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Characterization of fungi (*Aspergillus aculetus*) isolated from fermented *Ogi* (*Zea mays*) and sorghum (*Sorghum vulgar*) fortified with Tigernut (*Cyperus esculentus*)

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ABSTRACT

Fermentation of traditional Nigerian foods like *Ogi* plays a vital role in shaping their safety, nutritional value, and sensory attributes through complex microbial interactions. This study evaluates the microbial and physicochemical aspects of fermented maize, sorghum, and tigernut mixtures to enhance their quality and health benefits. The investigation carried out includes: microbial count, cultural growth, pH level determination, proximate composition, vitamins, minerals, and organoleptic property assessment. The microbial count analysis of isolated *Ogi* fungi revealed varying growth patterns across different substrates. Notably, maize on Potato Dextrose Agar (PDA) at a 10^{-7} dilution exhibited robust fungal colonies, reflective of its nutrient-rich nature. Conversely, lower fungal counts were observed at higher dilutions on Sabouraud Dextrose Agar (SDA), indicating effective microbial load reduction. Cultural growth characteristics exhibited a diverse array of shapes, colors, textures, and growth rates among the fungi isolates. The identification of suspected organisms based on morphological features revealed the presence of *Saccharomyces* sp. and *Aspergillus* sp., known for their roles in fermentation and spoilage, respectively. The study tracked pH levels over three days, indicating progressive acidity and ongoing fermentation typical of lactic acid fermentation processes. Proximate and vitamin analyses showcased the nutritional enhancements in maize-sorghum-tigernut mixtures, with elevated fat, fiber, protein, and vitamin content. Organoleptic evaluations demonstrated consumer preference for maize-sorghum *Ogi* due to its appealing appearance and aroma. In conclusion, the study highlights that strategic blending of maize, sorghum, and tigernut enhances the microbial safety, nutritional composition, and sensory appeal of *Ogi*. These findings support the development of nutritionally improved, consumer-acceptable fermented foods with potential health benefits and extended shelf life.

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1. Introduction

Traditional fermented staple foods play a vital role in The diet offers nutrient-rich options characterized by unique flavors and aromas that reflect cultural heritage and enhance food diversity.

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They help maintain food texture and are common across various cultures globally. These fermented products will continue to be relevant and are used in the production of alcoholic beverages, vinegar, pickled vegetables, sausages, cheeses, yogurts, vegetable proteins, sauces, and breads (1).

Microorganisms have been used in food production for thousands of years. Fermented foods are not only appreciated for their taste but also for the acids produced during microbial metabolism, which inhibit spoilage organisms and food-borne pathogens (2). Thus, fermentation remains a critical food preservation method, particularly in the absence of modern conveniences such as refrigeration. However, achieving a consistent, high-level fermentation is often difficult and unpredictable in terms of duration and product quality. In some cases, undesirable end products may result from poor fermentation processes (3).

To achieve high-quality fermentation, it is essential to isolate and purify the dominant microorganisms present in desirable products, as recommended by Marshall (4).

Foods are typically consumed for their nutritional value, including the provision of energy, vitamins, amino acids, and essential nutrients such as potassium, sodium, calcium, magnesium, manganese, zinc, and iron. Fermentation may lead to a reduction in some nutrients, especially from maize. The degree of nutrient loss depends largely on the method of maize fermentation with sorghum and tigernut. Additionally, if undesirable microbes dominate the fermentation environment, they can negatively alter the organoleptic properties of the final product (5).

Cereals are major sources of energy and protein in the diets of most Africans. Examples include maize (*Zea mays*), sorghum (*Sorghum vulgare*), and tigernut (*Cyperus esculentus*) are key crops widely grown in Nigeria, particularly in the southern regions (6). Maize plays a significant role in the production of traditional fermented products such as *ogi* (Yoruba) or *akamu* (Igbo), and serves as an essential dietary source of carbohydrates, proteins, B-complex vitamins, and minerals. However, it lacks sufficient levels of lysine and tryptophan—two essential amino acids—making its protein content of low biological value (7). Lactic acid fermentation has been found to enhance the amino acid composition of cereal-based foods by promoting microbial synthesis and protein breakdown, thereby increasing essential amino acids such as lysine and tryptophan (8). Moreover, incorporating tiger nut into these blends helps to balance the amino acid profile by contributing additional nutrients, including higher levels of tryptophan, thus improving the overall protein quality (9). Maize porridge (*ogi*) is a dietary staple, especially for infants during weaning, nursing mothers, and young adults (10).

