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Shelf stability of processed cocoyam flour during storage at room temperature (28.0 ± 2°C) for a period of four months

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The shelf life study of dry milled cocoyam flour packaged in low density polyethylene was carried out for a period of 4-month. Microbiological, nutritional, physicochemical quality characteristics and aflatoxin content were evaluated. The total viable bacterial counts ranged from 1.6×10^3 - 4.8×10^5 cfu/g while the total viable fungal count increased from 5.0×10^1 - 3.8×10^5 sfu/g. The bacteria isolated include *Bacillus* species, *Bacillus subtilis, Proteus* species, *Staphylococcus epidermidis, Streptococcus pyogenes, Micrococcus luteus, Klebsiella* species, *Staphylococcus aureus, Pseudomonas* species and *Staphylococcus saprophytic.* Fungal genera isolated include *Penicillum* species, *Aspergillus flavus, Aspergillus niger, Fusarium*, *Mucor* and *Rhizopus* species. Gradual decrease in pH (6.40 ± 0.001 to 4.17± 0.01) and noticeable increase in titrateable acidity (0.024 ± 0.003 to 1.17 ± 0.01%) were observed during storage. There was an increase in moisture content while carbohydrate, protein, fat, crude fibre and ash were found to decrease during storage. Aflatoxin B1 and B2 content from 0 h to the 4th month were (0.020, 0.006) and (0.097, 0.063) µg/kg respectively. The presence of aflatoxin B_1 and B_2 is of public health concern. There is need for improved processing, handling techniques and good hygiene practices to ensure safety of the finished product.

Key words: Cocoyam, shelf life, room temperature, aflatoxin content, nutritional analysis.

INTRODUCTION

Cocoyam is an herbaceous perennial plant which belongs to the family Araceae. Cocoyams are originally from the tropical and sub-tropical countries and studies reveal that cocoyam is among the least studied root plants. Some species include *Xanthosoma sagittifolium, Amorphophallus titanum* and *Colocasia esculenta*. This species, *Xanthosoma sagittifolium*, is food for over 400 million people worldwide and is the most consumed in West Africa (Boakye et al., 2018). According to Onyeka (2014), Africa is the major producer with West and Central Africa, notably, Nigeria, Ghana, and Cameroon contributing to over 60% of the total African production. Nigeria is the world's largest producer of cocoyam. The average production figure for Nigeria is 5.400 Metric Tonnes which accounts for about 37% of total world's output of cocoyam (FAO, 2012). It is nutritionally

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superior to yam and cassava in terms of its digestibility, contents of crude protein and essential minerals, such as Ca, Mg, and P (Chukwu et al., 2012). All parts of the cocoyam (corm, cornel, leave and flower) are edible and the corms contain approximately 25% starch and eaten mainly as thickeners, purees or whole (Ejoh et al., 2013).

It is used in the treatment of diabetes, prevention of cancer and as food for the aged people, individuals and children (Kundu et al., 2012).

The crop has assumed nutritional and industrial significance in flour industries (Onwubuya and Ajani, 2012). Cocoyam flour can be used in the production of bread, cakes and also in chin-chin production.

Cocoyam is a great source of dietary fibre and starch that can generate energy to the body (Adekiya et al., 2014); also an adequate source of potassium (Nnabuk et al., 2012).

Previous works have been carried out on the functional properties of raw and precooked taro (*C. esculenta*) flour (Tagodoe and Nip, 1994), starch structure and some properties of cocoyam (Sefa-Dede and Sackey, 2002), production of ethanol from cocoyam (*C. esculenta*) (Braide et al., 2011) and effects of processing on energy value, nutrient and anti-nutrient components of wild cocoyam (Olajide et al., 2011). Despite the increasing demand and usage of cocayam in Nigeria, there is little scientific information on the quality characteristics, shelf stability and packaging existing in literature, hence the need for this present work.

MATERIALS AND METHODS

Healthy red coloured cocoyam (*C. esculenta*) tubers (100 kg) were purchased from different vendors at Iruekpen market, Edo State, Nigeria, and placed in two sterile Hessian bags of 50 kg each. Thereafter, it was taken to Ambrose Alli University Microbiology laboratory where it was immediately processed, packaged and analyzed.

Preparation of sample

The cocoyam tubers were peeled, washed, sliced thinly, and oven dried at a temperature of 65°C for 2 h. Thereafter, it was ground into powdered form with a milling machine and immediately packaged in a low density polyethylene (LDPL) (50 g per pack), sealed, labeled and kept on the shelf at ambient temperature (28 ± 2°C) for further analyses.

