Journal of Food Processing & Technology
ISSN: 2157-7110

Jonathan et al., J Food Process Technol 2019, 10:1
DOI: 10.4172/2157-7110.1000773

Research Article Open Access

Analysis of Nutrient, Fungal and Aflatoxin Compositions of Ogi Processed with Stored and Fresh Maize

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Abstract

Ogi is a gruel that is widely consumed throughout Nigeria. Stored maize is often used in processing this fermented product while fresh maize is used in some cases. In this study, the incidence of fungi and aflatoxin at different stages of ogi production using stored and fresh maize was investigated. Samples of stored and fresh maize were collected from different points in the market and processed into ogi in the laboratory. Fungi were isolated at different stages of ogi production i.e. maize, steep water, and ogi samples. The incidence of each fungus was determined while their overall percentage incidence was determined at the end of production. Aflatoxin contents (AFB_1, AFB_2, AFG_1 and AFG_2) of the samples were analyzed using Thin Layer Chromatography (TLC) using AOAC procedures while their nutrient was analyzed using AOAC method. The pH and TTA of the samples were also determined. The result showed that the pH of the steep water decreased with the length of fermentation while the TTA increased. The isolated fungi include *Aspergillus niger, A. tamarii, A. flavus, A. fumigatus, Penicillium chrysogenum, Penicillium* sp., *Fusarium* sp., *Rhizopus nigricans,* and *Saccharomyces cerevisiae. Aspergillus niger* had the highest percentage incidence followed by *Penicillium sp.* and *A. flavus* respectively. There was a significant high content of aflatoxin in stored maize (18.48 μgkg^{-1}) ($p>0.05$) compared to fresh maize (6.20 μgkg^{-1}) with the corresponding significant higher content of aflatoxin content in stored maize ogi (2.41 μgkg^{-1}) compared to fresh maize ogi (0.17 μgkg^{-1}) ($p>0.05$). In addition, the crude protein and carbohydrate contents of fresh maize ogi ($3.24 \pm 0.03, 46.68 \pm 0.05$) were significantly higher than that of stored maize ogi ($3.13 \pm 0.04, 46.52 \pm 0.05$) ($p>0.05$). It was deduced that due to fungal activities in stored maize there was a reduction in the nutritional composition of its ogi sample. This study also affirms that various processing methods employed during the production of ogi had a significant effect on the reduction of aflatoxins in maize grains. Consumers are enjoined to consider the use of fresh maize in making ogi because it was found to be better for ogi production due to its higher nutritional content with lowering fungal and aflatoxin incidence. However, maize traders are advised to employ proper storage methods for storing their maize grains in order to reduce fungal and aflatoxin contamination of maize products and selling lower quality maize grains to producers of maize foods to the barest minimum thereby curbing the risk of malnutrition in infants and children.

Keywords: Maize; Ogi; Fungi; Aflatoxin; Fungal incidence; Processing methods; Nutritional composition

Introduction

Maize is very critical to the diet of the general populace in Nigeria. Several products are derived from maize one of which is ogi [1]. Ogi is a well-known fermented cereal product which serves many communities in Nigeria [2,3]. It is often consumed by children and infants and also serves as a dietary food for adults including the elderly and the sick ones [4-6]. Traditionally, the processing of ogi from maize involves soaking the maize in clean water and allowed to ferment for 1 to 3 days. After the period of fermentation, the steep water is decanted and the fermented maize grain is washed with clean water and wet milled. The maize slurry is later wet-sieved in order to remove the bran and the sievate is allowed to settle down for two days. Solid starchy 'ogi' sediment is formed at the end of the process [2,7,8]. In Nigeria, Maize is commonly used for ogi production especially in the Southwest due to its affordability and availability [9]. While most producers of ogi use stored maize for its production, fresh maize is not commonly used.

Fungi have also been found to be associated with the fermentation of various cereals products. Yeasts and Moulds have been reported to be the major fungi that aid the fermentation process of maize products [7,10]. For instance, *Rhizopus* and *Mucor* which belong to order Mucorales aids the saccharification of the cereals substrates while Yeasts notably *Saccharomyces cerevisiae* aids the fermentation of cereals products during the souring stage [2,11]. On the other hand, Fungi have been found to be the major microorganisms to be responsible for the contamination of maize especially during storage [12-14]. Fungi such as *Fusarium* spp. and *Penicillium* spp. contaminates maize grains while they are in the field through their spores while *Aspergillus* spp. contaminates them during storage [15]. However, Fungi do not only contaminates maize, but they also contaminate various maize products [14,16,17] (Table 1).

Fungi produce toxic metabolites known as mycotoxins which are released into various foods. Two species of *Aspergillus* namely *Aspergillus flavus* and *A. parasiticus* produce a mycotoxin known as aflatoxins in various foods. Aflatoxins are toxic secondary metabolites which have a carcinogenic and mutagenic effect on humans [15,18]. They have been described as an unavoidable contaminant in various foods during their production, harvest, storage, and processing [19]. *Aspergillus* spp. and aflatoxins have been reported to persist during the processing of maize into ogi as they were not removed totally at the end of the process [19,20]. Although several studies have been carried out on the microbial contamination of maize and ogi, it is, however, imperative

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Received October 26, 2018; **Accepted** December 07, 2018; **Published** December 12, 2018

Citation: Jonathan GS, Ogunsanwo OB, Asemoloye MD (2018) Analysis of Nutrient, Fungal and Aflatoxin Compositions of Ogi Processed with Stored and Fresh Maize. J Food Process Technol 10: 773. doi: 10.4172/2157-7110.1000773

Samples	Moisture Content (%)	Crude Protein (%)	Crude Fat (%)	Crude Fibre (%)	Ash (%)	Carbohydrate (%)
Stored maize ogi	47.84 ± 0.01[a]	3.13 ± 0.04[b]	2.10 ± 0.01[c]	0.23 ± 0.01[a]	0.17 ± 0.01[b]	46.52 ± 0.05[b]
Fresh Maize ogi	47.56 ± 0.01[b]	3.24 ± 0.03[a]	2.02 ± 0.01[b]	0.23 ± 0.01[a]	0.29 ± 0.01[a]	46.68 ± 0.05[a]
Means with the same alphabets down the column are not significantly different at p>0.05						

Table 1: Proximate compositions of fresh ogi samples.

to study the fungal and aflatoxin incidence during the production of ogi using the two kinds of maize (fresh and stored maize) that are used for its production (Table 2).