Sorghum, a genus comprising around 30 grass species, is cultivated both for grain production and as animal fodder. It thrives in tropical and subtropical climates and is adapted to high temperatures. Certain varieties may accumulate toxic substances like hydrogen cyanide, hordenine, and nitrates—especially during early growth or under environmental stress such as drought or excessive heat (11).

Tigernut, often called earth almond and classified under the Cyperaceae family, develops edible tubers with a peanut-like appearance. It is rich in essential

nutrients, including phosphorus, potassium, vitamins E and C, sugars, oleic acid, dietary fiber, and unsaturated fats. It also provides moderate protein content and bioactive compounds such as starch and various phytochemicals (12). Despite its nutritional advantages, tigernut can contain anti-nutritional factors such as oxalates, phytates, and tannins, and may be susceptible to fungal contamination and mycotoxin development during improper storage, posing health concerns (13).

Nigeria is richly endowed with a range of fermentable indigenous staple foods that support small-scale agro-processing industries. *Ogi*, a fermented maize product, is one such example. While fermentation improves product shelf life, the microorganisms involved also play roles in spoilage. Therefore, this study aims to isolate and characterize fungal species present in maize fermented with sorghum and tiger nut.

2. Materials and Methods

2.1. Media preparation

Thirty-nine grams (39 g) each of Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) were measured and dissolved in 1 litre of distilled water by boiling. The media were sterilized in an autoclave at 121 °C for 15 min under 15 psi pressure (1.05 kg/cm^2). Following sterilization, the media were transferred to a water bath maintained at 45 °C to keep them in a liquid state, and chloramphenicol was subsequently added to suppress bacterial growth.

For sample preparation, 1 g of each sample was introduced into a test tube containing 9 mL of sterile distilled water and subjected to serial dilution up to 10^{-6}. From the final dilution, 1 mL was aseptically dispensed into sterile Petri dishes, which had been previously dried and sterilized in a hot air oven at 160 °C for 1 h. The molten media (PDA or SDA) were then poured into the plates in duplicates. After the agar solidified, all plates were incubated in an inverted position at 37 °C. This procedure was carried out for both types of media to facilitate fungal isolation (14).

2.2. Isolation of fungi from the fermented products

Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) were prepared according to the manufacturer's protocol (15). After sterilization, the media were dispensed into sterile Petri dishes and allowed to cool and solidify under aseptic conditions. Fermented product samples suspected to contain microorganisms were then inoculated onto the surface of the solidified agar using a sterile loop or spreader. The inoculated plates were incubated under suitable conditions to promote microbial growth. Throughout the incubation period, plates were regularly inspected for the appearance of colonies. Key observations such as colony morphology, texture, and growth patterns were carefully documented.

2.3. Characterization of the fungi

Morphological Characterization: Macroscopic examination of surface colonies on each solid medium was conducted to determine colony shape, size, surface characteristics, margin, elevation, colour, opacity, growth intensity, consistency, and emulsifiability.

2.4. Macroscopic examination

This was done based on the nature of the morphological characteristics of the isolated fungi using the color Atlas of Diagnostics microbiology (16)

Microscopic slides of the mycelium from various fungal isolates were prepared using the following method: A few drops of lactophenol cotton blue stain were placed in the center of clean, grease-free glass slides. A small portion of each unidentified fungal isolate was collected using a sterile wire loop and gently mixed into the stain to achieve even dispersion. A cover slip was carefully placed over the preparation to avoid air bubbles. The slides were examined under a microscope to study the structural features, including the morphology of the mycelia, spores, fruiting bodies, and the type of hyphae. Identification was aided by reference to the Color Atlas of Diagnostic Microbiology (16).

2.5. Proximate composition

Moisture content, crude protein, total ash, crude fibre, crude fat, and carbohydrate in fermented products were determined using the modified procedure of AOAC (17).

2.6. Moisture content determination

Moisture content was assessed following the procedure outlined by AOAC (17). Approximately 5 g of the sample was weighed into a clean, pre-dried, and pre-weighed moisture can. The can and its contents were placed in a hot air oven and dried at 105 °C for three hours. After drying, the can was removed, cooled in a desiccator, and reweighed. It was then returned to the oven for an additional 30 min, followed by cooling and weighing. This cycle was repeated until a constant weight was achieved. Moisture content was calculated based on the loss in weight using the formula:

$$\text{Moisture content (\%)} = X\frac{(W_1-W_2)}{W}\ 100$$

where:

W_1 = weight of pan + fresh sample

W_2= weight of pan + dried sample

W= weight of sample AOAC (2005).

