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(RESEARCH ARTICLE)

Rhizoctonia solani, Aspergillus niger, Streptococcus pyrogenes, Alcaligenes faecalis and *Proteus vulgaris* selectively associated with two varieties of banana and effects of storage conditions on nutritional composition of banana

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Abstract

This study was carried out to identify organisms associated with banana deterioration and effects of storage on nutritional compositions of banana. There is a high cultivation and consumption of banana in Nigeria. However, high post-harvest loss due to microbial rot remains an albatross. *Aspergillus niger* and *Alcaligenes faecalis* were isolated from rotten *M. sapientus* fruit while *Rhizoctonia solani* and Proteus vulgaris were isolated from *M. acuminata* fruit. However, *Streptococcus pyrogenes* was isolated from both *M. sapientus* and *M. acuminata*. The percentage proximate content of the macro nutrients, moisture, carbohydrate, ash, crude protein, crude fibre and lipid were carried out on banana fruits stored for 5 days. The mean proximate composition of *M. sapientum* on the fifth day of storage showed decrease in carbohydrate (from 18.807 to 5.334%) and lipid (from 3.292 to 2.874%) content, while an increase was observed in the moisture content (from 75.493 to 81.987%), protein (1.713 to 1.947%) and ash contents (1.937 to 1.969%). The results obtained showed that fruits stored at 4°C+1 in a refrigerator for 20 days stayed for a longer time before initiation of ripening, compared with fruits stored in moistened sawdust for 7 days and fruits stored in polyethylene bag moistened with $KMnO_4$ solution for 3 days. The results obtained from this work showed that refrigeration was the most effective storage condition for prolonging the shelf life of banana.

Keywords: Bananas; Shelf Life; Rot Organisms; Nutrient Composition

1 Introduction

Banana is a popular fruit that is highly nutritious and delicious. It can be eaten raw or cooked as a tropical delicacy. Usually, ripe banana is soft and sweet while unripe banana contains a lot of starch and fibre. It is a cheap source of carbohydrate and a rich source of potassium, calcium, antioxidants and other micronutrients. The sugar rich and low-fat banana has varied uses as infant food, functional food, carbohydrate based staple food and many more diversified food/feed uses (Debabandya *et al*., 2010). Dessert banana and plantain (*Musa spp*.) are the fourth most important staple food crops in the world after rice, wheat and maize (Salvador *et al*., 2007). They are important sources of income for many smallholders Sub-saharan African farmers (FAOSTAT, 2012). Demand and increase consumption for this fruit has grown particularly faster in the developing countries of this fruit (CSA 2004).

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For international trading, banana fruits are usually harvested before ripening, and stored at relatively low temperature during transportation and market process. Long distance transportation and extended storage period in the market may make banana susceptible to disease infection. Banana fruits are exposed to contamination by microbes through contact with soil, dust, water and by handling at harvest or during postharvest processing. These make them to harbour a wide range of microorganisms including plant and human pathogens (Eni *et al.*, 2010). Microbial spoilage of fruits may be due to bacteria or fungi, causing the fruits to be undesirable, reducing the market value and may also cause some side effects such as gastroenteritis when consumed. Post-harvest diseases can cause huge loss of fruits both in terms of quantity and quality, thus reducing their market value. Therefore, it is necessary to improve the post-harvest quality of banana fruit in an attempt to extend their shelf life, thus storage life of banana fruit can be improved using low temperature, 90% humidity, removal of ethylene, storage in 5% CO_2 and 3% oxygen at 28^oC, use of chemicals, irradiation, use of packaging materials and fruit processing. There is a high consumption of banana in Nigeria coupled with high loss due to spoilage. This study was centered on identification of microorganisms associated with post-harvest banana deterioration and extension of banana fruits shelf life.

2 Material and methods

2.1 Collection of samples

Infected and healthy (green) mature banana fruits (*M. sapientum* and *M. acuminata* var. dwarf Cavandish) were obtained from a retailer outlet in Akure metropolis. The fruits were placed in separate sterile polyethylene bag and transferred to the laboratory of the Department of Microbiology, Federal University Technology, Akure (FUTA), Ondo State, Nigeria

2.2 Determination of proximate analyses

Proximate analyses of the banana fruits were carried out on the fresh and stored fruits for five days. The percentage proximate content of the macro nutrients, moisture, carbohydrate, ash, crude protein, crude fibre and lipid were carried out using the method published by Association of Official Analytical Chemists (AOAC, 1995).

