THE EFFECT OF ETHANOL VAPOUR ON SHELF LIFE OF FRESH BANANA FRUIT

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Abstract
Banana (Musa species) are not only sources of food, they are sources of beverages, feeds, medical, ritual and ornamentals. However, its availability has been seriously hindered by its short shelf life due to environmental and microbial influences. This study therefore evaluated the effects of ethanol vapour pretreatment on proximate composition, shelf life and sensory attribute on storage of banana fruit. Fresh banana fruits were pretreated with 20cm^{3}ethanol vapour at concentrations of 0%, 25%, 95% and stored for two weeks in a 30-litre plastic bucket at ambient temperature. Results showed that pretreatment of banana with ethanol vapour prolonged the shelf life of fresh banana fruits by slowing down ripening and reduced the percentage spoilage to 20%, 50% and 80% at 95%, 25% and 0% concentration of ethanol vapour respectively. The moisture and the carbohydrate contents of ethanol vapour pretreated banana after 2weeks of storage significantly ($p<0.05$) increase with decrease in concentration of ethanol vapour while crude fat, crude fiber and protein contents significantly ($p<0.05$) decreased with decrease in concentration of ethanol vapour. Also the firmness of all ethanol vapour pretreated banana was significantly reduced compared to the fresh banana. The aroma and overall acceptance was higher in ethanol vapour pretreated banana compare to 0%. A total of three fungal genera from Saccharomyces spp., Aspergillus spp. and Rhizopus spp. were isolated. Untreated banana fruits have the highest abundance of the three genera. This research revealed that ethanol vapour prolonged the shelf life of fresh banana by slowing down ripening, enhanced the nutritional and sensory attribute of fresh banana at higher concentration.

Keyword: *Banana, shelf life, ethanol, proximate, sensory*

Introduction
Bananas are monocotyledonous plants in the genus *Musa* which belongs to the family Musaceae (Wong *et al.*, 2002). Banana plant is a large perennial herb with leaf sheaths that form trunk-like pseudostems. Fruits mature in about 60 - 90 days after the appearance of flowers (Khair, 2012).

Bananas are cultivated in more than 100 countries of the world and provide food for millions of people (De Langhe *et al*., 2009). Total banana and plantain production all over the world is 95.8 million metric tons (FAOSTAT, 2009). Bananas are not only the source of food, they also have other purposes. They are the sources of beverages, feeds, medical, ritual and ornamental materials. Alcohol, beer, vinegar and wine are usually produced from fruits of banana (Nelson *et al*., 2006). The diuretic and disinfectant effects of *Musa* potash and the strong astringent effects of *Musa* sap make its use relevant in traditional medicine. In Africa, *Musa* plants has different and various uses in ceremonies related to birth, funeral rites and religion. Banana is used as a treatment for gastric ulcer and diarrhoea and also beneficial for preventing cancer and heart disease because of high vitamin A and B6 contents (Emery, 1997). Its potassium content reduces cardiovascular disease and thus controls blood pressure (Blade, 2003).

Banana, being a tropical fruit with perishable nature, post-harvest losses of the fruits due to rotting in the market are innumerable (Pillay & Tripathi, 2007). Its availability has been seriously hindered by its short shelf life due to environmental and microbial influence. Different biochemical and physiological changes take place within a short span of time during ripening (Batra & Manna, 2007). Increasing its shelf - life, therefore, will not only increase its availability but as well improve the market value and the quantity of the fruit. Recently, there has been

substantial interest in non biological control agents to replace the existing chemical applications. Non-biological control involves chemicals product such as ethanol that are Generally Regarded as Safe "GRAS". For a long time, ethanol has been used as a fungicide treatment because it is an environmentally friendly disinfectant. Its vapour has been reported to provide antimicrobial properties and prolong the shelf life of fruits such as apple. Ethanol is a familiar constituent of many beverages and is considered to be the least toxic of the straight-chain alcohols. The effectiveness of ethanol vapour has been reported to improve the shelf life of fruits such as mango and apple, however, there is limited information on its effects on shelf life of banana. Ethanol dips have been reported to control post-harvest decay of cherry, table grapes, peaches and nectarines. It was comparable with other applications made in commercial like 1-MCP (Smith *et al*., 2000; Russell, 2001; Seiler. 2008)). On this note, this studied aimed at improving the shelf life of fresh banana fruit using ethanol vapour. It is hoped that the results obtain from these study will help in developing the strategy for preventing the loss of banana fruit and spoilage.