2.7. Determination of crude protein content of the fermented products

The crude protein content was estimated following the AOAC method (17). 1 g of the sample was introduced into a Kjeldahl digestion flask, followed by the addition of a catalyst tablet and 20 mL of concentrated sulfuric acid (H_2SO_4). The flask was mounted on a digester and heated at 410 °C for approximately 6 h until a clear digest was obtained. After cooling, the digest was transferred into a 100 mL volumetric flask and diluted to volume with distilled water.

The distillation unit was assembled and flushed for 10 min post-boiling. Into a conical flask, 20 mL of 4% boric acid solution was added, along with five drops of methyl red indicator. Then, 10 mL of the digest was introduced into the distillation flask, followed by the addition of 20 mL of 40% sodium hydroxide (NaOH) via a funnel. The mixture was distilled, and the released ammonia was collected into the boric acid solution for 15 min, during which the solution changed from pink to green. The distillate was titrated using 0.05 N hydrochloric acid (HCl), and nitrogen content was calculated using the formula below.

$$\%\text{Nitrogen (W/W)} = \frac{14.01\times(\text{Sample titre}-\text{blank titre})\times\text{Normality of acid}}{10\times\text{weight of sample}}$$

$$\%\text{Crude protein (W/W)} = \%\ \text{Nitrogen} \times 6.25 \quad \text{AOAC (2005)}$$

2.8. Determination of crude fat content

Crude fat content was determined using the Soxhlet extraction method (17). The extraction flask was oven-dried to constant weight. Four grams (4 g) of each dried sample were placed in a fat-free thimble, plugged with cotton wool, and inserted into the extractor. A pre-weighed, oven-dried 250 mL Soxhlet flask was filled to three-quarters with petroleum ether (b.pt. 40–60 °C) and attached to the extractor and reflux condenser.

The setup was heated for six hours with constant water flow for condensation. Ether refluxed 10–12 times with gentle boiling. After extraction, the thimble was removed and dried. The setup was reassembled and distilled further until the flask was nearly dry. The flask containing the extracted fat was detached, cleaned, and oven-dried to a constant weight.

$$\text{Percentage crude fat} = \frac{W_1 - W_o}{\text{Weight of sample}} \times 100$$

W_o= initial weight of dry Soxhlet flask

W_1= final weight of oven-dried flask + oil AOAC (2005).

2.9. Determination of crude fibre

The crude fibre content was determined using the method described by AOAC (17). Three grams (3g) of the product were weighed and extracted with petroleum ether. It was allowed to boil (under a reflux condenser) for 40 min. Filter paper was placed in the funnel, and the sample was drained by applying suction. The insoluble material was washed first with boiling water, then with 1% HCl, twice with alcohol, and thrice with ether. The residue was dried in an electric oven at 100^{o}C to a constant weight. The residue was incinerated to ash, cooled and weighed. The difference between the weight of the ash-less filter paper plus the insoluble material and that of the ash represents the fibre content.

$$\% \text{ crude fibre} = \frac{W2 - W3}{W1} \times 100$$

W_1 = weight of sample used,

W_2 = weight of crucible plus sample,

W_3 = weight of sample crucible + ash. AOAC (2005).

2.10. Mineral composition determination

The mineral contents of the samples were determined by the procedure of AOAC (17). Manganese, copper, phosphorus, potassium, magnesium, calcium, iron, selenium, zinc, and copper elements were measured with an Atomic Absorption Spectrophotometer (Thermo scientific S Series Model GE 712354) after digesting with a perchloric–nitric acid mixture. Prior to digestion, 0.50 g of the samples was weighed into a 125 mL Erlenmeyer flask with the addition of perchloric acid (4 mL), concentrated HNO_3 (25.00 mL), and concentrated sulphuric acid (2.00 mL) under a fume hood. The contents were mixed and heated gently in a digester (Buchi Digestion unit K-424) at low to medium heat on a hot plate under a perchloric acid fume hood, and heating was continued until dense white fume appeared. Heating was continued strongly for half a min and then allowed to cool, followed by the addition of 50 mL distilled water. The solution was allowed to cool and filtered completely with a wash bottle into a Pyrex volumetric flask and then made up with distilled water. The solution was then read on the Atomic Absorption Spectrophotometer.

2.11. Nutritional composition of the fermented products

2.11.1. Determination of vitamins

Vitamins were quantified by high-performance liquid chromatography (HPLC) using the AOAC Official Method 2016.04 (18). Method validation was carried out in line with ICH Q2(R1) guidelines, assessing linearity ($R^2 \geq 0.999$), accuracy (recoveries of 95–105%), precision (intra- and inter-day relative standard deviations <5%), limit of detection (LOD), and limit of quantification (LOQ).