2.3 Determination of moisture content

The moisture dish was washed and dried in the oven for an hour; this was later transferred into a desiccators to cool. The empty dish was weighed on the analytical balance. Ten grams (10g) of the sample was transferred into the moisture dish and re-weighed. The moisture dish was later put into an oven and heated at 105 ^oC for 3 hours to dry the content. This process was repeated at an hour interval and weighed until constant weight was observed.

$$\% \text{ Moisture} = \frac{\text{W2} - \text{W3}}{\text{W2} - \text{W1}}$$

- W_1 =Weight of empty can
- W_2 =Weight of can + sample before drying
- W_3 = Weight of can + sample after drying

2.4 Determination of Crude Protein

The samples were blended to homogenized form. Each sample (2.5g) was accurately weighed and placed in the Kjeldahl flask; 25 ml of conc. H_2SO_4 and catalyst was added to the flask sequentially and digested until colourless solution was obtained. The content was allowed to cool, and then 200 ml of water was cautiously added to the content. This was transferred to the distillation unit and neutralized with 50% NaOH solution which was slowly dispensed into the side of the Kjeldahl flask until the solution became dark. The ammonia formed was distilled into 15 ml of 2% Boric acid solution containing mixed indicator. The distillation process was stopped when the volume of distillate reached 150 ml and the distillate titrated with 0.05M HCl. A reagent blank was run to subtract reagent nitrogen from the sample nitrogen.

$$\% \text{ Crude Protein} = \frac{\text{Conc. of HCl x titre of blank x 0.004 x 100 x 6.25 (protein conversion factor}}{\text{Gram of sample}}$$

2.5 Determination of Crude Fibre

The crude fibre was determined using gravimetric method, 2g of each sample was weighed and the fat extracted with hexane. The sample was later transferred into an oven and dried at 70^oC overnight. After drying, the sample was

transferred into the flask of the Reflux apparatus and digested with 200ml of 1.25% H_2SO_4 for 30 minutes after which it was filtered and washed with distilled water. After draining, the sample was again transferred into the flask and digested with 200ml of 1.25% NaOH for 30 minutes. It was later filtered and washed with distilled water. This was later transferred into a pre-weighed crucible and dried in the oven at 105°C until constant weight was achieved. The crucible was then placed in the muffle furnace and ignited until fully burnt.

$$\% \text{ Fibre } = \frac{\text{Fibre} - \text{Ash}}{\text{Weight of sample}} \text{x } 100$$

2.6 Determination of Ash Fibre

The ash was determined according to the method of AOAC, (1995). The crucible was prepared by igniting in a muffle furnace at 550°C for 12 hours and later transferred into a desiccators containing active desiccant while still hot to cool. After cooling, the crucibles were then placed in the muffle furnace and ignite until all the organic components have burned off remaining ash. The crucible is transferred into a desiccators when the furnace temperature reduced to about 200°C and allowed to cool. This was weighed after cooling to get the weight of ash.

$$\%\text{Crude Ash } = \frac{\text{Ash x } 100}{\text{Weight of sample}}$$

2.7 Determination of Fat

The fat was determined using acid hydrolysis method. Two grams (2g) of the sample was weighed into 100ml beaker and 2ml of ethanol was added together with 7ml of conc. HCl and 3ml of H_2O. All these were heated on water bath for about 15 minutes. After cooling, it was separated into the separating funnel and the beaker washed with 100ml ethanol and transferred into the separating flask. This was extracted three times with 30ml of diethyl ether and once with 30ml of light petroleum ether (40°C-60°C). The solvent and the extract were combined and filtered through cotton plug into a clean, dried and pre- weighed 100ml beaker. The solvent was removed by evaporating on a water bath; the beaker and the residue were transferred into an oven, this was dried for about an hour. This was later transferred into desiccators for cooling and residue weighed as fat.

$$\% \text{ Crude Fat} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \text{x } 100$$

2.8 Determination of Carbohydrate

Carbohydrate is obtained thus:

$$\% \text{ Carbohydrate } = 100\,\% - (\% \text{ Moisture } + \% \text{ Protein} + \% \text{ Fibre})$$

2.9 Identification of Spoilage type

Symptoms shown by the infected fruits were critically studied. Various stages of disease development were noted. The nature of rot and extent of tissue damages in fruits were carefully observed.