Microbiological analysis

Samples of the packaged cocoyam flour were analyzed microbiologically. At intervals (1 week, 2 weeks, 1 month, 2 months, 3 months and 4 months), 10 g of each sample was homogenized in 90 ml of sterile distilled water for 2 min to obtain stock solution. A further ten-fold serial dilution was done up to 10^{-8} for colony counts. Aliquots (1 ml) of appropriate dilutions were aseptically pour-plated in nutrient agar for isolation of bacteria and on acidified potato dextrose agar for isolation of fungi under aseptic conditions. The nutrient agar plates were aerobically incubated at 37°C for 24 to 48 h, while the potato dextrose agar plates were incubated at room temperature (28.0 ± 2°C) for 3 to 5 days. At the end of incubation, the colonies were enumerated and recorded. The bacterial isolates were purified, characterized and identified using series of cultural and biochemical tests as described by Ochei and Kolhatkar (2008). The fungal isolates were identified microscopically using lacto-phenol cotton blue test.

Physicochemical and nutritional analysis

The moisture content of the samples was determined according to Cole (2002). This is an oven dry method in which weight of the various samples was placed in previously weighed watch glass and the initial weight noted. Thereafter, it was placed in a vacuum oven (Gallenkamp) at 95±2°C. At intervals, the sample was brought out and weighed, until the weight became constant (final weight). The difference between the initial and the final weight was recorded as the moisture content. The pH readings were obtained using a digital Jenway Model 3510 benchtop pH meter. The available carbohydrates, proteins, lipids, ash, crude fibre and titratable acidity (TA) were also determined according to AOAC (2008) methods.

Aflatoxin determination

The extraction, detection and quantification of aflatoxin were done according to the method of Jonathan and Esho (2010). Known weight (5 g) of sample was added to 7 ml of distilled water and 25 ml of chloroform, the mixture was shaken and left for 30 min after which the solution obtained was filtered using a Whatman No. 1 filter paper. Extract was obtained and evaporated to dryness to a volume of 5 ml on a hot water bath (Gallenhamp, England). 0.5 ml of the reconstituted extract with chloroform was spotted on a pre-coated 20 × 20 cm^2 thin layer chromatography (TLC) plate along with aflatoxin standard of known concentration. Developed TLC plate was air dried at ambient temperature (28 ± 2°C) and aflatoxins were detected under UV light at wavelength of 360 nm (Cecil Instrument CE505). The preparative TLC plates employed in the quantification were 0.5 µm thick. On detection of the area containing the toxin of interest, it was scrapped off, eluted with chloroform and filtered using Whatman No 1. filter paper. The extract was evaporated to dryness and reconstituted with 3 ml chloroform. Alongside with aflatoxin standard of 20 µg/ml concentration, the absorbance was determined on an ultraviolet spectrophotometer (Cecil Instrument CE505) at a wavelength of 360 nm.

This approach as used by Jonathan and Esho (2010) was calculated as follows:

$$\text{Aflatoxin concentration, C (µg/kg)} = \frac{\text{Absorbance of sample} \times \text{Concentration of standard} \times \text{Dilution Factor}}{\text{Absorbance of Standard}}$$

RESULTS AND DISCUSSION

The total viable bacterial counts of the cocoyam flour samples during storage for four months ranged from 1.6×10^3 - 4.8×10^5 cfu/g (Table 1). There was gradual but steady increase in the bacterial count throughout the

Table 1. Total viable counts (TVC) of cocoyam flour stored at Room Temperature (28.0 ± 2°C) for a period of four (4) months.

Period of storage	Total Viable Count	
	Bacteria (cfu/g)	Fungi (sfu/g)
0 h	Nil	5.0×10^1
1 week	1.6×10^3	7.0×10^2
2 weeks	3.5×10^3	6.8×10^3
1 month	1.0×10^4	1.5×10^4
2 months	6.0×10^4	1.7×10^4
3 months	9.1×10^4	2.7×10^5
4 months	4.8×10^5	3.8×10^5

Each value is the mean of triplicate determinations.

Table 2. Bacteria isolated from cocoyam flour during storage at Room Temperature (28.0 ± 2°C) for a period of four (4) months.