Materials and Methods

Collection of samples

Stored and fresh (dried) corn samples were collected from four different shops in the market. These samples were later put into an airtight aseptic polythene bags and taken to the laboratory for further analysis.

Production of ogi

A method described by Awoyale et al., [5] was used to produce ogi samples. The maize grains were carefully sorted by removing various contaminants in it. They were later rinsed and steeped in distilled water using a clean container. After 72 hours, the fermented water was drained from the maize samples, washed thoroughly and milled using an aseptic warring blender. Distilled water and a sterilized muslin cloth were used to wet-sieve the maize slurry of fresh maize and stored maize. The filtrate was allowed to settle and ferment for 24 hours to form starchy sediment of ogi. The filtrate was bagged and dewatered in order to obtain fresh ogi. The obtained samples were immediately analyzed for their pH, proximate composition, fungal incidence and aflatoxin contents (Figure 1 and Table 3).

Determination of pH and TTA of steep water and the pH of the ogi samples

The hydrogen potential (pH) and Total Titratable Acidity (TTA) of the steep water of each maize samples were taken at 24 hours interval during the 72 hours of steeping period. After processing, the pH and TTA of the fresh samples of ogi were determined. The pH of the steep water samples was determined using the method of Oluwafemi and Ikeowa, [19]. The pH values of the steep water were taken at 0,24,48, and 72 hours using Digital pH meter (JENWAY 3016). The electrode was standardized using buffer 7 and 4. Three readings were taken for each sample and the average of the readings were recorded. The method described by Akinleye et al., [7] was used to determine the pH of the ogi sample. 1 g of ogi sample was weighed using weighing balance into a beaker and dissolved in 10 ml of distilled water. The pH of the ogi samples was determined manually, using JENWAY 3016 digital pH meter. Standardization of pH meter was done using a buffer solution of pH 7 and 4. The electrode of the pH was dipped into the beaker that contained the ogi solution, three readings were taken for each sample and the average of the readings was recorded (Figure 2 and Table 4).

The TTA of steep water was determined by using the method described by Mbata et al., [21] 10 ml of steep water (three replicates) were pipetted into small beakers and two drops of phenolphthalein solution were added to each prepared samples of steep water in the beakers. The burette was filled with already prepared 0.1N NaOH. The base was titrated against the steep water of maize samples until a light pink colour was observed and three readings were taken while the average of the readings was recorded. The percentage titratable acidity

	Maize		Steep Water		Ogi		Total Occurrence	% Incidence
Mycoflora	**S.M**	**F.M**	**F.M**	**S.M**	**S.M.O**	**F.M.O**		
Penicillium chrysogenum	1	2	1	2	0	0	6	6.6
Penicillium sp.	5	3	2	1	0	0	11	12.1
Aspergillus niger	6	2	5	3	0	0	16	17.6
Saccharomyces cerevisiae	0	1	2	1	3	2	9	9.9
A.tamarii	3	1	0	2	0	0	6	6.6
Fusarium sp.	4	1	0	2	0	0	7	7.7
Rhizopus nigricans	4	3	1	1	0	0	9	9.9
Mucor sp.	5	2	0	1	0	0	8	8.8
A. flavus	4	2	1	3	0	0	10	10.9
A. fumigatus	5	1	2	1	0	0	9	9.9
Total	37	18	14	17	3	2	91	100
Values are means of three replicates								

Table 2: Occurrence of fungal isolates on maize samples and ogi.

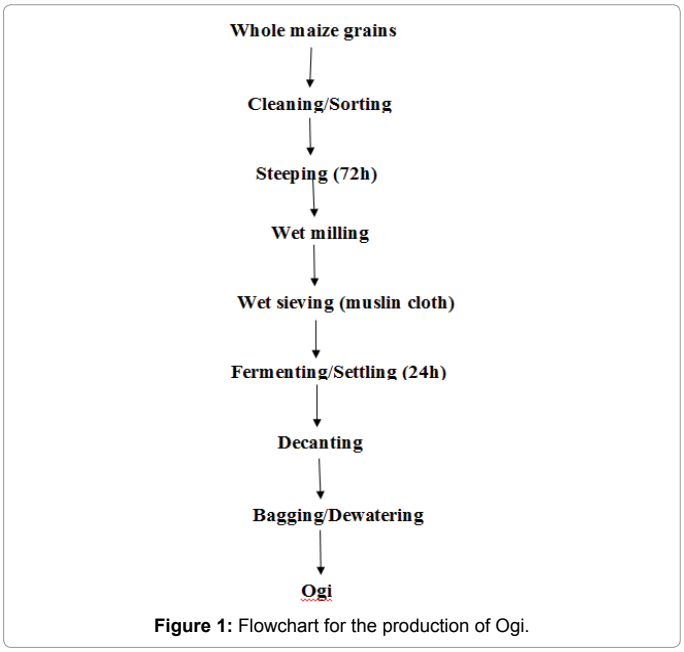


Figure 1: Flowchart for the production of Ogi.

