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## ISOLATION OF MICROORGANISM ASSOCIATED WITH DETERIORATION OF BANANAS FRUITS

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### Abstract:

Synopsis *Musa sapientum* and *Musa acuminata* var. dwarf Cavendish, two microorganisms linked to banana fruit degeneration, were isolated. *Aspergillus niger* was recovered from *M. sapientum*, and *Rhizoctonia solani* was obtained from the *M. acuminata* var dwarf Cavendish. *Alcaligenes faecalis* and *Streptococcus faecalis* were recovered from *M. sapientum*, while *Streptococcus pyogenes* and *Proteus vulgaris* (from *M. acuminata* var. dwarf Cavendish) were the bacteria that were isolated. On the fifth day of storage, the mean proximate composition of *M. sapientum* showed a rise in the contents of moisture (73.919 to 85.425%), protein (1.375 to 1.554%), and ash (1.008 to 2.431%), but a decrease in the contents of fat (3.475 to 2.852%) and carbohydrates (18.807 to 5.334%). On the fifth day of storage, the *Musa acuminata* var. dwarf Cavendish mean proximate composition showed an increase in the contents of moisture (75.493 to 81.987%), protein (1.713 to 1.947%), and ash (1.937 to 1.969%), but a decrease in the contents of lipid (3.292 to 2.874%) and carbohydrates (16.919 to 8.334%). Various methods were used to store the fruits. The findings indicated that fruits kept in a refrigerator at 4°C±1 had a longer period of time before starting to ripen (20 days) than fruits kept in wet sawdust (7 days) or in polythene bags soaked with KMnO<sub>4</sub> solution (3 days). The fruits' rate of decay was also looked at. Fruits kept in moist sawdust did not show any signs of

deterioration over the 31-day storage period. On the tenth day, mould was found in fruits that had been stored at 4°C±1. Fruits kept in polythene bags that had been wet with KMnO<sub>4</sub> solution started to grow contaminates on the seventh and eighth day, respectively. The work's findings indicate that wet sawdust seemed to be the best option for extending the shelf life of the banana fruits, while chilling (4°C±1) seemed to be the best condition for delaying the start of ripening of the varieties of bananas utilised.

**Keywords:** Ripening, Storage, Deterioration, Proximate Analysis, Contamination, Shelf Life

### 1. Introduction

Banana (*Musa* species) is a major staple crop of considerable importance in the developing world. They are consumed as an energy giving food and as dessert[1].

Unlike most plantain, banana is usually eaten without further preparation[2].

The colour of banana, cooking banana and plantain probably contributes more to the assessment of quality by the consumer than any other single factor. The colour of the fruit could give an indication of state of deterioration, disease infestation and/or contamination. The market quality and consumer acceptability of banana, cooking banana and plantain are significantly influenced by the colour of the fruit[1]. The peel colour is often the major post-harvest criterion used by researchers, growers



and consumers to determine whether the fruit is ripe or unripe[3]. In some countries, (e.g. Ghana, Nigeria, Honduras, etc), consumers have developed distinct correlations between colour and the overall quality of specific products[1].

Cooking banana or plantain should be green or yellow, anything which falls short of that (e.g. red plantain) would be difficult to sell[1]. Hence, colour is critical as the first visual assessment of the quality of cooking banana or plantain. Consumers associate the colour of the peel with specific tastes or uses and they will usually buy cooking banana or plantain if the colour is suited to the required purpose or desire [1]

quickly and have a very short shelf life . Once harvested, the fruits ripen [4]. Traditionally, the stage of ripening of banana, cooking banana and plantain have been closely linked with the changes in peel colour and the matching of the peel colour against a set of standard colour plates is a common method used to assess the ripeness of the fruit[1].

During ripening of banana, cooking banana and plantain, there is a tremendous increase in the amount of ethylene produced. This increase is usually accompanied by an increase in respiration rate of the fruit (a phenomenon which is called the climacteric). The rate of respiration and ethylene production usually depends on storage temperature, age of fruit and cultivar/hybrid[5].

In the trade, seven ripening stages of bananas are generally recognized: stage 1: green; Stage 2: green, traces of yellow; stage 3: more green than yellow; stage 4: more yellow than green; stage 5: green tip and yellow; stage 6: all yellow, and stage 7: yellow, flecked with brown[6]. Beyond the full yellow stage, degenerative changes occur on the skin with the appearance of brown flecks

which ultimately coalesce until the skin is blotchy brown[2]. The loss of green colour is due to degradation of the chlorophyll structure[1].

