

Effect of Hexanal as a post-harvest treatment to extend the shelf-life of banana fruits

(*Musa acuminata* var. sweet banana) in Kenya

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 $25 \pm 1^\circ\text{C}$ and $\text{RH } 60 \pm 5\%$ to ripen. Hexanal treatment maintained the fruits quality and prolonged the shelf-life by 6 days in the dipped fruits, 6 and 3 days 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 $^\circ\text{Brix}$, 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 [11]. 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% [8]. 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 [11]. However, most of it is consumed locally with only a small percentage of approximately 7.2% being exported [11]. 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 [8]. 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

39 range of vitamins, minerals, anti-oxidants and it is naturally low in fats [21]. However, once ripe the fruits
40 have a short shelf life of approximately 3-4 days and this limits their utilization, postharvest handling and
41 marketing [1].

42 Banana is a climacteric fruit which is often harvested at the physiological maturity stage and then ripened
43 before marketing. During ripening, the fruit undergoes different biochemical and physiological changes that
44 transforms the fruit to edible state. Some of these changes include fruit softening, changes in peel color,
45 degradation of starch to sugars, changes in concentration of aroma volatiles and acids. According to
46 Maduwanthi and Marapana [19], sugar levels increases from of an initial of 2% in green banana to
47 approximately 15% -20% in the ripe fruit making it sweeter. However, once the fruit is fully ripe, it becomes
48 very delicate and if not properly handled high postharvest losses can be incurred. In order to increase
49 storage life of the fruits, appropriate post-harvest technologies aimed at reducing the deterioration rate
50 have been developed over the years. These technologies are used to slow down fruits metabolic processes
51 to deliver enhanced shelf-life and optimal quality without compromising on the consumer safety. Recently,
52 efforts have been made to develop new and biological post-harvest technologies for extension of banana
53 shelf-life while retaining quality [33-35]. Use of hexanal and its formulations is one of the new innovations
54 which have been proved effective in enhancing the post-harvest shelf life of banana fruits [33, 34]. Hexanal,
55 is an aldehyde compound produced naturally by plants as a defence response to different biotic stresses
56 and has an odour similar to that of freshly cut grass or cucumber [18]. The United States Food and Drug
57 Administration Agency has approved the use of hexanal as a GRAS compound [22]. Hexanal use offers a
58 human-safe post-harvest preservation product that is environmentally friend and economically viable.
59 Hexanal is oxidized to
60 hexanoic acid in the body after consumption and further oxidized to carbon dioxide and water during respir
61 ation through the tricarboxylic acid cycle [18]. It has also been noted that hexanal, has antimicrobial
62 properties against several post-harvest pathogens such as *Alternaria alternate* and *Botrytis Cinerea* [29]. A
63 biochemical formulation of an artificially synthesized version of hexanal (Enhanced Freshness Formulation)
64 has been developed which delays fruit ripening [26]. This formulation can be applied in different ways such
65 post-harvest dip, pre-harvest spray or as a vapor. Being a relative new technology, there is need to test its
66 suitability to enhance banana shelf-life while preserving post-harvest quality in Kenya. A previous study in
67 'Grand naine' variety [34], showed promising results of hexanal extending fruits shelf life by nine days
68 without compromising on quality. However, since hexanal's effect is physiological, it is possible that its
69 efficacy might vary between varieties. The objective of this study was therefore, to determine the effect of
70 hexanal treatment on the post-harvest shelf-life and quality of 'sweet banana' fruits, a very popular variety
71 in Kenya.

72

73 2. MATERIALS AND METHODS

74 2.1. Study area

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

79 **2.2. Experimental setup**

80 For the pre-harvest spray mode of application, 15 banana trees at flowering stage in each study site were
81 randomly selected and tagged in the farmer's field. Two concentrations of hexanal (2% and 3%) and a
82 control (clean, plain water) were sprayed twice at 30 and 15 days before harvest. The dosing range used
83 was informed by a previous study done on 'Grand naine' variety [34]. Since hexanal is immiscible with
84 water, Tween 20 and ethanol were added to increase its solubility [22]. **Tween 20, ethanol and hexanal**
85 **were added in the ratio of 10:10:1.** The stock solutions were mixed with water and diluted accordingly to
86 provide the required hexanal concentrations. Using a knapsack sprayer, the fruits were sprayed to the point
87 of dripping with the solution. Spray contamination was avoided by using alternate rows of trees for the
88 experiment and a 4 tree gap between treatments in the same row of trees. The fruits were left on the tree
89 until approximately 20% per bunch had ripened. The fruits were then harvested and only the middle hands
90 were used in the post-harvest analysis.

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

97 **2.3. Sample preparation**

98 In the post-harvest laboratory, the fruits were cleaned, dried, and selected for uniformity and freedom from
99 mechanical injuries. Pre-harvest spray-treated fruits were left to undergo normal ripening under ambient
100 room conditions of $25 \pm 1^\circ\text{C}$ and $\text{RH } 60 \pm 5\%$. Fruits for post-harvest treatment were dipped in one of the
101 two hexanal concentrations (2%, 3%) or plain water (control) for 5 minutes. The hexanal solution was
102 mixed with Tween 20 and ethanol to increase its solubility. The hexanal concentrations and application time
103 used was informed by a previous study done on 'Grand naine' variety [34]. All the fruits were left to undergo
104 normal ripening under ambient room conditions. Five banana hands from each treatment combination were
105 randomly sampled at 3-day intervals to evaluate respiration and ethylene evolution rates. Three fruits were
106 also randomly sampled to evaluate other ripening related parameters including pulp firmness, °Brix,
107 titratable acidity, ascorbic acid, simple sugars and sensory analysis evaluation.

108 **2.3.1. Shelf life**

109 The time taken by the fruits from harvesting to reach the optimal, edible ripe stage was counted and
110 reported in days. This was defined as stage 7 according to the standard banana ripening chart by Soltani *et*
111 *al.* [27].

