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Effect of microorganisms and storage environments on ripening and Spoilage of Plantain (*Musa paradisiaca*) fruits sold in Eke Awka market

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Abstract
Activity of microorganisms and effect of storage environments on ripening and spoilage of plantain (Musa paradisiaca) fruits were investigated. It was observed that it took ripe fruits stored in the fridge longer time to develop mould spots and final spoilage. Also unripe fruits stored in the refrigerator stayed for 29 days to ripe and 28 days more to spoil. Unripe fruits stored in jute bags ripe faster in 8 days and spoil in the next 12 days. Bacteria isolated from the pulp of the spoilt ripe plantain fruits include; Lactobacillus sp, Streptococcus sp, Staphylococcus sp, Bacillus sp, and Proteus sp. Those isolated from the peel are; Streptococcus sp, Enterobacter sp, and Pseudomonas sp. The following fungi were isolated from the peels as well as the mould spot regions of the ripe fruits; Candid sp, Aspergillus sp, Saccharomyces sp, and Rhizopus sp. The bacterial organisms associated with the spoilt unripe fruits include; Streptococcus sp, Aeromonas sp, and Escherichia coli and fungi isolated are; Aspergillus sp, Candida sp and Rhizopus sp. There is no specific role played by these organisms on the ripening time of the fruits rather they accumulate to cause spoilage. The rate of accumulation of these organisms was low when the fruits were stored in the refrigerator maintained at 4^{o}C. It is therefore advised that ripe and unripe plantain fruits be stored in the refrigerator or in a very low temperature environment devoid of microorganisms to achieve long time storage.

Keywords: Food spoilage, Plantain, food storage, low temperature, spoilage organisms, ethylene gas

Introduction
Plantains (Musa paradisiaca) are perennials and herbaceous plant which belong to the family Musaceae. They are Musa cultivars with firmer, starchier fruits which are mostly intended to be consumed only after cooking or other processing, rather than being eaten raw [1]. The plants are grown primarily for their fruits and to a lesser extent to make fiber and as ornamental plants [2]. In Africa, plantains provide more than twenty-five percent of the daily energy requirements by the people [2, 3]. Fruits of plantain are either taken raw or processed into various products at various stages of ripening [4]. They play major roles in the nutrition and well-being of the people of the tropical and sub-tropical regions of the world. They are good source of income to the farmers in their respective growing regions [3, 4, 5]. Green plantains are easier to store and can stay for some days without spoiling unlike the ripe ones which are more prone to microbial attack when stored in a place suitable for microbial growth. These microbial attacks on ripe plantain results to the deterioration of the plantain fruit tissue, resulting to spoilage. Green plantain stored to attain ripening produces ethylene which is commonly called ethane gas (C_2H_4) which indirectly affects its flavour due to the stimulation of an organic compound called isoamyl acetate [3, 6]. The presence of the ethane gas in the stored bunches can also stimulate microorganisms to produce amylase which breaks down the starch in the fruit to sugar. This increases the sugar content of the fruit thereby rendering them prone to microbial deterioration. Also, the presence of the gas can cause the pectinase producing microbes to produce pectinase which breaks the pectin of the peel and the inner fruit to soften and ripen. The softness of the ripe fruits facilitates the invasion and penetration of microorganisms also causing spoilage [1]. Healthy plantain fruits are mostly green (unripe) and yellow (ripe) and the presence of black patches or spots

indicates onset of spoilage in the fruit. Sometimes spoilage Healthy plantain fruits are mostly green (unripe) and Healthy plantain fruits are mostly green (unripe) and yellow (ripe) and the presence of black patches or spots indicates onset of spoilage in the fruit. Sometimes spoilage in plantains is identified by appearance of whitish moulds on the plantain skin. Microorganisms like the bacteria and fungi can cause plantain fruit spoilage and this spoilage can be prevented most times.

Awka main market is known for abundance agricultural products which plantain is one of them. As other agricultural products, storage of both ripped and unripped fruits is a big problem. Traders pack the bunch inside their shop after days of business, lock their shops and go. Deterioration of the bunch is rampant and this brings about a lot of wastages in the farmers effort and the sellers income. The aim of this study is to check the best storage environment for both ripe and unripe plantain fruits. Also to isolate and identify microorganisms that is associated with the ripening and spoilage of the plantain fruits thereby advising the farmers and sellers on how to handle and store the fruits to last longer.

