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Microbial and Fungal Contamination of Different Dried Cocoyam Flakes during Storage

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HIGHLIGHTS:

- The samples absorbed a significant level of moisture during storage period.
- *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, and *Staphylococcus aureus* were isolated from the samples.
- *Aspergillus flavus* and *A. niger* were isolated from all the samples.
- There was an emergence of *Rhyzopertha dominica* only in the sun-dried samples.

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Acronyms and abbreviations
CFU=Colony Forming Unit
MC=Moisture Content
NSPRI=Nigerian Stored Products Research Institute

ABSTRACT

Background: Contamination of food by microorganisms from the processing to the consumption stage has become a major health concern in this era, where a better approach for the elongation of food shelf life is explored. This study aims to evaluate the microbial and fungal contamination of different dried cocoyam flakes during storage.

Methods: The cocoyam (*Colocasia esculenta*) samples were properly cooked, peeled, sliced into thin shapes, and dried to constant weights. The drying methods used were sun, hot-air oven, and Nigerian Stored Products Research Institute (NSPRI) multi-crop dryer; samples were divided into three groups A, B, and C, respectively, and the dried samples were stored for three months. Moisture Content (MC) was conducted using the standard method. Colonial morphology, Gram staining, and biochemical test were used to identify and characterize microorganisms. Statistical analyses were performed using SPSS 20.0.

Results: There was a significant increase in the MC of all the samples after the storage duration. The sun-dried sample had the highest MC (13.60%) while the oven-dried sample had the lowest MC (10.82%). The sun-dried samples had the highest viable bacteria count (7.2×10^5 Colony Forming Unit (CFU)/g) and the oven-dried sample had the lowest count (5.1×10^5 CFU/g). The four bacterial isolates identified were *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, and *Staphylococcus aureus*. The heterotrophic fungal number was the highest (0.5×10^3 CFU/g) regarding sun-dried samples, whereas the samples dried with NSPRI multi-crop dryer and oven-dried samples had the same value (0.3×10^3 CFU/g). *Aspergillus flavus*, *A. niger*, *Rhizopus*, *Penicillium*, and *Mucor* were isolated from the samples. *Rhyzopertha dominica* was identified in sun-dried samples.

Conclusion: The increase of MC and the nature of the storage material may have contributed to high bacterial and fungal counts of stored dried cocoyam flakes, especially sun-dried ones, thereby exposing consumers to potential health risks.

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Introduction

In recent times, the processing and consumption of dried foods and food ingredients have been appreciated in many countries due to convenient storage and transportation (Hammond et al., 2015). Food drying is a preservation method that works by dehydrating water from food, thus suppressing the growth of microorganisms (Deng et al., 2019). Fresh products and seafoods are dried by sun drying or convective drying to maintain their quality for prolonged storage. Dried food products could be contaminated with pathogenic bacteria through a wide variety of channels during processing, storage, transportation, and distribution. The contamination was occurred because of improper storage conditions including storage temperature, relative humidity, and packaging materials (Guo et al., 2023). Several studies have reported that dried foods and food ingredients were contaminated with pathogenic bacteria such as *Cronobacter* sp. (Cechin et al., 2022), *Escherichia coli* (El-Prince et al., 2023), and *Salmonella* sp (Makinde et al., 2020). Particularly, pathogenic spore-forming bacteria and molds have been identified as significant hazards in dried foods. Spores of *Bacillus cereus* and *Clostridium perfringens* have been detected in dried foods including rice cereal (Jovanovic et al., 2021), spices (Beuchat et al., 2013), and foods stored for long periods; they can germinate and grow in reconstituted products which are not properly processed or stored. Moreover, some of these molds can produce aflatoxin, ochratoxin, and other mycotoxins (Salman and Mudalal, 2022). Dried foods and food ingredients are advantageous in controlling microbial growth due to low water activity. However, they tend to absorb water as the relative humidity of the surrounding air is increased (Zhang et al., 2020). In particular, some species of spoilage molds and osmophilic yeasts can grow at low water activity values (Alp and Bulantekin, 2021; Plotnikova et al., 2022). Thus, it is important to maintain the recommended storage temperatures and relative humidity to ensure optimal product quality and safety (Elik et al., 2019).

