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Contamination of fermented foods in Nigeria with fungi

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ABSTRACT

This study assessed the safety and quality of some fermented foods in Nigeria. Cluster sampling was used to obtain different fermented foods: maize gruel (*ogi*), locust beans (*iru*), sorghum meal (*ogi baba*), dried locust beans (dried *iru*), African oil bean seed (*ugba*) and melon (*ogiri*) from Southwest Nigeria. Moisture content, Total Titratable Acidity, pH, and fungal diversity within each sample were determined. The identity of the isolates was established through macroscopic, microscopic and molecular biology means. The moisture content and pH of analysed samples ranged from 12 to 56% and 3.60 to 8.08, respectively. The overall data on the mycobiota of the fermented foods revealed that total fungal loads of *ugba* and *ogiri* were 1.05×10^5 and 7.9×10^5 cfu/g, respectively. Generally, fungal isolates belonged to 17 genera including *Aspergillus*, *Fusarium*, *Candida*, *Saccharomyces* and *Penicillium*. The dominant fungi detected were *A. flavus* and all analysed samples were contaminated with *F. verticillioides* except for *ogi baba*. The study led to the discovery of new fungal strains and previously unreported fungal species in the selected fermented foods. The analysed fermented foods were highly contaminated with different fungal species that could potentially be toxigenic in producing various types of mycotoxins.

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1. Introduction

Fermented foods are one of the major food groups in Africa. The process of fermentation involves the breakdown of organic compounds into acids or alcohol through enzymatic action of microorganisms particularly yeasts and bacteria under anaerobic conditions (Chilton, Burton, & Reid, 2015). Fermentation has been found to enhance the nutritional, health promoting, organoleptic and preservative properties of food (Oyewole & Isah, 2012). Fermentation plays a significant role in developing economies as a technology that increases income sources, food availability, food diversity and reduces post-harvest losses. In Africa, fermented foods are mostly produced traditionally which brings about variation in the substrate used, processing conditions, packaging materials, handling and storage practices (Babajide, Oyewole, & Obadina, 2006). Examples of such foods include *iru, gari, amasi, banku, ogi, injera, mahewu* and *meriss*. *Iru* from fermented African locust bean (*Parkia biglobosa*), *ugba* from African oil bean seed (*Pentaclethra macrophylla*) and *ogiri* from melon (*Colocynthis citullus*) are important condiments used to flavor soups and stews in West Africa particularly in Nigeria. Enujiugha, Akanbi, and Adeniran (2008) delineated that *ugba* is consumed by more than 40 million people in Africa and asides from being a condiment, *ugba* also served as a snack (Olotu, Enujiugha, & Obadina, 2014). *Ugba*, *iru* and *ogiri* are also examples of vegetable proteins that are used as meat substitutes in diets and are processed with methods of wild solid-state fermentation, which gives rise to extensive hydrolysis of its carbohydrate and protein constituents (Achi, 2005). In addition to their protein content, they are rich in fat, carbohydrates and are good sources of vitamins and minerals. Their typical processing method involves dehulling, boiling, draining, fermentation, salting, drying (optional) and packaging. Maize and sorghum grains are converted to *ogi* and *ogi baba* by steeping in water (±3 days), washing, wet milling and sieving. The products are the resultant sediments from this process. *Ogi* and *ogi baba* are products of lactic acid fermentation used mostly for infant feeding and often supplemented with proteinaceous foods because of their high carbohydrate content (Oloyede, Ocheme, Chinma, & Agbejule, 2015).

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The microbial quality of these foods is influenced by intrinsic and environmental factors such as hygiene during processing, composition of substrates etc. The fermentation of melon, African oil bean seed and locust bean into *ogiri*, *ugba* and *iru*, respectively is predominantly by *Bacillus species* (Olasupo, Okorie, & Oguntoyinbo, 2016). Other bacteria species that can be isolated during the fermentation process are *Staphylococcus*, *Escherichia*, *Micrococcus*, *Leuconostoc*, *Pseudomonas* and *Corynebacterium* (Achi, 2005). However, fungal species have not been associated with the fermentation of *ugba*, *iru* and *ogiri*. *Lactobacillus* species such as *L. plantarum* and yeasts have been reported to be the fermenting organisms for *ogi* production (Okeke et al., 2015). Irrespective of the benefits derived from fermented foods, there are concerns about their safety because of the continuous and unpredictable pre- and post-processing contamination by pathogenic microorganisms some of which can be toxigenic fungi that produce harmful secondary metabolites including mycotoxins.