Banana fruits can be contaminated by microorganisms through skin penetration, natural opening or mechanical damage. 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[7],[8]. Post-harvest diseases can cause serious losses of fruits both in terms of quantity and quality. Fruits microorganisms have no market value. The storage life of banana fruits can be improved using low temperature, 90% humidity, removal of ethylene, storing in 5% CO<sub>2</sub> and 3% oxygen at 28o C, use of chemicals and irradiation[9], use of packaging materials and fruit processing[10].

The objectives of this study are to isolate microorganisms associated with deterioration of banana fruits in storage and to assess common ways that have used to improve the shelf

## 2. Materials and Methods

### Collection of Samples

Fresh, ripe and green samples of banana fruits (*M. sapientum* and *M. acuminata* var. *dwarf Cavendish*) were obtained from the retailer's outlet in Akure, Ondo State, Nigeria. The fruits were collected in a polythene bag and transferred into the laboratory of the Department of Biology, Federal University of Technology, Akure, Nigeria.

### Identification of Spoilage Types

The fresh fruits were placed on sterile glass support in sterile desiccators. The spoilage types were identified by physical examination of the samples. The color changes and mold appearance were observed and recorded.



### Preparation of Media

Media used for the work were nutrient agar, and potatoes dextrose agar for the isolation of bacteria and fungi respectively. The media were prepared using manufacturer's instruction and sterilized using autoclave at 121o C for fifteen minutes.

### Determination of Proximate Analysis

The proximate analysis of the banana fruits was carried out on the fresh fruits and fruits stored for five days. The percentage proximate content carried out include: moisture, carbohydrate, ash, crude protein, crude fibre and lipid using methods described by Peason[11].

### Isolation and Characterization of Microorganisms

Streak plate method was used for the isolation of bacteria while a portion of the fruit was aseptically inoculated onto the centre of potatoes dextrose agar for the isolation of fungi.

The microorganisms were subcultured into freshly prepared media for pure culture isolation. The bacteria were characterized using methods described by Cowan[12] while the fungi were identified using cultural and microscopic examination as described by Domsch and Gams[13].

### Pathogenicity Test

Healthy, fresh and unripe fruits were each inoculated with isolates in order to establish the spoilage symptoms of each isolate. The fruits were surface disinfected using cotton wool moistened with ethanol. Each isolate was inoculated into 9mls of sterile water using inoculating loop and needle for bacteria and fungi respectively. One milliliter (1ml) of the inoculum was inoculated into each fruit using a sterile syringe.

### The Effects of Different Storage Conditions on the rate of ripening banana fruits

Unripe, matured banana fruits were stored using refrigerator (4o C±1), polythene bag moistened with KMnO<sub>4</sub> solution, and moist sawdust to examine the rate of ripening of the fruits. A control was also setup at ambient temperature in a storage cabinet. Ripeness was currently assessed visually by comparing the color of the peel to standardized color charts that describe the seven ripening stages[6]. The ripening progression of the fruits were scored using number 1 for green, 2 for traces of yellow, 3 for more green than yellow, 4 for more yellow than green, 5 for full yellow, 6 for yellow with traces of brown and 7 for more brown than yellow

### Determination of Rate of Deterioration under Different Storage Conditions

Unripe, matured banana fruits were stored using refrigerator (4o C±1), polythene bag moistened with KMnO<sub>4</sub> solution, and moist sawdust to examine the rate of deterioration of the fruits. A control was also setup at ambient temperature in a storage cabinet. The observed deterioration progression of the fruits were scored using number 1 for green, 2 for traces of yellow, 3 for more green than yellow, 4 for more yellow than green, 5 whitish tip with for full yellow colour, 6 for yellow with traces of brown and 7 for more brown than yellow, 8 for all brown flecks with soft flesh.

## 3. Results

### Mean Proximate Composition of *M. sapientum* and *M. acuminata* var *dwarf Cavendish*

**Table 1.** Mean Proximate Composition of *M. sapientum* and *M. acuminata* var *dwarf Cavendish*

Parameters	<i>M. sapientum</i>		<i>M. acuminata</i> var <i>dwarf Cavendish</i>	
	Fresh	5days of storage	Fresh	5 days of s
% Moisture	73.919	85.425	75.493	81.98
% protein	1.375	1.554	1.713	1.947
% carbohydrate	18.807	5.334	16.917	8.334
% crude fiber	1.416	2.454	1.702	2.889
% lipid	3.475	2.852	3.292	2.874
% ash	1.008	2.431	1.937	1.969