Cocoyam flake is one of the most popular delicacies made in southeastern Nigeria. It is usually called "*Achicha ede*". It involves cooking corms, peeling, and slicing them into flakes. Cocoyam (*Colocasia esculenta*), a root and tuber crop, is a staple food in many countries and is considered a good and inexpensive source of energy in diets (Lebot et al., 2013). They are often produced with very low input but contribute greatly to food security, which are culturally held in high esteem (Okwu et al., 2021). In Nigeria, cocoyam is majorly cultivated for subsistence purposes, particularly by smallholder farmers (Ukwu et al., 2022). Their corms can be boiled, baked, or partly boiled and fried before consumption. The corms are sometimes ground into flour for pastry and can be stuffed with meat or other fillings and sometimes into flakes. The young leaves can be boiled and used as vegetables similar to spinach (Ramawat and Mérillon, 2013).

The flakes are dried under the sun for some days and stored for a very long time. It is subsequently pounded and prepared into a delicacy whenever the need arises, particularly in times of food scarcity. During cocoyam flake processing, some unwholesome practices such as poor handling and processing methods which might introduce some microorganisms, especially during drying and storage, may have been involved. Therefore, the present study aims to investigate the growth of microorganisms in different dried cocoyam flakes during storage.

Materials and methods

Collection of samples

Cocoyam corms were purchased from a smallholder farm in Onicha-Igboeze, Onicha L.G.A, Ebonyi state, and brought to Nigerian Stored Products Research Institute (NSPRI) Port-Harcourt zonal station, River State for laboratory analysis in October 2022.

Sample preparation

The corms weighing about 30 kg were sorted, washed, and boiled for 4 h. They were cooled, peeled, and sliced into thin shapes using a clean kitchen knife. The sliced flakes were divided into three groups of A, B, and C. Group A was sundried to a constant weight between 9:30 am and 4:00 pm daily for six days. B was dried in the oven to a constant weight at 60 ^{o}C for 72 h, and C was dried using NSPRI multi-crop dryer for 24 h. Afterwards, the samples were stored in zip-loc bags at an ambient temperature for three months, while analyses were carried out once a month for the three months storage duration.

Moisture content (MC) determination

The MC of samples was determined according to the standard methods of the Association of Official Analytical Chemists (AOAC, 2019). Two g of each sample was weighed into containers for measuring moisture and dried at 130 °C for 1 h. The samples were cooled in a desiccator and weighed using a sensitive analytical weighing balance.

Enumeration and isolation of bacteria

Enumeration of total heterotrophic bacteria was determined by inoculating the surface of Nutrient Agar (Titan Biotech, India) plates with 0.1 ml of 10^{-3}-10^{-5} dilutions in triplicates. The aliquot was evenly spread using

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a sterile bent glass rod followed by incubation of the inoculated plates in the incubator at 37 °C for 24 h. After incubation, colonies that grew on the respective plates were counted using standard plate count to determine the bacterial loads of each sample (Prescott et al., 2011).

Identification of bacterial isolate

The identification of bacterial isolates was determined based on their morphological and cultural characteristics. Morphological characteristics which were adopted included color, shape, texture, size of colonies, and Gram staining technique while the cultural characteristics adopted were sugar fermentation tests, Methyl Red (MR) test, Voges Proskauer (VP), indole, catalase, oxidase, and citrate tests. Identities of bacterial isolates were further authenticated by referencing their characteristics with those presented in Bergy's manual of systematic bacteriology (Prescott et al., 2011).

