound preservative. This study was conducted to determine the effect of hexanal on enhancing the postharvest shelf-life and quality of 'sweet banana' fruits. Two hexanal concentrations (2% and 3%) were applied as either a pre-harvest spray or a post-harvest dip. Fruits were obtained from two different agro ecological zones of Kenya (AEZs II and IV). The treated fruits were kept under ambient room conditions of 25 ± 1°C and RH 60 ± 5% to ripen. Hexanal treatment maintained the fruits quality and prolonged the shelf-life by 6 days in the dipped fruits, 6 and 3 days

**Corresponding author: E-mail: muenipenny8756@gmail.com;*

in the sprayed fruits from the drier AEZ IV and colder AEZ II respectively compared to the untreated controls. Hexanal treatments significantly (P = .05) delayed or reduced the rate of most of the physicochemical parameters analysed irrespective of the concentration and mode of application used. Fruit firmness was significantly (P = .05) maintained up to day 6 and 9 of storage in the treated fruits compared to the controls which softened drastically as from day 3 and 6 in the sprayed and dipped fruits respectively. Hexanal treatment delayed ethylene and respiratory peaks by 3 days in both modes of application and significantly delayed progression of other ripening related changes such as ^{0}Brix, titratable acidity, simple sugars and vitamin C. Sensory evaluation showed no significant differences in the various quality attributes analysed between the hexanal treated and control fruits. The results of this study indicate that, use of hexanal is a potential technology that could be adopted by banana farmers to enhance post-harvest shelf-life without compromising on quality.

Keywords: Hexanal; fruit quality; shelf life; postharvest loss; sweet banana.

1. INTRODUCTION

Kenya is endowed with good climatic conditions which favours production of different types of horticultural crops among them fruits, vegetables and cut flowers. Fruits are a key component of horticultural subsector in Kenya and come third in terms of income contribution after flowers and vegetables [1]. However, the full commercial potential of fruits such as banana has not been realized due to various challenges along the value chain among them high postharvest losses estimated at 40% [2]. The huge postharvest losses are mostly attributed to the highly perishable nature of the produce and further aggravated by failure to use appropriate post-harvest technologies.

In Kenya, banana is the most popular fruit crop often consumed as a dessert while the cooking varieties serve as a staple food in different regions of the country [1]. However, most of it is consumed locally with only a small percentage of approximately 7.2% being exported [1]. Production of banana is mostly dominated by small scale farmers though few medium and large scale growers are found in the major banana growing areas [2]. Banana is a security crop at the household level and the surplus is sold to provide the much-needed income for farmers. Nutritionally, banana contains high levels of calorie, a wide range of vitamins, minerals, anti-oxidants and it is naturally low in fats [3]. However, once ripe the fruits have a short shelf life of approximately 3-4 days and this limits their utilization, postharvest handling and marketing [4].

Banana is a climacteric fruit which is often harvested at the physiological maturity stage and then ripened before marketing. During ripening, the fruit undergoes different biochemical and physiological changes that transforms the fruit to edible state. Some of these changes include fruit softening, changes in peel color, degradation of starch to sugars, changes in concentration of aroma volatiles and acids. According to Maduwanthi and Marapana [5], sugar levels increases from of an initial of 2% in green banana to approximately 15% -20% in the ripe fruit making it sweeter. However, once the fruit is fully ripe, it becomes very delicate and if not properly handled high postharvest losses can be incurred. In order to increase storage life of the fruits, appropriate post-harvest technologies aimed at reducing the deterioration rate have been developed over the years. These technologies are used to slow down fruits metabolic processes to deliver enhanced shelf-life and optimal quality without compromising on the consumer safety. Recently, efforts have been made to develop new and biological post-harvest technologies for extension of banana shelf-life while retaining quality [6-8]. Use of hexanal and its formulations is one of the new innovations which have been proved effective in enhancing the post-harvest shelf life of banana fruits [6,7]. Hexanal, is an aldehyde compound produced naturally by plants as a defence response to different biotic stresses and has an odour similar to that of freshly cut grass or cucumber [9]. The United States Food and Drug Administration Agency has approved the use of hexanal as a GRAS compound [10]. Hexanal use offers a human-safe post-harvest preservation product that is environmentally friend and economically viable. Hexanal is oxidized to hexanoic acid in the body after consumption and further oxidized to carbon dioxide and water duri ng respiration through the tricarboxylic acid cycle [9]. It has also been noted that hexanal, has antimicrobial properties against several post-

harvest pathogens such as *Alternaria alternate* and *Botrytis Cinerea* [11]. A biochemical formulation of an artificially synthesized version of hexanal (Enhanced Freshness Formulation) has been developed which delays fruit ripening [12]. This formulation can be applied in different ways such post-harvest dip, pre-harvest spray or as a vapor. Being a relative new technology, there is need to test its suitability to enhance banana shelf-life while preserving post-harvest quality in Kenya. A previous study in 'Grand naine' variety [7], showed promising results of hexanal extending fruits shelf life by nine days without compromising on quality. However, since hexanal's effect is physiological, it is possible that its efficacy might vary between varieties. The objective of this study was therefore, to determine the effect of hexanal treatment on the post-harvest shelf-life and quality of 'sweet banana' fruits, a very popular variety in Kenya.

2. MATERIALS AND METHODS

2.1 Study Area

The experiment was conducted on 'sweet banana' fruits from two contrasting agro ecological zones (AEZs) in Kenya. Meru County is a high potential AEZ II that lies at an elevation of 1980–2700 m above sea level and receives an annual average rainfall of 1500 mm. Machakos County is a semi-arid AEZ IV that lies at an elevation of 1000-1600 m above sea level with an annual average rainfall of 600 mm.

2.2 Experimental Setup

For the pre-harvest spray mode of application, 15 banana trees at flowering stage in each study site were randomly selected and tagged in the farmer's field. Two concentrations of hexanal (2% and 3%) and a control (clean, plain water) were sprayed twice at 30 and 15 days before harvest. The dosing range used was informed by a previous study done on 'Grand naine' variety [7]. Since hexanal is immiscible with water, Tween 20 and ethanol were added to increase its solubility [10]. Tween 20, ethanol and hexanal were added in the ratio of 10:10:1. The stock solutions were mixed with water and diluted accordingly to provide the required hexanal concentrations. Using a knapsack sprayer, the fruits were sprayed to the point of dripping with the solution. Spray contamination was avoided by using alternate rows of trees for the experiment and a 4 tree gap between treatments in the same row of trees. The fruits were left on the tree until approximately 20% per bunch had ripened. The fruits were then harvested and only the middle hands were used in the post-harvest analysis.

For the post-harvest dip mode of application, fruits were harvested at the mature green stage based on degree of fullness of the fingers, as indicated by the disappearance of angularity and the number of days after anthesis which was approximately 104 days. Only the middle hands of each banana bunch (a cluster of fruits attached together at the stalk) were used in the analysis. The harvested fruits were packed in cushioned crates, covered with wet magazine papers to reduce water loss, and immediately transported to the post-harvest laboratory at Jomo Kenyatta University of Agriculture and Technology (JKUAT).