Isolate	Period of Storage						
	0 h	1 Week	2 Weeks	1 Month	2 Months	3 Months	4 Months
Bacillus subtilis	-	+	+	+	+	+	-
Staphylococcus epidermidis	-	-	-	+	+	+	+
Staphylococcus saprophyticus	-	+	+	+	-	-	-
Klebsiella species	-	+	+	+	+	+	+
Proteus species	-	+	+	+	+	-	-
Micrococcus luteus	-	-	-	-	+	+	+
Streptococcus pyogenes	-	-	-	-	-	+	+
Pseudomonas species	-	-	-	-	+	+	+

+ = Present; - = Absent.

of storage.

The total viable fungal counts of cocoyam flour samples ranged from 5.0×10^1 to 3.8×10^5 sfu/g. There was also a gradual increase in the fungal counts with increase in storage period (Table 1).

The increase in microbial load might be due to increase in moisture content during storage. These results are in line with Modupe et al. (2016) who showed that a high moisture content has been reported to potentiate biodeteroriation. The cocoyam flour is hygroscopic and can absorb moisture from the environment. Furthermore, the increase in moisture could be attributed to the type of packaging material used. Low density polythene packaging material has the ability to absorb moisture from the environment.

A total of ten bacterial species were isolated from the cocoyam flour sample during the four months of storage (Table 2), they include: *Staphylococcus epidermidis, Staphylococcus saprophytic, Staphylococcus aureus, Micrococcus luteus, Streptococcus pyogenes,* species of *Bacillus, Klebsiella, Pseudomonas* and *Proteus.* While six fungal genera were isolated (Table 3). The fungal isolates include *Aspergillus niger, Aspergillus flavus,* species of *Rhizopus, Fusarium, Penicillium* and *Mucor.* Bacterial species isolated have been associated with food handlers, equipment and raw materials and they play important role in the spoilage of food and some of them (*Staphylococcus aureus*, *S. pyogenes* and *Bacillus cereus*) are pathogenic (Moretro and Langsrud, 2017). The processing of cocoyam flour involves lot of manual handling and this might be one of the sources of contamination.

S. aureus grows well in protein and carbohydrate rich foods and it is tolerant to high levels of salt (Moretro and Langsrud, 2017). According to Sachindra et al. (2005), the processing conditions such as drying and heat treatment might reduce microbial levels, but recontamination could take place during the post-processing handling or storage practices. Processing and storage conditions may influence the presence and number of microorganisms present in the processed cocoyam flour. The growth conditions for microorganisms are dependent on specific intrinsic and extrinsic factors such as temperature, water activity, pH, oxidation-reduction potential, microbial interactions and nutrient content (Jay, 2000).

The predominant fungi were *Fusarium* spp., *A. niger* and *A. flavus*. *A. niger, A. flavus* and *Penicillium* spp.

Table 3. Fungi associated with cocoyam flour during storage at Room Temperature (28.0 ± 2°C) for a period of four (4) months.

Isolate	Period of storage						
	0 h	1 Week	2 Weeks	1 Month	2 Months	3 Months	4 Months
Rhizopus species	-	+	+	+	+	+	+
Aspergillus niger	+	-	-	+	+	+	+
Aspergillus flavus	+	+	+	+	+	+	+
Fusarium species	+	+	+	+	+	+	+
Penicillium species	+	+	+	+	+	+	+
Mucor species	-	-	-	-	+	+	+

+ = Present; - = Absent.

Table 4. Biochemical (Nutritional) quality of cocoyam flour stored at Room Temperature (28.0 ± 2°C) for a period of four (4) months.

Parameter	% Composition	
	0 h	4th Month
Moisture	5.27 ± 0.01	6.90 ± 0.02
Ash	3.28 ± 0.04	2.92 ± 0.03
Protein	15.84 ± 0.02	14.95 ± 0.01
Fat	1.93 ± 0.05	1.86 ± 0.02
Carbohydrate	67.76 ± 0.003	58.08 ± 0.005
Crude Fibre	0.76 ± 0.01	0.67 ± 0.04

Each value is the mean ± standard deviation of triplicate determinations.

were isolated throughout the storage of the flour samples. *Mucor* spp. emerged after two months of storage. This indicated that ecological succession may have occurred during the storage of the cocoyam flour.

High number of fungi in the final product may indicate poor handling during processing and storage conditions (temperature and humidity) which allowed the growth and proliferation of these organisms (Mandeel, 2005).

Fungi are widely distributed in air and in the soil (Braide et al., 2008). *Aspergillus* and *Penicillium* spp. are frequently isolated from food and may have contaminated the products through the soil during processing and storage (Abbey, 2007). *Rhizopus* and *Mucor* spp. are less fastidious and are frequently involved in the deterioration and spoilage of food with low moisture content (Braide et al., 2011).