**Table 2.** Microorganisms isolated and their spoilage symptoms

<i>M. sapientum</i>	<i>M. acuminata</i> var. <i>dwarf Cavendish</i>
<i>Aspergillus niger</i> (black moldy spot)	<i>Rhizoctonia solani</i> (white moldy spo
<i>Streptococcus pyogenes</i> (brown rot)	<i>Streptococcus pyogenes</i> (brown rot)
<i>Alcaligenes faecalis</i> (brown rot)	<i>Proteus vulgaris</i> (brown rot)

Table 1 shows the proximate composition of fresh *M.*

*sapientum* and *M. acuminata* var *dwarf Cavendish*. 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* var. *dwarf Cavendish* 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 contents (from 1.937 to 1.969) between the fresh fruits and fruits stored at five days of storage while there was a decrease in the percentage (%) carbohydrate contents (from 16.917 to 8.334) and lipid contents (from 3.292 to 2.874).

#### Microorganisms isolated and their spoilage symptoms

Table 2 shows microorganisms isolated from the banana fruits and their spoilage symptoms. Fungi isolated from *M. sapientum* was *Aspergillus niger* with a characteristic black moldy spot while bacteria isolated were *Streptococcus pyogenes* with a characteristic brown rot and *Alcaligenes faecalis* with brown rot symptom. Fungus isolated from *M.*

*sapientum* was *Rhizoctonia solani* with a characteristic white moldy spot while bacteria isolated include *Streptococcus pyogenes* with a characteristic brown rot and *Proteus vulgaris* with brown rot symptom.

#### Colour ratings of *M. sapientum* stored under different storage conditions

Figure 1 shows the colour ratings of *M. sapientum* stored under refrigeration temperature (4°C±1), polythene bags moistened with KMnO<sub>4</sub> solution and moist sawdust for thirty (30) days. The control (fruits stored under ambient temperature of 28°C±2) had the least day (14days) before attaining the last stage of colour rating (stage 7), followed by fruits stored in polythene bags moistened with KMnO<sub>4</sub> solution and fruits embedded in sawdust (18days) while fruits stored in refrigeration temperature 4°C±1 took 28days before reaching the last stage of colour ratings chart (stage 7).

#### Colour ratings of *M. acuminata* var. *dwarf Cavendish* stored under different storage conditions

Figure 2 shows the colour ratings of *M. acuminata* var. *dwarf Cavendish* stored under refrigeration temperature (4°C±1), polythene bags moistened with KMnO<sub>4</sub> solution and moist sawdust for thirty (30) days. The control (fruits stored under ambient temperature of 28°C±2) had the least day (12.5days) before attaining the last stage of colour rating (stage 7), followed by fruits stored in polythene bags moistened with KMnO<sub>4</sub> solution and fruits embedded in sawdust (15days) while fruits stored in refrigeration temperature 4°C±1 took 26days before reaching the last stage of colour ratings chart (stage 7).

#### Deterioration of *M. sapientum* stored under different storage conditions

Figure 3 shows the deterioration of *M. sapientum* stored under refrigeration temperature (4°C±1), polythene bags moistened with KMnO<sub>4</sub> and moist



sawdust for thirty (30) days. Fruits stored in polythene bag moistened with  $KMnO_4$  was the first to deteriorated (13 days of storage), followed by the control (17days of storage) and refrigeration temperature ( $40C\pm 1$ ) (28days). Fruits embedded in moist sawdust was not deteriorated throughout the storage period of 30days.

#### Deterioration of *M. acuminata* var. *dwarf Cavendish* stored under different storage conditions

Figure 4 shows the deterioration of *M. acuminata* var. *dwarf Cavendish* stored under refrigeration temperature ( $40C\pm 1$ ), polythene bags moistened with  $KMnO_4$  and moist sawdust for thirty (30) days. Fruits stored in polythene bag moistened with  $KMnO_4$  was the first to deteriorated (13 days of storage), followed by the control (16days of storage) and refrigeration temperature ( $40C\pm 1$ ) (30days). Fruits embedded in moist sawdust was not deteriorated throughout the storage period of 30days.