2.3 Sample Preparation

In the post-harvest laboratory, the fruits were cleaned, dried, and selected for uniformity and freedom from mechanical injuries. Pre-harvest spray-treated fruits were left to undergo normal ripening under ambient room conditions of 25 ± 1^{o}C and RH 60 ± 5%. Fruits for post-harvest treatment were dipped in one of the two hexanal concentrations (2%, 3%) or plain water (control) for 5 minutes. The hexanal solution was mixed with Tween 20 and ethanol to increase its solubility. The hexanal concentrations and application time used was informed by a previous study done on 'Grand naine' variety [7]. All the fruits were left to undergo normal ripening under ambient room conditions. Five banana hands from each treatment combination were randomly sampled at 3-day intervals to evaluate respiration and ethylene evolution rates. Three fruits were also randomly sampled to evaluate other ripening related parameters including pulp firmness, ^{o}Brix, titratable acidity, ascorbic acid, simple sugars and sensory analysis evaluation.

2.3.1 Shelf life

The time taken by the fruits from harvesting to reach the optimal, edible ripe stage was counted and reported in days. This was defined as stage 7 according to the standard banana ripening chart by Soltani et al. [13].

2.3.2 Analysis of physiological parameters

Rate of respiration and ethylene production were determined using gas chromatographs models GC-8A and GC-9A, Shimadzu Corp., Kyoto,

Japan, respectively. The gas chromatograph to determine rate of respiration was fitted with a thermal conductivity detector and a Poropak N column while that for ethylene determination was fitted with an activated alumina column and a flame ionization detector. Five banana fingers were randomly sampled from each treatment, numbered and their weights taken using a digital balance, Model Libror AEG-220, Shimadzu Corp. Kyoto, Japan. Each of the five fingers was incubated for two hours in air tight containers fitted with self-sealing rubber septa. Gas samples were taken from the headspace using an airtight 1 mL hypodermic syringe and injected into the respective gas chromatographs. The rate of carbon dioxide production (used to estimate respiration rate) was expressed as mL/Kg/h while ethylene production was expressed as µl/Kg/h.

2.3.3 Pulp firmness

Pulp firmness was measured along the equatorial region of the fruit using a penetrometer (CR-100D, Sun Scientific Co. Ltd, Japan) fitted with an 8 mm probe. Four locations along the equatorial zone of the fruit were used and average value of firmness calculated. The banana was peeled first, before allowing the probe to penetrate the flesh to a depth of 8mm and the corresponding force required to penetrate this depth determined. Firmness was expressed as Newton.

2.3.4 Total soluble solids (TSS)

Total soluble solids content of banana fruit pulp was determined using digital hand held refractometer (Model PAL-1, Atago, Tokyo, Japan). Five grams of banana paste extracted from three different fruits in each treatment by use of mortar and pestle was placed on the prism of the refractometer and TSS content was recorded as % Brix from direct reading of the instrument.

2.3.5 Total titratable acidity (TTA)

Total titratable acidity was determined by titration in which 5 grams of the fruit pulp was macerated and diluted with 20 ml of distilled water. Ten mL of the diluted solution was obtained, mixed with 3 drops of phenolphthalein indicator and titrated with 0.1N Sodium hydroxide until the solution changed color to faint pink. The titer volume was recorded and the results expressed as percent malic acid, the predominant organic acid in banana fruits.

2.3.6 Ascorbic acid content (Vitamin C)

Ascorbic acid content was determined by use of high performance liquid chromatography (HPLC) method. Five grams of sample was weighed and extracted with 0.8% meta-phosphoric acid under subdued light conditions. The extract was made to 20 mL of juice and centrifuged at 10000 rpm at 4^{o}C for 10 minutes. The supernatant was filtered and diluted with 10 mL of 0.8% meta-phosphoric acid. This was passed through 0.45 micro filters. The samples were then set as a post-run into HPLC machine (Model LC- 10AS, Shimadzu Corp., Kyoto, Japan) where 20 µL of the micro filtered sample was automatically injected into the HPLC machine on the same day of extraction. Various concentrations of ascorbic acid standards were prepared at 10, 20, 40, 60, 80 and 100 ppm and a blank containing only degassed meta-phosphoric acid and used to obtain a calibration curve. HPLC analysis was done using Shimadzu UV-VIS detector fitted with phenomenex 250mm*4.6mm*5µl C-18 ODS column. The mobile phase was 0.8% meta-phosphoric acid, at 1.2 mL/min flow rate and wavelength of 266.0 nm.

2.3.7 Simple sugars

Simple sugars were analysed using a high performance liquid chromatography (HPLC) (Model LC-20AS, Shimadzu Corp., Kyoto, Japan) fitted with phenomenex 250mm*4.6mm*5µl Amino NH2P column. Five grams of the banana pulp was macerated and 96% ethanol added. Refluxing was done for one hour at 100^{o}C and then cooled under running water. The solution was then filtered using 42 mm whatman filter paper. Rising was done using 5 ml of 96% ethanol. The solution was rotary evaporated to dryness at 60^{o}C. 5 ml of 50% acetonitrile was then added and finally micro-filtered (0.45 µ). The HPLC was running under the following conditions: oven temperature: 30^{o}C, Flow rate: 1.0 ml/min, Injection volume: 20 uL, Column: NH_2 (5.0 µl) Mobile phase: Acetonitrile: water (75:25). Sugars present in the solution including sucrose, glucose and fructose were identified and their individual concentration calculated using the standards.

2.3.8 Sensory analysis

Sensory quality evaluation was performed on the hexanal treated and untreated fruits once they were fully ripe; stage 6, according to the standard banana ripening chart by Soltani et al. [13]. The

fruits were washed with clean water, dried and diced into approximately equal-sized slices, avoiding the extreme ends. Three slices were placed on white plates which were anonymously coded based on treatment to ensure objectivity. A panel of 36 untrained judges from the faculty of Agriculture student population at the University of Nairobi was guided on the scoring procedure of the various sensory attributes that included: fruit colour, aroma, texture, flavour, mouth feel and the general acceptability. The panellists scored for these attributes on a five point hedonic scale where 1 = dislike (worst), 2 = (dislike moderately), 3 = (neither like nor dislike), 4 = (like moderately) and 5= Like extremely (Best). This was adapted from Galan et al. [14], but with few modifications.

2.4 Statistical Analysis

Data collected was subjected to analysis of variance (ANOVA) using Genstat statistical package (version 15). The means were separated by Least Significance Difference (LSD) at $p \leq .05$ using Fisher's protected test. The sensory quality evaluation data was analyzed using Statistical Package for the Social Sciences (SPSS) version 20.

3. RESULTS

3.1 Shelf Life

Hexanal treatment enhanced shelf life by 6 days (Plate 2) in the post-harvest dipped fruits, 6 and 3 days in the sprayed fruits (Plate 1) from the drier AEZ IV and the wetter AEZ II respectively as compared to the controls, irrespective of the production zone and hexanal concentration used.

3.2 Rate of Ethylene Production

In the pre-harvest spray mode of application, the control fruits had significantly *(P* = .05) high levels of ethylene production with the climacteric peaks of approximately 10 nL/kg/hr occurring 3 days earlier compared to the hexanal treated fruits (Fig. 1A & B). Hexanal treatment significantly (*P* = .05) reduced the rate of ethylene production in both AEZ and delayed the climacteric peaks by 3 days compared to the untreated fruits. The reduced climacteric peaks of 4.8- 6.3 nL/kg/hr and 5.6 – 6.3 nL/kg/hr in the hexanal treated fruits from the drier AEZ IV (Fig. 1A) and wetter AEZ II fruits (Fig. 1B) occurred at day 6 of storage, then ethylene levels drastically declining till the end of storage exhibiting a true climacteric pattern.