112 **2.3.2. Analysis of physiological parameters**

113 **Rate of respiration and ethylene production were determined using gas chromatographs models GC-8A**
114 **and GC-9A, Shimadzu Corp., Kyoto, Japan, respectively. The gas chromatograph to determine rate of**
115 **respiration was fitted with a thermal conductivity detector and a Poropak N column while that for ethylene**
116 **determination was fitted with an activated alumina column and a flame ionization detector. Five banana**

117 fingers were randomly sampled from each treatment, numbered and their weights taken using a digital
118 balance, Model Libror AEG-220, Shimadzu Corp. Kyoto, Japan. Each of the five fingers was incubated for
119 two hours in air tight containers fitted with self-sealing rubber septa. Gas samples were taken from the
120 headspace using an airtight 1 mL hypodermic syringe and injected into the respective gas chromatographs.
121 The rate of carbon dioxide production (used to estimate respiration rate) was expressed as mL/Kg/h while
122 ethylene production was expressed as $\mu\text{l/Kg/h}$.

123 **2.3.3. Pulp firmness**

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

129 **2.3.4. Total soluble solids (TSS)**

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

134 **2.3.5. Total titratable acidity (TTA)**

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

140 **2.3.6. Ascorbic acid content (Vitamin C)**

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

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153 2.3.7. Simple sugars

154 Simple sugars were analysed using a high performance liquid chromatography (HPLC) (Model LC-20AS,
155 Shimadzu Corp., Kyoto, Japan) fitted with phenomenex 250mm*4.6mm*5µl Amino NH2P column. Five
156 grams of the banana pulp was macerated and 96% ethanol added. Refluxing was done for one hour at
157 100°C and then cooled under running water. The solution was then filtered using 42mm whatman filter
158 paper. Rising was done using 5ml of 96% ethanol. The solution was rotary evaporated to dryness at 60°C.
159 5ml of 50% acetonitrile was then added and finally micro-filtered (0.45µ). The HPLC was running under the
160 following conditions: oven temperature: 30°C, Flow rate: 1.0 ml/min, Injection volume: 20 µL, Column: NH₂
161 (5.0µl) Mobile phase: Acetonitrile: water (75:25). Sugars present in the solution including sucrose, glucose
162 and fructose were identified and their individual concentration calculated using the standards.

163 2.3.8. Sensory analysis

164 Sensory quality evaluation was performed on the hexanal treated and untreated fruits once they were fully
165 ripe; stage 6, according to the standard banana ripening chart by Soltani *et al.* [27]. The fruits were washed
166 with clean water, dried and diced into approximately equal-sized slices, avoiding the extreme ends. Three
167 slices were placed on white plates which were anonymously coded based on treatment to ensure
168 objectivity. A panel of 36 untrained judges from the faculty of Agriculture student population at the
169 University of Nairobi was guided on the scoring procedure of the various sensory attributes that included:
170 fruit colour, aroma, texture, flavour, mouth feel and the general acceptability. The panellists scored for
171 these attributes on a five point hedonic scale where 1 = dislike (worst), 2 = (dislike moderately), 3 = (neither
172 like nor dislike), 4 = (like moderately) and 5= Like extremely (Best). This was adapted from Galan *et al.* [9],
173 but with few modifications.

