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FERMENTATION AND PRODUCTION OF COCOYAM (*Colocasia esculenta*) FLOUR FORTIFIED WITH SOYBEAN POWDER

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ABSTRACT

Cocoyam (*Xanthomonas sagittifolium*) is consumed by millions of people in the West African sub-region and in Nigeria in particular, regardless of ethnicity and socio-economic class. However, production and handling methods of cocoyam flour have not been standardised, resulting in a product with varying quality and safety indices and hence varying public health concerns. Fermentation and production of cocoyam flour fortified with soybean powder were carried out to determine its suitability or otherwise for consumption. Ten tubers of cocoyam were purchased from *Eke*-Awka market, Awka South Local Government Area, Anambra State. The microorganisms isolated are *Aspergillus fumigatus, Aspergillus flavus, Rhizopus stolonifer, Penicillium sp., and Geotricium sp.,* which belong to the fungi family, while *Bacillus sp. and Streptococcus sp.* belong to the bacteria family. The result also revealed that the longer the storage time, the higher the pH and moisture content. Proper storage is recommended owing to the public health concerns due to endotoxins, food safety, shelf life, and biostability of this product.

Keywords: *Aspergillus fumigatus*, *Aspergillus flavus*, *Rhizopus stolonifer*, flour, cocoyam

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INTRODUCTION

Cocoyam (*Colocasia esculenta) is* an edible, highly nutritious, and underutilised crop that belongs to the family *Araceae*. Two types of crops are known by the name cocoyam" and are both herbaceous plants. The most common one found on the market is formally known as new cocoyam (*Xanthomonas sagittifolium*), and its leaves are used as vegetables (kontomire). The second type is known as old cocoyam (*Colocasia esculenta*) or taro; it grows in marshy areas, and unlike the new cocoyam, its leaves are not eaten (Niba, 2003). Cocoyam flour is traditionally processed by peeling, sometimes slicing, and parboiling in hot water (650 °C) for a varied time, followed by steeping for 13–24 hours by sun drying to give a dry yam, which is milled into flour. Cocoyams are highly cultivated in West Africa. They are an important food crop for more than 400 million people worldwide. Cocoyams produce corms with about 25% starch on a wet weight basis, primarily consumed as purees mixed with other ingredients. Current statistics indicate cocoyam production increased over 5 years in the 1990s from 5.6 million to 8.8 million metric tonnes. Nutritionally, the tubers contain easily digestible starch and are known to contain substantial amounts of protein, fibre, vitamin C, thiamine, riboflavin, potassium, sodium, phosphorus, magnesium, calcium, and niacin. The leaves are rich in iron, folic acid, and beta-carotene (FAO, 1990; Eka, 1990; Niba, 2003).

It is estimated that in the tropics each year between 25% and 40% of stored agricultural products are wasted because of inadequate farm and village-level storage (Agu *et al.*, 2015a; Agu *et al.*, 2015b; Okigbo *et al.*, 2015; Agu *et al.*, 2016a; Agu *et al.*, 2016b). Cocoyams are susceptible to attack by pests and diseases. A typical pest is the slugs that wound corms, providing entry points for spoilage microorganisms. Losses can reach 60% of corms. Prevention is achieved through the use of disease-free planting material, weeding and hilling, and treatment with copper-based pesticides (Opara, 2000). Storage loss of root and tuber crops has plagued farmers because more than 40% of their harvests are lost as a result of deterioration (Frank and Kingsley, 2013). Fungal rot is the principal cause of root and tuber loss in storage (Frank and Kingsley, 2013; Agu *et al.*, 2014). Microorganisms that cause rotting and deterioration have been identified. These include

