Fungi associated with selected fermented foods in Sierra Leone

Felixtina E. Jonsyn

Department of Biological Sciences, Njala University College, University of Sierra Leone, Private Mail Bag, Freetown, Sierra Leone

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Introduction

Fermentation is the oldest method of processing and preserving food for human consumption. The technology is worldwide. It is important that the essential microorganisms in each indigenous fermentation process be isolated, identified, and studied with respect to the individual products, growth conditions and enzymology (Steinkraus 1982). In Sierra Leone, local fermentation processes depend upon chance inoculation with indigenous microbes and as such, the microorganisms involved are both unknown and uncontrolled. Current knowledge of some of the toxic metabolites (mycotoxins) produced by fungi necessitates extensive investigation into their role in fermentation. Two popular fermented foodstuffs were used in this study, foofoo from cassava (Manihot esculenta) and ogi from maize (Zea mays). Foofoo constitutes one of the two staple foods of the creoles in Sierra Leone although it is rapidly being discovered by the other tribes. Ogi, when supplemented with high protein foods like milk or eggs, makes an excellent weaning baby food, an ideal food for the sick and convalescent and an important breakfast cereal for old and young alike.

For the traditional preparation of foofoo, fresh cassava tubers are washed, peeled and soaked in tap water for about 3 to 4 days. The hydrated tubers are then grated and packaged in either straw or wire mesh baskets lined internally and covered in large leaves of Mitragyna stipulosa Kuntze, thus creating anaerobic conditions. A piece of metal or stone considered to be sufficiently heavy is placed on top of the leaf-sealed baskets to expel excess water and air. The cassava is then allowed to ferment for a period of 4 days or more. The characteristic foofoo odour and texture is an indication of the readiness of the product for marketing. In the case of ogi, maize is cleaned, steeped (1 to 3 days), wet milled into a paste, sieved into a plastic bucket, fermented (1 to 7 days) and marketed.

This investigation was carried out to determine the role; if any, of fungi in these two fermentation processes.
Materials and methods

Isolation of fungi on samples before fermentation. Maize and cassava were purchased from the local markets. Maize (3 g) was surface sterilized in 1% sodium hypochlorite at room temperature for 2 min. Using a sterile forcep, seeds were picked up, shaken rapidly to remove excess solution and then plated on malt extract agar (MEA) (Oxoid). Fresh cassava (2 g) was washed, peeled with a clean knife and rewashed twice with sterile distilled water and plated on MEA. All plates were incubated for 5 d at room temperature. Each fungi colony was monocultured in Czapek agar (Oxoid) and pure colonies sent to the Commonwealth Mycological Institute (CMI), UK, for identification.

Isolation of fungi during fermentation. Samples were obtained from traditional ogi and foofoo fermentations as follows: at daily intervals, duplicate spatula-full samples, obtained by inserting a sterile spatula into the basket or plastic bucket, were plated on MEA and incubated at room temperature for 5 d.

Isolation of fungi from fermented product. Laboratory-prepared foofoo and foofoo purchased from the local market (3 g each) were plated directly on potato dextrose agar (PDA) (Oxoid). A spatula-full of ogi sample was removed and plated as above. Triplicate samples were incubated at room temperature for 5 d. Colonies produced from single spores were transferred to PDA slants and sent to the CMI for identification.

Screening samples for aflatoxin. Samples (20 g) of visible mouldy foofoo and maize respectively and 10 g of laboratory prepared ogi were each put in a 500-ml separating funnel. Celite 545 (20 g) was added to each funnel and the contents shaken vigorously. Chloroform (200 ml) and 20 ml water were added successively and further vigorous shaking by hand continued for 30 min. Extracts were filtered through Whatman No. 1 filter paper. The chloroform (100 ml) was collected in a 150-ml Erlenmeyer flask, wrapped with aluminium foil and evaporated to near dryness in a water bath at 40°C under a hood. This process normally takes between 5 and 8 h depending on the room temperature. Extract cleaning was done using the procedures of Kellert & Spott (1980). The resulting extracts were screened for aflatoxin by spotting 10 to 15 μl standards and 20 to 50 μl of extracts on commercially purchased aluminium silica gel 60 thin-layer chromatography (TLC) plates (Merck 5553). For TLC, the procedure of Scott et al. (1970) was followed, using toluene/ethyl acetate/formic acid (5:4:1, by vol.). The plates were dried at room temperature under a hood and viewed under long- and short-range ultraviolet light. The presence of these aflatoxins were confirmed using two methods. In the first, the developed TLC plate was sprayed with 0.01 M sulphuric acid (Przybylski 1975). In the second, (Coker et al. 1984) pairs of 5 μl and 10 μl portions of standard aflatoxin and extracts containing aflatoxin were spotted on the baseline of a TLC plate; 2 μl of trifluoroacetic acid was superimposed on one of the 5 μl and 10 μl portions of the extracts and on the 5 μl of the standard. The plate was heated at 105°C for 1 min and developed as described above (see Table 1).

Screening samples for other mycotoxins. The method of Nowotny et al. (1983) was adopted with some modifications: mouldy maize, mouldy foofoo and laboratory prepared ogi (10 g) were mixed in a Waring blender with 100 ml methanol. The extract was filtered through two layers of Whatman No. 1 filter paper and the solvent evaporated (as previously described) to approximately 10 ml; 10 ml of 1 N HCl was added, mixed and extracted with n-hexane to remove fatty residue. The aqueous methanol phase was then extracted with 3 × 10 ml dichloromethane and the dichloromethane extract was dried over Na₂SO₄ and the solvent then subsequently evaporated. The residue was dissolved in 500 μl dichloromethane and 10 to 20 μl were spotted on oxalic acid-impregnated TLC plates together with standard ochratoxin and citrinin. The plates were developed in chloroform:acetone (9:1 v/v), dried at room temperature (10 min) and viewed under long- and short-range ultraviolet light. The presence of ochratoxin and citrinin were confirmed by spraying with p-anisaldehyde reagent (Scott et al. 1970) and heating (see Table 1).