A significant difference (*P* = .05) was observed between the two modes of hexanal application where post-harvest mode of application delayed the climacteric peaks by 6 days compared to the pre-harvest spray (Fig. 1A & B). However, zone of production did not have any significant effect on the rate of ethylene production.

3.3 Respiration Rate

Respiration rate followed a similar pattern to the ethylene production. In both zones of production, hexanal treatment significantly (*P* = .05) reduced the rate of respiration, with a post-harvest dip mode of application exhibiting lower rates compared to pre-harvest spray (Fig. 2A & B). Just like in ethylene production, fruits from the pre-harvest spray mode of application had higher respiratory rate compared to the post-harvest dipped ones. The high respiratory peaks of 61 mL/kg/h and 69 mL/kg/hr in the controls occurred at day 3 of storage, compared to 41 -47 mL/kg/h and 44 -48 mL/kg/h in the hexanal treated fruits, 3 days later in drier AEZ IV and wetter AEZ II respectively (Fig. 2A & B). A similar trend was observed in the post-harvest dip mode of application experiment, where the treated fruits had lower levels of respiration compared to the pre-harvest spray experiment with respiratory peaks of 49 mL/kg/h and 34 -39 mL/kg/h, in drier AEZ IV and colder AEZ II, respectively, occurring 6 days later.

3.4 Pulp Firmness

A general reduction in pulp firmness was observed in both the hexanal treated and control fruits as ripening progressed (Fig. 3A and B). Hexanal treatment applied either as a pre-harvest spray or post-harvest dip significantly (*P* = .05) delayed pulp softening in both AEZ. Interaction between mode of application and zone of production had a significant (*P* = .05) effect on the rate of softening with fruits from the drier AEZ IV (Fig. 3A) softening faster compared to those from the colder AEZ II (Fig. 3B). The control fruits drastically lost their pulp firmness by 96% in fruits produced in both AEZ, after 6 and 9 days of storage in the drier AEZ IV and wetter AEZ II, respectively in the pre-harvest spray mode of application. Similarly, in the post-harvest dip mode of application, the untreated control fruits had lost approximately 95% of their pulp firmness after 12 days of storage in both zones.

By the 9th day of storage, pre-harvest sprayed fruits had lost approximately 72% -75% and 82%- 85% of their firmness compared to 50% - 76% and 60% – 71% in the post-harvest dip treated fruits in the drier AEZ IV and wetter AEZ II, respectively (Fig. 3A and B).

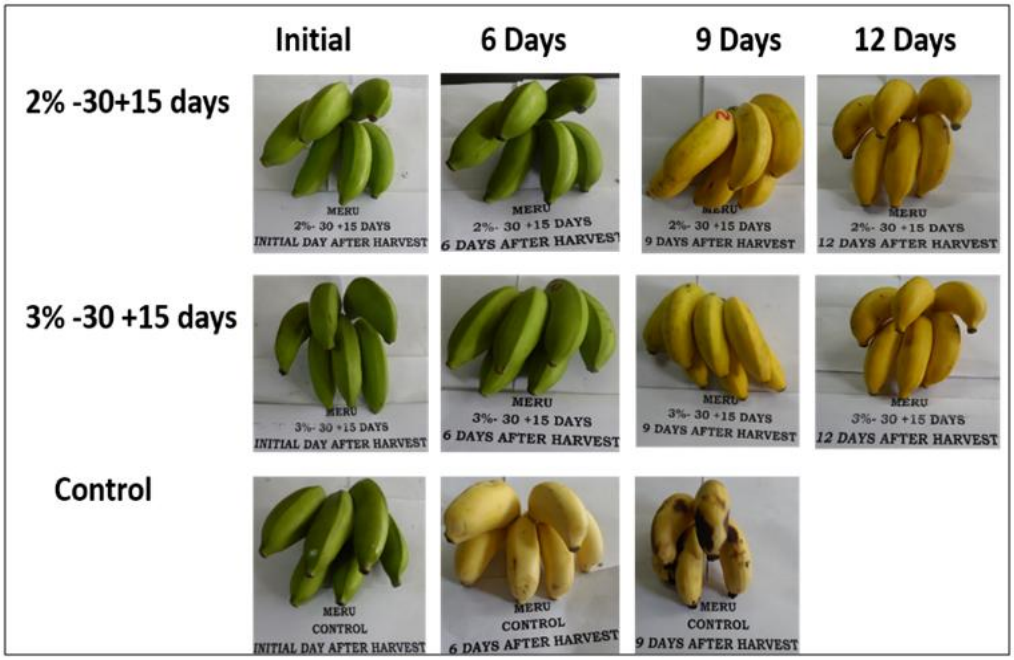

Plate 1. Ripening changes of 'sweet banana' fruits sprayed with 2% and 3% Hexanal and controls fruits during post-harvest storage (from initial day to day 12)

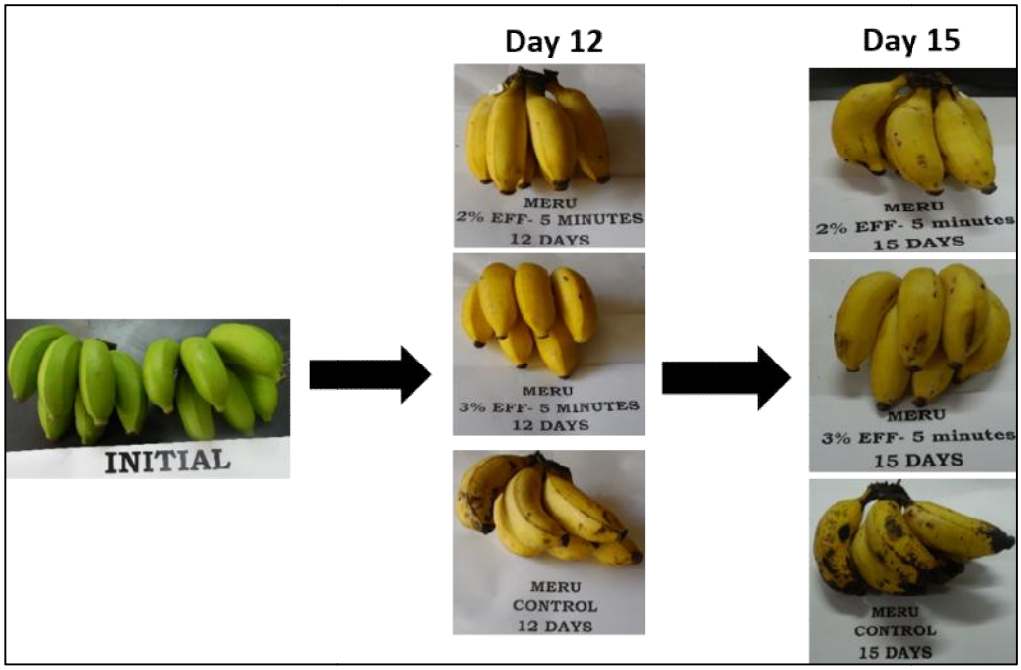

Plate 2. Ripening changes of 'sweet banana dipped in 2% and 3% Hexanal for 5 minutes and controls fruits during post-harvest storage (from initial day to day 15)