Materials and Methods

Sample collection

Ripped and unripe fruits of plantain (Musa paradisiaca) were purchased at random from different plantain sellers in Eke Awka market. Some unripe ones with mechanical abrasions were also bought. All were carried in a polythene bag to the laboratory of the Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka for the analysis.

Media Preparation

The media used were Nutrient agar for bacterial isolation, Tomato juice agar (TJA) for lactic acid bacteria, Sabouraud's dextrose agar for mould and Malt yeast extract agar for yeast isolation. The media were prepared according to the manufacturer's instructions.

Storage of the fruits in the laboratory

The ripped and unriped fruits were divided into three portions of four fingers each. The mechanical bruised unripe ones were also divided in the same manner. Swabs were taken from the fingers from each group. After that they were washed, cleaned with cotton wool moistened with 70% ethanol and stored in three different places. One group each was kept one the bench, another in a jute bag and third group in the refrigerator. Swabs were taken on the bruised portions also before washing and cleaning the bruised fruits. The ripe ones were monitored daily for signs of physical spoilage such as colour change, mould spot, peel deterioration and degree of softness. The unripe samples were monitored for signs of ripening then colour change and spoilage. Swabs were taken from the peels and the developed spots. The bruised ones were also monitored for physical deterioration, ripening and other changes which developed at storage. The swabs were shaken in sterile water in different test tubes and cultured in nutrient agar plates for bacteria, Tomato juice agar for lactic acid bacteria, Sabouraud dextrose agar for moulds and Yeast extract agar for Yeasts. The number of days taken for the ripe fruits to develop black or white spots and spoil was noted. The days taken for the unripe fruits to ripe, develop signs of spoilage and spoil completely was also checked. Ripeness was assessed visually by comparing the colour of the peel to the standard colour charts that describe the seven ripening stages as postulated [7]. The ripening progression of the plantain 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 dark (more brown than yellow). Swabs were taken from the peels after spoilage and assessed as the above.

Spoilt pulp preparation

After spoilage, the peels of the ripe samples were removed and 10 grams the pulp was macerated in 100 mls of sterile water. The mixture was homogenized and serial dilutions carried out on each sample using the tenfold dilution method. The 10^{-5} and 10^{-6} dilutions cultured as above and growth was observed after 24 hrs for bacteria, 48 – 72 hrs for yeast and mould and 48 hrs under reduced oxygen condition for lactic acid bacteria.

Microbial Isolation and identification

The pour plate method as described by Collee and Miles [8] was used. All the plates were incubated at their respective incubation conditions. Isolation and identification of the bacterial isolates were as stipulated by Cheesebrough [9]. Fungal isolates were identified using the method of Barnett et al. [10].

Effect of storage methods on spoilage of the fruits

The ripe and unripe plantain fruits stored in different conditions (on the shelf, in jute bag and refrigerator at 4^{o}C were checked for complete ripening and deterioration. The spoilage effects were assessed using the ratings of Oyewole [11].

Pathogenicity test

In order to confirm that the isolated organisms were responsible for the spoilage of the plantain fruits, pathogenicity tests were carried out. The plantain fruits both ripe and the unripe ones were washed with sterile water and cleaned with cotton wool moistened with 70% ethanol. A loopful of each isolate were inoculated in 9 mls of saline and mixed thoroughly. One milliliter of the inoculums was gently inoculated into the peel of the plantain fruits using sterile syringe and needle. They are kept in their different storage places and the formation of the spoilage signs checked after 7 days [11].

Open and Close controls.

Some Petri dishes containing sterile media without addition of samples were used to check the presence of contaminants through the media, water used in preparing media or the environment. The close control plates remain closed while open control is left open for the duration of inoculation and is closed later and incubated.