2.10 Preparation of media

Media used for the work were nutrient agar (NA) and potatoes dextrose agar (PDA). NA and PDA for culturing bacteria and fungi respectively.

The nutrient agar was prepared by dissolving 28g of the powder in 1litre of distilled water and autoclaved at 121°C for 15mins. The PDA was prepared by dissolving 39g of commercial PDA powder in 1lire of distilled water and autoclaved at 121°C for 15 mins. Then, 15cm^3 of the media was poured into each Petri dish.

2.11 Isolation of the microorganisms

Isolation of pathogenic fungi was carried out at the point of progression/development of banana fruit rot according to the methodology described by Diedhiou *et al* (2007). The streak plate method was used for culturing of bacteria; this was performed after a piece of banana fruit was cut from the front progression of developing disease symptom. The inoculating loop was sterilized with flame for a few second and used to pick up the isolated colony from the agar plate culture and spread it gently over the first quadrant. The process was repeated for the second, third and fourth quadrant

respectively. The plates were then incubated at 37°C for 24 hours. The streak plate method was repeated on a freshly prepared media to achieve a uniform and general appearance of the colony. The fruits were first soaked in a 2% solution hypochlorite for 1 minute. A sterilized scalpel was used to cut a small fragment of pulp and peal at the front progression of diseased spot of the banana fruit. The fragment is placed in a Petri dish containing PDA supplemented with 100ppm of chloramphenicol. The Petri dishes were incubated at room temperature for 7 to 10 days to allow sporulation. The microorganisms were sub-cultured into freshly prepared media for pure culture isolation.

2.12 Pathogenicity test

In order to establish disease causing quality of each isolate, the fruits were disinfected with ethanol to eliminate surface contaminants. Fresh, healthy and unripe banana fruits were inoculated with each identified pure isolate. Suspension of each isolate was prepared by introducing a loop full of the isolate into 9ml of distil water in a test tubes and shook for even distribution of the isolate in the medium. Then, 1ml of the suspension was inoculated into each fruit using a sterile syringe and observed for rot symptom after 5days. Each inoculated fruit was labeled appropriately.

2.13 The effect of different storage conditions on the rate of ripening of banana fruits

Unripe and matured banana fruits were stored in a refrigerator (4°C+1), the polythene bag was moistened with $KMnO_4$ solution, and moist sawdust to examine the rate of ripening of the fruits. A control experiment was setup at ambient temperature in a storage cabinet. Ripening was visually observed by comparing the colour of the peel to the standardized colour chart that described the five ripening stages. The ripening progressions of the fruits were correlated with number for easy assessment. The ripening progressions of the fruits were correlated with number for easy assessment using the key thus: 1-5, where Green=1, Traces of yellow =2, More green than yellow =3, More yellow than green=4, Yellow 5.

3 Results

Table 1 Mean Proximate Composition of *M. sapientum*

Parameters	Fresh (%)	5 days storage (%)
Moisture	73.92	85.44
Crude protein	1.38	1.55
Carbohydrate	18.81	5.33
Crude fibre	1.42	2.45
Lipid	3.48	2.85
Ash	1.01	2.43

Table 2 Mean Proximate Composition of *M. acuminata*

Parameters	Fresh (%)	5 days storage (%)
Moisture	75.49	81.98
Crude protein	1.71	1.95
Carbohydrate	16.92	8.53
Crude fibre	1.70	2.89
Lipid	3.29	2.87
Ash	1.94	1.97

Table 1 and Table 2 show the proximate composition of fresh and after five days of storage of *M. sapientum* and *M. acuminata*. There was an increase in the percentage (%) moisture contents (from 73.919 to 85.425), protein

contents (from 1.375 to 1.554), crude fibre contents (from 1.416 to 2.454) and ash contents (from 1.008 to 2.431) between the fresh fruits and fruits at five days of storage while there was a decrease in the percentage (%) carbohydrate contents (from 18.807 to 5.334) and lipid contents (from 3.475 to 2.852). At 5 days of storage, *M. acuminata* recorded an increase in the percentage (%) moisture contents (from 75.493 to 81.987), protein contents (from 1.713 to 1.947), crude fibre contents (from 1.702 to 2.889) and ash content (from 1.937 to 1.969) between the fresh fruits and fruits stored for five days, while there was a decrease in the percentage (%) carbohydrate content (from 16. 917 to 8.334) and lipid contents (from 3.292 to 2.874).