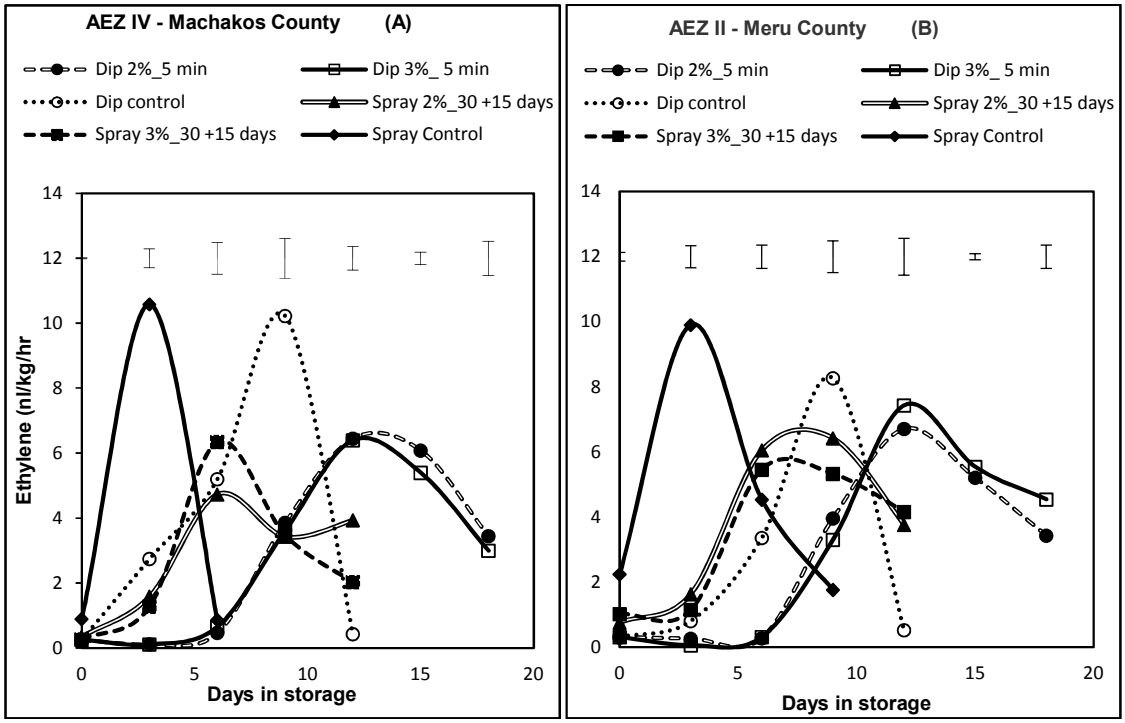

Fig. 1. Effect of pre and post-harvest application of Hexanal on rate of ethylene production in 'sweet banana' fruits from drier AEZ IV (A) and wetter AEZ II (B). Bars indicate least significant difference (LSD) between means at p < 0.05

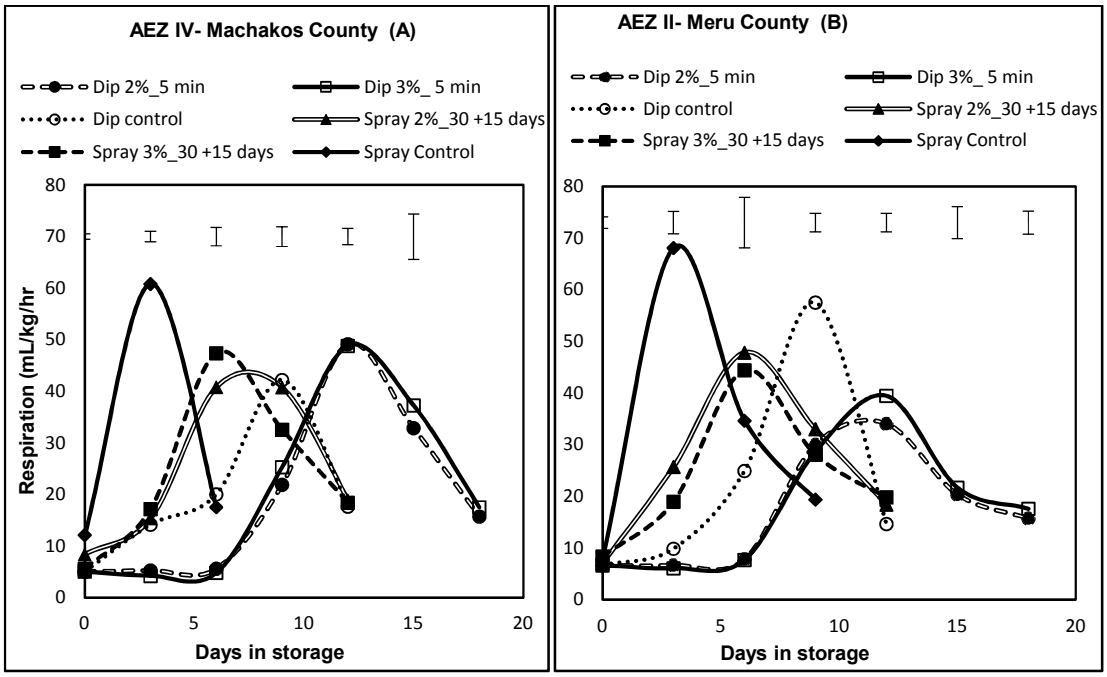

Fig. 2. Effect of pre and post-harvest application of Hexanal on the rate of respiration in 'sweet banana' fruits from drier AEZ IV (A) and wetter AEZ II (B). Bars indicate least significant difference (LSD) between means at p < 0.05

3.5 Total Soluble Solids (TSS, °Brix)

Total soluble solid (TSS) levels were significantly (P = .05) affected by the interaction between zone of production and hexanal treatment. Generally, fruits from the drier AEZ IV (Fig. 4A) had significantly high TSS levels throughout storage compared to those from the colder AEZ II (Fig. 4B). The °brix levels of the untreated fruits from the drier AEZ IV, increased rapidly from an initial value of 1.3 and 9.5^{o} brix to a peak value of 33.81^{o} and 31 ^{o}brix on day 12 and 3 of storage in the post-harvest dip and pre-harvest spray mode of treatments respectively (Fig. 4A). On the other hand, TSS levels increased gradually from initial of 1.1^{o} brix to peak of 29^{o} brix at day 9 of storage in the post-harvest dip mode of application in the wetter AEZ II (Fig. 4B).

Hexanal treatment significantly (P = .05) reduced the rate of TSS increase in both zones and mode of application. However, at the end of storage, the hexanal treated fruits attained almost the same TSS level of approximately 28^{o}- 32^{o} brix compared to the untreated controls.

3.6 Total Titratable Acidity (TTA)

As ripening progressed, total titratable acidity (TTA) increased up to a peak level then gradually decreased till the end of storage (Fig. 5A & B). A significant (P = .05) interaction was observed between zone of production and hexanal treatment with fruits from the colder AEZ II (Fig. 5B) having high TTA levels throughout the storage period compared to those from the drier AEZ IV (Fig. 5A). Hexanal treatment significantly (P = .05) slowed the rate of TTA increase in both zones of production, irrespective of the mode of application used (Fig. 5A & B).

3.7 Ascorbic Acid Content

The ascorbic acid content decreased gradually during storage in all the fruits except in the hexanal treated fruits pre-harvest spray) from the wetter AEZ II, where an increase was observed up to day 3 of storage (Fig. 6B). The ascorbic acid levels were significantly (P = .05) affected by the interaction between zone of production and hexanal treatment. Generally, fruits from the wetter AEZ II had significantly (P = .05) high ascorbic acid levels (Fig. 6B) compared to those from the drier AEZ IV (Fig. 6A). Hexanal treatment significantly (P = .05) slowed the rate of ascorbic acid reduction with the treated fruits maintaining relatively higher levels throughout the storage period compared to the controls in both AEZ (Fig. 6A & B).