Two grams of the flour sample were accurately weighed and homogenized with 20 mL of n-hexane in a test tube for 10 min. The mixture was then centrifuged for another 10 min. After centrifugation, the supernatant was filtered, and 3 mL of the clear filtrate was dispensed in duplicate into dry test tubes. These were evaporated to dryness using a boiling water bath. Subsequently, 2 mL of 0.5 N alcoholic potassium hydroxide was added to each tube, and the contents were heated in a water bath for 30 min. After cooling, 3 mL of n-hexane was introduced, and the tubes were vigorously shaken. The hexane layer was carefully transferred into a fresh set of test tubes and again evaporated to dryness. To the resulting residue, 2 mL of ethanol was added, followed by 1 mL of 0.2% ferric chloride solution in ethanol, and then 1 mL of 0.5% 1,1-dipyridyl solution in ethanol. Finally, 1 mL of ethanol was added to make up the volume to 5 mL. The mixture was thoroughly mixed, and its absorbance was measured at 520 nm against a blank solution.

2.11.2. Organoleptic properties of the fermented products

Sensory characteristics of the coded gruel were evaluated for different sensory attributes by twenty students from Crescent University, Abeokuta, to assess the appearance, taste, flavour, texture, colour, aroma. Sensory evaluation of the samples was carried out using a five-point hedonic scale with the following as categories: Excellent=5; Very Good=4; Good=3; Fair=2 and Poor=1. Colour (appearance), flavor (aroma), texture, taste and overall acceptability of the fortified samples.

2.11.3. pH

The pH of the fortified fermented maize samples was monitored over a 3-day fermentation period to assess acidification and determine the product's potential shelf life and market acceptability. The rate of pH reduction served as an indirect indicator of microbial activity and fermentation progress. In addition, the visual appearance and signs of spoilage—such as discoloration, off-odours, or gas formation—were used alongside pH values to evaluate the suitability of the product for continued consumption and sale.

2.12. Statistical analysis

All experiments were conducted in triplicate. Data were expressed as mean ± standard deviation (SD). One-way Analysis of Variance (ANOVA) was used to determine significant differences among the samples (Maize – M, Maize with Sorghum – MS, and Maize with Sorghum and Tigernut – MST). Statistical analysis was performed using SPSS version 25.0. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Microbial count of fungi

Table 1 shows the microbial count of fungi isolated from fermented maize, sorghum, and tigernut on sabouraud dextrose agar (SDA) and potato dextrose agar (PDA). All seven isolates had microbial growth.

The isolate from maize grown on Potato Dextrose Agar (PDA) at 10^{-7} dilution showed a relatively high occurrence of fungal colonies. While maize and Sorghum grown on Potato Dextrose Agar (SDA) at 10^{-6} dilution, maize, sorghum and tigernut grown on Sabouraud Dextrose Agar (SDA) at 10^{-6} dilution and maize, sorghum and tigernut grown on Potato Dextrose Agar (SDA) at 10^{-6} dilution recorded the lowest fungi colonies compared to other isolates respectively.

3.2. Cultural characteristics of fungi from the fermented maize and sorghum fortified with tigernut

Table 2 shows the cultural growth characteristics of the fungi present in the isolates. The fungi exhibit different shapes, including circular and ovoid forms. This variation in shape can be indicative of different fungal species or stages of growth. The fungi display a range of colors such as white, brown, grey, and black. Fungal coloration can be influenced by factors like the presence of pigments or the type of substrate they are grown on. The reverse side of fungal colonies shows colors like cream and black. This characteristic can also be used to identify certain fungal species. The texture of the fungi colonies varies from powdery to smooth to cotton-like. Texture can provide clues about the composition and growth pattern of the fungi. The growth rate of the fungi is categorized as rapid or medium. Rapid growth may indicate favorable conditions for fungal proliferation, while medium growth could suggest a slower development rate.