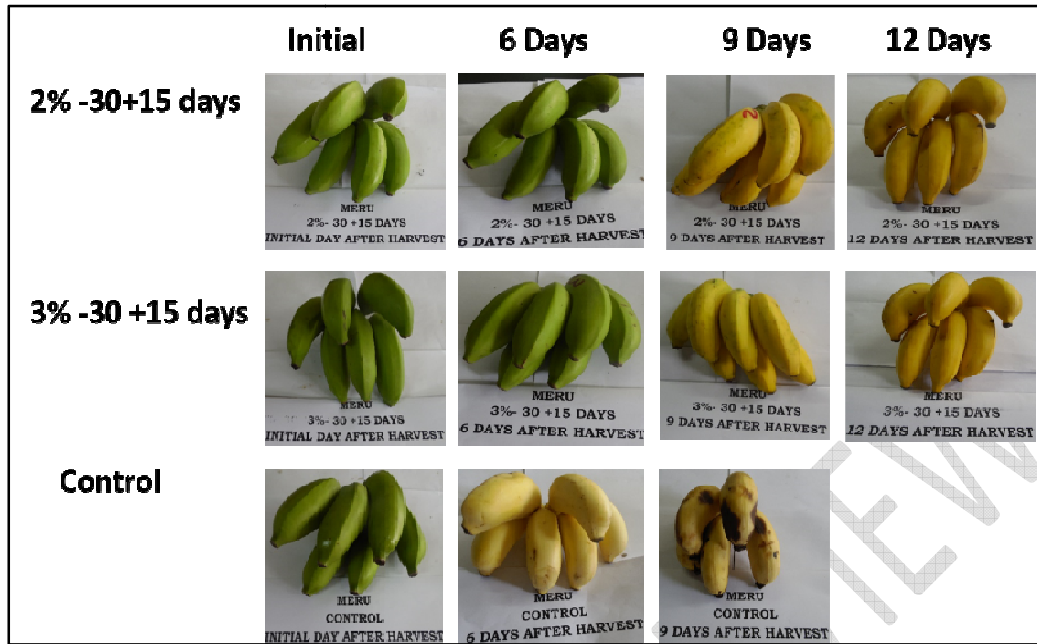
175 2.4 Statistical analysis

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

180 3.0 Results

181 3.1. Shelf life

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

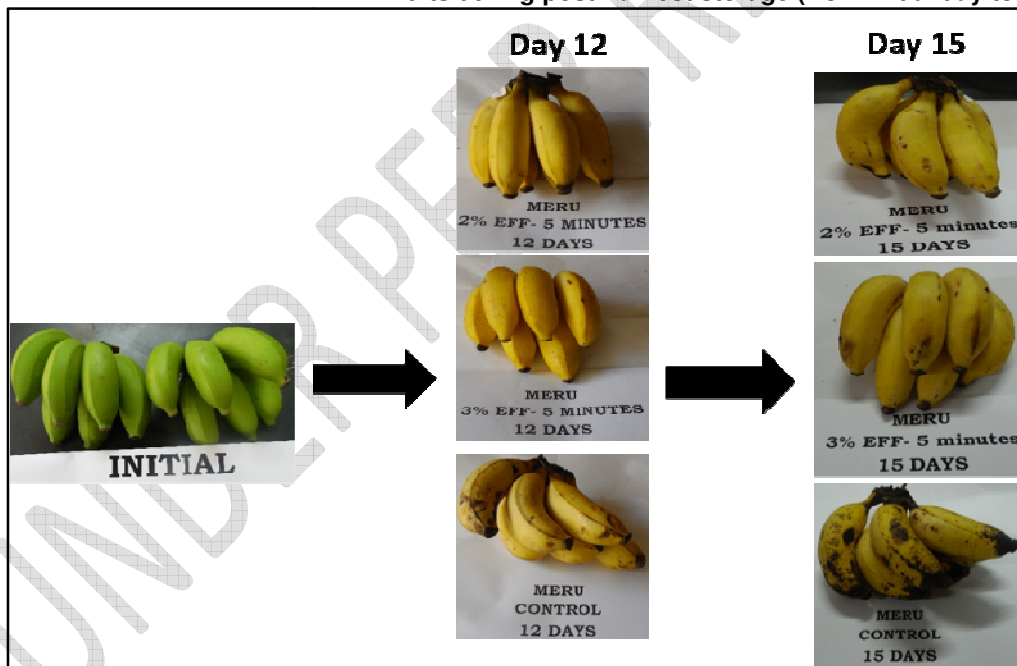


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Plate 1: Ripening changes of 'sweet banana' fruits sprayed with 2% and 3% Hexanal and controls fruits during post-harvest storage (from initial day to day 12)



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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)

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192 3.2 Rate of ethylene production

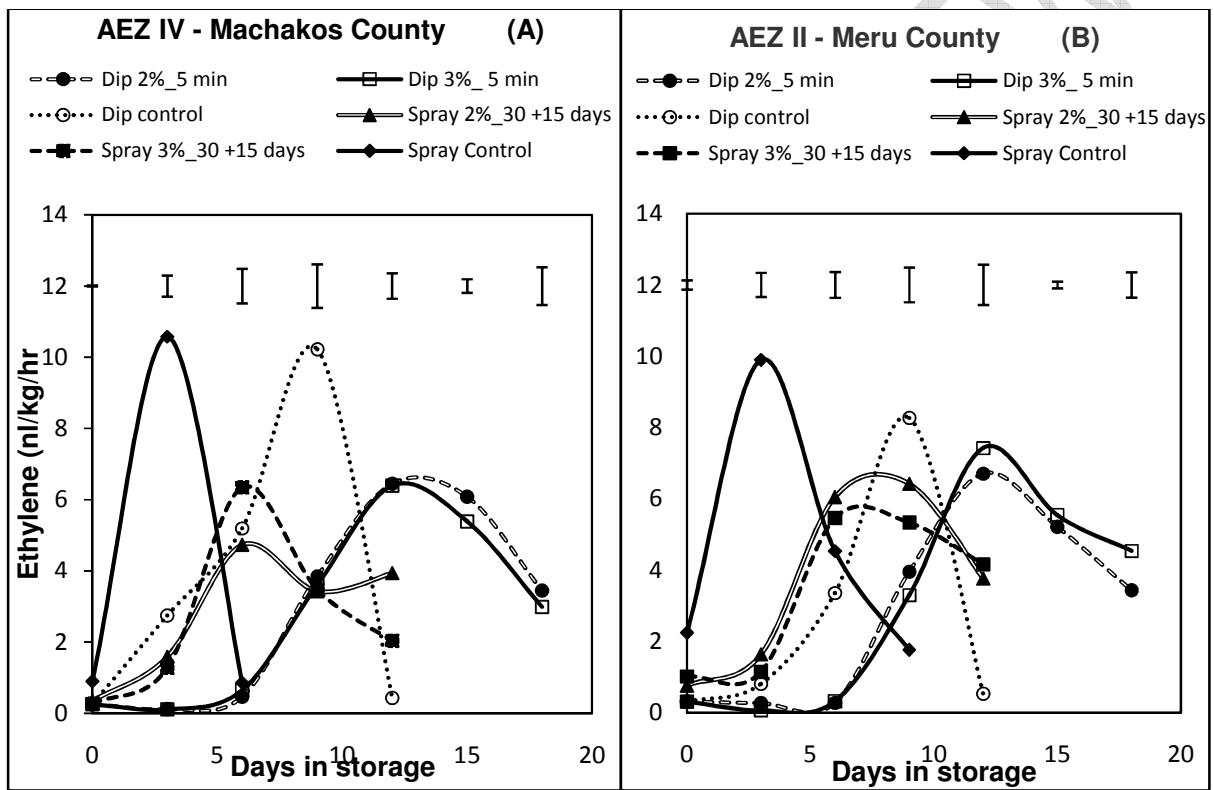
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In the pre-harvest spray mode of application, the control fruits had significantly ($P = .05$) high levels of ethylene production with the climacteric peaks of approximately 10 nL/kg/hr occurring 3 days earlier compared to the hexanal treated fruits (Fig. 1A & B). Hexanal treatment significantly ($P = .05$) reduced the