Enumeration and isolation of fungi

Sabouraud Dextrose Agar (SDA; Titan Biotech Ltd., India) which contained antibiotics to inhibit bacterial growth (Kpormon and Douglas, 2018) was used to determine the total heterotrophic fungi count. The spread plate technique was adopted (Prescott et al., 2011). An aliquot (0.1 ml) of 10^{-3} regarding the serially diluted samples was inoculated in duplicates on the surface of SDA plates, and then, spread evenly with a flame glass spreader. The plates were incubated at 25 °C for 72 h after which the colonies were counted, and the mean of the count was recorded (Douglas and Robinson, 2019).

Identification of fungal isolates

Spores of the fungal isolates were picked using a flamed inoculating pin, placed on a clean glass slide, and gently spread with inoculating pin and a drop of Lactophenol Cotton Blue (LPCB). The preparation was covered with a cover slip, and then, observed with a microscope (OPTIKA, Italy). The presence or absence of septa in the mycelium, type of spore, presence of primary or secondary sterigmata, and other microscopic characteristics as well as cultural characteristics were used in the identification of the fungal isolates. These isolates were ascertained by comparison with a fungal atlas (Kidd et al., 2016).

Entomological inspection of samples

The samples were also examined for any form of insect infestation within the period of study.

Data analysis

One-way analysis of variance and then Duncan's pairwise comparison test were performed using SPSS 20.0 software.

Results

MC

The results of MC percentage of dried stored cocoyam flakes processed by sun, oven, and NSPRI multi-crop dryer are shown in Table 1. It ranged from 11.22-13.60 (A), 8.80-10.82 (B), and 10.38-12.13% (C). After drying, group A retained the highest (11.22%) MC level, whereas group B had the lowest level (8.80%). There was an increase in the value of moisture in all the groups from the beginning to the end of the storage duration. Group A had the highest percentage of MC (13.60%) and B had the lowest (10.82%) in the 3rd month of the storage period.

Total viable bacteria count

Table 2 displays the total heterotrophic bacteria count, measured in units of 10^5 Colony Forming Units (CFU)/g), at different times (month 0, month 1, month 2, and month 3) for three different drying methods: sun dried (A), oven dried (B), and NSPRI dried (C). It ranged from 2.70±0.40 to 7.20±0.50, 1.90±0.20 to 5.10±0.60, and 0.90±0.30 to 5.70±0.90 CFU/g, respectively. A total of four bacterial species were isolated from the samples during the period of storage as shown in Table 3. They included *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, and *Staphylococcus aureus*.

Heterotrophic fungal count

Table 4 displays the total heterotrophic fungi count, ($\times 10^3$ CFU/g), at different time points (month 0, month 1, month 2, and month 3) for three different drying methods: sun dried (A), oven dried (B), and NSPRI dried (C). It ranged from 0.20±0.10 to 0.50±0.20 CFU/g (A), 0.10±0.00 to 0.40±0.00 CFU/g (B), and 0.10±0.10 to 0.30±0.00 CFU/g (C). After the third month of storage, the sun-dried sample showed the highest mean fungal count of 0.5×10^3 CFU/g while the sample dried with NSPRI multi-crop dryer had the lowest amount at 0.3×10^3 CFU/g. Moderate growth of *Aspergillus flavus*, *A. niger*, *Penicillium*, *Rhizopus*, and *Mucor* were isolated from the samples.

Entomological inspection of samples

No insects were reported after 30 days of storage. However, emergence of adult insects was observed in the second month (60 days after storage) in sample A (sun-dried); they were examined and identified as *Rhyzopertha dominica.* The population of insects increased in the third month (90 days after storage). No insect was dried with a hot air oven and the NSPRI multi-crop dryer.

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Table 1: Percentage of Moisture content (MC) of stored dried cocoyam flake sample

Group	0th Month	1st Month	2nd Month	3rd Month
A	11.22±0.04 [c]	11.63±0.13 [c]	12.13±0.17 [c]	13.60±0.05 [c]
B	8.80±0.03 [a]	9.13±0.03 [a]	10.00±0.10 [a]	10.82±0.22 [a]
C	10.38±0.07 [b]	10.57±0.07 [b]	11.43±0.12 [b]	12.13±0.06 [b]

Values are Mean±SEM (n=3). Means with the same letters along the same column are not significantly different at $p<0.05$.
A=Sun-dried; B=Oven-dried; C=Nigerian Stored Products Research Institute (NSPRI) dried.