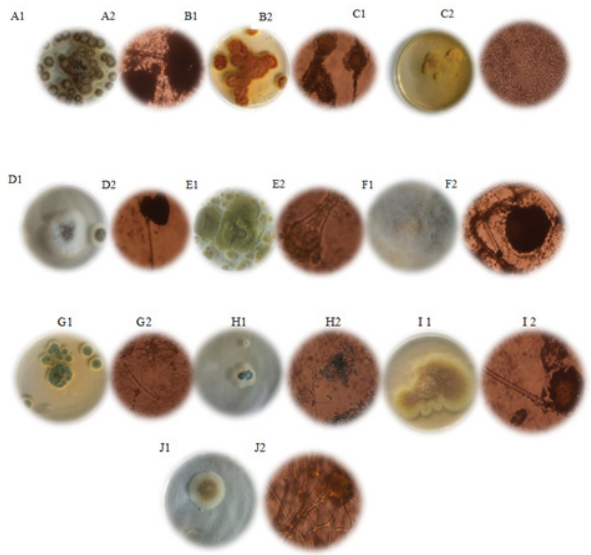
was calculated by using the formula:

$$\%acid = \frac{[mls\ \text{NaOH used}] \times [0.1\ \text{N NaOH}] \times [0.009\ (\text{miliequivalent factor})] \times [100]}{Grams\ \text{of sample}}$$

Inoculation maize samples and steep water into the prepared growth media

Seeds of maize samples were plated out using the modified method of Amusa et al., [22]. The seeds were first surface sterilized in 2% NaOCl for 30 s and then rinsed in three successive changes of sterile distilled water. The grains were then blotted dry in between sterile Whatman

filter paper. For each of the maize samples (stored and fresh maize), four maize seeds (about 0.1 g each) were picked using a sterile forceps and placed aseptically at a regular distance from one another in each Petri dish containing Potato Dextrose Agar (PDA). This was done in three replicates making a total of twelve seeds per sample. The seed samples in Petri dishes were then incubated at room temperature of (35°C ± 2) for 5 days under standard growth condition. The incubated seeds were observed daily for fungal growth (Table 5).



A=*Aspergillus niger* (A1:The plate view; A2:Microscopic view); B=*Aspergillus tamari* (B1:The plate view; B2:Microscopic view); *Saccharomyces cerevisiae* (C1:The plate view; C2:Microscopic view); *Rhizopus nigricans* (D1:The plate view; D2:Microscopic view); *A. flavus* (E1:The plate view; E2:Microscopic view); *Mucor sp.* (F1:The plate view; F2:Microscopic view); *Penicillium chrysogenum* (G1:The plate view; G2:Microscopic view); *Penicillium* sp. (H1:The plate view; H2:Microscopic view); *A. fumigatus* (I-1:The plate view; I-2:Microscopic view); *Fusarium* sp. (J-1:The plate view; J-2:Microscopic view).

Figure 2: Aflatoxin analysis of samples of maize and ogi.

Inoculation of steep water and ogi samples into the prepared growth media

Samples of both steep water and ogi were serially diluted and 0.1 ml dilution factor at 10^{-4} were plated on PDA media. Steep water samples were however plated out at 0 h, 24 h, 48 h, and 72 h. Samples were incubated for 3-4 days at 30°C [6]. The Petri dishes were examined daily for fungal growth noted in each replicate of the sample.

Fungal cultures obtained were subsequently sub-cultured. The average Yeast-mould colonies obtained from the triplicates were determined and expressed as colony forming unit per gram (CFU/g) [23] using the formula:

(*CFG/g*)=*Number* of colonies/Volume transferred to plate (1 ml) × (dilution factor)

Slide culture of the fungi isolates were prepared and viewed under light microscope. They were later identified based on the following taxonomical keys and morphology: spore colour on PDA, plate surface, growth rate, vesicle and microscopic features [14,24] (Table 6).

Nutrient analysis of the samples

Moisture, ash, crude protein, crude fat and fibre of ogi samples were determined using the procedures of AOAC [25] as follows:

Crude protein determination: Protein content was determined by semi-micro Kjedahl procedure and multiplied total nitrogen estimated by the factor 6.26.

Crude fat determination: The crude fat content of the sample was determined by ether extraction using a soxhlet method.

Dry matter and moisture determination: Moisture content was determined by heating the sample in a hot air circulating Gallenkamp Hotbox oven at 105°C for 4 hours until the weight is constant.

Time		0 h	24 h	48 h	72 h
pH	Stored maize	6.14 ± 0.00^{a}	4.54 ± 0.00^{c}	4.37 ± 0.00^{e}	3.37 ± 0.00^{g}
	Fresh maize	6.01 ± 0.00^{b}	4.52 ± 0.00^{d}	4.35 ± 0.00^{f}	3.36 ± 0.00^{h}
TTA	Stored maize	0.04 ± 0.00^{e}	0.05 ± 0.00^{d}	0.05 ± 0.00^{d}	0.11 ± 0.00^{b}
	Fresh maize	0.03 ± 0.00^{f}	0.04 ± 0.00^{e}	0.04 ± 0.00^{e}	0.90 ± 0.00^{a}
Fungal count (cfu/ml)	Stored maize	$8.33 \pm 1.15^{a} \times 10^{4}$	$4.67 \pm 0.58^{ab} \times 10^{4}$	$2.77 \pm 0.58^{ab} \times 10^{4}$	$2.67 \pm 0.58^{ab} \times 10^{4}$
	Fresh maize	$6.67 \pm 0.58^{a} \times 10^{4}$	$3.67 \pm 0.58^{b} \times 10^{4}$	$2.67 \pm 0.58^{b} \times 10^{4}$	$2.00 \pm 1.00^{b} \times 10^{4}$
Means with the same alphabets down the column are not significantly different at p>0.05					

Table 3: Effect of fermentation on pH, TTA and fungal count of the steep water of maize samples.