196 rate of ethylene production in both AEZ and delayed the climacteric peaks by 3 days compared to the
 197 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
 198 treated fruits from the drier AEZ IV (Fig. 1A) and wetter AEZ II fruits (Fig. 1B) occurred at day 6 of storage,
 199 then ethylene levels drastically declining till the end of storage exhibiting a true climacteric pattern.
 200 A significant difference ($P = .05$) was observed between the two modes of hexanal application where post-
 201 harvest mode of application delayed the climacteric peaks by 6 days compared to the pre-harvest spray
 202 (Fig. 1A & B). However, zone of production did not have any significant effect on the rate of ethylene
 203 production.
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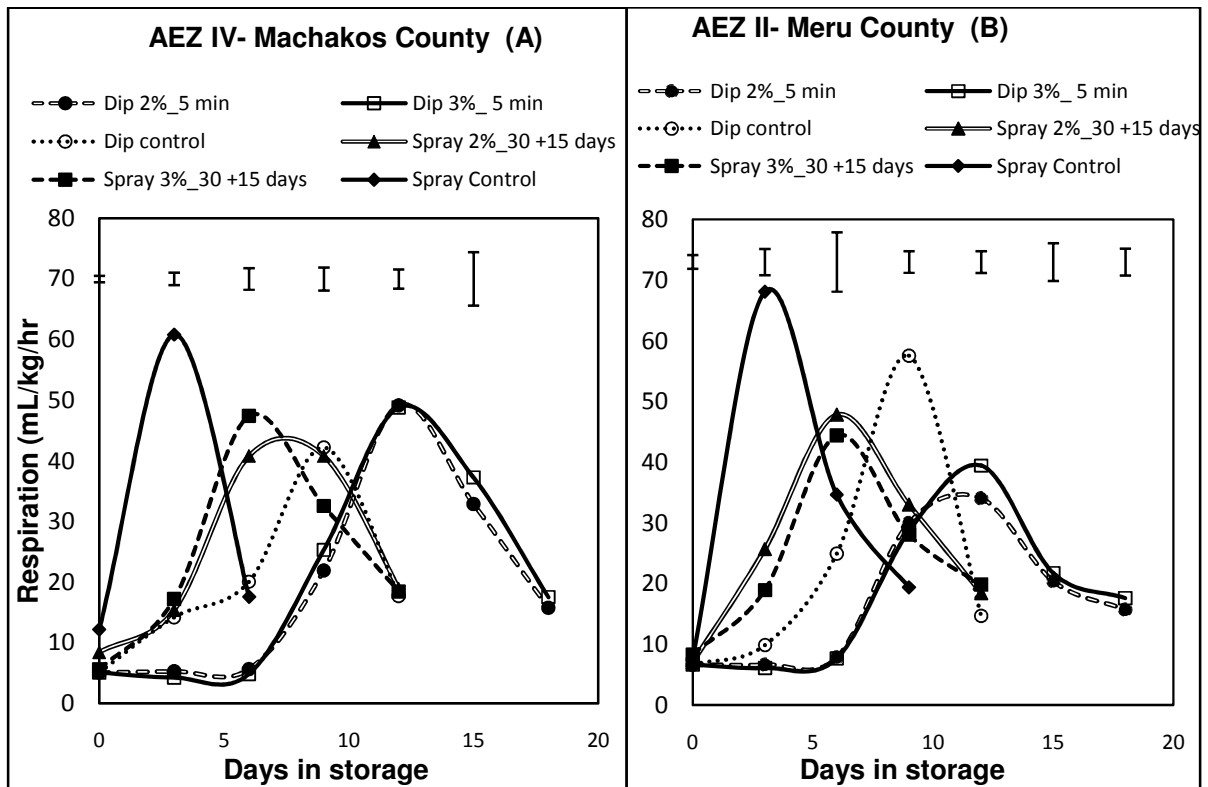


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 207 **Fig. 1. Effect of pre and post-harvest application of Hexanal on rate of ethylene production in 'sweet**
 208 **banana' fruits from drier AEZ IV (A) and wetter AEZ II (B). Bars indicate least significant difference**
 209 **(LSD) between means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

210 3.3 Respiration rate

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

218 mode of application experiment, where the treated fruits had lower levels of respiration compared to the
 219 pre-harvest spray experiment with respiratory peaks of 49 mL/kg/h and 34 -39 mL/kg/h, in drier AEZ IV and
 220 colder AEZ II, respectively, occurring 6 days later.

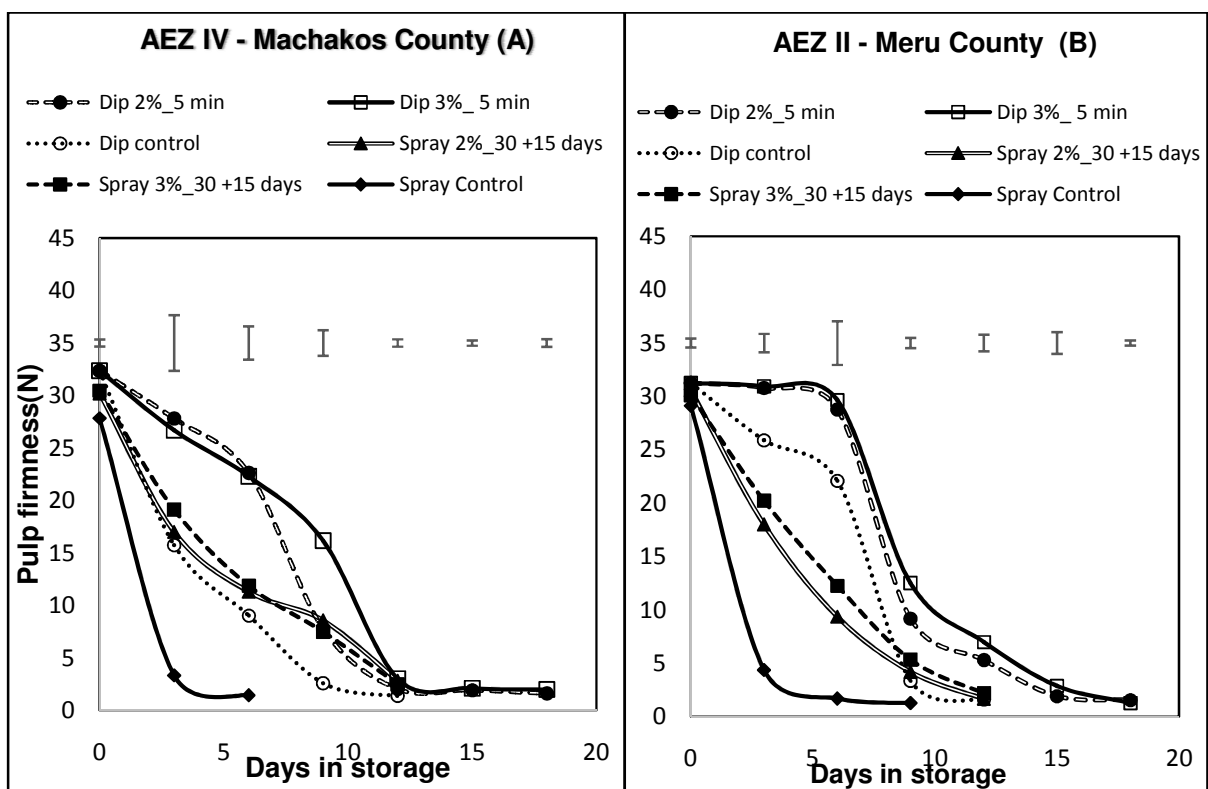


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222 **Fig. 2. Effect of pre and post-harvest application of Hexanal on the rate of respiration in 'sweet**
 223 **banana' fruits from drier AEZ IV (A) and wetter AEZ II (B). Bars indicate least significant difference**
 224 **(LSD) between means at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