Some fungal species belonging to the *Penicillium, Mucor, Geotrichum,* and *Rhizopus* genera have been used in the fermentation of cheese and milks (William & Dennis, 2011, chap. 4), while others produce undesirable toxins and their presence in food has been attributed to their sporulating ability, which makes them easily contaminate the environment (Frisvad & Samson, 2007) and the food products therein. Hence, the assumption among consumers that fermented foods - especially those processed traditionally - are safe is a dangerous one as such foods could be potential sources of mycotoxin exposure and accompanied health complications. There has also been evidence of multiple mycotoxins in different food categories including fermented foods. Chilaka, De Boevre, Atanda, and De Saeger (2016) reported the co-occurrence of *Fusarium* mycotoxins such as fumonisins, nivalenol, HT-2 and deoxynivalenol-3-glucoside in fermented maize from Nigeria. Colak et al. (2012) detected Aflatoxin B_1 within the range of 0.2 and 13.2 μg/kg in *Tarhana*, a Turkish fermented cereal. Nevertheless, most microbiological studies on African fermented foods have been based on the isolation and characterisation of microorganisms that enhance the fermentation processes and only studies with limited scope have been conducted to establish the incidence of pathogenic organisms like fungi (Olasupo et al., 2016). Even though some studies have reported the presence of fungi in some fermented foods consumed in Nigeria, there is little adequate information on the spectrum of microorganisms associated with these foods since most of these organisms were identified to genus level and only a few to species level. It is worthy to note that the safety of fermented food is largely influenced by some quality parameters such as moisture content, pH and Total Titratable Acidity (TTA) and these needs to be considered during safety studies. This research was therefore aimed at assessing the fungal diversity in some Nigerian fermented foods in order to gain insight into their safety.

2. Materials and methods

2.1. Sampling

Cluster sampling was used to obtain fermented foods that included fermented: maize gruel (*ogi*), locust beans (*iru*), sorghum meal (*ogi baba*), dried locust beans (dried *iru*), African oil bean seed (*ugba*) and melon (*ogiri*) that are indigenous to Nigeria. A total of 108 samples (18 each) with an equivalent weight of 30 ± 5 g were purchased from some selected fermented food sellers in South West, Nigeria between Feburary and March 2015. Upon their collection, samples were placed into sterile containers in cooler boxes and shipped to the University of Johannesburg, South Africa where they were analysed. *Iru* and *ugba* samples were milled using a sterile mechanical blender prior to analysis.

2.2. Methodology

2.2.1. Moisture content, pH and Total Titratable Acidity (TTA) determination of fermented foods

Moisture content of the samples was determined using the method described by AOAC (2005). The pH of the food samples was measured using a pH meter (Jenway, Model 3510, Essex, UK) after calibration using standard buffers. TTA was determined using the AOAC (2005) method whereby the amount of acid (lactic acid) in each sample was determined by using the following equation:

$$\%\,Lactic\ acid = \frac{\text{Volume of base used (ml)} \times \text{Normality of alkali} \times \text{Molecular equivalent of lactic acid (90)}}{\text{Sample weight (g)} \times 10}$$

2.2.2. Isolation and identification of fungi

The samples were blended using a sterile laboratory blender for 60 s. 1 g of each sample was diluted in 9 ml of sterile 0.1% peptone water solution, vortexed and serially diluted to 10^{-10}. Solidified Rose Bengal Chloramphenicol, Czapek Yeast Extract and Malt Extract Agar plates were inoculated with an aliquot of 0.1 ml. The inoculated plates were checked for fungal colonies after incubation at 25 °C for 5 days. The colonies were counted with a colony counter, mean of fungal colonies were calculated, and results were expressed as cfu/g. Cultures were streaked onto Czapek Yeast Extract Agar and Malt Extract agar and plates were incubated at 25 °C for 5 days. Pure isolates were identified based on their macroscopic and microscopic characteristics according to keys of Klich (2002), Samson and Varga (2007) and Pitt and Hocking (2009), chap. 1. To this end, fungal isolates were stained with lactophenol blue, mounted on slides, overlaid with cover slides and placed on the stage of an optical microscope (Olympus CX40, Micro-Instruments, New Zealand) to observe the micro morphological attributes for identification at species level.

2.2.3. Molecular studies

Genomic DNA was extracted from each fungal culture using the ZR fungal DNA kit (Zymo Research, D6005, California, USA). After DNA extraction, Polymerase Chain Reaction was performed to amplify the DNA of interest within the Internal Transcribed Spacer (ITS) region using EconoTag Plus Master Mix (Lucigen) and ITS 1 forward and ITS 4 reverse primers with sequences TCCGTAGGTGAACCTGCGG and TCCTCCGCTTATTGATATGC. After amplification, the PCR products were run on a gel and the gel extracted using ZymoClean Gel DNA recovery clean-up kit (Zymo Research, D4001). The extracted fragments were sequenced in the forward and reversed directions (Applied Biosystems, Thermofisher Scientific, Big Dye terminator kit v3.1, Carlsbad, California, USA) and purified using ZR-96 DNA sequencing clean-up kit (Zymo Research, D4050). The purified fragments were run on an ABI 3500xL Genetic Analyser (Applied Biosystems, Thermofisher Scientific) for each reaction for every sample. CLC Bio Main Workbench v7.6 was used to

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