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Microbial rotting and preservation of banana fruits (*Musa sapientium* L.) in Nigeria

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Abstract

The bacterial and fungal pathogens associated with the watery rot of banana fruits were isolated and identified. The bacterial pathogens were *Pediococcus* sp., *Propionibacterium* sp., and *Pseudomonas aeruginosa.* The fungal pathogens which also showed cellulolytic activities were *Botryodiplodia theobromae, Aspergillus niger* and *Rhizopus* sp. These organisms caused rot at room temperature but were unable to cause rot at 5°C and 10°C. Banana fruit rot was generally prevented at relative humidities of 10, 50, 80, 90 and 100%, except that *Botryodiplodia theobromae* caused rot at 10, 50 and 80% RH. Microbial infection of healthy fruits resulted in a depletion of the total carbohydrate, crude protein, reducing sugar, free fatty acid and ascorbic acid contents. There was an increase in the total lipid and moisture contents of spoilt fruits. Preservation of banana fruits with fungicides and chemical preservatives was investigated. The use of fungicidal wax emulsion was observed to delay fruit ripening and prevented moisture loss thereby controlling the onset of rot.

Introduction

Edible banana (*Musa sapientium* L.) is widely distributed in the tropics especially in the West Indies, India, West and Central Africa, where it is consumed directly by man or used as livestock feeds and in the preparation of alcoholic drinks (Simmonds, 1966).

Banana is an important source of carbohydrates for man. It is also a rich source of protein although the amount varies between 1.3% and 1.6% of total weight (Oyenuga, 1968). Banana fruits also have a high content of potassium, chlorine, sodium, phosphorus, magnesium, and vitamins such as carotene, thiamine and ascorbic acid.

Despite the nutritional status of the banana fruit, its perishable nature has limited its distribution. One of the biggest problems in the banana trade is storage losses due to fungal infection as a direct consequence of mechanical damage (Shillingford, 1970). The growth of these pathogens is favoured by the high temperatures and humidities in the tropics. The common storage diseases of bananas include crown rot caused by a mixture of fungi, bacteria and yeasts (Greene and Goos, 1963; Lukezie *et al.*, 1967; Meredith, 1971), anthracnose disease caused by *Colletotrichum musae* (Meredith, 1960) and *Botryodiplodia theobromae* (Slabaugh and Grove, 1982), finger and main stalk rot, black end disease and squitter disease caused by *Nigrospora sphaerica* (Meredith, 1961).

The current emphasis on food production in Nigeria has necessitated the development of adequate preservative techniques. The work reported in this paper describes the micro-organisms associated with the spoilage of a local variety of banana fruit in Nigeria, the effects of microbial infection on nutritive value, and the various post-harvest treatments aimed at prolonging the storage life.

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Materials and methods

Collection of samples

Bunches of mature, unripe and spoilt banana fruits were purchased from the Tejuosho market in Lagos, Nigeria. They were transported to the laboratory in clean polythene bags and were washed and surface-sterilized with ethyl alcohol (70% v/v) before they were used for various experiments.

Isolation of spoilage microflora

Fruits showing obvious signs of spoilage were selected and homogenized in sterile distilled water. Serial dilutions of homogenates were spread on nutrient agar and potato dextrose agar plates for the isolation of bacteria and fungi, respectively. The plates were incubated at room temperature (28 ± 2°C) for 48 h. The isolates were subsequently identified on the basis of their morphological, staining and biochemical characteristics.

Pathogenicity tests

The abilities of bacterial and fungal isolates to cause spoilage were established using the methods of Meredith (1960) and Greene and Goos (1963). Healthy, unwounded fruits were surface-sterilized with mercuric chloride (0.1% w/v), streptomycin (0.1% w/v) and absolute alcohol. They were subsequently washed with sterile distilled water to remove the residual chemicals. Bacterial cells and fungal spore suspensions were introduced into scalpel wounds on the banana skin near the crown, the middle of the finger and near the cigarette butt of the finger. Controls were inoculated with sterile distilled water and all wounds were sealed with petroleum jelly.

The inoculated fingers were kept in sterile Kilner jars and kept at room temperature until visible signs of rot appeared. Ten banana fingers were inoculated with each isolate and the degree of rot was scored according to the arbitrary disease index of Shillingford (1977). The diseased tissues were cut, washed in two changes of sterile distilled water and then crushed aseptically in sterile distilled water (10 ml). Loopfuls of the resulting suspensions were streaked on nutrient agar and potato dextrose agar plates in order to re-isolate the pathogens.