3.3. Morphological characteristics of fungi

Table 3 revealed the results of the study on the morphological characteristics of fungi isolated from fermented maize and sorghum fortified with tiger nut reveals that M/PDA/10^{-7} and M/SDA/10^{-6} had smooth cream or whitish colonies with white or cream reverse color, oblong spheroids with a prominent central vacuole and nucleus, with suspected organism *Saccharomyces* sp. MS/PDA/10^{-7} had a black colony with white reverse color. Slightly roughened globose conidia with a hyaline unbranched conidiophore, with *Aspergillus* sp. as the suspected organism. MS/PDA/10^{-6} had a white cotton colony with a greyish center, branched conidiophores with cylindrical metulae, and bottle-shaped philiads. with *Penicillium* sp. as the suspected organism. MST/PDA/10^{-7} had a white cottony colony with cream reverse color, Filamentous, non-septate hyphae with rhizoid characteristics, and *Rhizopus* sp. as the probable organism. MST/SDA/10^{-6} had smooth whitish colonies with cream reverse color, oblong spheroids with a prominent central vacuole and nucleus, with *Saccharomyces* sp. as the suspected organism, while MST/PDA/10^{-6} had a cottony white colony with a brownish center, globose conidia with non-septate conidiophore with *Aspergillus* sp. as the probable organism.

Table 1. Microbial count of fungi isolated from fermented Maize, Sorghum, and Tigernut on Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA)

Isolate ID	Medium	Dilution Factor	Microbial Count
M/SDA	SDA	10^{-6}	5×10^{6}
MS/SDA	SDA	10^{-6}	2×10^{6}
MST/SDA	SDA	10^{-6}	1×10^{6}
M/PDA	PDA	10^{-7}	9×10^{7}
MS/PDA	PDA	10^{-7}	2×10^{7}
MS/PDA	PDA	10^{-7}	1×10^{7}
MST/PDA	PDA	10^{-7}	3×10^{7}

Keys:
M = Maize
MS = Maize, Sorghum
MST = Maize, Sorghum, and Tigernut
SDA = Sabouraud Dextrose Agar
PDA = Potato Dextrose Agar

Table 2. Cultural characteristics of fungi found in isolation from fermented Maize and Sorghum fortified with Tigernut.

Sample code	Shape	Colour	Reverse	Texture	Growth
M/PDA	Circular	White	Cream	Powdery	Rapid
M/SDA	Circular	White	Cream	Smooth	Medium
MS/PDA	Ovoid	Brown	White	Powdery	Rapid
MS/PDA	Ovoid	Grey	Black	Cotton	Rapid
MST/PDA	Ovoid	White	Cream	Cotton	Rapid
MST/SDA	Circular	White	Cream	Smooth	Rapid
MST/PDA	Circular	Brown	White	Powdery	Medium

Keys: M= Maize;
MS= Maize, Sorghum
MST=Maize, Sorghum and Tigernut
SDA= Sabouraud Dextrose Agar
PDA= Potato Dextrose Agar

Table 3. Morphological characteristics of fungi found in isolates from fermented Maize and Sorghum fortified with Tigernut.

Isolate ID	Cultural Characteristics	Morphology and Microscopic Characteristics	Suspected Fungi
MS-1	Smooth cream colonies with white reverse colour	Oblong spheroids with a prominent central vacuole and nucleus	*Saccharomyces* sp.
MSS-1	Colony appears black with white reverse colour	Slightly roughened globose conidia with a hyaline unbranched conidiophore	*Aspergillus* sp.
MSS-1	White cotton colony with a greyish center	Branched conidiophores with cylindrical metulae and bottle-shaped philiads	*Penicillium* sp.
MSTS-1	The white colony appears cotton with cream reverse colour.	Colonies are filamentous, non-septate hyphae, rhizoid.	*Rhizopus* sp.
MSTS-2	Smooth whitish colonies with cream reverse colour	Oblong spheroids with a prominent central vacuole and nucleus	*Saccharomyces* sp.
MSTS-3	Cottony white colony with a brownish center	Globose conidia with a non-septate conidiophore	*Aspergillus* sp.

Keys: MS= Maize Sample
MSS= Maize, Sorghum Sample
MSTS=Maize, Sorghum, and Tigernut Sample