Materials and Methods

Sample Collection

Freshly harvested matured healthy banana fruit were purchased from main market Lapai, Niger State in the month of July, 2014. The samples were collected in a sterile poly bag and taken to the laboratory in the Department of Biological Sciences Ibrahim Badamasi Babangida University Lapai Niger State Nigeria for further study. Fruits were surface sterilized by rinsing in 2.0mM ($150mgKg^{-1}$) Sodium hypochlorite (commercial bleach).

Preparation of Ethanol

Various strengths of ethanol 95%, 25% and 0% were prepared from absolute alcohol. Twenty five percent (25%) ethanol was prepared by taken $25cm^3$ of absolute alcohol in measuring cylinder and $75cm^3$distilled water was added. Ninety five percent (95%) of ethanol was prepared by taken $95cm^3$ of absolute alcohol in measuring cylinder and $5cm^3$ of distilled water. 0% ethanol was prepared by using ordinary distilled water without any addition of alcohol (Gabler *et al*., 2005).

Pretreatment of Banana

Ethanol vapour pretreatment was done by incubating five gram(5g) of banana samples in a 30-L plastic bucket containing 20 cm^3 alcohol per kilogram of banana in a beaker and a filter paper wick to aid evaporation at various strength of 95%, 25% and 0%. The containers were sealed at the top and kept for 24 hrs at ambient temperature ($28\pm2°C$). Zero percent (0%) served as the control. Three replicate of each was made. After 24 hrs, the beaker and the wick were removed and banana were kept at ambient temperature for 14 days (Plotto *et al*., 2006).

Proximate Analysis

Proximate analysis of banana was carried out based on the separation of food components into groups or fractions in accordance with their nutritional value. Samples of the fresh banana fruits were collected earlier for proximate analysis before storage and after 14days of storage in alcohol vapour. Samples were analysed using AOAC (2005) methods to determine moisture, carbohydrate, crude proteins, crude fat, crude fibre. Three replicate of each experiment was made.

Percentage Spoilage

Percentage spoilage was calculated thus:

$$\frac{\text{Number of spoilt banana}}{\text{Total number of banana}} \times 100$$

Organoleptic Evaluation

For organoleptic evaluation, the colour, flavor, taste and firmness were assessed. Five trained and experienced panel were involved to assess the freshness parameter which involved complete familiarization with the food in its most desired state - fresh, uncontaminated, and with the most desirable qualities - followed by familiarization with a range of samples at diminishing stages of freshness. The 0-5 organoleptic hedonic scale is used (1=dislike very much; 2= dislike; 3=neither dislike nor like; 4= like; 5=like very much) (Feng.*et al.,* 2012).

Fungal Isolation and Identification

Banana fruits were assayed for microbial growth. One representative fruit pieces per bucket was taken from each experimental group and placed in sterile 1 mL conical flask, one flask for each bucket and 99 mL of sterile phosphate buffer was added to the flasks and the fruit pieces were gently agitated for 2 min. Small aliquots of buffer (2mL) were then taken and put in a petri dish containing potato dextrose agar (PDA) for the isolation of fungi (Plotto *et al*., 2006). Fungal isolates were identified using fungal family of the World Mycological Monographs (Cannon and kirk, 2007; Amadi and Adebola, 2008).

Statistical Analysis

Analysis of Variance (ANOVA) was used to analyze the data generated and Duncan New Multiple Range Test (DNMRT) was used to separate the means.

