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Research Article

Assessment of the Quality and the Susceptibility Profile of fungi in Peanut Butter Hawked in Lafia Metropolis

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Abstract

Peanuts are a high-nutritional food source containing unsaturated fats, high-quality protein, carbohydrates, minerals and vitamins. The aim of the study was to determine the mycological quality of peanut butter consumed in Lafia and determine the antifungal susceptibility profile of fungal isolates. Peanut butter samples were collected from Lafia markets from which fungi were isolated, and the *In vitro* antifungal susceptibility profile was determined. The highest fungal count was from Shinge market (28.0x10^3 cfu/g), and the lowest from the College Village market (3.0x10^3 cfu/g). Of the fourteen fungal species isolated, genus *Aspergillus* had four species followed by genera *Fusarium* with three species. *A. flavus* was obtained from all the markets while *A. carbonarius, A. versicolor, A. parasiticus, Alternaria alternata, Saccharomyces cerevisiae, Candida* sp. and *Trichophyton verrucossum* were found in one market each. Antibiotics sensitivity test showed the highest inhibitory activity against *F. graminearum* and *A. parasiticus* from the Roundabout market (100% inhibition). Nystatin was the most effective antibiotic with inhibition of 1.87±0.23 cm against *Alternaria alternata*, and inhibiting all the isolates, while fluconazole was the least effective antibiotic tested. The study concludes that peanut butter consumed in Lafia has high concentrations of microorganisms making it unsafe for consumption.

Keywords: *Food, Contamination, Antibiotics, Aspergillus, Susceptibility*

1. Introduction

Peanuts (*Arachis hypogaea* L) or groundnut is a popular food crop. It is a generally acceptable nut crop with economic and high nutritional value due to its unsaturated fatty acids, protein, carbohydrates, minerals and vitamin contents (Settaluri et al. 2012). It is consumed roasted, cooked uncooked and in the form of paste (butter and candies). Peanut butter is a semi-perishable product traditionally produced on a small scale in Nigerian homes. To increase acceptance, different ingredients are added to improve the taste and texture during production. The consumption rate is higher in the northern part of Nigeria where the crop is mostly grown. Peanut butter has beneficial importance among which are control of blood pressure, reduction of the risk of cardiovascular disease, weight control and reducing risk of diabetes.

The shelf life of the paste is greatly affected by the addition of ingredients and water in the production chain. Other factors include the quality of groundnut, which is dependent on the agricultural practice engaged in by the farmer, storage and transportation conditions (Carminati et al. 2016). Spoilage of the butter or paste starts with the putrefaction of the paste's protein component and the unsaturated fat's rancidity when exposed to air (Okello et al. 2010). Bacterial action initiates the process and it is promoted by water; sunlight action during marketing, which accelerates rancidity. Foodborne pathogens burden the food production industry, causing heavy losses, and reducing food quality and scarcity. Bacteria and fungi contaminate groundnut before and during harvesting, and after harvesting (post-harvesting processes) (Odeniyi et al. 2019). Roasting by heating has not been effective in decontaminating microorganisms because of groundnuts' high fat content and low water activity (Ma et al. 2009).

Fungi isolated from peanuts have been shown to produce mycotoxins, which are secondary metabolites with carcinogenic, teratogenic, and immunosuppressive activities (Uzeh et al. 2021). Mycotoxicoses, salmonellosis and other disease conditions have been associated with the consumption of peanut butter (Sharma et al. 2018; CDC, 2013). Despite associated negative conditions, measures are not in place in Nigeria to monitor and control the production, storage, retailing, and consumption of peanut butter in Nigeria.

Control over production processes, packaging, storage and marketing is lacking in Nigeria despite the rate of consumption. This leads to the products being contaminated by microorganisms from water sources, spoilt or infected seeds, human contamination and pathogenic fungi in peanuts. The poor quality of preparation materials and the environment also call for concern. The aim of the study was to assess the mycological quality of peanut butter hawked in the Lafia metropolis, Nigeria.