There was notable increase in the moisture content (5.27%-14.90%) at the end of storage period as presented in Table 4. However, decreases were observed in percentage Ash (3.28 - 2.92%), Protein (15.84 - 14.95%), Fats (1.93 - 1.86%), Crude Fibre (0.76 - 0.67%) and Carbohydrates (67.76 - 58.08%) contents at the end of four months.

The decrease in carbohydrate, protein and fat could be attributed to high microbial activities potentiated by the high moisture content. Also, the drying process applied may have denatured the protein structure leading to decrease with storage period. Presence of microbial contaminants may have encouraged utilization of the nutrients in the stored cocoyam flour for their growth and proliferation. Increase in moisture content could also be attributed to the low density polythene packaging material which has the ability to absorb moisture from the environment. High moisture content encourages prolific growth of bacteria and mould in foods (Kordylas, 1991).

There was a notable decrease in pH (6.40 - 4.17) and increase in percentages TA (0.024 - 1.116%) during storage period (Table 5). The low pH observed may be related to the activities of associated microbes which may have increased the release of some organic acids and other metabolites; thereby increasing the titratable acidity.

The mean aflatoxin concentration of the sample obtained is shown in Table 6. Aflatoxin G1 and G2 were not detected in the samples analyzed at 0 h, but B1 and B2 were detected and also as the month of storage increased, the level of aflatoxin content gradually increased. *Aspergillus, Penicillium* and *Fusarium* spp. produce various mycotoxins in food under storage (Efiuvwevwere, 2000; Abbey, 2007). *A. flavus* elaborate aflatoxins that may induce hepatocellular carcinoma.

Toxins produced by *Penicillium* spp. may be nephrotoxic and carcinogenic, *Fusarium* spp. toxins give

Table 5. Physico-chemical quality of packaged cocoyam flour stored at Room Temperature (28.0 ± 2°C) for a period of four (4) months.

Period of storage	pH	TA (as % Lactic acid)
0 h	6.40 ± 0.001	0.024 ± 0.003
1 Week	5.97 ± 0.03	0.09 ± 0.001
2 Weeks	5.70 ± 0.01	0.109 ± 0.003
1 Month	5.60 ± 0.02	0.128 ± 0.05
2 Months	5.07 ± 0.04	0.162 ± 0.03
3 Months	4.50 ± 0.03	0.17 ± 0.01
4 Months	4.17 ± 0.01	1.17 ± 0.01

Each value is the mean ± standard deviation of triplicate determinations.

Table 6. Aflatoxin content (µg/kg) in samples of packaged cocoyam flour stored at Room Temperature (28.0 ± 2°C) for a period of four (4) months.

Period of storage	Aflatoxin content (µg/kg)			
	B_1	B_2	G_1	G_2
0 h	0.020	0.006	0.00	0.00
1 month	0.041	0.029	0.011	0.009
2 months	0.046	0.033	0.017	0.010
3 months	0.078	0.057	0.026	0.019
4 months	0.097	0.063	0.031	0.025

rise to allergic symptoms or are carcinogenic in long term consumption (Pitt, 2000; Abbey, 2007). *Rhizopus* and *Mucor* spp. also produce mycotoxins associated with various mycotoxicoses (Abbey, 2007). The presence of mycotoxins in our food systems and tissues has enormous public health significance because these toxins are nephrotoxic, immunotoxic, teratogenic and mutagenic. They are also capable of causing acute and chronic effects in man and animals ranging from death to disorder of central nervous, cardiovascular, pulmonary systems and intestinal tract (Bhat and Vasanthi, 2003).

The values of aflatoxin determined were insignificant when compared with the values provided by National Agency for Food and Drug Adminstration and Contol (NAFDAC), Nigeria. NAFDAC has recently given a permissible limit for AFB1 of 4 - 5µg for beans, wheat and flours (Makun et. al., 2010).

Conclusion

The study has revealed that obvious microbiological, physico-chemical and biochemical changes took place during processing and storage of cocoyam flour packaged in low-density polyethylene (LDPE) under tropical temperature. The presence of aflatoxin B_1 and B_2 in the stored product is of public health concern. Therefore, there is need for improved processing and handling techniques, hygiene practices and safety of the finished product. Findings obtained may be useful in the handling and storage of cocoyam flour.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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