Results

Proximate Compositions

The results on the proximate compositions of ethanol vapour pretreated banana (*Musa species*) are presented in Table 1. The percentage moisture contents of fresh untreated banana (12.01±0.01%) was significantly ($p<0.05$) lower as compared to moisture contents of 25% ethanol vapour pretreated banana (15.0±0.01) and 0% ethanol vapour pretreated banana (25.00±0.11). However, there was no significant difference in the moisture contents of fresh banana as compared to moisture of 95% ethanol vapour pretreated banana (10.43±0.01).

The ash contents significantly ($p<0.05$) increased with decrease concentration of ethanol vapour (Table 1). The ash contents of fresh banana (12.63±0.05) was significantly ($p<0.05$) lower as compared to ash contents of 25% ethanol vapour pretreated banana (13.10±0.01) and 0% ethanol vapour pretreated banana (15.20±0.10). However, there was no significant difference in the ash contents of fresh banana as compared to ash of 95% ethanol vapour pretreated banana (12.71±0.01).

The crude fat contents (Table 1) significantly ($p<0.05$) increased with increase concentration of ethanol vapour after 14 days of storage. The crude fat contents of fresh banana (9.35±0.15) was significantly ($p<0.05$) higher as compared to crude fat contents of 25% ethanol vapour pretreated banana (8.60±0.15) and 0% ethanol vapour pretreated banana (6.20±0.05). But there was no significant difference in the crude fat contents of fresh banana as compared to crude fat contents of 95% ethanol vapour pretreated banana (9.25±0.05).

The crude fibre contents significantly ($p<0.05$) increased with increase concentration of ethanol vapour after 14 days of storage (Table 1). The crude fibre contents of fresh banana (11.80±0.005) was significantly ($p<0.05$) higher as compared to crude fiber contents of 25% ethanol vapour pretreated banana (10.20 ±0.015) and 0% ethanol vapour pretreated banana (8.90±0.15). But there was no significant difference in the crude fiber contents of fresh banana as compared to crude fiber contents of 95% ethanol vapour pretreated banana (11.73±0.15).

The results revealed that the crude protein contents of fresh banana (4.10±0.10) was significantly ($p<0.05$) higher as compared to crude protein contents of 95%, 25% & 0%

(3.85±0.05, 3.20±0.25& 2.90±0.05) ethanol vapour pretreated banana (Table 1). However, there was no significant difference between the crude protein contents of 95% & 25% ethanol vapour pretreated banana.

The carbohydrate content (Table 1) of ethanol vapour pretreated banana significantly ($p<0.05$) increased with decrease in concentration of ethanol vapour. The carbohydrate content of ethanol vapour pretreated banana 52.03±0.005(95%),53.10±0.11(25%) and 55.10±0.12 (0%) after 14 days of storage was significantly ($p<0.05$) higher than carbohydrate content of fresh banana (50.11±0.01).

Organoleptic Scoring

The result of the organoleptic assessment of ethanol vapour pretreated banana after 14 days of storage are shown in Table 2.

The flavour scores of ethanol vapour pretreated banana increased with increase concentration of ethanol vapour. The scores of ethanol vapour pretreated banana 3.50±0.21 and 3.00±0.31 for 95%, and 25% concentration respectively after i4 days of storage was significantly ($p<0.05$) higher as compared to aroma scores fresh banana (2.50±0.32).

There was no significant difference in the taste scores of ethanol vapour pretreated banana 3.50±0.01, & 3.10±0.11 (for 95% and 25% concentration after i4 days) as compared to taste scores fresh banana (3.50±0.10). However, the taste score (2.50±0.10) of banana pretreated with 0% ethanol vapour after 14 days of storage was lower compare to the taste scores of fresh banana and other concentrations. The taste steadily decreased from fresh banana and 95% concentration to 25% and 0%.