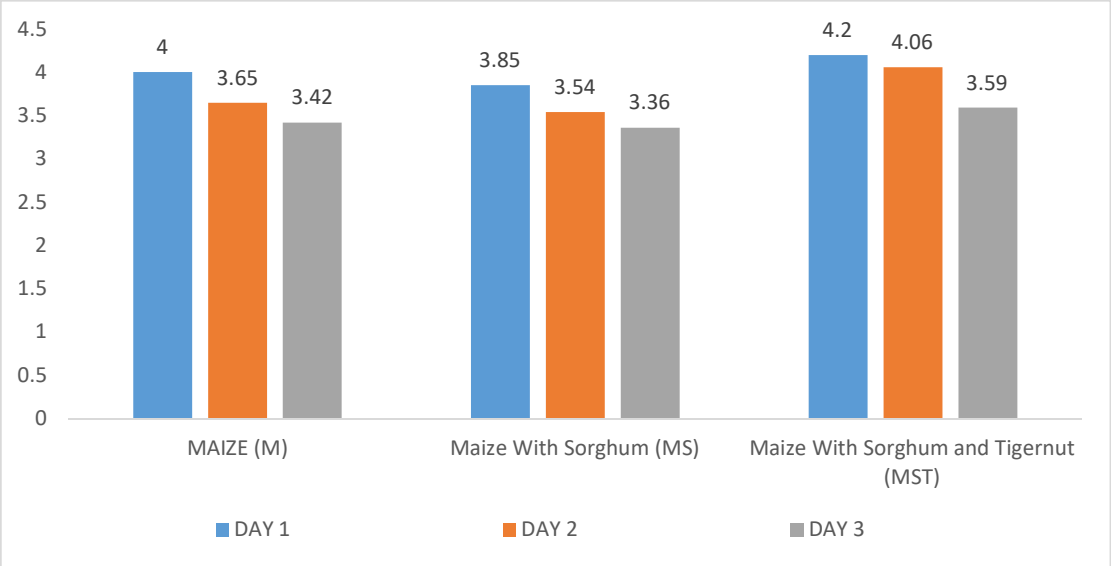

Figure 1. pH result of the fermentation medium over different days

Table 4. Proximate analysis of Pap from Maize, Maize with Sorghum and Maize with Sorghum and Tigernut

Sample	Moisture Content %	Fat Content %	Ash Content %	Crude Fibre %	Crude Protein %	Carbohydrate %
Maize (M)	50.43	2.27	1.43	1.25	3.34	41.28
Maize with Sorghum (MS)	52.58	4.43	1.56	1.23	3.99	36.21
Maize with Sorghum and Tigernut (MST)	48.58	4.98	1.07	1.89	4.55	38.93

Note: All the values of results are in g/100g sample.

Table 5. Vitamins analysis of Pap from Maize, Maize with Sorghum and Maize with Sorghum and Tigernut.

Vitamins	Result		
	Maize (M)	**Maize With Sorghum (MS)**	**Maize With Sorghum and Tigernut (MST)**
A	10.238(ug/100g)	21.867(ug/100g)	126.734(ug/100g)
B1	0.364(mg/100g)	0.528(mg/100g)	0.824(mg/100g)
B2	0.258(mg/100g)	0.361(mg/100g)	0.356(mg/100g)
B3	2.092(mg/100g)	2.311(mg/100g)	2.588(mg/100g)
C	6.963(mg/100g)	10.456(mg/100g)	15.118(mg/100g)

All the values of results are in mg/100g sample.

Table 6. Mineral analysis of Pap from Maize, Maize with Sorghum and Maize with Sorghum and Tigernut.

Minerals	Result		
	Maize (M)	Maize With Sorghum (MS)	Maize With Sorghum and Tigernut (MST)
Potassium(K)	190.321(mg/100g)	245.336 (mg/100g)	218.634(mg/100g)
Phosphorus(P)	143.689(mg/100g)	112.834(mg/100g)	86.391(mg/100g)
Calcium (Ca)	21.168(mg/100g)	38.691(mg/100g)	41.832(mg/100g)
Magnesium (Mg)	56.348(mg/100g)	62.591(mg/100g)	60.121(mg/100g)
Iron (Fe)	2.866(mg/100g)	4.186(mg/100g)	9.126(mg/100g)

Note: All samples were in the same condition as submitted when analysed.
All the values of results are in mg/100g sample.

Table 7. Frequency and percentage of sensory characteristics of fermented Maize and Sorghum fortified with Tigernut (n = 20)

Sample	Texture	Appearance	Taste	Odour	Remark	Frequency (n)	Percentage (%)
Maize (M)	Smooth	Cream	Sour	Pleasant	Like	14	70.0%
Maize with Sorghum (MS)	Smooth	Light brown	Slightly sour	Pleasant	Extremely Like	16	80.0%
Maize with Sorghum and Tigernut (MST)	Smooth	Whitish brown	Slightly sour	Pleasant	Extremely Like	8	40.0%
Maize with Sorghum and Tigernut (MST)					Like	12	60.0%