2. Material and Method

The study to determine fungal contaminants in peanut butter consumed in Lafia, Nasarawa State, Nigeria was carried out at the Federal University of Lafia, Nigeria. Hawked peanut butter was collected from Lafia markets (Modern market and Kasuwa tomato market).

One hundred and sixty samples were collected from markets in the Lafia metropolis (twenty samples per market from different hawkers/sellers). The collected peanut butter individually weighed about 300 g per sample and was kept inside transparent sealed containers. The collected samples were light to dark brownish coloured with a friendly smell.

One gram of each sample from the different markets, was individually weighed and dissolved in distilled water to $x10^5$ dilution and plated on Potato Dextrose Agar (PDA) supplemented with chloramphenicol (50 mg). Incubation was at 37°C for 72 h. Plates were prepared in duplicates per sample. The colonies were counted and the CFU per gram of each sample was recorded.

The isolated bacteria were identified based on morphology, gram reaction, and biochemical tests Fungal colonies in the cultured plates with distinct morphologies were counted. They were subcultured by transferring pieces of the mycelia onto fresh sterile Sabouraud Dextrose Agar (SDA) plates to obtain pure isolates and incubated at 37°C for 4 days. Taxonomic identification of the genera was carried out macroscopically, and microscopic characteristics of the colonies were recorded. Fruiting structures and spores of the fungus were observed using a microscope. The characteristics of the isolates were recorded.

An in vitro assay for antagonism was employed to test for the inhibitory effect of some antifungal drugs against the isolated fungi. Three classes of antibiotics namely polyene (nystatin 200 mg), triazole (fluconazole 200 mg) and imidazole (clotrimazole 200 mg) were tested. Using the McFarland standard, 108 CFU of the fungal isolates were prepared and inoculated unto a Petri dish containing PDA, and the disks soaked with antibiotics were placed on the cultured plates and incubated at 25°C for 5 days. The inhibition zones were determined by measuring the diameter of the inhibition. Plates were prepared in triplicates and those without the endophytes served as control. The antifungal drugs were dissolved in 4 mL of distilled water and a concentration equal to 2 mg/mL of stock was prepared. Sterilized disks were incorporated with the different antifungals, air-dried and kept for use against the fungal isolates. The antifungal activity of the test drugs was determined by measuring the diameter of the zone of clearance. The readings are recorded in millimetres (mm).

3. Results and Discussion

3.1. Results

Samples of peanut butter collected from Alamis market had the highest bacteria count of 160.0 x 10^5 CFU/g (Table 1) followed by samples collected from the market located around the roundabout (120.0 x 10^5 CFU/g). Fungal counts were highest at the Shinge market (28.0 x 10^3 CFU/g) and lowest in samples collected from the College Village market (3.0 x 10^3 CFU/g). Seven bacterial genera were isolated from peanut samples collected from various markets located within and around the Lafia metropolis.

Table 1. Bacterial and fungal population in peanut butter

Sample ID	Market	Bacterial count	Fungal count
		x10^5 CFU/g	x10^3 CFU/g
K1	Tomato market	24.6	7.0
K2	Shinge market	57.6	28.0
L1	Modern market	64.0	5.0
L2	Shabu market	70.4	4.0
M1	Alamis market	160.0	6.0
M2	Roundabout market	120.0	10.1
N1	College village market	72.0	3.0
N2	Mararaba student market	40.0	8.0

Table 2 presented the fourteen (14) fungal species isolated and identified in the course of the project. *Aspergillus* with four species were the most isolated (*A. niger, A. flavus, A. carbonarius, A, parasiticus*) followed by genera *Fusarium* with three species. Other isolates were single species. As shown in Table 2 fungal distribution was highest in

Lafia Modern market and the Shinge market. *Aspergillus flavus* was isolated from all the markets where samples were obtained closely followed by *Aspergillus niger* as the second most spread isolate. Isolates such as *Aspergillus carbonarius, A. versicolor, A. parasiticus, Alternaria alternata, Saccharomyces cerevisiae, Candida* sp and *Trichophyton verrucossum* were found in one market each.