Samples	pH	Fungal count(cfu/ml)
Fresh Maize Ogi	6.11 ± 0.02^{a}	$2.00 \pm 2.12^{a} \times 10^{4}$
Stored Maize Ogi	5.78 ± 0.00^{b}	$3.00 \pm 1.41^{a} \times 10^{4}$
Means with the same alphabets down the column are not significantly different at p>0.05		

Table 4: pH and fungal count of fresh ogi samples.

Sample	Sample Code	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2	Total Aflatoxins
maize (μgkg^{-1})	Fresh Maize	2.85 ± 0.03^{c}	1.95 ± 0.03^{d}	0.75 ± 0.01^{d}	0.65 ± 0.01^{b}	6.20^{c}
	Stored Maize	9.84 ± 0.01^{b}	6.11 ± 0.01^{b}	1.40 ± 0.02^{b}	1.11 ± 0.02^{b}	18.48^{b}
ogi (μgkg^{-1})	Stored maize ogi	1.21 ± 0.01^{d}	1.11 ± 0.01^{de}	0.05 ± 0.00^{e}	0.04 ± 0.00^{b}	2.41^{d}
	Fresh maize ogi	0.08 ± 0.00^{d}	0.07 ± 0.00^{e}	0.01 ± 0.00^{e}	0.01 ± 0.00^{b}	0.17^{e}
Means with the same alphabets down the column are not significantly different at p>0.05						

Table 5: Aflatoxin analysis of samples of maize and ogi.

Isolates	Spore Colour on PDA	Plate surface	Growth rate	Vesicle	Microscopic structures	Fungi Isolates
1	Black	Powdery	Rapid	Globose	Long and smooth Conidiophores	*Aspergillus niger*
2	Brown	Powdery	Rapid	Semi-globose	Long and smooth Conidiophores	*A. tamarii*
3	Green	Powdery	Rapid	Semi-globose	Long and smooth Conidiophores	*A. flavus*
4	Yellowish black	Smooth	Rapid	Globose	Long and smooth Conidiophores	*A. fumigatus*
5	Leafy Green	Smooth	Rapid	Globose	Long and smooth Conidiophores	*Penicillium* sp.
6	Greenish with yellow ring	Smooth	Rapid	Globose	Long, smooth and branched conidiophores	*P. chrysogenum*
7	Spongy wooly dark	Wooly	Rapid	Globose	Simple, long and branched sporangiophores	*Mucor* sp.
8	Spongy wooly dark	Wooly	Rapid	Globose	Simple, long and branched sporangiophores	*Rhizopus* sp.
9	White	Rough	Rapid	Globose	Long and smooth Conidiophores	*Fusarium* sp.
10	Cream	Smooth and Glistering	Slow	-	Ovoid Budding	*Saccharomyces cerevisiae*

Table 6: Cultural and morphological characteristics of fungi isolated.

%Moisture=100 - M

%Dry Matter (DM)=W_2 - W_1/W_1 - W_0 × 100

Ash determination: Two grams of the samples was heated at 550°C until the difference between two successive weight was less than 1 mg.

Ash content=Weight of ash/original weight of sample × 100

Fiber determination: Crude fibre extraction was determining using fibretec extraction. Fibre content was estimated using the formula below:

%Fiber=W_1-W_2/Weight of sample × 100

Carbohydrate (CHO): The carbohydrate content was determined by differences:

100-(%Crude%fat+%Crude Protein+%Crude Fiber+%Ash)

Aflatoxin extraction, detection and quantification

Extraction: 1 g of sample was pipetted into a 100 ml conical flask. 2.5 ml of distilled and 25 ml of Chloroform water was added. The flask was covered with a stopper and shake in a shaker for 30 minutes after which the solution obtained was filtered using a Whatman no.1 filter paper. 10 ml of each extract or filtrate was collected and evaporated to dryness to a volume of 5 ml on a hot water bath. Five ml extract was stored in dark bottles in a freezer for detection and quantification [26].

Detection of aflatoxin: 1 ml chloroform and 0.2 ml of the reconstituted extract was spotted on a precoated 20 × 20 cm TLC plate along with aflatoxin standards of known concentration. The spotted TLC plate was developed in an equilibrated tank containing chloroform: acetone (9:1 v/v). The developed TLC plate was air-dried at ambient temperature (28 ± 2°C) and aflatoxins were detected under UV light at a wavelength of 360 nm. A colour change from blue to yellow upon exposure to aqueous sulphuric acid (50:50 v/v) confirmed the presence of Aflatoxin B1. Aflatoxin B_2 was derived from Aflatoxin B_1 as dihydro derivative which experienced a colour change from pale blue to deep yellowish colour upon exposure to aqueous sulphuric acid (50:50) to confirm its presence. Aflatoxin G_1 fluoresced yellowish green upon exposure to UV light while Aflatoxin G_2 fluoresced pale yellowish green upon exposure to same UV light [26].

Quantification of aflatoxin: 0.5 µm thick preparative TLC plates was employed for the quantification of stored extract after aflatoxin extraction was applied to the plate as a band rather than a spot to chromatograph, the maximum amount of sample at the same time. The preparative TLC plates were developed in an equilibrated tank as an aflatoxin extraction. When the solvent front had risen to about ¾ of the total length of the plate, the plate was taken out of the tank and examined under UV light. The area containing the toxin of interest was located and scrapped off, elute with chloroform and filtered using Muslin cloth. The extract was evaporated to dryness over a hot water bath and reconstituted with 3 ml chloroform. The 3 ml reconstituted solution and aflatoxin standard of 20 µg/ml concentration was used to read Absorbance or Optical Density on an ultraviolet Spectrophotometer (Cecil Instrument CE505) at a wavelength of 360 nm [26].