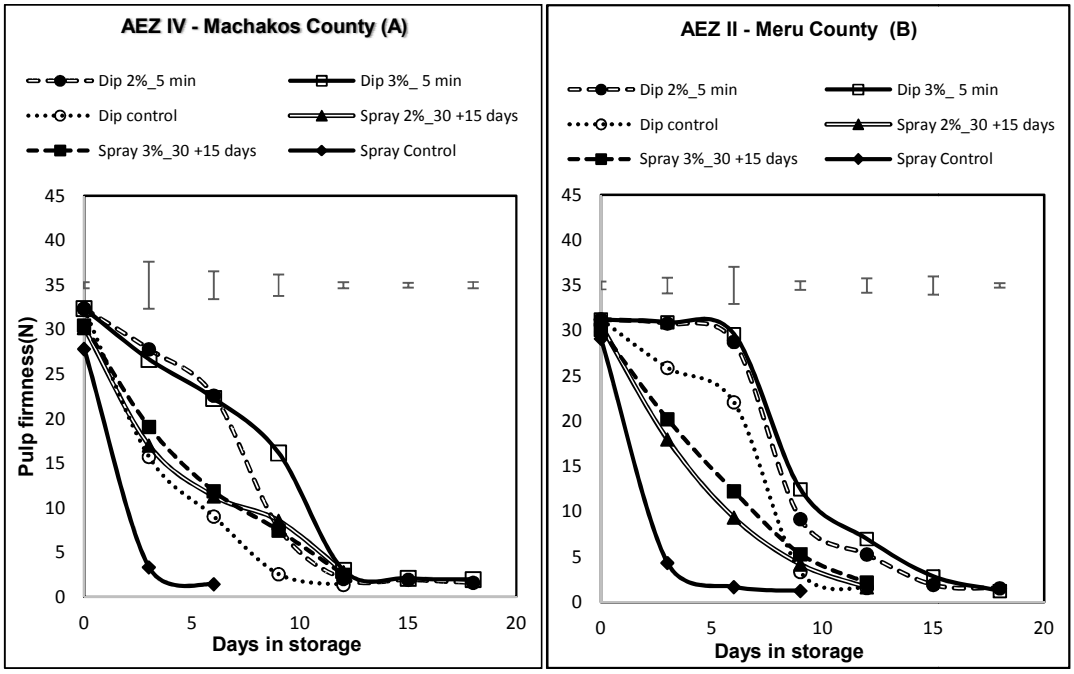

Fig. 3. Effect of pre and post-harvest application Hexanal on pulp firmness in 'sweet banana' fruits from drier AEZ IV (A) and wetter AEZ II (B). Bars indicate least significant difference (LSD) between means at p < 0.05

Ascorbic acid levels decreased rapidly in the control fruits from an initial of 14.5 mg/100 g and 11.7 mg/100 g to an average of 8.8 mg/100 g and 9.2 mg/100 g in the drier AEZ IV and wetter AEZ II respectively, by the end of storage (day 9) in the pre-harvest spray mode of application (Fig. 6A & B). Contrasting results were observed in the hexanal treated fruits where 2% concentration was more effective in the drier AEZ IV fruits where else in wetter AEZ II, 3% concentration was more effective. In the post-harvest dip experiment, the ascorbic acid levels decreased from initial values of 15.9 mg/100 g and 13 mg/100 g to 7.4 mg/100 g and 7.3 - 9.6 mg/100 g in the treated fruits at the end of storage (day 18), 6 days later compared to the controls in AEZ IV and AEZ II, respectively.

3.8 Simple Sugars (Sucrose, Glucose and Fructose)

Sucrose, glucose and fructose gradually increased with ripening in all the fruits regardless of production zone and hexanal treatment (Tables 1 and 2). Sucrose was the most abundant sugar in banana fruits compared to glucose and fructose irrespective of zone of production and hexanal treatment. A significant interaction (P = .05) was observed between hexanal treatment and zone of production in both glucose and fructose (Tables 1 and 2) with the drier AEZ IV fruits compared to ones from the wetter AEZ II. The increase in glucose, fructose and sucrose content was significantly (P = .05) affected by hexanal treatment, were the increase was lower in the treated fruits compared to the controls throughout the storage period. However, no significant differences were observed between 2% and 3% hexanal concentrations evaluated.

3.9 Sensory Quality Evaluation

Generally, there was no significant (P = .05) differences observed in all the quality attributes scores in both zones between the hexanal treated and control fruits (Fig. 7A & B). The treated and control fruits from both AEZ scored almost the same scores for peel color, texture in AEZ IV fruits (Fig. 7A) and aroma in AEZ II (Fig. 7B). On the other hand, hexanal treated fruits scored slightly high for taste/flavour in both AEZs (Fig. 7A & B) while general acceptability and aroma scored highest in AEZ IV (Fig. 7A) fruits though this was not significantly different.

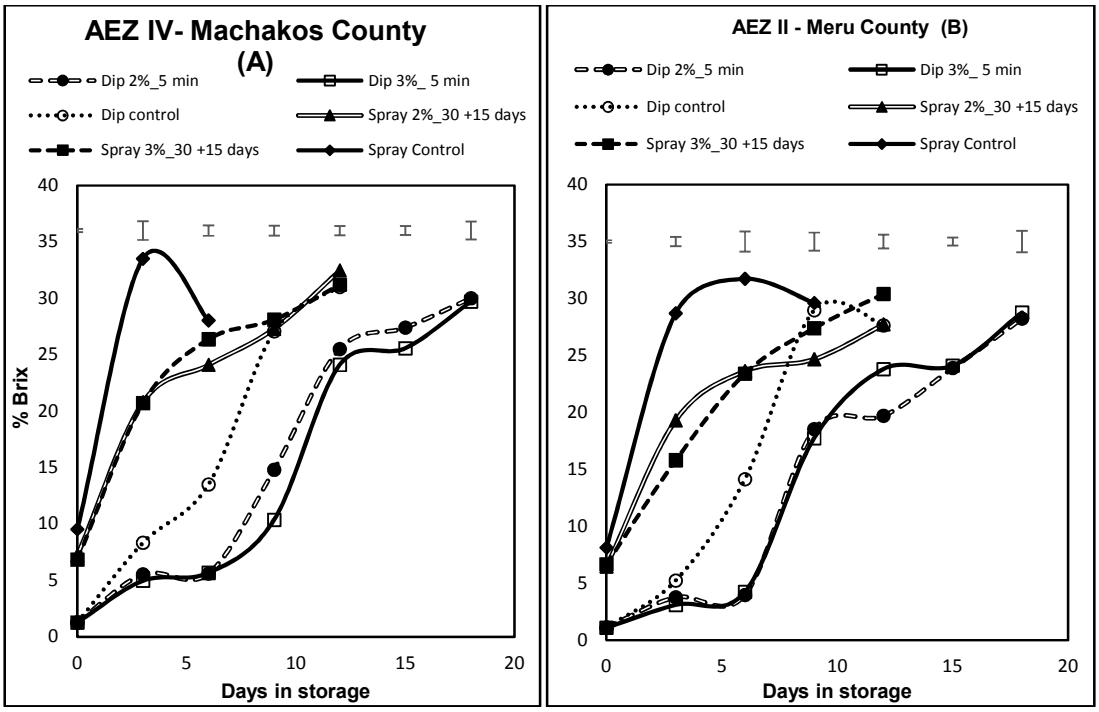

Fig. 4. Effect of pre and post-harvest application of Hexanal on Total Soluble Solids (TSS) in 'sweet banana' fruits from drier AEZ IV (A) and wetter AEZ II (B). Bars indicate least significant difference (LSD) between means at p < 0.05