Results

Colour change of the fruits and time of spoilage

The ripe plantain fruits showed a colour change from beautiful yellow to deep yellow and brown to black on spoilage which starts from the tail end of the fruits. Spoilage starts first on the ripe fruits on the bench,

followed by the ones in jute bags and finally on the one in the refrigerator. Mould spots appeared on the sample in the bench on the 3rd day, 5th day on the jute bag and 18th day in the fridge. Total blackness and softness which showed complete spoilage appeared on the 10th, 12th and 28th days for the bench, jute bag and refrigerator respectively. The wounded unripe fruits developed blackness around the wounds and ripe faster than the unwounded ones. They also deteriorate faster than the other samples. The unripe fruits on the bench ripped after 10 days, those in the jute bag ripe faster in 8 days and spoiled within 12 days while the ones in the refrigerator took a longer time of 29 days to ripe and 28 days more to spoil.

Organisms isolated from the fruits

The same types of organisms were isolated from the different storage conditions. Bacteria isolated from the pulp of the spoilt ripe plantain fruits include; Lactobacillus sp, Streptococcus sp, Staphylococcus sp, Bacillus sp, and Proteus sp. Those isolated from the peel are; Streptococcus sp, Enterobacter sp, and Pseudomonas sp. The following fungi were isolated from the peels as well as the mould spot regions of the ripe fruits; Candid sp, Aspergillus sp, Saccharomyces sp, and Rhizopus sp. Bacteria associated with the spoilt unripe fruits include; Streptococcus sp, Aeromonas sp, and Escherichia coli and fungi isolated are; Aspergillus sp, Candida sp and Rhizopus sp. The morphological and biochemical characteristics of these bacteria and fungi were as shown in Tables 1, 2 and 3. Table 4 showed the type of spoilage caused by the different organisms after the pathogenicity tests.

Table 1: Some morphological and biochemical characteristics of the bacterial isolates from the plantain fruits

Isolates	Colony Morphology	Gram Stain	Spore test	Motility test	Ura Se test	Cata lase	Citrate	MR	VP	Indole	H_2S	Gel atine	KCN	Coagulase	Glucose	Lactose	Maltose	Sucrose	Manitol	Probable Organism
1	Cream, Rough, Opaque & Circular.	+ve Long rod in chains	+	+	-	+	+	+	-	-	-	-	-	-	AG	-	-	-	-	*Bacillus Sp*
2	Creamy Smooth Raised	-ve rods in chains	-	+	-	+	-	nd		nd	nd	nd		nd	-	-	-	-	+	*Aeromonas sp*
3	Discoid Colony	+ve cocci in chains	-	-	-	Haemolysis in blood agar														*Streptococcus sp*
4	Raised Whitish Swarming	-ve short rods	-	+	+	+	+	+	-	+	+	-	+	+	AG	-	-	-		*Proteus*
5	Smooth Mucoid Circular	-ve short rods	-	+	-	+	+	-	+	-	-	-	+	-	A	A	-	A		*Enterobacter sp*
6	Cream white, non-viscous flat	-ve short rods	-	+	-	-	-	-	-	+	-	-	-	-	A	A	-	A		*Escherichia coli*
7	Blue to dirty green & convex	-ve rods	-	+	-	+	+	-	-	-	-	-	+	-	AG	-	-	-		*Pseudomonas aeruginosa*
8	Creamy, smooth, circular	+ cocci in clusters	-	-	-	+	+	-	-	-	-	-	-	+	A	-	-	-		*Staphylococcus aureus*

9	**Gray to white on TJA**	**+ ve long rods**	**+**	**-**	**-**	**-**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**Nd**	***Lactobacillus sp***

Key: + = positive, - = negative, A = acid, AG = acid and gas, nd = not determined, TJA = Tomato juice agar

Table 2: Morphology and biochemical properties of the yeast isolates

S/ no	Culture Characteristics	Cell Morphology	Sugar fermentation							Sugar assimilation							Probable Organism
			Glucose	Maltose	Lactose	Galactose	Sucrose	Dextrose	Mannitol	Glucose	Maltose	Lactose	Galactose	Sucrose	Dextrose	Mannitol	
1	**Cream white and smooth**	**Budding cells and pseudohyphae**	-	-	-	-	+	+	-	+	+	-	-	+	-	-	***Candida sp***
2	**Smooth, creamy white**	**Budding cells**	+	+	-	-	+	-	+	+	+	-	-	+	-	-	***Saccharomyces sp***