Assay of cellulolytic enzymes

Fungal isolates were grown in a mineral salts medium containing carboxymethyl-cellulose (2.0% w/v) as sole carbon source according to Pyc *et al.* (1977). Cellulase activity was determined in culture filtrates using avicel (cellulose microcrystalline), carboxymethyl-cellulose and native cellulose as substrates. The assay tubes contained the substrate solution (2.0 ml, 0.5% w/v), acetate buffer (0.2 M, 2.0 ml) and the enzyme solution (2.0 ml). The mixtures were incubated at 45°C for 30 min. Progressive changes in cellulase levels in fungal cultures were assayed on a daily basis for 12 days. Reducing sugar released in the assay tubes, was measured by the Nelson-Somogyi method (Nelson, 1944). Enzyme activity was expressed as the amount of reducing sugar released per ml of culture filtrate.

Chemical analyses of banana fruits

Total carbohydrate content of fresh and rotted fruits were determined by the anthrone reagent method (Plummer, 1978) whilst the reducing sugar content was determined by the Nelson-Somogyi method using glucose as standard. Moisture content per g of fruit was determined by heating up the pulp in a crucible at 103°C for 2 h. The percentage of moisture was calculated from the weight difference. Lipid content was estimated by refluxing a known weight of fruit pulp in petroleum spirit. The oil extract was separated from solvent by distillation whilst the residue was weighed as the amount of lipid in the sample. The free fatty acid level was determined by titration with potassium hydroxide (0.1 M) using oleic acid as standard. Crude protein content was measured by the semi-micro Kjedal distillation method.

Effects of environmental conditions on rot development

The effect of temperature on rot development was studied by incubating inoculated fruits at 5, 10 and 30°C for a period of 2 weeks. For the effect of relative humidity, inoculated fruits were kept in desiccators containing different concentrations of NaOH corresponding to relative humidities of 10, 50, 80, 90 and 100% at ambient temperature.

Effects of fungicides on banana rot control

Freshly-prepared solutions of benlate and brestan were used at the concentrations of 0.1, 0.5, 1.0, 2.0 and 3.0% (w/v). Washed banana fingers were sprayed with spore suspensions of fungal isolates. When the fingers had dried, they were dipped in the appropriate fungicide solution, drained and kept in sterile Kilner jars. Ten fingers were used for each treatment and were kept at room temperature for 2 weeks after which the severity of rot was assessed. Untreated fingers served as controls.

Effect of fungicides on spore germination and vegetative growth of fungal isolates

Spore suspensions were prepared from sporing cultures and added in aliquots (2.0 ml) to tubes containing different concentrations of benlate and brestan (0.1—3.0% w/v). Germination inhibition tests were carried out on PDA plates at room temperature. The number of colonies which developed on PDA plates were scored relative to the initial spore density. To test the effect of fungicides on vegetative growth, they were added in 2.0 ml aliquots to molten PDA (18.0 ml), mixed and poured into sterile Petri dishes and allowed to solidify. The plates were subsequently inoculated at the centre with a culture disc (3.0 mm size) of each fungus and incubated at room temperature. The colony diameter was measured after 7 days. The percentage inhibition of growth was computed as described by Arinze *et al.* (1975).

Preservation of banana fingers with fungicidal wax coating

Green, unripe banana fingers were washed, surface sterilized with absolute alcohol and dipped in a wax emulsion with or without the fungicides benlate and brestan (1.0, 2.0 and 3.0% w/v) for 1 min. They were subsequently stored in sterile jars at room temperature for 20 days during which the weight loss and disease incidence was monitored.

Application of chemical preservatives

Chemical preservatives, including sodium hypochlorite, sodium chloride, sodium benzoate, sodium sulphite, sodium orthophenylphenate and zinc carbonate, were prepared at different concentrations (0.1, 0.5, 1.0, 2.0, 2.5 and 3.0% w/v) and applied to the skin of healthy, green banana fingers for 5 min. They were then packed loosely in clean sterile polythene bags for daily observations. The numbers of spoilt banana fingers from each treatment were recorded as a percentage of the original number used.

Results

Micro-organisms associated with banana rot

Some bacteria and fungal strains were isolated from spoilt banana fruits and pathogenicity tests confirmed their involvement in causing spoilage. The bacteria were characterized on the basis of their cultural, biochemical and physiological properties as *Pseudomonas aeruginosa, Pedicoccus* sp. and *Propionibacterium* sp. The fungal pathogens were identified as *Botryodiplodia theobromae, Aspergillus niger* and *Rhizopus* sp. The fungal isolates were observed to cause more extensive rotting of the fruits relative to bacteria.