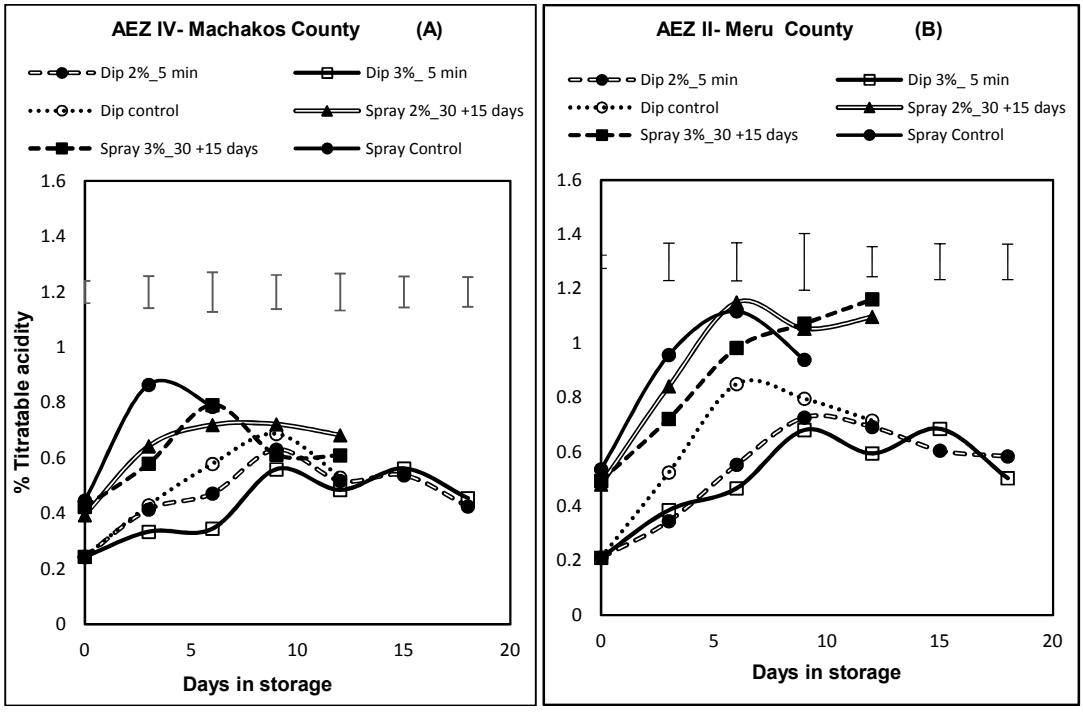

Fig. 5. Effect of pre and post-harvest application of Hexanal on Total Titratable Acidity (TTA) in 'sweet banana' fruits from drier AEZ IV (A) and wetter AEZ II (B). Bars indicate least significant difference (LSD) between means at p < 0.05

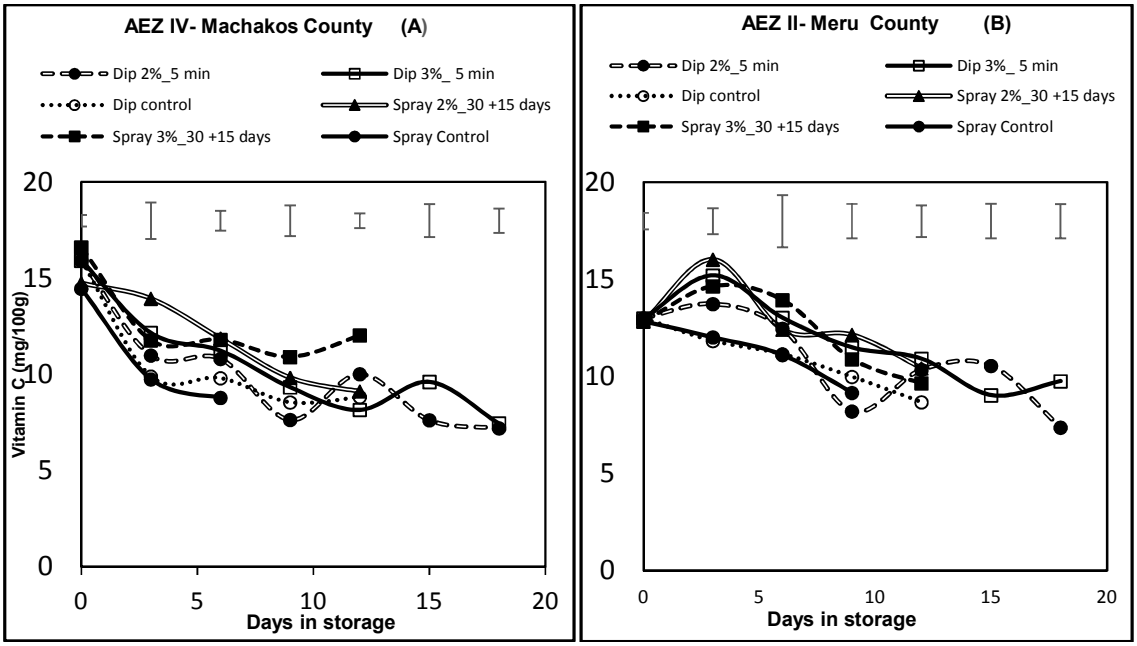

Fig. 6. Effect of pre and post-harvest application of Hexanal on ascorbic acid content in 'sweet banana' fruits from drier AEZ IV (A) and wetter AEZ II (B). Bars indicate least significant difference (LSD) between means at p < 0.05

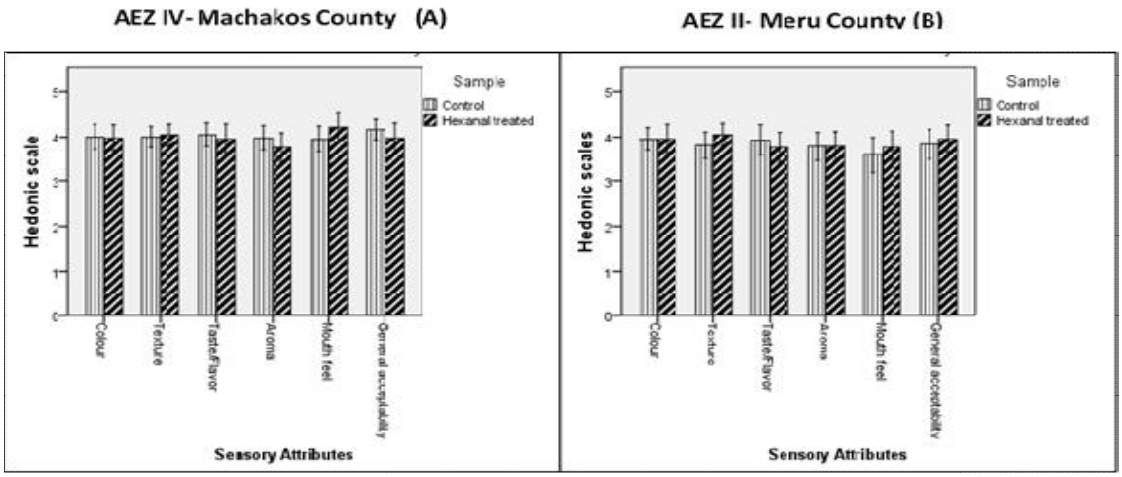

Fig. 7. Hedonic scores for sensory quality attributes of 'sweet banana' variety harvested from Machakos and Meru Counties and treated with Hexanal or left untreated to act as the control. The values on Y-axis represent scores on a 5-point hedonic scale (1 = dislike (worst), 2 = (dislike moderately), 3 = (neither like nor dislike) 4 = (like moderately) and 5= (Like extremely/best)). The vertical bars represent means ± SE