Table 3: Morphological characteristics of the mould isolates

S/no	Young culture morphology	Old culture morphology	Microscopy	Texture	Days	Probable organisms
1	Whitish with yellow reverse	Blue-green to dark-green	Double branching septate hyphae, short conidiophores	Powdery and velvety	3-4	Aspergillus sp
2	Dense grayish cottony	Green to brown, to black filling the plate	Oval non-septate hyphae with sporangiophores	Fluffy and cottony	2-3	Rhizopus sp
2	Light whitish with discrete head	Black on aging	Septate hyphae and short conidiophores	Powdery and fluffy	3-4	Aspergillus sp

Discussion

Ripe and unripe plantain fruits (Musa paradisiaca) were stored in different methods, environments and temperature. The ripe ones were allowed to overripe and spoil while the unripe ones were stored to ripe, overripe and deteriorate. Fruits with mechanical injuries from the farm, as a result of transportation or handling were also examined for the type of organisms that entered the wounds thereby spoiling the fruits. The wounded fruits ripe faster than the unwounded ones and at the same time spoil faster. This may be as a result of enzymatic activities of the peel enzymes and the invaded microorganisms [11].

During ripening of cooking 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). Climacteric fruits like plantains and bananas produce ethylene gas during ripening [3, 12]. This gas increases considerably as ripening progresses [3, 13]. The accumulation of the ethylene gas helps in the ripening process. The fruits on the bench liberate their ethylene into the surrounding air and this may be the reason why they did not ripe faster. In the jute bag, once the gas is produced, they accumulate inside the bag and the bag prevents their escape. This makes the fruits in the bag to ripe faster. This is in line with the findings in [3] which also found out that fruits of Musa species stored in jute bags had a higher percentage rate of fruit ripening.

Fruits stored in the fridge took a longer time to ripe and spoil. The findings in [11] also showed the same effect on another Musa specie (banana) which he stored in the refrigerator at 4^{o}C and found that it ripped faster than those stored using other methods. This also supports the findings of Medlicott *et al.*, [14] that refrigerator ripening took longer time, by reducing the environmental temperature, reducing the rate of respiration thereby delaying ripening. Unripe plantain fruits stored in the refrigerator can stay longer to ripe as well as to spoil.

Bacteria isolated from the pulp of the spoilt ripe plantain fruits include; Lactobacillus sp, Streptococcus sp, Staphylococcus sp, Bacillus sp, and Proteus sp. Those isolated from the peel are; Streptococcus sp, Enterobacter sp, and Pseudomonas sp. The following fungi were isolated from the peels as well as the mould spot regions of the ripe fruits; Candid sp, Aspergillus sp, Saccharomyces sp, and Rhizopus sp. The bacterial organisms associated with the spoilt unripe fruits include; Streptococcus sp, Aeromonas sp, and Escherichia coli and fungi isolated are; Aspergillus sp, Candida sp and Rhizopus sp. (Tables 1, 2 and 3). Some of these organisms were reported by Fajinmi et al., [3] as the organisms associated with spoilage of Musa species. The ability of fungi to hydrolyze sugar in the plantain fruits may be due to the presence of intracellular hydrolyzing enzyme [11]. Another researcher [15] reported that microorganisms cause undesirable changes and reduction in nutrient and market values of banana fruit and this is also the case with plantain [11].

Spoilage of plantain fruits can cause poor quality of plantain fruits. This brings about poor market value and reduction of profit for farmers and traders. They also cause food borne diseases and food poisoning. It can be assumed that the spoilage of foods can be influenced by the method of storage or processing, temperature of storage, environment and the nutrient availability for the microbial growth. This spoilage can be reduced by storing plantain in lower temperatures (refrigerator) which can reduce the rate of ripeness which brings about pectinase action and subsequent microbial attack. Improved processing and

packaging can help reduce the easy availability of the nutrients to microorganisms. Mechanical injuries should also be avoided during harvesting and transport to reduce microbial attack on foods. The refrigeration remains the best method of plantain storage for it to last longer. In the absence of the fridge, efforts should be made to provide very low temperature for storage of plantain fruits and other Musa fruits.

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