Microbial contribution to spoilage of African breadfruit (Artocarpus communis, Forst) during storage

Olusegun B. Ajayi¹ & Tinuola T. Adebolu²

¹Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria
²Department of Microbiology, Federal University of Technology, Akure, Nigeria

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Abstract
The contributions of microorganisms in the deterioration of African breadfruit during storage were investigated in this study. Matured fruits of the seedless variety of the African breadfruit (Artocarpus communis, Forst) were stored under different temperature conditions and morphological changes observed at 24-h intervals for 120 h. Spoilage of breadfruit was observed after 72 h with microbial growth. Although all the fruits in the different media deteriorated by the 72nd hour (this was revealed in morphology and confirmed by the proximate analysis which showed an increase in %crude protein in all the stored fruits), microbial growth was observed only in those fruits stored at room temperature and in water, and there was no significant microbial growth in fruits stored in refrigerator, freezer, and vinegar. A higher rate of deterioration (i.e., higher %crude protein) was observed in morphology of fruits which had microbial growth during storage (i.e., those stored in the room, under water, and refrigerator) than in those stored fruits with no significant microbial growth. The difference between the %crude protein in fruits where there is microbial growth and that of the fruits where there is no microbial growth (i.e., freezer and vinegar) proved to be significant (P < 0.05). The study thus reveals that microorganisms play a substantial role in the spoilage of African breadfruit. A strain of the Aspergillus sp., two strains of the Penicillium sp., and a strain of the Molinia sp. were isolated as fungal spoilage organisms. Bacillus sp. and Pseudomonas sp. strains were isolated as bacteria spoilage organisms.

Introduction

Breadfruit
Breadfruit is an important energy food containing starch and sugars, especially common in the Pacifics (Secretariat of the Pacific Community [SPC] 2006). The levels of starch and sugars vary according to the stage of ripeness at which the fruit is eaten (SPC 2006). The Pacific varieties of breadfruit are reputed to be rich in vitamin C, thiamin, and the precursor of vitamin A – carotenoids (a fair source of vitamin B and niacin); the amount of these varies with ripeness. It is also rich in fiber and the mineral calcium (Ragone et al. 1996). It contains 5.3 g/100 g of protein (SPC 2006).

African breadfruit varieties
The African breadfruit is of two varieties – the seeded (Artocarpus altilis) and the seedless (Artocarpus communis). These varieties of the African breadfruit contain on average a higher amount of dietary fiber and protein – 36 g/100 g and 6.1 g/100 g, respectively – than that of the Pacific (Ejidike and Ajileye 2007). Moreover, having compared the nutrient value of the African breadfruit with that of existing carbohydrates sources, such as yam, potato, and cassava, several scholars have concluded that African breadfruit (A. communis) have the nutrient potential to replace several of these carbohydrate sources in food consumed on the continent, especially flour-based foods (Ravindram and Sivalcanessan 1995; Steve et al. 2007).
The media used for the isolation of the microorganism were Sabouraud’s dextrose agar and nutrient agar. These agars were prepared according to manufacturer’s instructions (BDH). The media were sterilized at a temperature of 121°C for 15 min in an autoclave.

Preparation of the breadfruit samples

Twenty-five healthy fruits were harvested at the matured, but not fully ripened, stage from a breadfruit tree from a location in Akure, Ondo State, Nigeria. These fruits were washed in chlorinated water, rinsed with sterile water, and blotted dry with a clean towel. Five of the fruits were cut, dried, and subjected to proximate and mineral content analyses, which included ash content, carbohydrate, crude fiber, crude fat, crude protein, and moisture compositions. This was to ascertain their proximate and mineral content from the field. Ten of the remaining fruits were cut into halves to observe the morphological changes and microbial growth in the pulp of the breadfruit as time progresses in the course of the study, whereas the remaining 10 fruits were left whole to observe the changes that occur on the bark of the fruit.

Growth of microbial spoilage organisms

The fruits that were not dried in the oven were then divided into five groups of four fruits each. Each group comprised two whole fruits and four halves. The five groups were then kept under different storage conditions for 120 h as follows:

- The first group was placed on sterile trays laid with aluminum foil and stored in a temperature-controlled room at 28 ± 2°C. The second group was stored in water at a big water bath with temperature regulated and maintained at 20 ± 2°C. The third and fourth groups were kept in a refrigerator and freezer at temperatures of 10 ± 2°C and −6 ± 2°C. The last group was kept in a big jar of vinegar (10% acetic acid). After 120 h, the microbial load was determined and the isolation of spoilage organism determined.