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Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Penicillium citrinum, Fusarium solani, Mucor spp., and Rhizopus spp. for fungi, and Bacillus sp. and *Streptococcus sp. for* bacteria (Onyimonyi, 2002). Cocoyam flour is used in soup thickeners and baking flours, in beverages, as porridge, and in producing foods for people with gastrointestinal disorders (Hussain *et al*., 1984; Ihekoronye et *al*., 1985). Corms can start rotting as early as two weeks after harvest, with tannia suffering less than taro (Passam, 1982), which recorded 90% losses in six months of storage. Such microbial decay can be controlled by pre-storage fungicide and sodium hypochlorite applications as dips, normally within 24 hours after harvest. Damage to cocoyam tissue is followed by enzymatic browning reactions from polyphenol oxidases catalysing the oxidation of polyphenols, resulting in complex formation leading to the production of pigments that cause discolorations. Sprouting and chill injury at low storage temperatures also reduce quality in stored corms (Opara, 2000).

Soyabean (*Glycine max*) is a high-protein legume grown as food for both humans and livestock. Although indigenous to Eastern Asia (Dinakin, 1995), it is known by different names in different parts of the world: soyabeans (Nigeria), Chinese peas (China), and churia beans (Manchuria) (Keshunet et *al*., 1999). The use of soy beans can be traced back to the Orient, where they were consumed in the form of fermented foods. Today, its uses range from the preparation of tofu, tempeh, and natto to the fortification and enrichment of foods such as soy-fortified wheat bread and coy soy blends, soy sauces, soy yoghurt, and soy cream cheese (Crowley and Dale, 1995; Michael, 1996). Soybeans, which have been cultivated and considered a miracle bean by many people, are the main source of protein for all of East Asia, particularly vegetarians. In Nigeria, although the cultivation of soyabeans has been successfully established, a greater percentage of the whole produce has until now been cultivated and exported as cash crops. Presently, soyabeans are incorporated into so many food formulations for both children and adults to enhance the nutritional value of foods (1996), in preparations such as ‘’dawadawa’’, allele, moi-moi, akara, soy-ogi," and most recently, "soymilk (Dinakin, 1995). Other commercial uses in

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animal feed production and the vegetable oil industry cannot be overemphasised (Kochlair, 2006).

Fermentation of cocoyam flour can be a useful tool that can be used to improve the nutritional quality and safety profile of cocoyam flour as a raw material for food product development (Okpalla *et al*., 2012; Agu *et al.*, 2013). Thus, fermentation and other elaborate processing can be carried out to enhance consumption, improve nutritional safety, storability, palatability, and convenience both in handling and utilisation (Iwuoha *et al*., 1995). Natural fermentation processes are increasingly attracting the attention of scientists and policymakers as a vital part of food security strategies (Abegazet et *al.*, 2002).

The aim of this work is to isolate and characterise the organisms involved in the fermentation and production of cocoyam (*Colocasia esculenta*) flour as well as to fortify cocoyam flour using soyabean powder.

MATERIALS AND METHODS

Sample collection

A variety of Cocoyam (*Colocasia esculenta)* cultivated in south Eastern part of Nigeria was used in the study. A total of ten tubers were obtained from *Eke*-Awka market Awka South Local Government Area, Anambra state. The cocoyams were packed in sterile cellophane bags and transported to the laboratory of the Department of Applied Microbiology and Brewing, NnamdiAzikiwe University and was assessed microbiologically.

Processing of cocoyam to cocoyam flour

Cocoyam tubers were processed to cocoyam flour in the laboratory following the method described by Babajide *et al* (1981) with some modifications. The white tubers were thoroughly washed with clean water to remove adhering soil and other undesirable materials from the cocoyam and to reduce microbial growth on the final product. Peeling was done using a sharp