Table 2: Total bacterial counts (Colony Forming Unit (CFU)/g) for dried cocoyam flake samples

Sample	Total Heterotrophic Bacteria Count (× 10^5 CFU/g)			
	Month 0	Month 1	Month 2	Month 3
A	3.10±0.40	2.70±0.40 [c]	4.70±0.40	7.20±0.50
B	2.40±0.30	1.90±0.20 [ab]	3.10±0.60	5.10±0.60
C	1.70±0.30	0.90±0.30 [a]	3.40±0.20	5.70±0.90

*Values are the mean and standard error of duplicate
a-c: Different characters in the same column indicate values with significant differences ($p<0.05$)
A=Sun-dried; B=Oven-dried; C=Nigerian Stored Products Research Institute (NSPRI) dried

Table 3: Characterization of bacterial isolates

Morphological characteristics	Biochemical characteristics										Sugar fermentation						
Colonial/Cell characteristics	Gram reaction	Spore Formation	Coagulase	Catalase	Oxidase	Indole	M R	V P	Citrate	Motility	Sucrose	Mannitol	Lactose	Glucose	Galactose	Fructose	Organism
Creamy, Round, Opaque, Entire, Small, Elevated, Moist.	+ Rod	+	-	-	+	-	+	-	+	+	+	+	+	+	-	+	*Bacillus subtilis*
Golden yellow, Raised, Translucent, Moist	+ Cocci	-	+	+	-	-	+	+	+	-	+	+	+	+	+	+	*Staphylococcus aureus*
Greenish yellow, Smooth	- Rod	-	-	+	+	-	-	-	+	+	+	+	-	+	-	+	*Pseudomonas aeruginosa*
Yellow, Round, Opaque, Small	+ Cocci	-	+	+	+	-	-	-	+	-	-	+	-	+	+	+	*Micrococcus luteus*

Keys: (-)=Negative; (+)=Positive; MR= Methyl Red; V.P=Voges Proskauer

Table 4: Total fungal counts (Colony Forming Unit (CFU)/g) for the dried cocoyam flakes samples

Sample	Total Heterotrophic Fungi Count (×10^3 CFU/g)			
	Month 0	Month 1	Month 2	Month 3
A	0.00±0.00	0.20±0.10	0.30±0.10	0.50±0.20
B	0.00±0.00	0.10±0.00	0.40±0.00	0.30±0.20
C	0.00±0.00	0.10±0.10	0.30±0.00	0.30±0.00

* Values are the mean and standard error of duplicate
a-c: Different characters in the same column indicate values with significant differences ($p<0.05$)
A=Sun-dried; B=Oven-dried; C=Nigerian Stored Products Research Institute (NSPRI) multi-crop dried sample

Discussion

In many studies, MC in food samples has been described as the amount of water contained in a food product. Shelf-life of food products are determined by the quantity of water in that product (Liu et al., 2022). The MC of the sample after drying ranged from 8.80-11.22%. The sun-dried sample was 11.22%, and the oven-dried sample was 8.80%. The result was in agreement with the work of Ilondu (2017) who observed that sun-dried samples of *C. esculenta* chips retained a high percentage of moisture when compared to the oven-dried ones. The high percentage of moisture in the sun-dried sample may be

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attributed to the temperature and relative humidity of the environment. The low MC of the cocoyam flakes through drying may help enhance the sample's shelf-life during storage. The minimum quantity of moisture at which the product is safe for long time storage is considered a safe moisture level (Afolabi, 2014). According to Liu et al. (2022), dried, desiccated, or low-moisture foods are those that generally do not contain more than 25% moisture. Analysis of variance shows that there was a significant increase in the MC of the stored cocoyam flake samples. The MC after the storage duration ranged from 10.82-13.60%, with sun-dried samples being the highest and oven-dried samples the lowest. The significant increase in the MC of the samples might affect the shelf-life of the sample.