Aflatoxin concentration in µg/kg was calculated using the formula:

$$\frac{Absorbance \text{ of sample} \times Conc. \text{ of Standard} \times dil.factor}{Absorbance \text{ of Standard}}$$

Determination of fungal incidence

Fungal incidence was determined by using the formula:

$$\text{Percentage Incidence} = \frac{\text{Number of Each Fungus}}{\text{Total Number of Fungi}} \times 100$$

Statistical analysis

Statistical significance of the data generated from this study was evaluated by analysis of variance (ANOVA) using SAS 9.3 version. Means were separated at 5% test of significant using Duncan's Multiple Range Tests (DMRT).

Result

Proximate analysis of fresh ogi

There was a significant difference ($p<0.05$) in the moisture content, crude protein, crude fat and ash content of both stored and fresh maize ogi (Table 1) while the ash content of both samples, however, shows no significant difference ($p>0.05$). Higher content of moisture (47.84%) and crude fat (2.01%) was recorded for stored maize ogi. Fresh maize ogi had higher crude protein (3.24%), ash (0.29%) and carbohydrate (46.68%) content.

The Occurrence of fungi isolates in maize and ogi

Fungi isolated from maize samples (Stored maize and Fresh maize) and ogi (Stored Maize ogi and Fresh Maize Ogi) includes *Aspergillus niger, A. flavus, A. fumigatus, Penicillium chrysogenum, Penicillium* sp., *Fusarium* sp., *Rhizopus nigricans* and *Saccharomyces cerevisiae* as shown in (Table 4). *Saccharomyces cerevisiae* was isolated from all the samples. Seven different isolates of fungi were found to be associated with stored maize. These fungi include *Aspergillus niger, A. flavus, A. fumigatus*, *Fusarium* spp., *Penicillium chrysogenum*, *Rhizopus nigricans,*

and *Saccharomyces cerevisiae*. Five fungi were isolated from fresh maize they are: *Aspergillus niger*, *A. flavus*, *Penicillium chrysogenum*, *Penicillium* sp. and *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* was the only fungi isolated from the ogi samples i.e. fresh maize ogi and stored maize ogi.

Fungi isolated from the steep water during the fermentation of maize

Aspergillus niger, A. flavus, Penicillium chrysogenum, Penicillium sp., *Rhizopus nigricans,* and *Saccharomyces cerevisiae* were isolated from the steep water of both fresh and stored maize at the initial stage of fermentation (Table 4). *Aspergillus niger, A. flavus, Penicillium* sp., *Rhizopus nigricans,* and *Saccharomyces cerevisiae* were isolated from the steep water of fresh maize. While *Aspergillus niger, A. flavus, Penicillium chrysogenum, Penicillium* sp., *Rhizopus nigricans* and *Saccharomyces cerevisiae* were isolated from the steep water of stored maize. *Saccharomyces cerevisiae* persisted throughout the period of fermentation.

Effect of pH (hydrogen ion potential) and TTA (titratable acidity) on the fungal count of the steep water of fresh maize and stored maize during the 72 hours of fermentation

The pH (hydrogen ion) and fungal count decreased while the TTA (total titratable acidity) increased as the fermentation period progressed. After 24 hours of fermentation, no growth of mold was observed in the steep water of fresh maize while *Aspergillus niger* and *Penicillium* sp. were isolated from the steep water of stored maize. However, after 48 hours and for the rest of the fermentation period no growth of molds was observed in the steep water of both stored and fresh maize. *Saccharomyces cerevisiae* was however present in the steep water of the maize samples throughout the fermentation period. There was a progressive decrease in the fungal count of the steep water as the hours of fermentation increased. In the steep water of stored maize, the fungal count decreased from 8.33×10^4 at 0 hour to 2.67×10^4 after 72 hours while the fungal count in fresh maize it decreased from 6.67×10^4 at 0 hours to 2.00×10^4 at 72 hours.

However, there was no significant difference ($p>0.05$) in the fungal count of the steep water of both stored and fresh maize throughout the fermentation period. The pH of the steep water of the maize samples decreased with the period of fermentation. A decrease of pH values from 6.14 to 3.37 was observed in the steep water of stored maize and while a decrease of 6.01 to 3.36 was observed in the steep water of fresh maize. On the other hand, the TTA (Total Titratable Acidity) of the steep water of the maize samples. An increase of 0.04-0.11 was recorded in the steep water of stored maize and 0.03-0.90 in the fresh maize steep water as the fermentation progressed. It was observed that the pH and the total titratable acidity of the steep water of the stored maize was significantly ($p<0.05$) higher than that of the fresh maize throughout the period of fermentation.

The levels of pH (hydrogen ion potential), TTA (total titratable acidty) and fungal count in ogi samples.

The pH of the stored maize ogi was significantly higher than that of the fresh maize ogi. The only fungus that was isolated from stored maize ogi and fresh maize ogi was *Saccharomyces cerevisiae*. However, there was no significant difference in the fungal count of both samples.

Effect of fermentation on pH, TTA and fungal count of the steep water of maize samples.

Aflatoxin analysis of maize and ogi samples

There was a drastic decrease in the total aflatoxin levels in ogi samples compared to maize samples. The aflatoxin levels reduced from 6.20 μgkg^{-1} in fresh maize to 0.17 μgkg^{-1} in fresh maize ogi while the aflatoxin levels in stored maize reduced from 18.48 μgkg^{-1} to 2.41 μgkg^{-1} in stored maize ogi. There was a significant difference ($p<0.05$) between aflatoxin B_1, B_2 and G_1 of stored maize and fresh maize while aflatoxin G_2 shows no significant difference ($p>0.05$). Total aflatoxin in stored maize (18.48 μgkg^{-1}) was significantly ($p<0.05$) higher than that of fresh maize (6.20 μgkg^{-1}). There was no significant difference ($p>0.05$) in the aflatoxin levels of the ogi samples however there was a significant difference ($p<0.05$) in the total aflatoxin.