The firmness of banana was found to decrease from fresh banana to 95%, 25% & 0% concentrations of ethanol vapour. The firmness at 95% ethanol vapour pretreated banana was significantly higher than that of 25% and 0% concentration.

The Colour scores of ethanol vapour pretreated banana increased with increase concentration of ethanol vapour. The colour scores of ethanol vapour pretreated banana 2.70±0.10 and 2.30±0.02 for 25%, and 0% concentration after i4 days of storage was significantly ($p<0.05$) lower as compared to colour scores of fresh banana (3.50±0.02). However, there was no significant difference in the colour scores (3.50±0.13) of 95% ethanol vapour pretreated banana as compare to the colour score of fresh banana.

The overall acceptance score (3.70±0,11) of 95% ethanol vapour pretreated banana was significantly ($p<0.05$) higher as compared to overall acceptance score of 25% ethanol vapour pretreated banana (3.20±0,11) and fresh banana (3.00±0.11). But banana pretreated with 0% ethanol vapour had the least overall acceptance score (2.00±0.11).

Shelf life

The pretreatment of banana with ethanol vapour prolonged the shelf life (Fig. 1) of fresh banana and reduce the percentage spoilage. The banana pretreated with 95% ethanol vapour produce the lowest spoilage of 20% followed by 25% ethanol vapour with spoilage of 50% while the 0% ethanol vapour cause the highest percentage spoilage 80%.

Fungal Isolates

Three different genera of fungi *Saccharomyces* spp.*, Aspergillus* spp. *and Rhizopus* spp. were isolated from fresh, pretreated and untreated banana (Table 3). The most frequently occurred in all was genus *Saccharomyces* spp. in the entire banana. However, the untreated banana has more of genera *Aspergillus* spp. *and Rhizopus* spp.

Discusion

The fruit quality is determined by size (finger length and thickness), evenness of ripening, absence of blemishes defects, and the arrangement of the clusters (Khair, 2012). In the present study, storage of banana results in deterioration of most of the physical features of banana as observed in banana pretreated with 0% ethanol vapour. The skin of the banana which was initially yellowish green turned to black, the softness of banana was also found to increase after 14days of storage. However, banana pretreated with ethanol vapour at 95% concentration and to a lesser extends 25% concentration was found to be in good condition after 14 days of storage. The skin colour was found to remain the same as it was on the first day. The findings were an indication of shelf life extending properties of ethanol vapour. These results were in agreement with earlier reports that ethanol dips and vapors controlled postharvest diseases of peaches, citrus fruit, and table grapes (Gabler and Smilanick, 2001; Gabler *et al.*,2002; Karabulut *et al*., 2003). Ethanol vapors applied to whole apples reduced ethylene and CO2 production of fresh-cut apples, and their shelf life was increased due to maintenance of visual quality (Bai *et al*., 2004).

It was also found that the pretreatment resulted in more pleasant aroma than the fresh untreated banana. This result was in contrary to the perception of undesirable flavor in fruit tissue when exposed to ethanol for 24 h reported by Bai *et al.,* (2004); Plotto *et al*., (2003).

Analysis of proximate composition gave a quantitative idea of moisture, ash, protein, fat and carbohydrate composition in a food. Water is required for many biochemical reactions in the body (Ashifat *et al*., 2012), However, high moisture content will increase the susceptibility of fruit to microorganism attacked. The decrease in moisture contents of fresh banana compared to ethanol vapour pretreated banana and at 0% (control) could be as a results of increase availability of water with ripening of the banana. However, the insignificant difference in the moisture contents of fresh banana as compared to moisture of 95% ethanol vapour pretreated banana indicated the delayed ripening of banana caused by 95% ethanol vapour. This delayed ripening will favour its preventive properties against microbial contamination. Thus the storage time of 95% ethanol vapour will be high.