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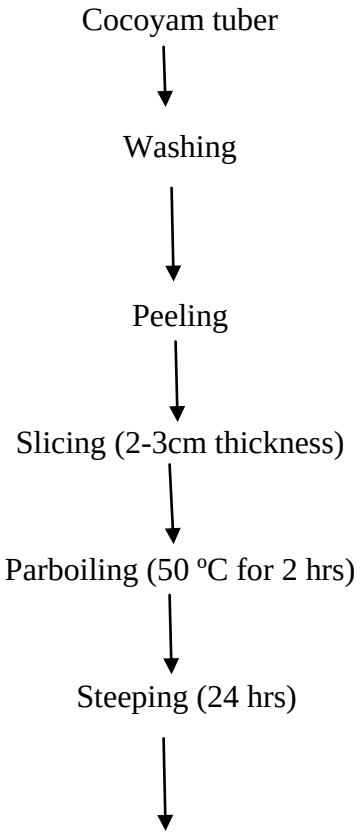
knife. The peeled cocoyamtubers were sliced into size of 2 to 3 cm in thickness so as to hasten the process of drying. The sliced cocoyam was parboiled at 50°C for 2hours in water baths, (Clifon, England), then left in the parboiling water for 24 h in order to become flabby, after which they were drained and dried at 60°C for minimum of 3 days in the hot air oven, the dried samples were weighed at intervals to obtain a constant weight. The dried cocoyam was then grounded into powder as shown in figure 1.

pH determination

The pH was determined for each processing water sample using Jenway pH meter (Model 3015, Serial no. 1647, U.K).



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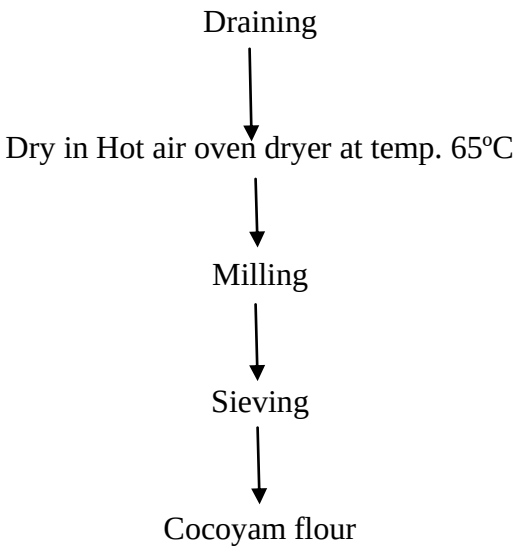

FIG 1: Flowchart of the Production of Cocoyam Flour

Source: (Babajide*et al*, 2006)

Microbiological analysis

Microbiological analyses of cocoyam flour and parboiling water were microbiologically analysed. The microbiological procedures were those recommended in the International Commission onMicrobiological Specification for Foods (1996). Culture media were those of Oxoid and Difco. Microbiological analyses included total aerobic and viable count.

Enumeration of microorganisms

Each sample container was cleaned externally with 70% ethanol to disinfect it. An appropriate serial dilution of all the samples was carried out and 0.1 ml of the selected dilution was spread onduplicate plates using sterile glass spreader. This technique was used for the enumeration of

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total aerobic viable count on Nutrient agar (NA) and Sabouraud Dextrose Agar (SDA). The cultures were incubated at 37°C for 24 hours for bacteria and 72hours for fungi. Media used were prepared according to the manufacturer's instructions.

Characterization and Identification of bacteria

Identification of the bacterial isolates was accomplished by the observation of colonial characteristics, Gram reaction and biochemical tests (Chessbrough, 1984). The characterization of the isolates were performed, by employing Gram staining reaction, Catalase test, Citrate test, Sugar fermentation test, Spore test, Motility test, Indole test, Methyl Red and Vogesproskauer test as described by Cheesbrough (2002) matched against Bergey's Manuel of Determinative Bacteriology, 9th edition (1994).

Gram Staining

Smear of the isolates were made onclean, dust and grease free slides, dried and heat fixed by passing the slides quickly several times over the flame of a Bunsen burner. The fixed smears were stained for one minute using aqueous solution of crystal violet. This was flooded by water after which the smearswere flooded with iodine solution and left to stand for one minute. This was also washed off with sterile water and the smears were then treated with ethanol, which was left for 5-8 seconds after which it was washed off with water. The smear were then counter stained with safranin, left 30 seconds and then washed off with running water. The stained smears were then allowed to dry and then viewed under the microscope using the oil immersion lens (×100). The Gram positive cells remain purple while gram negative cells appear red.