Table 2. Fungal species isolated from the different market locations

Fungal Isolates	L1	L2	M1	M2	N1	N2	K1	K2
Aspergillus flavus	+	+	+	+	+	+	+	+
Aspergillus niger	+					+	+	+
Aspergillus carbonarius					+			
Aspergillus versicolor							+	
Aspergillus parasiticus								+
Alternaria alternata						+		
Saccharomyces cerevisiae							+	
Saccharomyces sp.		+		+				
Fusarium oxysporum							+	+
Fausarium verticillioides							+	+
Fusarium graminearum							+	+
Candida sp.							+	
Trichophyton verrucosum								+
Rhizopus sp.			+	+				

Key: K1 – Tomato market, K2 – Shinge market, L1 – Modern market, L2 – Shabu market, M1 –Alamis market, M2 – Round about market, N1 – College village market, N2 – Mararaba student market

The three antibiotics drugs tested against the fungal isolates presented in Table 3 showed that the antibiotics were most potent against *Fusarium graminearum* isolates and *Aspergillus parasiticus* isolated from the Roundabout market with 100% inhibition of fungal growth. Nystatin was the most effective antibiotic tested with an inhibition of 1.87 ± 0.23 mm against *Alternaria alternata* as the highest, and it inhibited all the fungi isolates, while Ketoconazole also gave promising result inhibiting all the isolates. Fluconazole was the least effective antibiotic tested and it was resisted with no inhibition by *Fusarium oxysporum, F. graminearum, Aspergillus parasiticus* and *A. niger.*

Table 3. Antifungal Susceptibility Profile of isolated fungal species

Sample collection venue	Name of isolate	Antibiotics		
		Ketoconazole	Nystatin Inhibition (cm)	Fluconazole
Tomato market	*A. flavus*	0.57 ± 0.06	1.03 ± 0.15	0.60 ± 0.10
	F. oxysporum	0.80 ± 0.56	1.40 ± 0.20	0.00
	F. graminearum	4.00	4.00	4.00
Shinge market	*A. parasiticus*	0.70 ± 0.15	1.13 ± 0.25	0.00
Alamis market	*A. niger*	0.77 ± 0.15	1.53 ± 0.47	0.00
	F. graminearum	4.00	4.00	4.00
Modern market	*F. graminearum*	0.57 ± 0.07	0.90 ± 0.20	0.00
Roundabout market	*A. parasiticus*	4.00	4.00	4.00
	A. niger	0.00	0.00	0.00

Shabu market	*A. niger*	0.97 ± 0.12	1.33 ± 0.06	1.73 ± 0.15
	F. oxysporum	0.93 ± 0.06	1.27 ± 0.15	1.07 ± 0.23
College village market	*Alternaria alternata*	1.30 ± 0.44	1.87 ± 0.23	0.90 ± 0.30
	A. niger	0.40 ± 0.17	1.50 ± 0.10	0.00

Key: NG – No Growth, R – Resistant (No Inhibition)

3.2. Discussion

The assessment of the quality and safety of food is important for human health. The samples collected showed a rich diversity of filamentous fungi, non-filamentous fungi and bacteria. The sanitary conditions of the environment of these markets may also lead to contamination of food and food products. Findings about fungal and bacterial contamination of the buttered cream corroborated the findings of Carminati *et al*. (2016) and Uckun and Var (2018) who in their respective studies isolated *Salmonella, E. coli* and *Listeria* spp. The International Microbiological Standards recommended a limit of bacterial contamination in the range of 10-10^2 CFU/g for total aerobic plate count (ICMSF, 2011). Since large numbers, typically >106 CFU/g, are required for the production of enough toxins to cause illness, contamination is necessary but is not sufficient for an outbreak to occur.