The increase in carbohydrate content of ethanol vapour pretreated banana with decrease in concentration of ethanol vapour after 14 days of storage may be linked to the delayed ripening of banana caused by ethanol vapour. The high content of carbohydrate in this study is comparable with the value reported for Garden Egg fruit (Auta *et al*., 2009). This high carbohydrate content in is beneficial since carbohydrate constitute a major class of naturally organic compounds that are essential for the maintenance of plant and animal life and also provide raw material for many industries (Ebun-Oluwa & Alade, 2007).

This study also revealed that increased ethanol vapour pretreatment cause increase in protein fiber, protein and fat contents of banana. This is a very good attribute because high fibre content of banana (11.80±0.005) is an indication of it good preventive properties against constipation and metabolic disorders such as diabetes.

The decrease in fat content with increase concentration of ethanol vapour after 14 days of storage and the decrease observed between fresh and pretreated banana (9.35±0.15) is a desirable property as excess fat consumption is implicated in etiology of many degenerative disease.

High protein diet support growth, movement, body defense in both human and livestock (Eddy, 2004) thus the low protein content of banana observed in this study is an indication that its consumption will have little or no effect on improving the body defense system The ash content give a measure of the total amount of inorganic compound like minerals present in plants. Banana was found to contain considerably high level of ash (12.65 to 15.20). This finding is an

indication that Banana is a good source of minerals and thus can be used as a mineral supplementing fruit.

In the present study, the prevention of spoilage of banana with increase concentration ethanol vapour and the prolonged shelf life of ethanol vapour pretreated banana may be attributed to inhibitory effect in ethylene production resulting in delayed ripening, the mode of action of the ethanol in the banana would be by conversion of acetyldehyde which in turn act as inhibitors of ethylene production (Plotto *et al*., 2003). Similar prolong shelf life due inhibitory effect of methanol vapour on ripening has been previously reported for banana (Jiang *et al*., 1999) and mango (Plotto *et al*., 2003). And also the prolonged shelf life may be linked to the antimicrobial activities of ethanol vapour, which reduce the microbial load of pretreated banana and thus result in increase shelf life.

In the present study, banana preserved with ethanol vapour showed increase in organoleptic score as compared to untreated banana (control). The increase in flavour scores of pretreated banana may be attributed to the accumulation of ethanol and some methyl esters. These findings agreed with earlier data published by Jinhe *et al*. (2005) where ethanol pretreatment of apple increase the aroma contents.

The softening of fruit during ripening is a major attribute that often dictates shelf life. During ripening partial disassembly of the fruit cell wall is largely responsible for softening and textural changes (Alexander and Grierson, 2002). Therefore, in the present study the significant decrease in the texture scores of pretreated banana as compared to the control group may also be attributed to the delay of ripening process.

Conclusion

In conclusion, this study revealed that pretreatment of fresh banana with ethanol vapour prolonged the shelf life of fresh banana by slowing down ripening, enhance the sensory attribute, increase the carbohydrate and decrease the moisture contents of fresh banana. It is therefore recommended that the use of ethanol vapour as food preservatives should be encouraged. However, evaluation of the safety and toxicity of ethanol vapour in pretreated banana is of paramount important.

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Table 1: Proximate compositions of ethanol vapour pretreated banana (*Musa species*)

		After 14 days		
PARAMETERS	Fresh Banana	95%	25%	0%
MOISTURE	12.01 ± 0.01^{a}	10.43 ± 0.01^{a}	15.0 ± 0.01^{b}	25.00 ± 0.11^{c}
ASH	12.63 ± 0.005^{a}	12.71 ± 0.01^{a}	13.10 ± 0.01^{ab}	15.20 ± 0.10^{b}
CRUDE FAT	9.35 ± 0.15^{b}	9.25 ± 0.05^{b}	8.60 ± 0.15^{ab}	6.20 ± 0.05^{a}
CRUDE FIBRE	11.80 ± 0.005^{b}	11.73 ± 0.15^{b}	10.20 ± 0.015^{ab}	8.90 ± 0.15^{a}
CRUDE PROTEIN	4.10 ± 0.10^{c}	3.85 ± 0.05^{b}	3.20 ± 0.25^{b}	2.90 ± 0.05^{a}
CARBOHYDRATE	50.11 ± 0.01^{a}	52.03 ± 0.005^{a}	$53.10\pm0.11a^{b}$	55.10 ± 0.12^{c}