Discussion

Maize and maize products become an excellent substrate for the growth of fungi when they are not kept properly. Various kinds of fungi are found in maize grains as a result of some factors present in the environment which enables them to survive. Several reports have indicated that fungi mostly affect maize grains during storage, where they reduce their viability and their nutritional quality. Consequently, the nutritional composition of various maize products is also affected. In this study, the lower protein and Carbohydrate content in stored maize ogi can be as a result of the biodeteriorating effect of fungi on the stored maize. Nutritional compositions of stored maize have been reported to be prone to deterioration due to some factors. Coupled with some factors such as relative humidity, temperature, moisture, and insect attack fungi activities prominently enhances the deterioration of maize by making use of the moisture available in the grain to destroy the germ of the maize seeds [27]. According to the submission of Chuck-Hernandez et al., [28] when maize is infected by fungi during storage, it often leads to discoloration, dry matter loss, chemical, and nutritional changes and overall reduction of maize grain quality. All these factors could have resulted to the differences observed in the nutritional content of both samples of stored maize and fresh maize ogi. Ogi is an important gruel which serves both the old and young ones in Nigeria. Unfortunately, the consumers of this fermented food especially infants and children who need basic nutrient like protein for body building may not get enough of this nutrient from ogi processed with stored maize infected with storage fungi. This will make infants and children to be fed with an inferior diet which could result to malnutrition; a prevalent problem facing the diet that most children in sub-Saharan Africa are exposed to [23,29].

Grains are common substrates that support the growth of a variety of fungi especially during storage [14,15]. The isolation of different fungi from maize samples has been reported. In a separate study conducted by Amusa et al., [22] and Hussain et al., [30] they respectively reported the incidence of 6 and 12 species of fungi in maize seeds respectively. Fungi isolated from the maize samples used for this study comprised of the field and storage fungi [31]. Storage of maize is a common practice in the market where the maize samples were collected which is typical of most markets in Nigeria. This is usually done in order to preserve maize grains till the time that fresh ones will come out. However, maize grains are often contaminated by fungi during storage when fungal spores in the air enter the maize grains through the pores of the storage materials that are used to store the grains [32]. The isolation of *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp. and *Rhizopus* sp. from stored maize samples used for this study corroborates with the findings of Amadi and Adeniyi [33] and Sule et al., [14] where the

same set of fungi were isolated from stored maize samples. Moreover, *Aspergillus* and *Penicillium* have been reported to be prevalent in stored products. However, their prevalence and incidence depends on the suitable conditions available during storage. In a report by Atanda et al., [34], *Penicillium* spp. and *Aspergillus* spp. were identified as storage fungi which are capable of growing at lower water content and contaminate grains in silos and other storage places. According to the report of Egbuta et al., [13]. *Aspergillus* sp. and *Penicillium* sp. were the most prevalent fungi in maize samples collected from store houses in markets, having percentage incidences of 78.9% and 57.9% respectively.

Possible sources of fungal contamination is a major factor that must be considered before storing maize grains as they are often contaminated by fungal spores from unknown sources. International Rice Research Institute, [35] asserted that spores of fungi are introduced into grains either on the field, during harvest or through spores of fungi that are already present in storage equipment or storage structures. Of all the fungi isolated from the stored maize samples used in this study, *Aspergillus* species had the highest fungal incidence. This findings is in agreement with the work of Sule et al. [14] who reported that *Aspergillus* recorded the highest frequency distribution of all the fungi isolated from stored maize samples that was purchased from a local market in Kaduna. Similarly, Chauhan et al., [36] had also reported that of all fungi isolated from maize collected from Gedeo zone in Ethiopia, *Aspergillus* had the highest occurrence (75%).

The presence of *Aspergillus* in stored maize is known to produce mycotoxins during storage [37]. The occurrence of *Aspergillus flavus* in the stored maize samples was inevitable as it has been reported to be present in stored maize grains. Sule et al., [14] reported the presence of *Aspergillus flavus* in stored maize collected from a local market in Kaduna while Amadi and Adeniyi [33] isolated *Aspergillus flavus* as a storage fungus from samples of stored maize. It is a fungus that spreads its spores very fast during storage which makes it a fungus with a very high incidence in stored maize compared to other fungi. In the findings of Egbuta et al., [13] *Aspergillus flavus* recorded a high incidence in the samples of stored maize analysed. *Aspergillus flavus* is widely known for the production of aflatoxins [38].

The isolation of *Aspergillus niger* and *A. fumigatus* from stored maize sample agrees with the works of Makun et al., [39] and Egbuta, [40] who isolated the same set of fungi from stored maize. In the submission of Egbuta et al. [13] of all the fungi isolated from maize samples, *Aspergillus fumigatus* recorded the highest percentage incidence. *Fusarium* is a regular contaminant of maize grains [41]. However, *Fusarium* has also been identified as a common genus of fungi that infect maize in the field [31]. Isolation of *Penicillium* sp. from stored maize is consistent with the works of Makun et al., [39] Suleiman and Omafe, [42] and Chauhan et al., [36] where the same fungi was isolated from stored maize. The isolation of *Rhizopus* sp. from stored maize sample agrees with the findings of Makun et al. [39] and Masirevic et al., [43] that isolated *the* same fungus from maize samples.

The fresh maize sample was expected to have little or no fungal contamination since it had not gone into storage. However, the incidence of aflatoxin in the sample could be as a result of the contamination caused by the spores of aflatoxigenic fungi on the field during harvesting and packaging before going into storage. The isolation of the *Aspergillus* spp. and *Penicillium* spp. from the fresh maize samples, from the fresh maize sample, agrees with the work of Sule et al. [14] who isolated the same fungi from new maize purchased from local market in Kaduna. Though these two fungi have been earlier described as storage fungi however, their presence in the fresh maize samples could be as a result of their spore contamination during or before harvesting which may come from some sources on the field. According to Bankole and Adebanjo [15], *Penicillium* and *Aspergillus* are not frequently associated with crops in the field, but may be present in other sources of contamination such as plant debris, plant surfaces, atmosphere and other surfaces where water activity is low. For instance, *Aspergillus niger* is a fungus known to be found in soil, plant litter which can infect crops during harvest [44]. Small quantities of spores contaminate the grain as it is going into storage from the harvest during handling [35]. All these facts may be the reason for the possible source of contamination of fresh maize sample by these fungi.