225 3.4 Pulp firmness

226 A general reduction in pulp firmness was observed in both the hexanal treated and control fruits as ripening
 227 progressed (Fig. 3A and B). Hexanal treatment applied either as a pre-harvest spray or post-harvest dip
 228 significantly ($P = .05$) delayed pulp softening in both AEZ. Interaction between mode of application and
 229 zone of production had a significant ($P = .05$) effect on the rate of softening with fruits from the drier AEZ IV
 230 (Fig 3A) softening faster compared to those from the colder AEZ II (Fig 3B). The control fruits drastically
 231 lost their pulp firmness by 96% in fruits produced in both AEZ, after 6 and 9 days of storage in the drier
 232 AEZ IV and wetter AEZ II, respectively in the pre-harvest spray mode of application. Similarly, in the post-
 233 harvest dip mode of application, the untreated control fruits had lost approximately 95% of their pulp
 234 firmness after 12 days of storage in both zones. By the 9th day of storage, pre-harvest sprayed fruits had
 235 lost approximately 72% -75% and 82%- 85% of their firmness compared to 50% - 76% and 60% - 71% in
 236 the post-harvest dip treated fruits in the drier AEZ IV and wetter AEZ II, respectively (Fig. 3A and B).



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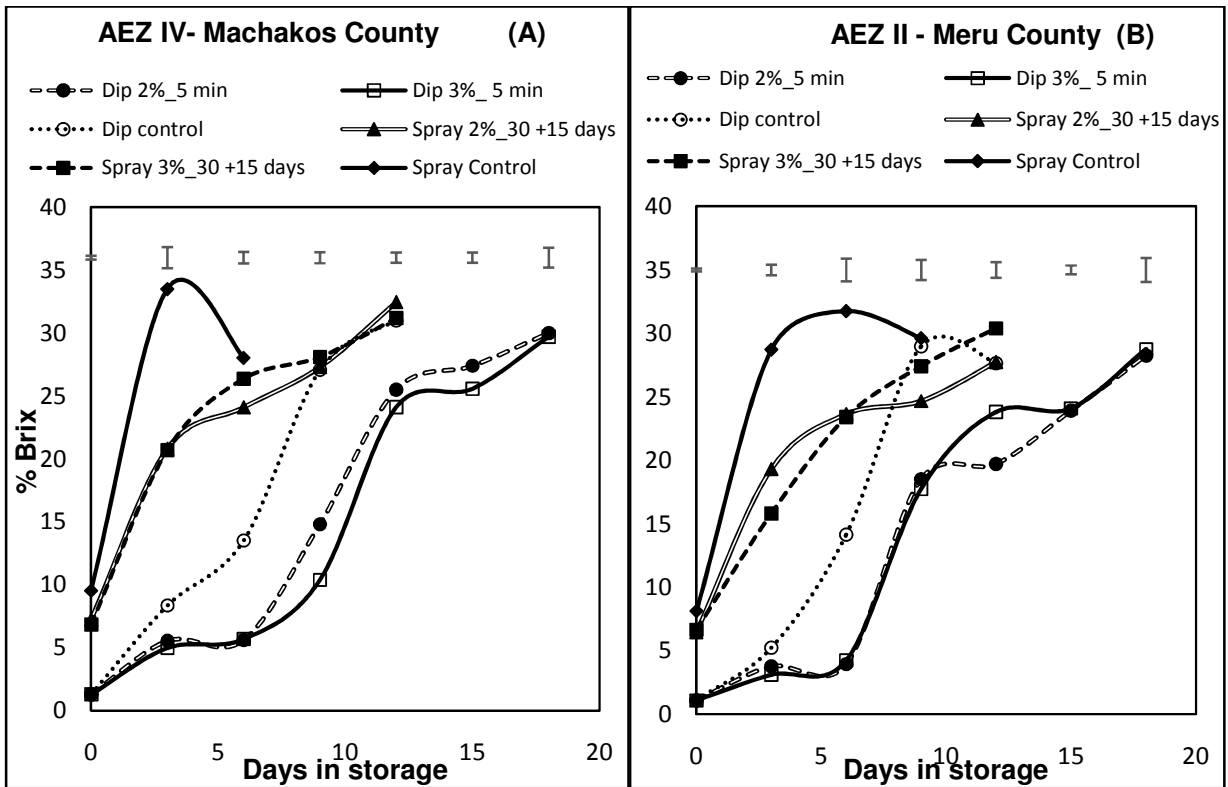
238 **Fig. 3. Effect of pre and post-harvest application Hexanal on pulp firmness in 'sweet banana' fruits**
 239 **from drier AEZ IV (A) and wetter AEZ II (B). Bars indicate least significant difference (LSD) between**
 240 **means at $p < 0.05$.**

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242 3.5 Total soluble solids (TSS, °Brix)