There was a significant increase in the MC of the cocoyam flakes during storage. Drying the flakes with oven resulted in lower MC; this was while drying using NSPRI technology was a better option than sun drying. This result was consistent with Okwu et al.'s study (2021). They observed that the MC of stored cocoyam flour increased during the storage period. Root and tuber flours are hygroscopic and can absorb moisture from the environment. Dried foods tend to absorb water as the relative humidity of the surrounding air is increased (Zhang et al., 2020). Furthermore, the increase in MC could be attributed to the type of packaging material used. Low-density polythene packaging materials could absorb moisture from the environment (Chitravathi et al., 2015).

Microorganisms are abundant in nature (Garcia, 2016), thus the ease at which they are freely transported can lead to contamination of food samples. Previous studies have reported that microbial contamination of food samples is influenced by various factors, including handling, processing, storage, and transportation (Guo et al., 2023). The total viable bacterial count, measured in units of 10^5 CFU/g, regarding cocoyam flake samples during storage ranged from 2.70±0.40 to 7.20±0.50 (A), 1.90±0.20 to 5.10±0.60 (B), and 0.90±0.30 to 5.70±0.90 (C). There was a gradual increase in the microbial load in each of the drying methods, possibly due to an increase in MC. These results were in line with Okwu et al.'s research (2021) who reported that absorption of moisture shortened the shelf-life of stored cocoyam flour. Ojewumi et al. (2016) in another study has been reported that high amount of MC potentiate biodeterioration of fermented *Parkia biglobosa* seeds.

Furthermore, the study revealed that lower bacteria counts were recorded in the NSPRI-dried cocoyam flakes when compared to other drying methods. This could be attributed to the aseptic nature and condition of the NSPRI dryer. Moreover, data suggested at the end of the study, the oven-dried flakes had lower counts than the NSPRI-dried sample. Four bacterial species were isolated from cocoyam flake samples during the period of storage. They included *B. subtilis*, *P. aeruginosa*, *M. luteus*, and *S. aureus*. This finding was in line with the work of Okwu et al. (2021); they reported isolating ten bacterial species including *M. luteus*, *Bacillus* species, *S. aureus*, and *Pseudomonas* from the stored cocoyam flour. They observed that the isolated bacterial species could have been associated with food providers, equipment, and raw materials which played an important role in food spoilage; some of them (*Staphylococcus* sp, *Pseudomonas* sp, and *Bacillus* sp) are pathogenic (Kharel et al., 2016). S. *aureus* and *B. subtilis* grow well in protein and carbohydrate-rich foods, and they are tolerant to high levels of salt (Møretrø and Langsrud, 2017; Ojewumi et al., 2016). According to Gavahian et al. (2018), processing conditions such as drying and heat treatment might reduce microbial levels, but recontamination could take place during the post-processing or storage practices. Okwu et al. (2021) also mentioned that the sun-dried cocoyam flour sample had a higher level of bacteria growth than the oven-dried and the sample dried in the cabinet. Similarly, this study showed that the bacterial count of the sun-dried sample increased steadily to 7.2×10^5 CFU/g in the 3rd month of the storage period. This increase was attributed to the uncontrolled settling of substances including dust debris on the surface of the sample during drying. The findings suggested that drying methods can influence the growth and survival of heterotrophic bacteria in the food product, which has implications for food safety and quality. Lower bacteria count recorded in NSPRI multi-crop and oven-dried cocoyam flakes in this study indicated that processing and storage conditions may influence the presence and number of microorganisms present in the processed stored cocoyam flakes. Some of these food-borne pathogens can survive for several months, even years, in low-water activity foods and in food preparation environments in case of inadequate drying as observed in sun-dried samples (Beuchat et al., 2013). The growth conditions for microorganisms depended on specific intrinsic and extrinsic factors such as temperature, water activity, pH, oxidation-reduction potential, microbial interactions, and nutrient content (Guo et al., 2023).