The isolation of *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Rhizopus* spp., and *Saccharomyces cerevisiae* from the steep water of stored maize and the isolation of *Aspergillus* spp. and *Penicillium* spp. from the steep water of fresh maize agrees with the findings of Oyelana and Coker [45] who isolated similar fungi from steep water of maize for ogi production. This report is also consistent with the view of Izah et al., [11] who concluded that yeast and mould participate in the steeped maize for ogi production. *Rhizopus* sp. a fungus belonging to order Mucorales has been shown to aid the saccharification of substrates during the initial phase of fermentation [46]. Furthermore, the isolation of *Saccharomyces cerevisiae* from the steep water of stored maize and fresh maize ogi affirms the reports of Oyedeji et al., [47] and Akinleye et al., [7] where *S. cerevisiae* was isolated from fermentation of steep water of maize during the production of ogi. Activities of various probiotics enhance the taste of ogi during the souring stage. *S. cerevisiae* has been identified as the predominant microorganism which enables the nutritional quality of ogi during the secondary fermentation of ogi [2,11,48]. *S. cerevisiae* increases with the fermentation period [2]. This assertion about *S. cerevisiae* affirms the reason why the fungus persisted throughout the fermentation period of both fresh and stored maize used for this study. The emergence of *Aspergillus niger* and *Aspergillus* spp as the fungus and the fungal genera with the highest incidence of all the fungi encountered in all the samples analysed in this study is in agreement with the reports of Sule et al. [14] who found out that among all the fungi isolated from samples of maize and maize flour *Aspergilus* species was the most dominant fungal genera. *A. niger* has also been reported to be the most dominant fungi isolates associated with a sample of ogi [49].

Generally, maize is usually fermented into ogi in acidic condition. However, the pH and TTA of steep water are the two physicochemical conditions that initiate the process of fermentation of maize during the steeping by gradually changing the state of maize grains and affect the growth of the microorganisms found in them. The decrease in pH and increase in TTA of the steep water of both stored and fresh maize between 0 to 72 hours agrees with the findings of Oluwafemi and Ikeowa [19], who reported that the pH of maize steep water decreased from 6.8 to 4.6 while its TTA increased from 0.124% to 0.169% after 72 hours of fermentation. Omemu, [2] reported that there was a significant decrease in the pH of steep water of maize during fermentation from 6.1cfu/g to 4.8 cfu/g between 0 to 48hours and an increase in titratable acidity from 0.01% to 0.4%. According to Egwim et al., [50] during fermentation, lactic acid bacteria produce high acidity into the fermenting medium which lowers its pH thereby inhibiting the growth of other non-desirable organisms especially fungi. The predominant lactic acid bacteria and fungus that have been mostly reported to aid the fermentation of maize for ogi production are *Lactobacillus plantarum* and *Saccharomyces cerevisiae* [11]. The presence of molds namely *Aspergillus niger, A. flavus, Penicillium* sp. and *Rhizopus nigricans* isolated from the steep water of both fresh and

stored maize at the initial stage of fermentation and their subsequent elimination is in agreement with the report of Omemu, [2].

The decrease in the fungal count of the steep water of stored maize from 8.33×10^4 at 0 hour to 2.67×10^4 at 72 hours and the steep water of fresh maize 6.67×10^4 at 0 hour to 2.00×10^4 at 72 hours agrees with the findings of Oluwafemi and Ikeowa, [19] who reported a decrease in the fungal count of steep maize from 12×10^4 to 2×10^4 after 72 hours of fermentation. From the foregoing, it can be deduced that the lowered pH of the steep water of maize inhibits the growth of food poisoning fungi that were present in the maize samples at the initial stage. However, higher fungal count in the steep water of stored maize compared to the steep water of fresh maize may be due to the higher fungal load present in the stored maize samples.

Maize is a cereal that has been severally reported to be susceptible to aflatoxins contaminations. The contamination of stored and fresh maize samples with aflatoxin is consistent with some reports. Jonathan and Esho [51] stated that certain environmental factors stimulate the extent to which mycotoxins are produced, which varies with geographic location, agricultural methods and the susceptibility of commodities to the penetration of fungi during storage and processing periods. Aflatoxins contamination of food products poses a serious threat to human's and animal's health, while its toxicity has remained a topic of debate in the international market as well as economic development of the country which are part of trade market [36,52]. Many researchers have widely reported high levels of aflatoxin contamination of maize and maize products in Nigeria. Maize as an important agricultural commodity throughout the world is considered as one of the best substrate for the growth of fungi and produce toxicogenesis [15, 36].

Aflatoxins have been reported to contaminate over 25% of the maize crops produced in Nigeria [32]. Among staple cereals in the Nigerian diet, maize was reported to have the highest level of aflatoxin contamination [53]. The first indicator that was suspected to be responsible for the contamination of the maize samples used for this study was the presence of *Aspergillus flavus*. Atehnkeng et al., [32] had identified *Aspergillus flavus* as the major fungi that produces aflatoxin in maize during storage. They however stated that maize without visible signs of *Aspergillus flavus* may also have a high amount of aflatoxin. The detection and quantification of lower aflatoxin content in fresh maize (6.20 $\mu g/kg^{-1}$) and higher content in stored maize (18.48 $\mu g/kg^{-1}$) samples used for this study affirms the findings of Sule et al., [14] who reported that new maize and old maize obtained from a local market in Kaduna had a mean aflatoxin level of 102 ppb and 177 ppb respectively. The result of the higher level of aflatoxin recorded in stored maize (18.48 $\mu g/kg^{-1}$) compared to fresh maize (6.20 $\mu g/kg^{-1}$) can be attributed to the long storage time of the stored maize coupled with other factors such as temperature, insect damage, and other environmental factors [14,32,34]. During storage, it has been discovered that aflatoxigenic fungi are able to grow and release aflatoxins into various food products [32,34].