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

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

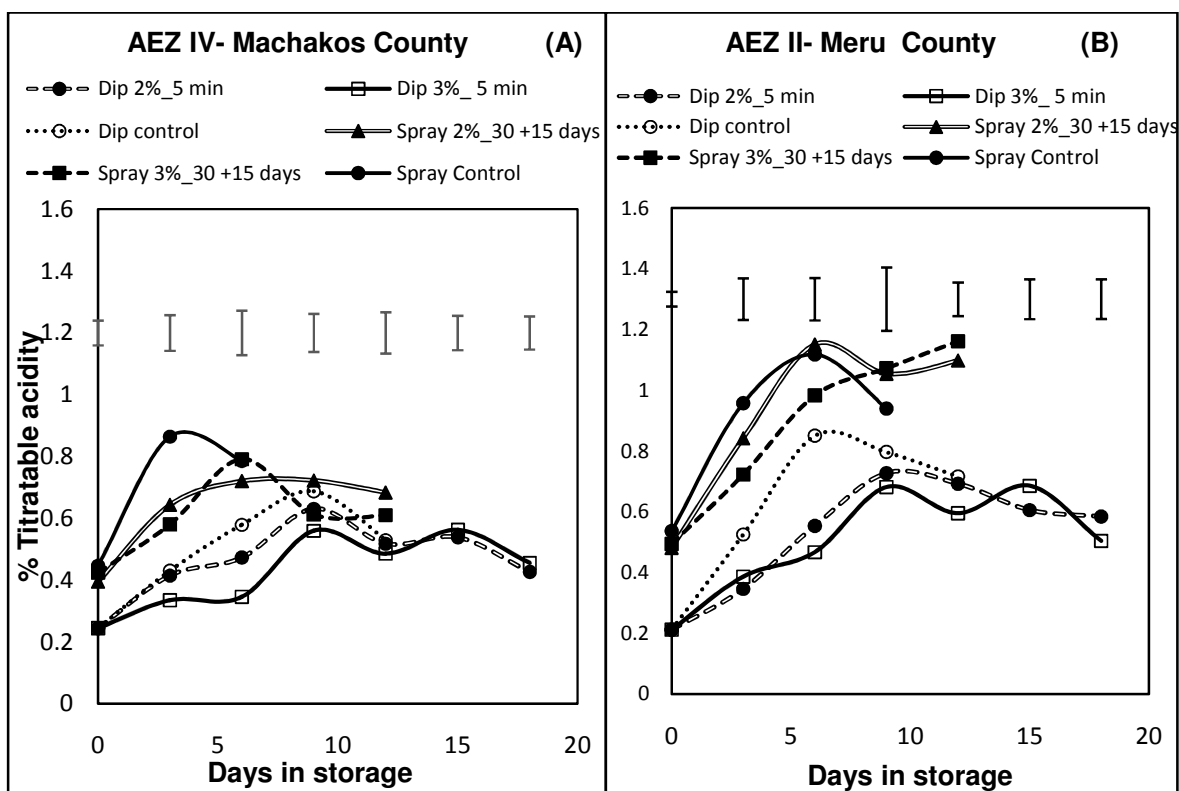


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255 **Fig. 4. Effect of pre and post-harvest application of Hexanal on Total Soluble Solids (TSS) in 'sweet**
 256 **banana' fruits from drier AEZ IV (A) and wetter AEZ II (B). Bars indicate least significant difference**
 257 **(LSD) between means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

258 **3.6 Total Titratable Acidity (TTA)**

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



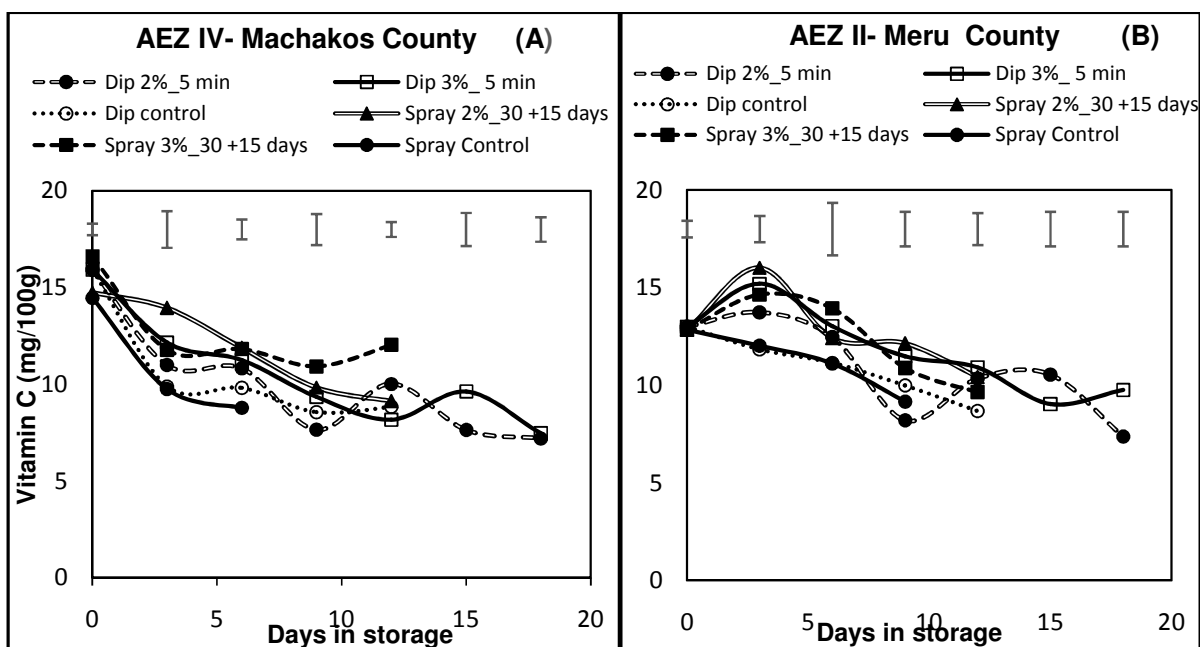
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266 **Fig. 5. Effect of pre and post-harvest application of Hexanal on Total Titratable Acidity (TTA) in**
 267 **'sweet banana' fruits from drier AEZ IV (A) and wetter AEZ II (B). Bars indicate least significant**
 268 **difference (LSD) between means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

269 **3.7 Ascorbic Acid content**

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

277 Ascorbic acid levels decreased rapidly in the control fruits from an initial of 14.5 mg/100g and 11.7 mg/100g
 278 to an average of 8.8 mg/100g and 9.2 mg/100g in the drier AEZ IV and wetter AEZ II respectively, by the
 279 end of storage (day 9) in the pre-harvest spray mode of application (Fig. 6A & B). Contrasting results were
 280 observed in the hexanal treated fruits where 2% concentration was more effective in the drier AEZ IV fruits
 281 where else in wetter AEZ II, 3% concentration was more effective. In the post-harvest dip experiment, the
 282 ascorbic acid levels decreased from initial values of 15.9 mg/100g and 13 mg/100g to 7.4 mg/100g and 7.3
 283 - 9.6 mg/100g in the treated fruits at the end of storage (day 18), 6 days later compared to the controls in
 284 AEZ IV and AEZ II, respectively.