Table 1. Effect of post-harvest dip application of hexanal on Fructose, Glucose and sucrose content (mg/100 g) of 'sweet banana' fruits from AEZ II and AEZ IV of Kenya

Days	Fructose			Glucose			Sucrose		
Zone IV	**Control**	**2%**	**3%**	**Control**	**2%**	**3%**	**Control**	**2%**	**3%**
0	4.8^{d}	4.8^{d}	4.8^{d}	5.4^{d}	5.4^{e}	5.4^{f}	6.4^{d}	6.4^{e}	6.4^{e}
3	5.5^{d}	2.9^{d}	2.2^{d}	6.9^{d}	3.6^{e}	3.1^{f}	32.4^{b}	5.2^{e}	6.5^{e}
6	18.4^{c}	4.5^{d}	5.1^{d}	15.2^{c}	7.1^{e}	6.7^{f}	45.7^{b}	19.4^{d}	28.7^{d}
9	41.6^{b}	6.9^{d}	7.6^{d}	29.5^{b}	18.6^{d}	19.6^{e}	75.8^{a}	44.7^{c}	42.7^{cd}
12	57.3^{a}	24.9^{c}	31.9^{bc}	51.1^{a}	33.2^{c}	39.2^{c}	69.2^{a}	64.9^{ab}	61.6^{ab}
15		37.8^{ab}	37.3^{b}		42.4^{b}	44.5^{bc}		76.7^{a}	64.7^{ab}
18		41.9^{a}	45.1^{a}		45.4^{a}	43.5^{bc}		64.2^{ab}	60.2^{ab}
Mean	25.5	17.7	19.1	21.6	22.2	23.1	45.9	40.2	38.7
Zone II									
0	4.4^{d}	4.4^{d}	4.4^{d}	4.1^{d}	4.1^{e}	4.1^{f}	3.8^{d}	3.8^{e}	3.8^{e}
3	4.5^{d}	3.0^{d}	3.6^{d}	7.5^{d}	5.0^{e}	5.6^{f}	27.5^{c}	3.4^{e}	5.8^{e}
6	15.7^{c}	6.6^{d}	3.7^{d}	29.1^{b}	5.9^{e}	3.7^{f}	$41.1b^{c}$	12.0^{de}	7.6^{e}
9	41.4^{b}	18.9^{c}	28.0^{c}	28.5^{b}	23.3^{d}	32.4^{d}	63.9^{a}	31.5^{c}	37.6^{d}
12	46.2^{b}	32.3^{b}	36.1^{b}	44.8^{a}	34.0^{c}	49.3^{ab}	61.9^{a}	52.9^{b}	72.9^{a}
15		31.8^{b}	42.4^{a}		34.1^{c}	53.3^{a}		62.2^{b}	60.9^{ab}
18		36.6^{ab}	33.7^{b}		51.7^{a}	32.2^{d}		59.1^{b}	54.4^{bc}
Mean	22.4	19.1	21.7	22.8	22.6	25.8	39.7	32.1	34.7
LSD*	7.6			6.3			14.1		

Values within each column followed by the same letter do not differ significantly at (p<0.05) between the treatments and zone of production across the storage period

4. DISCUSSION

Application of appropriate post-harvest technologies in banana fruits is of paramount importance in order to minimize losses after harvest and maintain the best possible quality. Over the past decades, different post-harvest technologies have been developed and tested in various fruits [3,4]. However, the adoption rate of most of these technologies depends on its appropriateness, cost, versatility and value of the commodity. Moreover, most of the consumers and other actors in the value chain in the recent past have high affinity for naturally-occurring

post-harvest preservative compounds which are environmentally friendly, pose no health hazard and are easy to use. Therefore, there is need to test the suitability of biological compounds such as hexanal to enhance banana shelf life while preserving its quality. The objective of this study was to evaluate the efficacy of hexanal, a naturally-occurring compound in enhancing shelf-life and quality of 'sweet banana' fruits in Kenya when applied as a pre-harvest spray or post-harvest dip.

Overall, zone of production had a significant effect on fruits shelf-life and quality. Fruits from the drier AEZ IV, ripened faster and had high content of ^{0}brix and simple sugars as compared to those from the wetter AEZ II. This could be as a result of differences on the prevailing environmental conditions such as temperatures and light as well as cultural practices which have all been reported to impact on the physiology and post-harvest quality of fruits [15]. Hexanal treatment significantly extended shelf-life by 6 days in the post-harvest dip mode of application in both zones compared to the controls. On the other hand, fruits sprayed with hexanal had a shelf life of 6 and 3 days in the drier AEZ IV and wetter AEZ II fruits respectively compared to the controls irrespective of the concentration used. This observed increase in shelf life is very significant especially to small scale farmers who will benefit by gaining an extra time to source for better market and minimize exploitation by middlemen along the value chain. Banana fruit especially the 'sweet banana' variety when ripe goes from marketable to unmarketable state rapidly, leading to huge post-harvest losses. The observed extended shelf-life of up to 6 days in this study could be as a result of the observed lower rates of ethylene production and respiration in the hexanal treated fruits. Physiologically, an increase in respiration rate leads to a quick utilization of substrates, such as free sugars that contributes to post-harvest losses as previously reported by [16]. Similar findings of extended shelf-life have been reported in other banana varieties such as 'Grand naine' [6,7] and in other fruits including mangoes [17], papaya [18], Lime [19] and tomatoes [20]. The observed reduced rate of ethylene evolution in the treated fruits may be as a result of hexanal being a weak inhibitor of ethylene as previously reported by Tiwari and Paliyath, [21]. A study at molecular level in tomato fruit by Tiwari and Paliyath, [21], showed that hexanal treatment in tomato fruit caused moderate down regulation of 1-aminocyclopropane-1-carboxylate synthase 6 (ACS6) and 1-aminocyclopropane-1-carboxylate synthase (ACS) genes. The expression of ACS6 and ACS genes are responsible for the biosynthesis of 1-Aminocyclopropane-1-carboxylic acid (ACC) synthase enzyme, which converts the S-Adenosyl-L-methionine (SAM) to ACC in the ethylene biosynthesis pathway. Hexanal inhibition of ACS genes will lead to a reduction in the evolution of ethylene, and this may explain the low levels of ethylene production observed in this study.