Effects of isolates on the nutrient composition of banana fruits

Table 1 shows the comparative effects of microbial infection on the nutrient status of banana fruits. There was a considerable decrease in the total carbohydrate content with *Aspergillus niger* showing the highest rate of depletion. The reducing sugar level in the unripe fruit was very low, whilst the level was higher in ripe infected, than in ripe uninfected, fruits. There was also a general decrease in the levels of crude protein, lipids, free fatty acids and vitamin C in infected fruits. However, the moisture content was higher in infected fruits.

Production of cellulolytic enzymes by fungal isolates

Figure 1 shows the progressive release of reducing sugars from three cellulosic substrates by culture filtrates of *B. theobromae, A. niger* and *Rhizopus* sp. Enzyme activities recorded for all the strains using avicel as substrate were generally higher than those for native cellulose and carboxymethylcellulose. Enzyme activities in culture filtrates increased with time and two peaks of maximum activity were observed between the third and fifth days and also between the eighth and twentieth days of incubation.

Control of fruit rot by fungicidal action

Benlate was found to control *B. theobromae*-induced rot at the lowest concentration of 0.1% (w/v) whilst it also controlled 67% of *A. niger*-induced rot at the same concentration. However, the fungicide was effective on *Rhizopus*-induced rot at concentrations between 2.0 and 3.0% (w/v).

Brestan on the other hand was effective for controlling *B. theobromae*- and *A. niger*-induced rots at concentrations of 1.0% and above. It gave 100% control of *Rhizopus*-induced rot at concentrations of 2.0% and above.

Table 1 Comparative effects of bacterial and fungal pathogens on the nutrient components of banana fruits

Parameters (%)*	Fresh unripe banana	Ripe uninfected banana	Banana fruits infected with strains of:						
			Pediococcus	*Propionibacterium*	*Pseudomonas*	*B. theobromae*	*A. niger*	*Rhizopus*	Water**
Total carbohydrates	30.65	30.45	20.20	12.50	13.75	14.38	11.88	21.88	31.25
Total reducing sugars	0.01	7.00	12.00	13.50	12.50	9.00	9.00	6.50	14.00
Crude protein	0.00	1.63	0.71	1.28	1.05	0.70	0.35	1.40	1.49
Total lipids	0.00	0.30	2.80	0.80	0.40	2.40	0.60	1.20	0.30
Free fatty acids	0.00	3.43	0.34	1.58	3.16	0.26	4.22	0.26	3.40
Vitamin C	0.00	0.10	0.07	0.07	0.07	0.04	0.04	0.03	0.08
Moisture	68.00	75.00	75.50	77.00	76.80	84.50	84.80	81.00	75.50

* Average of three replicates.
** Control of distilled water.

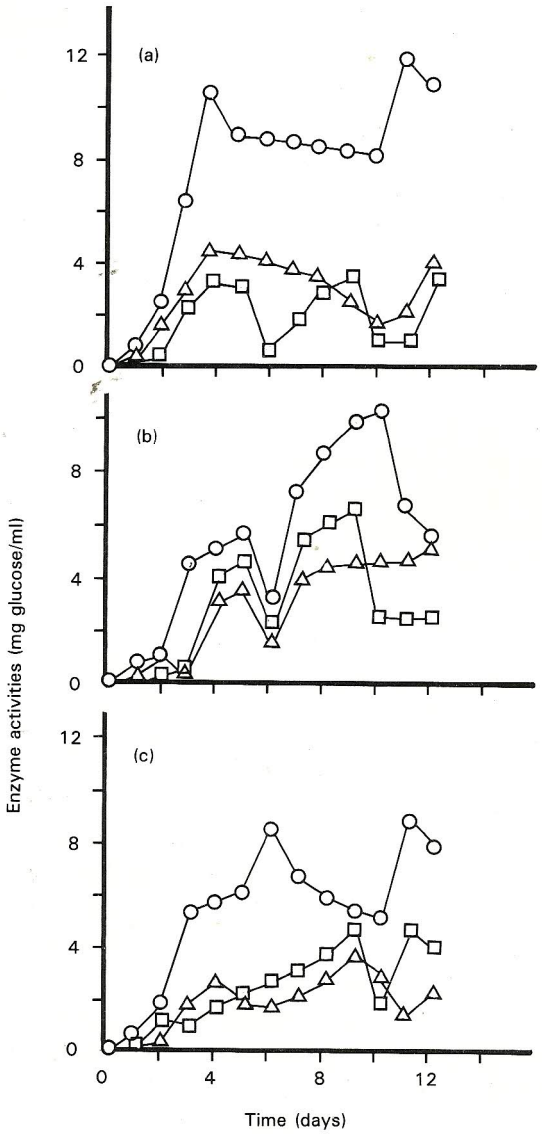

Figure 1 Progressive release of cellulolytic enzymes in cultures of fungi associated with banana spoilage using different substrates. (a) *Botryodiplodia theobromae*; (b) *Rhizopus* sp.; (c) *Aspergillus niger*. ○, Avicel; □, Carboxymethylcellulose; and △, native cellulose.