Table 3 Microorganisms associated with *M. sapientum* and the symptoms exhibited

Pathogens	Group	Symptoms
Aspergillus niger	Fungi	Black moldy spot
Streptococcus pyrogenes	Bacteria	Brown rot
Alcaligenes faecalis	Bacteria	Brown rot

Table 4 Microorganisms associated with *M. acuminata* and the symptoms exhibited

Pathogens	Group	Symptoms
Streptococcus pyrogenes	Bacteria	Black moldy spot
Rhizoctonia solani	Fungi	White moldy spot
Proteus vulgaris	Bacteria	Brown rot

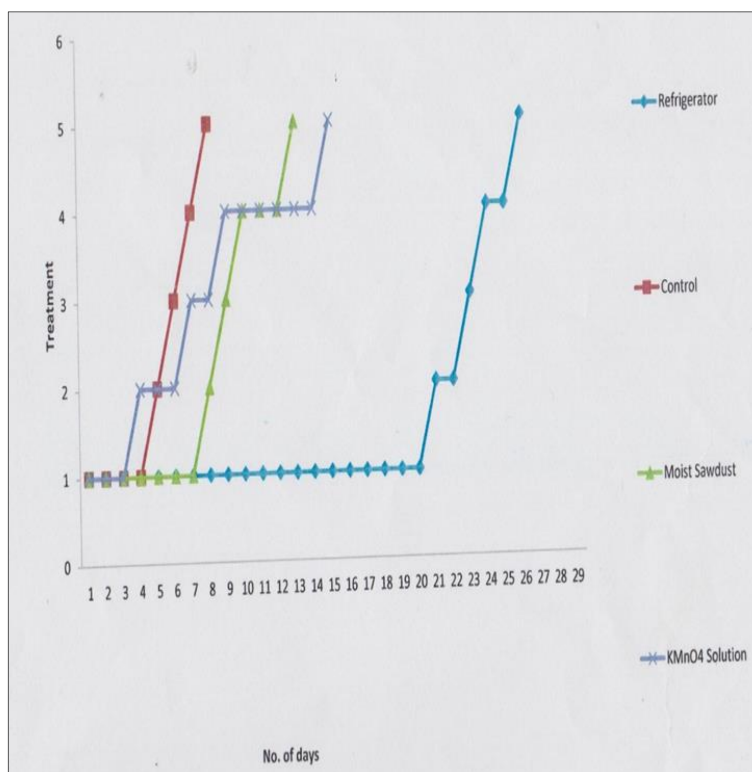

Figure 1 Effects of different condition on *M. Sopietum*

Table 3 and 4 show the microorganisms isolated from the banana fruits and their respective spoilage symptoms. Fungal isolate from *M. Sapientum* was *Aspergillus niger* with a characteristic black moldy spot while bacterial isolates were *Streptococcus pyrogenes* with a characteristic brown rot and *Alcaligenes facalis* with brown rot symptom. Fungal isolate

from *M. acuminata* was *Rhizoctonia solani* with a characteristic white moldy spot while bacterial isolates include *Streptococcus pyrogenes* with a characteristic brown rot and *Proteus vulgaris* with brown rot symptom. Figure 1 and 2 shows the effects of various storage conditions on shelf life of varieties of banana.