3.4. Nutritional analysis

3.4.1. Proximate analysis of Pap from Maize, Maize with Sorghum, and Maize with Sorghum and Tigernut

Table 4 shows the proximate analysis results for Pap from Maize, Maize with Sorghum, and Maize with Sorghum and Tigernut reveals that Maize with Sorghum has the highest moisture content, followed by Maize and Maize with Sorghum and Tigernut. Maize with Sorghum and Tigernut has the highest fat content, indicating a higher lipid content compared to Maize and Maize with Sorghum. Maize with Sorghum has the highest ash content, indicating mineral content, followed by Maize and Maize with Sorghum and Tigernut. Maize with Sorghum and Tigernut has the highest crude fibre content, suggesting a higher dietary fiber content compared to Maize and Maize with Sorghum. Maize with Sorghum and Tigernut has the highest crude protein content, indicating a higher protein content compared to Maize and Maize with Sorghum. Maize has the highest carbohydrate content, followed by Maize with Sorghum and Tigernut, and then Maize with Sorghum.

3.4.2. Vitamin analysis of Pap from Maize, Maize with Sorghum and Maize with Sorghum and Tigernut

Table 5 revealed the results of the vitamins analysis for Pap from Maize, Maize with Sorghum, and Maize with Sorghum and Tigernut, shows that Maize with Sorghum and Tigernut (MST) has the highest Vitamin A content, followed by Maize with Sorghum (MS) and Maize (M). Maize with Sorghum and Tigernut (MST) contains the highest Vitamin B1 content, followed by Maize with Sorghum (MS) and Maize (M). The Vitamin B2 content is similar across all samples, with Maize with Sorghum having a slightly higher content. Maize with Sorghum and Tigernut (MST) has the highest Vitamin B3 content, followed by Maize with Sorghum (MS) and Maize (M). Maize with Sorghum and Tigernut (MST) contains the highest Vitamin C content, followed by Maize with Sorghum (MS) and Maize (M).

3.4.3. Mineral Analysis of Pap from Maize, Maize with Sorghum and Maize with Sorghum and Tigernut

Table 6 shows the results of the mineral analysis for Pap from Maize, Maize with Sorghum, and Maize with Sorghum and Tigernut revealed that Maize with Sorghum has the highest potassium content, followed by Maize with Sorghum and Tigernut, then Maize. Maize has the highest phosphorus content, followed by Maize with Sorghum, then Maize with Sorghum and Tigernut. Maize with Sorghum and Tigernut has the highest calcium content, followed by Maize with Sorghum, then Maize. Maize with Sorghum has the highest magnesium content, followed by Maize with Sorghum and Tigernut, then Maize. Maize with Sorghum and Tigernut has the highest iron content, followed by Maize with Sorghum, then Maize.

3.4.4. Sensory characteristics of fermented Maize and Sorghum fortified with Tigernut

This table (7) presents a comparison of three types of *Ogi* based on their texture, appearance, taste, odor, and remarks: M *Ogi*: It has a smooth texture, a cream appearance, a sour taste, a pleasant odor, and is liked. MS *Ogi*: It also has a smooth texture, but with a light brown appearance. It has a slightly sour taste, a pleasant odor, and is extremely liked. MST *Ogi*: Similar to the previous two, it has a smooth texture. Its appearance is whitish brown, its taste slightly sour, its odor pleasant, and it is liked.

The sensory evaluation revealed that all three formulations had a smooth texture and pleasant odour. The maize–sorghum (MS) sample received the highest level of preference, with 80% of panellists rating it as "extremely like." The maize–sorghum–tigernut (MST) sample also scored well, with a combined 100% of responses indicating either "like" or "extremely like." The maize-only (M) sample had 70% of panellists indicating "like," suggesting a generally favorable reception, though slightly less preferred than the composite blends.

4. Discussion

Fermentation of foods like *ogi* (a traditional Nigerian fermented cereal pudding) involves complex microbial interactions that influence the product's safety, nutritional value, and sensory attributes. This study examined the microbial count, cultural growth characteristics, pH levels, molecular analysis, proximate composition, and vitamin and mineral content of fermented mixtures of maize, sorghum, and tigernut.

The microbial enumeration of fungi isolated from *ogi* revealed that all seven isolates exhibited microbial growth. The isolate from maize cultured on Potato Dextrose Agar (PDA) at a 10^7 dilution exhibited a relatively high colony count. This finding aligns with the study by Akinola and Ejechi (20), who reported high fungal loads in fermented maize products, likely due to the rich nutrient content of maize that supports fungal proliferation. Conversely, the isolates from maize and sorghum grown on Sabouraud Dextrose Agar (SDA) at a 10^6 dilution, as well as those from maize, sorghum, and tigernut (MST) on SDA, recorded the lowest fungal colonies. These reduced counts at higher dilutions underscore the efficacy of serial dilution in lowering microbial concentration, corroborating the observations of Ohenhen and Ikenebomeh (21).