Table 2 Cumulative losses in weight of wax-coated banana fingers during storage

Treatment	Mean wt of single fruit (g)	Weight loss in storage (%): 4 days	8 days	12 days	16 days	20 days
Uncoated fruits (control)	57.72	0.45	1.89	3.74	6.00	7.35
Wax emulsion only	68.01	0.50	0.74	1.69	2.21	2.71
Wax emulsion + 1% benlate	62.32	0.29	0.49	1.29	1.76	2.03
Wax emulsion + 2% benlate	62.67	0.13	0.31	1.23	1.54	2.00
Wax emulsion + 3% benlate	71.27	0.08	0.21	0.80	1.78	1.84
Wax emulsion + 1% brestan	65.96	0.32	2.23	3.34	4.26	5.18
Wax emulsion + 2% brestan	62.58	0.29	1.33	2.11	2.82	4.08
Wax emulsion + 3% brestan	62.27	0.17	1.50	2.00	2.95	3.67

Spore germination in *B. theobromae, A. niger* and *Rhizopus* sp. was completely inhibited by brestan at a concentration of 0.1% (w/v), whereas the spores of *Rhizopus* were slightly more susceptible to the inhibitory effects of benlate than those of *A. niger*. The fungicides also showed marked effects on the vegetative growth of the fungal hyphae. Benlate completely inhibited the mycelial growth of *B. theobromae* and *A. niger* at 1.0% (w/v) whilst *Rhizopus* was inhibited at concentrations above 1.0% (w/v). Brestan, however, completely inhibited the growth of all fungal strains at concentrations below and above 1.0% (w/v).

Fungicidal wax coating and storage behaviour of banana fruits

Coating of banana fruits with ordinary wax emulsion reduced the rate of weight loss from 7.35% observed in uncoated fruits to 2.71% (Table 2). The addition of benlate to the wax coating was more effective in preventing weight loss in banana fruits than the ordinary wax emulsion alone. Higher concentrations of benlate gave greater prevention of weight loss during storage. However, the addition of brestan to the wax coating did not show any added advantage over the use of wax emulsion alone. The ripening rate of wax-coated fruits was significantly reduced whereas the addition of benlate and brestan to the wax emulsion prevented ripening completely. Fungicidal wax coating ultimately preserved banana fruits by preventing wastage due to disease.

Effect of chemical preservatives on spoilage

Sodium chloride was observed to be the most effective preservative since it was able to effect a 100% control of spoilage after storage for a 2-week period. Sodium sulphite, sodium benzoate and sodium hypochlorite also gave 100% control of fruit rot but for a lesser duration of about 12 days.

Discussion

Banana fruit rot is evidently a direct consequence of microbial deteriorative activities. The pathogens were always present in rotten fruits although fungi appeared to be the major agents and most especially *Botryodiplodia theobromae* which has been associated with banana rot in many countries (Greene and Goos, 1963; Shillingford, 1970; Bhargava *et al.*, 1966). The occurrence of premature ripening of fruits inoculated with fungal isolates could be attributed to the production of amylases in these organisms which contributed to the ripening process thereby enhancing the rate of rotting. The spoilage organisms invaded the fruit tissues through the production of hydrolytic enzymes with a concomitant depletion of nutrients available.

It was possible in this study to delay ripening and thereby extend the storage life of banana fruits at high relative humidities of 80—100%. The early ripening of fruits at low relative humidities (10—50%) was prompted by water loss. The mechanism by which water stress reduces the green life of banana fruits has been attributed to the rapid accumulation of ethylene which results in a more rapid ripening (Littman, 1972). Thus, infected banana fruits kept in low relative humidity chambers ripened earlier and rotted at a faster rate.

The ripening rate of banana fruits treated with ordinary and fungicidal wax emulsions were slower than in untreated controls. Wax emulsion containing benlate was observed to increase the storage life of bananas by approximately 70% whilst brestan-containing wax coating increased the storage life by approximately 100%.

Fungicidal wax coating has similarly been used to increase the storage life of mangoes (Mathur and Subramanyan, 1956) and oranges (Lodh *et al.*, 1963). The inherent ability of wax coating to control fruit rot lies in its ability to delay ripening since fruits are more susceptible to spoilage in the ripened state. It is therefore evident that any preservative technique which inhibits ripening prolongs the storage life of banana fruits.

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