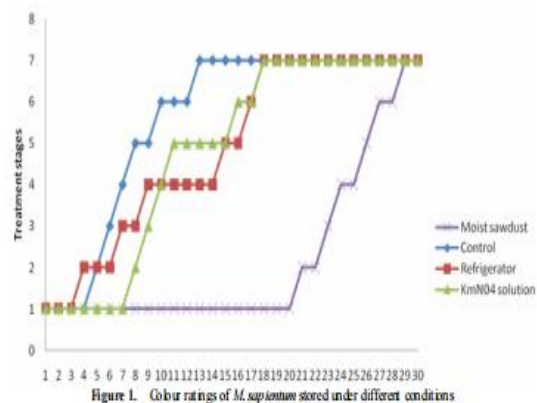


Figure 1. Color ratings of *M. sapientum* stored under different conditions

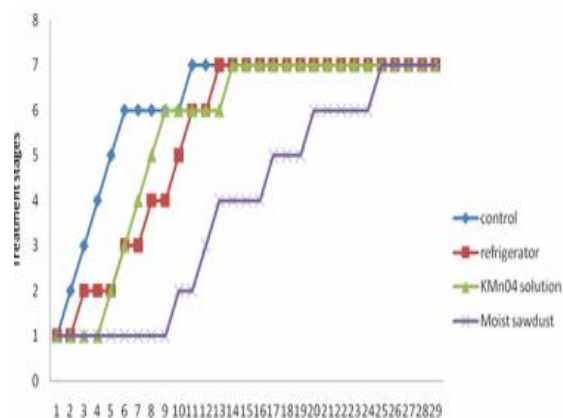


Figure 2. Color ratings of *M. acuminata* var. *dwarf Cavendish* under different storage conditions

#### 4. Discussion

Two fungi *A. niger* and *R. solani* were isolated from *M. sapientum* and *M. acuminata* var. *dwarf Cavendish* and respectively (Table 3). Hansen *et al.*[14] reported the occurrence of *R. solani* in banana ground parts and above ground. The ability of these fungi to hydrolyse the sugar in the banana fruits may be due to the present of intracellular hydrolyzing enzymes. Bacteria isolated include *Streptococcus pyogenes*, *Proteus vulgaris* and *Alcaligenes faecalis* (Table 3). Their presence may be as secondary pathogen. An increase in the moisture contents of the fruits on the 5th day (Tables 1) correspond with reports of [2] who stated that degenerative changes occurring banana deterioration including increasing watery and non-acceptability of fruits. A decrease in the percentage carbohydrate and fats composition may be due to their utilization by the microorganism *ms.* Al-Zaemey *et al.*[17] reported that microorganism *ms* cause



undesirable changes and reduction in nutrient and market values of banana fruits. Refrigeration temperature ( $4\text{ }^{\circ}\text{C}\pm 1$ ) had the longest time period before initiation of ripening compared with control, fruits stored in polythene bag moistened with  $\text{KMnO}_4$  solution and fruits embedded in moist sawdust (Fig. 1 and 2). This is in line with reports of [10] and [9] who stated that with refrigerator, ripening took a longer period, reducing the environmental temperature, reduces the rate of respiration thereby delaying ripening. Fruits embedded in moist sawdust showed no mold appearance throughout the course of storage (30 days), compared with fruits stored in refrigerator and polythene bag moistened with  $\text{KMnO}_4$  solution and control (no treatment) (Fig. 3 and 4). The results of this study showed that refrigeration temperature ( $4\text{ }^{\circ}\text{C}\pm 1$ ) appeared to be the most suitable condition for prolonging the ripening of banana fruits and embedding banana fruits in moist sawdust appeared to be the best condition for improving the shelf life of banana fruits.

## 5. CONCLUSION:

The conclusion of a study on the isolation of microorganisms associated with the deterioration of banana fruits would typically summarize the key findings and implications of the research. Here's an example of what such a conclusion might include:

**Identification of Deteriorating Microorganisms:** Through our study, we successfully isolated and identified several microorganisms from

deteriorating banana fruits. These included common fungal species such as *Aspergillus*, *Penicillium*, and *Fusarium*, along with bacterial strains like *Pseudomonas* and *Enterobacter*.

**Role in Banana Deterioration:** Our findings indicate that these isolated microorganisms play a significant role in the deterioration of banana fruits. Fungal species were particularly prevalent, suggesting their involvement in the decay process, while bacterial strains might contribute to secondary infections.

**Implications for Storage and Shelf Life:** Understanding the specific microorganisms responsible for banana deterioration is crucial for implementing effective storage and preservation strategies. By targeting these organisms, we can potentially extend the shelf life of bananas and reduce post-harvest losses.

**Potential for Biocontrol and Preservation Techniques:** Further research into the biology and behavior of these isolated microorganisms could lead to the development of biocontrol agents or preservation techniques tailored to mitigate banana spoilage. This could have significant implications



for both commercial banana producers and consumers.

Importance of Hygiene and Quality Control: Our study underscores the importance of stringent hygiene practices and quality control measures throughout the banana supply chain. Minimizing contamination by these deteriorating microorganisms at every stage, from cultivation to retail, is essential for maintaining fruit quality and reducing waste.

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