The result of the aflatoxin contamination levels in both fresh and stored maize samples is far below the acceptable aflatoxin level of 4 ppm for maize set by Standard Organization of Nigeria (SON) [14] thereby making them safe for consumption. However, due to the fact that aflatoxins accumulate in maize grains during storage, if the stored maize samples used for this study had spent more time in storage they could have accumulated a higher concentration of aflatoxins which could be above the acceptable level thereby making them unsafe for consumption [13,32]. According to FAO [54], high level of aflatoxin in food products is unacceptable and possible legal action to eliminate such products from the market may be taken by the government. The reduction in the aflatoxin level of both fresh and stored maize ogi compared to the raw maize samples that were used to process them could be as a result of various processing methods that were used during their production. According to the submission of Matumba et al., [55] traditional methods of processing maize products are effective in reducing Aflatoxin B_1 content in the final products. Findings from a similar study carried out by Olayiwola et al., [1] adduced to the fact that processing methods applied during the processing of maize into various food products reduced the aflatoxin in maize. The first processing method that the maize samples were subjected to was fermentation. Fermentation of maize has been proven to be effective in reducing aflatoxin in maize products [56].

Assohoun et al., [53] reported that the fermentation of maize samples during the processing 'Doklu' (a fermented maize product consumed in Cote d'lvoire), caused a significant reduction in the concentration of total aflatoxins (72%) of the maize samples with most aflatoxin B_1 (80%) after the soaking of maize grains for 72 hours. Also, Oluwafemi and Ikeowa [19] had reported that aflatoxins levels in maize grains significantly ($p<0.05$) reduced by 50% (from 50 $\mu g\ kg^{-1}$ to 25 $\mu g\ kg^{-1}$) after 72 hours of fermentation. Another factor that could be responsible for the reduction in the aflatoxin content in the ogi samples of both fresh and stored maize ogi is the ability of the Lactic acid bacteria such as *Lactobacillus plantarum* to detoxify the aflatoxin content of the maize during fermentation. According to the report of Oluwafemi et al., [57], among all the lactic acid bacteria tested for the reduction of aflatoxin in maize samples that were artificially contaminated with aflatoxin, *Lactobacillus plantarum*, the major lactic acid bacterium that participates in the fermentation of maize into Ogi was discovered to be the most effective as the highest rate of reduction of aflatoxin was recorded in the maize samples inoculated with the strains of the bacterium.

The second stage of the production of the Ogi samples which could have led to the reduction of aflatoxin content in the maize samples was sieving of maize bran from the fermented maize slurry. Pietri et al., [58] had reported that aflatoxin in maize grains concentrate in the bran. According to the report of Oluwafemi and Ikeowa, [19] they found out that after fermenting maize grains that contained aflatoxin level of 50 µg/kg and sieving its slurry to form ogi, the aflatoxin level in the ogi sample was found to be 25 µg/kg while the remaining aflatoxin content had adsorbed unto the bran. This is also affirmed by Sule et al., [14] who collected maize grains, maize flour and maize bran from a market in Kaduna state. They reported that among all the samples collected maize bran sample had the highest level of aflatoxin with a mean aflatoxin level of 213 ppb. In a similar report by Mutungi et al., [59], the dehulling of maize grains samples made the aflatoxins levels to significantly decrease from 10.7 and 270 ng/g with a mean value of 87.3 ng/g to 6.8 ng/g and 182 ng/g with a mean value of 57.3 ng/g. They also reported that the aflatoxin contents in the by-products, comprising hulls and fines, were 2-7 times higher than the levels in the whole-grain maize and ranged from 103 ng/g to 613 ng/g.

The incidence of aflatoxin however in fresh ogi although in minute form is the residual aflatoxin content of the sample that were not totally removed from the sample during the processing. Aflatoxins are stable metabolites that are able to persist in food products even after the aflatoxigenic fungus that produced them had died. Adegoke et al., [20] reported that the preformed aflatoxin in the aflatoxin contaminated maize persisted till the end of processing maize into ogi slurry. However,

the quantity of the aflatoxin in the fresh ogi obtained from fresh and stored maize poses no threat to the safety of the product.

Conclusion

This study showed that a total number of 10 fungal isolates belonging to 6 genera namely: *Aspergillus, Fusarium, Rhizopus, Penicillium, Mucor,* and *Saccharomyces* were isolated at various stages of ogi production. *Aspergillus niger* was the predominant fungi isolated from all the samples. Aflatoxin content of stored maize was significantly ($p<0.05$) higher than that of fresh maize. Changes in the physicochemical properties i.e. pH and TTA of the steeping water during fermentation led to the decrease in the fungal incidence as the fermentation period progressed. The fermentation of maize samples coupled with other processing methods such as milling, sieving, and souring led to the significant decrease of aflatoxin in the ogi samples compared to the raw maize samples. Ogi processed with fresh maize had a higher protein and carbohydrate content than the one processed with stored maize, this could be as a result of the biodeteriorating effect of fungi on the stored maize sample. Fresh maize is good for the processing of ogi owing to its higher nutritional content and lesser fungal and aflatoxin composition compared to stored maize as revealed by this study. Stored maize grains are commonly used for producing ogi all year round, therefore maize traders must always ensure that maize grains are properly stored using hygienic storage means so as to avoid their contamination by aflatoxin and selling such aflatoxin contaminated maize to the producers of maize foods. This will ultimately reduce the sale of lower quality maize grains to producers of ogi thereby preventing aflatoxicosis and malnutrition in children and infants; the major consumers of the fermented product.

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