Isolation of spoilage organisms

Samples of fruits stored under different conditions were taken and prepared for microbiological examination through the method of Fawole and Oso (1998) and Sanni et al. (2002a,b). The surface of the pulp and bark and core were scraped into a Petri dish and prepared for examination by 10⁻⁵ dilution. The 10⁻⁵ dilution was used as the inoculum and it was plated out using pour plate method. The plates were then incubated at 37°C for 24 h for bacteria and at room temperature for 72 h for fungi. The pure microbial isolates were streaked onto new culture plates and incubated for 48 h.
Preliminary identification tests

Various tests were carried out to identify and characterize the microorganisms that proliferate on the fruits during storage, according to Fawole and Oso (1998) and Sanni et al. (2002b). These tests include gram staining, motility tests, nitrate reduction, oxidase tests for bacteria, and microscopic morphological observations for fungal isolates.

Figure 1. Proximate composition of breadfruit before and after storage for 120 h.

Figure 2. Mineral contents of breadfruit before and after storage for 120 h.

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Proximate and mineral content analyses

Standard proximate analyses methods were used according to Osundahunsi et al. (2003) and Ogunbanwo et al. (2008). Breadfruit flour was produced by drying using Gallenkamp OV-160 laboratory oven drier and milling of the diced breadfruit using laboratory blender (Phillips). The moisture content was determined by oven-drying weighed samples and calculations using the standard American Organisation for Analytical Chemistry (A.O.A.C.) methods (Figs. 1 and 2). Other proximate analyses were carried out on the flour on dry matter basis according to conventional methods of A.O.A.C. as described by Ogunbanwo et al. (2008). Ash content was determined by dry ashing in a muffle furnace at 550°C, crude protein was determined using the micro-Kjeldahl technique, crude fiber was determined as described by Kirk and Sawyer (1991), and crude fat was determined using a Soxhlet extractor (Tecato, Ikeja, Lagos, Nigeria) with petroleum ether. The %carbohydrate is calculated as the difference between the total mass of the breadfruit sample taken and the %protein, %fat and the %minerals analysed from the sample. All determinations were performed in triplicate.

Mineral content analysis such as iron, calcium, potassium, sodium, manganese, magnesium, and zinc was conducted on the ash solution of the breadfruit flour using atomic absorption spectrophotometer (Perkin Elmer 500 and Varian AA 475).

Pathogenicity test

A test was conducted on fresh samples of fruits from the same breadfruit tree in the described location to confirm the pathogenicity of the organisms isolated from the stored fruits. This was performed by mixing a slightly turbid solution of the isolates in sterile water and inoculating the bark and pulps of the washed fresh fruits. The fruits were then placed under a laminar flow station at room temperature (i.e., 28 °C) for 120 h. At the expiration of the time, the inoculated fruits were checked for growth of the microorganisms.

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA), the means being tested for significance at 5% level using Duncan’s multiple range (DMR) test.

Results and Discussion

A strain of the Aspergillus sp., two strains of the Penicillium sp., and a strain of the Molinia sp. were isolated as fungal spoilage organisms. Bacillus sp. and Pseudomonas sp. strains were isolated as bacteria spoilage organisms. These species of microorganisms were suspected after preliminary microscopy and biochemical tests were conducted (Table 1). However, molecular analyses of the strains to determine their genetic component and confirm their identity were not covered by the scope of this study.

Pathogenicity test revealed proliferation of the microorganisms inoculated on the fresh sample indicating their spoilage activities. The test thus proved positive.

In this study, it was observed that there was significant growth of microorganisms on the breadfruit stored at room temperature (28 ± 2°C) and in water at 20 ± 2°C,
while no significant growth ($P \leq 0.05$) was observed for fruits stored in refrigerator, freezer at $10 \pm 2^\circ C$ and $-6 \pm 2^\circ C$, respectively; this was also true for vinegar (Table 2). It is interesting to note that morphological changes which indicate deterioration were observed in the fruits stored in the refrigerator, freezer, and vinegar (Table 3). This supports the established opinion that respiration causes spoilage in breadfruit. However, statistical analysis revealed significant difference between the deterioration observed in cases where microbial growth exists and where there was no growth. This is confirmed in the highest increase in %crude protein observed where there is high microbial load (i.e., in those fruits stored in the room and in water) (Fig. 1). The high difference in the %crude protein shows that microorganisms made significant contribution to the deterioration of breadfruit.

**Conclusion**

Although the African breadfruit (A. communis, Forst) deteriorates due to its high respiration rate in storage, this study has revealed that deterioration rate becomes significantly higher with presence of spoilage microorganisms. Provision of good storage conditions will substantially delay spoilage of breadfruit, the knowledge of which will contribute essentially to nutrition and economy of the Nigerian populace.

**Conflict of Interest**

None declared.

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