The cultural characteristics of the fungal isolates revealed diversity in colony shape, color, texture, and growth rate. Observed morphologies ranged from circular to ovoid, suggesting the presence of multiple fungal species or developmental stages. Variations in surface and reverse colony coloration (e.g., white, grey, brown, black, cream) supported the differentiation of fungal taxa, similar to findings by Okafor et al. (22), who reported varied pigmentation among fungi isolated from traditionally fermented foods. Colony textures ranged from powdery to cotton-like, and growth rates varied from medium to rapid. Rapid growth rates likely indicate optimal environmental and nutritional conditions, in agreement with Adebayo-Tayo et al. (23).

Morphological identification suggested specific fungal organisms associated with the isolates. For instance, M/PDA/10^7 and M/SDA/10^6 developed smooth cream or whitish colonies, with Saccharomyces spp. Suspected—organisms well-documented for their role in fermentation (24). The MS/PDA/10^7 isolate presented black colonies with a white reverse, indicative of Aspergillus spp., a genus often linked to spoilage and potential mycotoxin production (25).

The pH levels of the samples declined progressively over a three-day fermentation period, indicating increasing acidity as fermentation advanced. This is characteristic of lactic acid fermentation and corroborates the report of (26), who observed similar pH reductions in fermented maize products. Slight variations in pH among the maize-only, maize-sorghum, and maize-sorghum-tigernut samples reflect differences in fermentation dynamics and microbial community activities.

Proximate analysis revealed that the maize-sorghum-tigernut (MST) mixture had the highest contents of crude fat, crude fiber, and crude protein, indicating nutritional enhancement through fortification. This agrees with Adeyemi and Umar (27), who demonstrated that incorporating additional grains or legumes into maize-based products can significantly boost their nutritional profile.

Vitamin analysis revealed that the MST formulation had the highest levels of vitamins A, B_1, B_3, and C, indicating enhanced nutritional value through multigrain fortification. This aligns with the findings of Osungbaro et al. (28), who observed that incorporating multigrain fermentation can improve the micronutrient profile of traditional diets. Similarly, mineral analysis showed increased concentrations of calcium and iron in the MST samples, highlighting the potential health-

promoting effects of consuming such fortified formulations.

Lastly, the organoleptic evaluation (texture, appearance, taste, and odor) indicated that the maize-sorghum (MS) formulation was the most preferred by panelists, possibly due to its light brown color and pleasant aroma. These sensory qualities are critical to consumer acceptance, echoing the conclusions of Afolayan et al. (29), who highlighted the significance of appearance and aroma in the acceptance of fermented foods.

The maize–sorghum–tigernut blends in this study achieved significantly higher aroma, taste, mouthfeel and general acceptability scores than the unfortified control, particularly at 10–15% tigernut inclusion. These results are consistent with Oboh et al. (30), who showed that adding tigernut extract to *ogi* improved sensory quality—panelists "liked moderately" to "liked very much" the fortified samples without adverse effects on texture or colour. However, Kazeem et al. (31) reported a slight decline in colour acceptability at fortification levels > 15%, likely due to darker pigments in tigernut flour—mirroring our modest drop in colour scores at 20% inclusion. Taken together, these comparative studies justify our conclusion that up to 15% tigernut fortification optimally balances enhanced sensory appeal with nutritional enrichment in maize–sorghum fermented products.

5. Conclusion

This study highlights the complex microbial, nutritional, and sensory profiles of fermented maize, sorghum, and tigernut formulations. The isolation and molecular identification of specific fungal species, including *Aspergillus aculeatus*, and their genetic similarity to established reference strains, emphasize the critical need for rigorous monitoring and control of microbial populations in fermented foods. Such measures are essential to safeguard product quality, enhance nutritional value, and ensure consumer safety.

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Authorship contribution

JS – Lead researcher, Supervisor, Conception, Drafting of the manuscript, **EOO**- Revision of manuscript, Data interpretation, Correspondence **ROF**- Data analysis, Editing of the final version of the manuscript, **SA –** Statistics, **AOB –** Design, Literature review - Design, and Revision of manuscript

Declaration of competing interest

The authors affirm there are no conflicts of interest to disclose.

Data availability statement

All data supporting the findings of this study have been included in the manuscript and supplementary materials where applicable. No publicly archived datasets were used, but further inquiries can be directed to the corresponding author.

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