Table 2. Effect of pre-harvest spray application of hexanal on Fructose, Glucose and sucrose content (mg/100 g) of 'sweet banana' fruits from AEZ II and AEZ IV of Kenya

Days	Fructose			Glucose			Sucrose		
Zone IV	**Control**	**2%**	**3%**	**Control**	**2%**	**3%**	**Control**	**2%**	**3%**
0	16.6^{d}	6.6^{d}	4.2^{e}	14.7^{d}	4.7^{e}	4.3^{c}	4.0^{c}	1.9^{d}	2.6^{d}
3	50.8^{a}	9.0^{d}	6.3^{e}	55.7^{a}	15.6^{c}	11.3^{c}	46.4^{ab}	17.8^{c}	19.7^{c}
6	40.1^{b}	29.8^{b}	24.2^{c}	44.0^{b}	22.8^{c}	17.1b^{c}	35.0^{b}	36.1^{a}	32.5^{b}
9		41.1^{a}	37.8^{ab}		38.4^{a}	34.3^{a}		41.5^{ab}	34.3^{b}
12		27.3^{b}	43.1^{a}		42.5^{a}	36.4^{a}		44.6^{a}	46.0^{ab}
Mean	35.8	22.8	23.1	38.1	24.8	20.7	28.5	28.4	27.0
Zone II									
0	4.3^{e}	2.8^{d}	1.4^{e}	10.2^{d}	3.3^{e}	3.1^{d}	6.1^{c}	3.5d^{c}	5.1^{d}
3	25.3^{c}	20.6^{c}	14.8^{d}	34.3^{c}	14.6^{d}	7.8^{c}	37.2^{b}	15.7^{c}	11.5^{cd}
6	46.1^{ab}	16.2^{c}	18.0^{cd}	41.2^{b}	27.6^{bc}	23.2^{b}	49.9^{a}	47.6^{a}	36.9^{ab}
9	30.7^{c}	27.5^{b}	33.3^{b}	33.0^{c}	31.2^{b}	29.2^{a}	35.6^{b}	37.2^{ab}	50.6^{a}
12		30.7^{b}	35.3^{b}		43.3^{a}	35.1^{a}		30.5^{b}	38.3^{ab}
Mean	26.60	19.6	20.6	29.7	24.0	19.7	32.2	26.9	28.5
LSD*	6.5			7.9			13.96		

Values within each column followed by the same letter do not differ significantly at (p<0.05) between the treatments and zone of production across the storage period

Excessive softening is one of the main factors limiting fruit shelf life, transportability and storage in banana fruit resulting to high levels of post-harvest losses. In the present study, the rate of fruit softening was greatly delayed in the hexanal treated fruits compared to the controls throughout the storage period. Softening in banana fruits is majorly as a result of textural changes due to disassembly of the primary cell wall by various hydrolases such as pectin methyesterase, polygalacturonase and pectate lyase among others [22]. However, other mechanism may also be active in determining the overall textural characteristics of banana fruit such as loss of turgor and breakdown of starch to sugar [23]. The observed delayed softening in the treated fruits might be as a result of hexanal reducing the activity of the various enzymes involved in cell wall degradation and modification. A study in tomato [21], showed that hexanal treatment down regulates the expression of genes involved in pectin and hemicellulose degradation which are the major components of the plant cell wall. Additionally, the delay in fruit softening may also be as result of the observed low rate of ethylene production and respiration in the hexanal treated fruits. Ethylene, being a ripening hormone has a strong participation in modulating enzymes involved in fruit softening [24]. Degradation of starch during respiration in fruits such as banana results into pronounced texrtural changes. Similar results have been reported in banana fruits by Venkatachalam et al. [6] in India. Zone of production had a significant effect on fruit firmness with fruits from the drier AEZ IV (Machakos County), softening faster compared to those from the wet AEZ II (Meru County), irrespective of the treatment. This could be attributed to differences in temperatures and rainfall in the different zones; both having been reported to affect fruit softening [15].

Total soluble solids (TSS) increased gradually with ripening in all the fruits irrespective of zone of production and hexanal treatment. The observed increase in TSS during ripening may be associated with the breakdown of stored carbohydrates into simple sugars [23]. Fruits from the drier zone IV had higher TSS levels compared to those from the wetter zone II. This could be attributed to high temperatures and longer periods of exposure to sunlight characteristic of AEZ IV which led to increased accumulation of dry matter content. Similar results have been reported in papaya [18] and mangoes [25]. In general, the rate of TSS increase was significantly low in the Hexanal treated fruits throughout the storage duration and could be attributed to the observed low rate of respiration and ripening process. Low rate of respiration leads to a decrease in metabolic activity and slow conversion of starch to sugars, a possible explanation of delayed increase in TSS content in the hexanal treated fruits. Our results concur with those of Anusuya et al. [17], who reported similar results in mango fruits. Changes in simple sugars such as sucrose, glucose and fructose followed a similar trend to the one observed in TSS. In the present study, levels of this individual sugars increased drastically during the ripening process in all the fruits. However, hexanal treatment significantly slowed down the increase rate of glucose and fructose. This might be as a result of the observed delayed ripening and reduced rate of respiration in the hexanal treated fruits. During ripening process, starch, which is the major form of carbohydrates in banana fruit, is usually catabolized into simple sugars, which enters the metabolic pool where they are used as respiratory substrates or further converted to other metabolites. Similar findings have been reported in hexanal treated banana fruits by Venkatachalam et al. [6].

Banana is one of the few fruits whose TTA levels increases with ripening up to a maximum value then decreases in the fully ripe stage as reported by Lechaudel and Joas, [15]. This is as result of increase in malic acid from 1.8 meq/100 g to 6.2 meq/100 g during ripening [23]. In the present study, hexanal treatment delayed the rate of TTA increase as compared to the drastic increase in the control fruits which peaked at day 3 of storage. This could be attributed to the observed reduced rate of ripening in the hexanal treated fruits. Additionally, reduced activities of enzymes such as malate dehydrogenase, which influence the level of malic acid in banana could further explain the delayed rate of TTA increase by hexanal treatment.

Ascorbic acid is an important quality trait in fruits. In the present study, ascorbic acid levels decreased gradually in all the fruits as ripening advanced during storage. The decrease in vitamin C during ripening is partly due to degradation of ascorbic acid through oxidation [26]. The decrease in ascorbic acid was less rapid in the hexanal treated fruits compared to the untreated controls [27-29]. Higher retention of ascorbic acid observed in the hexanal treated fruits may be as a result of reduced enzymatic oxidation by hexanal [30,31].

Various quality attributes such as peel color, firmness, aroma, taste, mouth-feel and general acceptability were evaluated during the sensory evaluation analysis. The sensory evaluation results showed that, hexanal treatment did not have any significant effect on the various quality parameters scored [32,33]. Further, there was no significant difference on the general acceptability of the treated and the control fruits. This indicates that hexanal's effect on shelf life of banana fruit did not have detrimental effects on the various quality parameters [34,35]. These results are in agreement with a study by Siriboon and Banlusilp, [36], who reported that hexanal treatment does not affect the expression of genes involved in quality development pathway of tomato fruit.

5. CONCLUSION

Overall, results of this study indicated that, the use of Hexanal has the potential to increase 'sweet banana' shelf-life by at least 6 days in case of post-harvest dip, 6 and 3 days in pre-harvest sprayed fruits from drier AEZ IV and the wetter AEZ II respectively, without affecting the quality attributes. This results have also showed that hexanal efficacy might be influenced by zone of production and further studies need to be conducted to validate this. However, there was no significant difference between the 2% and 3% hexanal concentrations tested and both concentrations were equally effective. Therefore, this technology shows great promise in enhancing the shelf life while preserving quality attributes of banana fruits. This in turn can reduce the huge post-harvest losses currently being incurred in developing countries such as Kenya.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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