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

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291 **3.8 Simple sugars (Sucrose, glucose and Fructose)**

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

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Table 1. Effect of post-harvest dip application of hexanal on Fructose, Glucose and sucrose content (mg/100g) 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	41.1 ^{bc}	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		

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

314

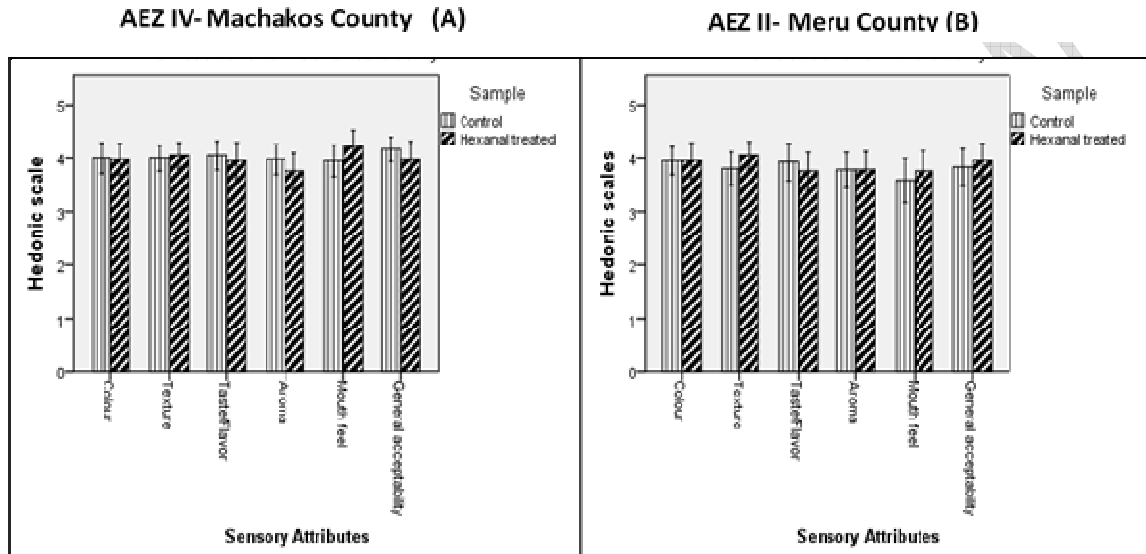
315 **Table 2. Effect of pre-harvest spray application of hexanal on Fructose, Glucose and sucrose**
316 **content (mg/100g) of 'sweet banana' fruits from AEZ II and AEZ IV of Kenya**

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	17.1 ^{bc}	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	3.5 ^d	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		

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

319 3.9 Sensory Quality Evaluation

320 Generally, there was no significant ($P = .05$) differences observed in all the quality attributes scores in both
321 zones between the hexanal treated and control fruits (Fig. 7A & B). The treated and control fruits from both
322 AEZ scored almost the same scores for peel color, texture in AEZ IV fruits (Fig. 7A) and aroma in AEZ II
323 (Fig. 7B). On the other hand, hexanal treated fruits scored slightly high for taste/flavour in both AEZs (Fig.
324 7A & B) while general acceptability and aroma scored highest in AEZ IV (Fig. 7A) fruits though this was not
325 significantly different.



326

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

332 4.0. DISCUSSION

333 Application of appropriate post-harvest technologies in banana fruits is of paramount importance in order to
334 minimize losses after harvest and maintain the best possible quality. Over the past decades, different post-
335 harvest technologies have been developed and tested in various fruits [1, 21]. However, the adoption rate
336 of most of these technologies depends on its appropriateness, cost, versatility and value of the commodity.
337 Moreover, most of the consumers and other actors in the value chain in the recent past have high affinity
338 for naturally-occurring post-harvest preservative compounds which are environmentally friendly, pose no
339 health hazard and are easy to use. Therefore, there is need to test the suitability of biological compounds
340 such as hexanal to enhance banana shelf life while preserving its quality. The objective of this study was to
341 evaluate the efficacy of hexanal, a naturally-occurring compound in enhancing shelf-life and quality of
342 'sweet banana' fruits in Kenya when applied as a pre-harvest spray or post-harvest dip.

343 Overall, zone of production had a significant effect on fruits shelf-life and quality. Fruits from the drier AEZ
344 IV, ripened faster and had high content of $^{\circ}$ brix and simple sugars as compared to those from the wetter
345 AEZ II. This could be as a result of differences on the prevailing environmental conditions such as

346 temperatures and light as well as cultural practices which have all been reported to impact on the
347 physiology and post-harvest quality of fruits [13]. Hexanal treatment significantly extended shelf-life by 6
348 days in the post-harvest dip mode of application in both zones compared to the controls. On the other
349 hand, fruits sprayed with hexanal had a shelf life of 6 and 3 days in the drier AEZ IV and wetter AEZ II fruits
350 respectively compared to the controls irrespective of the concentration used. This observed increase in
351 shelf life is very significant especially to small scale farmers who will benefit by gaining an extra time to
352 source for better market and minimize exploitation by middlemen along the value chain. Banana fruit
353 especially the 'sweet banana' variety when ripe goes from marketable to unmarketable state rapidly,
354 leading to huge post-harvest losses. The observed extended shelf-life of up to 6 days in this study could be
355 as a result of the observed lower rates of ethylene production and respiration in the hexanal treated fruits.
356 Physiologically, an increase in respiration rate leads to a quick utilization of substrates, such as free sugars
357 that contributes to post-harvest losses as previously reported by [25]. Similar findings of extended shelf-life
358 have been reported in other banana varieties such as 'Grand naine' [33], [34] and in other fruits including
359 mangoes [2], papaya [12], Lime [6] and tomatoes [5]. The observed reduced rate of ethylene evolution in
360 the treated fruits may be as a result of hexanal being a weak inhibitor of ethylene as previously reported by
361 **Tiwari and Paliyath**, [31]. A study at molecular level in tomato fruit by **Tiwari and Paliyath**, [31], showed that
362 hexanal treatment in tomato fruit caused moderate down regulation of 1-aminocyclopropane-1-carboxylate
363 synthase 6 (ACS6) and 1-aminocyclopropane-1-carboxylate synthase (ACS) genes. The expression of
364 ACS6 and ACS genes are responsible for the biosynthesis of 1-Aminocyclopropane-1-carboxylic acid
365 (ACC) synthase enzyme, which converts the S-Adenosyl-L-methionine (SAM) to ACC in the ethylene
366 biosynthesis pathway. Hexanal inhibition of ACS genes will lead to a reduction in the evolution of ethylene,
367 and this may explain the low levels of ethylene production observed in this study.