The heterotrophic fungal count ($\times10^3$ CFU/g) of cocoyam flake samples during storage ranged from 0.20±0.10 to 0.50±0.20 (A), 0.10±0.00 to 0.40±0.00 (B), and 0.10±0.10 to 0.30±0.00 (C). The fungal count remained low in all samples, indicating that the drying methods used were effective in controlling fungal growth. The results also suggested that the fungal population in the samples may be affected by environmental factors such as MC and nutrient availability, which can vary depending on the drying method used. Fungi are widely distributed in the air and in the soil (Abrego et al., 2020). These findings

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have implications for food safety and quality, as fungi can cause spoilage and produce toxins that can be harmful to human health. Moderate growth of *A. flavus*, *A. niger*, *Penicillium*, *Rhizopus*, and *Mucor* were isolated from the samples. The moderate growth of fungi in the final product may indicate poor handling during processing and storage conditions which allowed the growth and proliferation of these organisms (James et al., 2022). *Aspergillus* and *Penicillium* sp. were previously isolated from stored cocoyam flour and may have contaminated the products through the soil during the processing and storage (Okwu et al., 2021). *Rhizopus* and *Mucor* sp. are less fastidious and frequently involved in the spoilage of food with low MC (Anyanwu et al., 2023). *A. flavus* is famous for aflatoxin production (Ali et al., 2022). It produces a variety of enzymes which facilitate the growth of fungi on stored agricultural products such as corn, peanuts, soybeans, and groundnuts (James et al., 2022).

There were no insects after 30 days because this period was shorter than 36 to 42 days of developmental period reported by Estelle et al. (2019). However, adult insects were observed in the second month (60 days after storage) in sample A (sun drying); they were examined and identified as *R. dominica*, and this was similar to the findings of Isah et al. (2012) who identified *R. dominica* and other insects on cassava, cocoyam, yam, and plantain chips after 60 days. The high MC recorded for sample A could account for adult insects in A because it will enhance easy tunnelling by beetles (Omojasola and Sanu, 2013). The population of insects increased in the third month (90 days after storage), and this was similar to the findings by Saeed and Laing (2023) who observed an increase in *Sitophilus zeamais* after 90 days due to extensive tunneling of the grain (maize). The additional 30 days of storage exposed the flakes to an increase in population, and Isah et al. (2012) reported that in the presence of pests, chips kept for more than a month would be under serious threat. Omojasola and Sanu (2013) observed that *R. dominica* was one of the insects that greatly attacked sun-dried stored yam chips. Storage bags, varietal resistance, and biological control have been suggested as an important strategy against *Dinodenus porcellus* which attacks and spoils stored yam chips (Estelle et al., 2019)

Conclusion

This study revealed the health risk exposure of consumers regarding the samples assayed in the southeast of Nigeria, especially sun-dried cocoyam flakes, which had very high viable bacterial and fungal counts. The high number of *A. flavus* in these samples raises concerns about the susceptibility of consumers to mycotoxin-related diseases. Stakeholders in food industry and agricultural sector should review the food value chain from farm to people and identify critical control points in managing the situation. Open sun drying of cocoyam flakes should be discouraged as microorganisms, dust, and other obnoxious particles could be introduced into the flakes. Better storage materials such as Hermetic drum that suitably prevent moisture absorption and insect infestation should be explored.

Author contributions

O.O.O. designed the work; M.E.I. and F.E.N. carried out the microbial analysis; S.D.D. conducted MC determination; E.N. inspected the samples for entomological parameters; C.C.A. analyzed data; O.O.O. also searched the literature and wrote the manuscript. All authors read and approved of the final manuscript.

Conflicts of interest

The authors declared no conflict of interest.

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