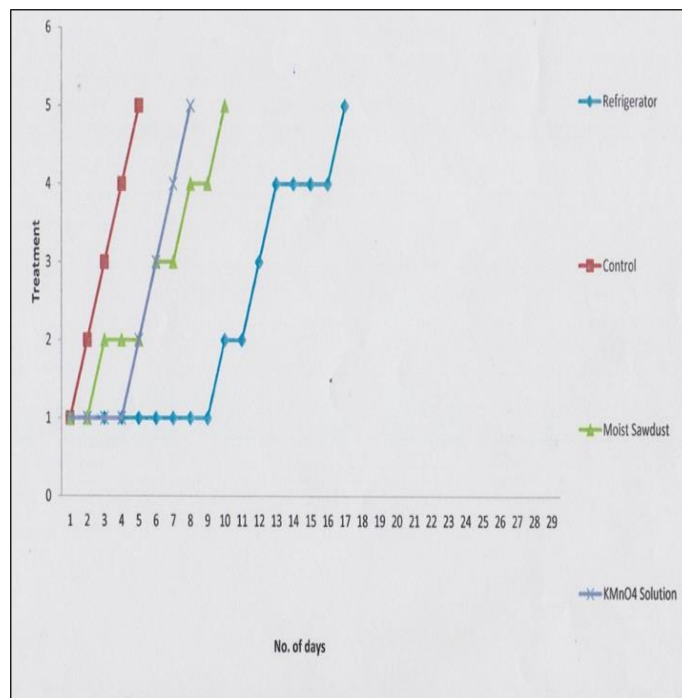

Figure 2 Effects of Different Conditions on *M. Acuminate* Var Dearf Cavendish

4 Discussion

The result from the proximate composition of the two species of banana examined revealed that the rate at which the macro-nutrients (moisture content, protein, carbohydrate, food fiber, lipid and ash) are being lost or gained in the process of ripening depends upon the species of banana. From the result, it showed that *Musa acuminata* although had lesser nutrients amount present, still showed reluctance in its nutritional content during ripening process when compared to *Musa sapientum.* This is in support of Watada *et al*., (1984) that stated that the degenerative changes occurring in banana differ from species to species. The rapid increase in the moisture content of these fruits could be as a result of micro-organisms present in these fruits. This is in line with the report of Miedes and Lorences (2004) that reported that spoilage microbes exploit the host using extra cellulose lytic enzymes that degrade the polymers to release water and the plant's other intra-cellular constituents for use as nutrients for their growth. The decrease in the carbohydrate content is as a result of the conversion of starch to sugar. This corresponds with contribution of Zhang *et al*., (2005) that during ripening process, the starch which consists of a long covalently bonded chain is converted into sugar. The increase in protein bound amino acids appears to be due to the association of fungal mycelium with fruit tissues. The presence of these pathogens in the banana fruit could be as a result of their infection on field. Hansen *et al*., (2003) reported the occurrence of *Rhizoctonia solani* in banana lower part which could have infected the fruits before harvesting. The presence of sugar and other nutrients support fungi pathogens (*Aspergillus niger* and *Rhizoctonia solani*) in infecting these fruits. This is in line with observation of Singh and Sharma (2007) which stated that high levels of sugar and nutrient elements and their low pH values made them particularly desirable to fungal decay. Studies by Li-Cohen and Bruhn (2002) showed that fungi can survive and/or grow on fresh produce, that the nutrient content (carbohydrate, protein and fats) in fresh produce support pathogens. The presence of bacteria pathogen like *Proteus vulgaris, Streptococcus pyrogenes* and *Alcaligenes faecalis* could result into secondary infections.

The result also indicated that refrigeration temperature (4°C+) had the longest time period before initiation of ripening compared with control, fruit stored in polyethylene bag moistened with KMnO4 solution and fruits embedded in moist sawdust. This support the assertion of Robinson, (1996) and Shaun *et al*., (1997) stated that with refrigerator, ripening took a longer period reducing the environmental temperature reduces the rate of respiration, thereby delaying ripening.

5 Conclusion

Banana is a wholesome tropical fruit with an important economic role in the global food trade. It is a rich source of various vitamins, minerals as well as starch. The role of banana in food product cannot be neglected both to the socio-economic growth of the country and its benefits to human. Banana is useful both as ripe and as unripe fruit due to its potential, hence, there is need to extend the shelf life of these produce in order to limit their wastage rate. Microbial invasion has been a challenging factor that has drastically reduced the rate of acceptability of banana fruits in the market as it shortens their shelf life. These microorganisms might have infected these fruits from their stock through wound in the course of harvesting, Since the ubiquity of these microorganisms cannot be overwhelmed, it is expedient to ease these microorganisms out of their comfort zone which may hinder their rate of reproduction. This can guarantee a longer shelf life of this fruit and reduce spoilage, though their importance cannot be totally circumvented, but with a better storage facility, its potential can be subdued to a minimal extent.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest.

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