368 Excessive softening is one of the main factors limiting fruit shelf life, transportability and storage in banana
369 fruit resulting to high levels of post-harvest losses. In the present study, the rate of fruit softening was
370 greatly delayed in the hexanal treated fruits compared to the controls throughout the storage period.
371 Softening in banana fruits is majorly as a result of textural changes due to disassembly of the primary cell
372 wall by various hydrolases such as pectin methylesterase, polygalacturonase and pectate lyase among
373 others [32]. However, other mechanism may also be active in determining the overall textural
374 characteristics of banana fruit such as loss of turgor and breakdown of starch to sugar [14]. The observed
375 delayed softening in the treated fruits might be as a result of hexanal reducing the activity of the various
376 enzymes involved in cell wall degradation and modification. A study in tomato [31], showed that hexanal
377 treatment down regulates the expression of genes involved in pectin and hemicellulose degradation which
378 are the major components of the plant cell wall. Additionally, the delay in fruit softening may also be as
379 result of the observed low rate of ethylene production and respiration in the hexanal treated fruits.

380 Ethylene, being a ripening hormone has a strong participation in modulating enzymes involved in fruit
381 softening [16]. Degradation of starch during respiration in fruits such as banana results into pronounced
382 textural changes. Similar results have been reported in banana fruits by **Venkatachalam et al.** [33] in India.
383 Zone of production had a significant effect on fruit firmness with fruits from the drier AEZ IV (Machakos
384 County), softening faster compared to those from the wet AEZ II (Meru County), irrespective of the

385 treatment. This could be attributed to differences in temperatures and rainfall in the different zones; both
386 having been reported to affect fruit softening [13].

387 Total soluble solids (TSS) increased gradually with ripening in all the fruits irrespective of zone of
388 production and hexanal treatment. The observed increase in TSS during ripening may be associated with
389 the breakdown of stored carbohydrates into simple sugars [14]. Fruits from the drier zone IV had higher
390 TSS levels compared to those from the wetter zone II. This could be attributed to high temperatures and
391 longer periods of exposure to sunlight characteristic of AEZ IV which led to increased accumulation of dry
392 matter content. Similar results have been reported in papaya [12] and mangoes [20]. In general, the rate of
393 TSS increase was significantly low in the Hexanal treated fruits throughout the storage duration and could
394 be attributed to the observed low rate of respiration and ripening process. Low rate of respiration leads to a
395 decrease in metabolic activity and slow conversion of starch to sugars, a possible explanation of delayed
396 increase in TSS content in the hexanal treated fruits. Our results concur with those of [Anusuya et al.](#) [2],
397 who reported similar results in mango fruits. Changes in simple sugars such as sucrose, glucose and
398 fructose followed a similar trend to the one observed in TSS. In the present study, levels of this individual
399 sugars increased drastically during the ripening process in all the fruits. However, hexanal treatment
400 significantly slowed down the increase rate of glucose and fructose. This might be as a result of the
401 observed delayed ripening and reduced rate of respiration in the hexanal treated fruits. During ripening
402 process, starch, which is the major form of carbohydrates in banana fruit, is usually catabolized into simple
403 sugars, which enters the metabolic pool where they are used as respiratory substrates or further converted
404 to other metabolites. Similar findings have been reported in hexanal treated banana fruits by
405 [Venkatachalam et al.](#) [33].

406 Banana is one of the few fruits whose TTA levels increases with ripening up to a maximum value then
407 decreases in the fully ripe stage as reported by [Lechaudel and Joas](#), [13]. This is as result of increase in
408 malic acid from 1.8 meq/100g to 6.2 meq/100g during ripening [14]. In the present study, hexanal treatment
409 delayed the rate of TTA increase as compared to the drastic increase in the control fruits which peaked at
410 day 3 of storage. This could be attributed to the observed reduced rate of ripening in the hexanal treated
411 fruits. Additionally, reduced activities of enzymes such as malate dehydrogenase, which influence the level
412 of malic acid in banana could further explain the delayed rate of TTA increase by hexanal treatment.

413 Ascorbic acid is an important quality trait in fruits. In the present study, ascorbic acid levels decreased
414 gradually in all the fruits as ripening advanced during storage. The decrease in vitamin C during ripening is
415 partly due to degradation of ascorbic acid through oxidation [3]. The decrease in ascorbic acid was less
416 rapid in the hexanal treated fruits compared to the untreated controls. Higher retention of ascorbic acid
417 observed in the hexanal treated fruits may be as a result of reduced enzymatic oxidation by hexanal.

418 Various quality attributes such as peel color, firmness, aroma, taste, mouth-feel and general acceptability
419 were evaluated during the sensory evaluation analysis. The sensory evaluation results showed that,
420 hexanal treatment did not have any significant effect on the various quality parameters scored. Further,
421 there was no significant difference on the general acceptability of the treated and the control fruits. This
422 indicates that hexanal's effect on shelf life of banana fruit did not have detrimental effects on the various
423 quality parameters. These results are in agreement with a study by [Siriboon and Banlusiip](#), [28], who

424 reported that hexanal treatment does not affect the expression of genes involved in quality development
425 pathway of tomato fruit.

426

427 **5.0. CONCLUSION**

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

436

437 **COMPETING INTERESTS**

438 Authors have declared that no competing interests exist regarding the publication of this paper.

439

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