Values follow by the same superscript along the rows are not differ significantly at $p<0.05$, values are Mean $\pm$ SEM of triplicate determination

Table 2: Organoleptic assessment of ethanol vapour pretreated banana (*Musa species*)

		After 14 days		
Sensory attribute	Fresh banana	95%	25%	0%
Flavour	2.50 ± 0.32^{b}	3.50 ± 0.21^{c}	3.00 ± 0.31^{c}	2.00 ± 0.32^{a}
Taste	3.50 ± 0.10^{a}	3.50 ± 0.01^{a}	3.10 ± 0.11^{a}	2.50 ± 0.10^{a}
Firmness	3.50 ± 0.01^{c}	2.90 ± 0.22^{b}	2.10 ± 0.21^{a}	2.00 ± 0.01^{a}
Colour	3.50 ± 0.02^{b}	3.50 ± 0.13^{b}	2.70 ± 0.10^{a}	2.30 ± 0.02^{a}
Overall acceptance	3.00 ± 0.11^{ab}	$3.70\pm0,11^{b}$	$3.20\pm0,11^{ab}$	2.00 ± 0.11^{a}

Values follow by the same superscript along the rows are not differ significantly at $p<0.05$, values are Mean $\pm$ SEM from Eight panelist

Table 3: Fungi isolated from fresh, ethanol vapour pretreated and untreated banana fruits

		Fungi	
Banana	Saccharomyces spp	Aspergillus spp	Rhizopus spp
Fresh banana	+++++	-	_
95% pretreatment	+++++	_	++
25% pretreatment	+++++	+ +	++
0% pretreatment	+++++	+++++	+++++

+++++ = Abundance ++ = slightly present - = not isolated

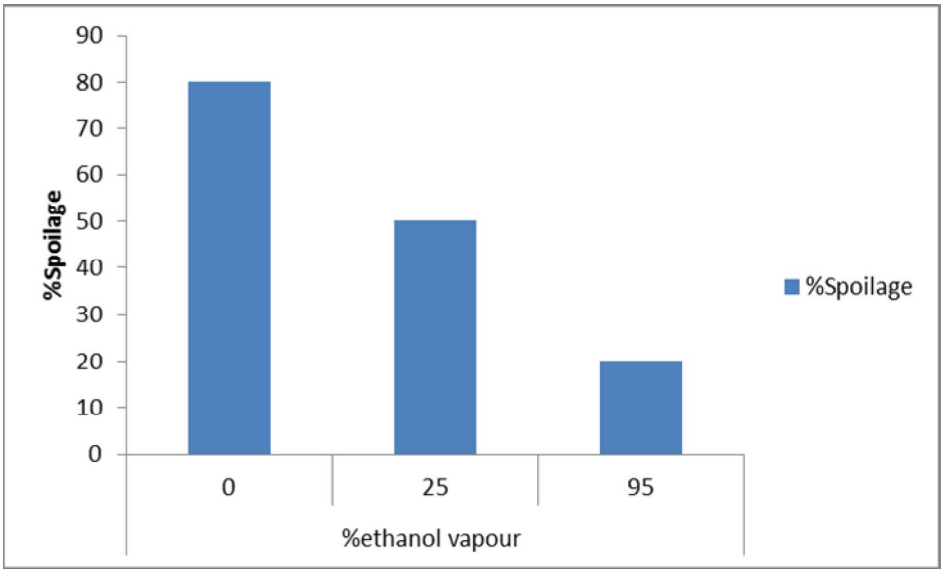

Fig. 1: The Shelf Life of ethanol vapour pretreated banana