Fungal species isolated were mostly of the pre-harvest fungi such as *Fusarium, Alternaria* and post-harvest fungi such as the *Aspergillus* and *Penicillium* genera. The prevalence obtained is dependent on the water availability for processing the cream, pre-harvest and post-harvest contaminations and poor hygienic conditions adopted. These fungal species are known producers of mycotoxins. Mycotoxins are poisonous chemicals with devastating health consequences in humans and animals. These metabolites are produced in minute quantities and present in food crops causing lower yield and quality of such food substances apart from reducing the income that is to accrue to farmers and cause export rejections. Some commonly produced mycotoxins are aflatoxin B1 and B2 by *Aspergillus fumigatus* and *Aspergillus flavus*, ochratoxins produced by the black *Aspergillus* species such as *A. niger*, *A. aculeatus* and *A. carbonarius*, while fumonisins and DON are produced by *Fusarium* species.

Pathogenic fungal strains are reported to be common to processed nuts and oils. Boli *et al.* (2020) presented results that showed that peanut paste samples from domestic milling are contaminated at the point of production. Milling introduces contaminants into the cream as the nutrient-rich residues in the milling machine make the mill a favourable environment for the proliferation of microorganisms (Adjou et al., 2012). In addition, Mutegi *et al*. (2009) reported that poor transport conditions and marketing of peanut products contribute to fungal growth. Fungal contaminants that were identified in peanut cream samples in the present studies were similar to the work of Ndung'u *et al*. (2013) and Boli *et al*. (2020) who both isolated *A. flavus*, *A. parasiticus*, *A. fumigatus*, *A. niger, Fusarium*, *Mucor* sp., *Penicillium* sp., and *Rhizopus* sp. The other fungal species isolated might have found their way into the peanut butter through soil contamination and unsanitary methods adopted in preparing the buttered cream. Pathogens can be transferred in several ways to food, such as through contaminated water and equipment, poor workers, hygiene, and pests (Chang et al., 2013). Other fungal species isolated in the course of the study might have contaminated the peanut butter from the environment. These include *Candida* sp., *Rhizopus* and *Mucor* species. While *Candida* sp has been implicated in causing ill health conditions known as candidiasis, *Aspergillus* species are known to cause aspergillosis which is another health condition in humans.

The results of the antifungal activity of the commonly used antifungal drugs showed varying degrees of inhibition. Nystatin and ketoconazole showed the best activities against the isolated fungal species in the study. Ketoconazole being an imidazole antifungal agent functions by preventing the synthesis of ergosterol, the fungal equivalent of cholesterol, thereby increasing membrane fluidity and preventing the growth of the fungus. While previously rarely considered the cause of infections, *Aspergillus* species are known to be the major cause of disease and mortality in immunocompromised patients. Most clinical strains of fungi are able to demonstrate resistance to antimycotic medications

Manikandan *et al*. (2013) reported that all the *Aspergillus* isolates in their study were susceptible to ketoconazole, which was similar to the result obtained in the present study. While Manikandan *et al*. (2013) reported that ketoconazole was more effective against *A. flavus*, our study had *A. niger* as the most susceptible. The antifungal drug gave better inhibition of growth against *Alternaria alternata* and *Fusarium oxysporium*. Patel *et al*. (2011) reported that the majority of fungi in their study were susceptible to fluconazole, followed by ketoconazole. This finding contradicted the results obtained in our study and supported by Yenişehirli *et al*. (2011) where most of the fungal isolates were resistant to the fluconazole. Fluconazole gave minimal activity against the fungi. The degree of antifungal susceptibility of *Aspergillus* strains varies from one isolate to another towards different antifungal drugs due to the level of immunity in patients and the toxicity of the antifungal drug (Yazdanpanah et al., 2007; Nucci and Anaissie, 2002).

Conclusion

Found This study showed that the microbiological quality of locally processed peanut butter creates a potential danger to public health. The presence of resistant fungi in the consumed cream calls for concern because some filamentous fungi of the genera Aspergillus and Fusarium are known to produce toxic secondary metabolites known as mycotoxins. The isolation of organisms like *Staphylococcus* sp, *Escherichia coli*, *Bacillus* sp, and *Salmonella* sp, which are of public health significance in traditionally processed peanut butter samples do not only pose health hazards to consumers.

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