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Biochemical Tests

Catalase Test

Catalasetest was carried out on the organisms that were grampositive cocci from the gram stainingthat was carried out on the culturedplate. This was done by pouring 2mlsof 3% hydrogen peroxide in asterile test tube. A stick applicator was used to pick a colony from the culture plate and placed in the test tube containing the solution. A negative test does not show any effervescence or gas once the organism is been introduced into 3% hydrogen peroxide solution, while a positive test is indicated by effervescence or gas where the organisms was able to break down hydrogen peroxide to water and gas. This is useful in differentiating *Staphylococcus* species (catalase positive) from *Streptococcus species* (catalase negative).

Coagulase Test

The coagulase test was performed to differentiate between pathogenic (S*taphylococcus aureus*) from non-pathogenic*Staphylococcus species***.**Coagulase test was carried out on organisms that were catalase positive. Two drops of normal saline was used to make a smear of the organism, later2 drops of human plasma was placed on the smear. The slide was agitatedfor 60 seconds a clumping caused by enzymes producing strains shows a positive result which shows agglutination while a negative result does not show any agglutination.

Citrate utilization Test

The citrate test determines whether an organism can use sodium citrate as a sole carbon source. The test medium contains ammonium salt as the sole nitrogen source. Bacteria able to use citrate would use the ammonium salt releasing ammonia.

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An inoculum from a pure culture was transferred aseptically to a sterile tube of Simmons citrate agar. The inoculated tube is incubated at room temperature for 24hours and the results were determined. Abundant growth on the slant and a change front green to blue in the medium indicated a positive test for growth using citrate.

Indole Test

This was done to determine which organism has ability to split indole from tryptophan presentin buffered peptone water. It aid in differentiating gram negative bacilli especially of *Enterobacteriaceae***.** The test was done by inoculating tubes of peptone water with young culture of the isolatefor 24hrs at room temperature. Two drops of Kovac'sreagentwas addedintolml of each the culture tubes. Positive test was indicated by the appearance of red colour at the upper part of the tube.

Methyl Red Test

Peptone broth was used to carry out the test. Fifteen grams of peptone powder was added to 1 litre of distilled water according to manufacturer's description and 5 ml each was added into test tubes and autoclaved at 121^0C for 15 minutes. The broth was allowed to cool at 42^0C, seeded with the isolates and incubated at 37^0C for 48 hours. After incubation, 5 drops of methyl red indicator was added to the broth culture. The presence of red colour indicated a positive result while yellow colouration indicated a negative result.

Voges-Proskaeur Test

The test organism was inoculated into sterile MR-VP broth medium and incubated at 37^0C for 48hours. The presence of a red colour indicated a positive test result.

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Sugar Fermentation Test

Each of the isolate was tested for its ability to ferment a specific sugar. 1g of the sugar and 1g of peptone water were dissolved in 100ml of water. Bromothymol blue which serves as an indicator was added until the colour changes. 5ml of the solution were pipette into clean test-tubes. The test-tubes containing peptone water and sugar were added Durham's tube which were placed inversely. These were sterilized for 10minutes and allowed to cool before inoculating the inoculums. The test-tubes were incubated for 48hours. The production of acid and gas or acid only indicates utilization of sugars. Acid production was indicated by change in colour of the medium from green to yellow while gas production was observed by presence of gas in the durhams tubes.

Motility

Sterile nutrient agar slant was inoculated by stabbing the slant with a sterile straight inoculating wire. Motility is indicated by the growth of the organism away from the vertical line.

Characterization of Fungal isolates

This was done based on the description of the gross morphological appearance of fungal colonies on the SDA culture medium and the improved slide culture technique of Agu and Chidozie (2021) was employed in this study. A sterile glass slide was placed on the bottom of a sterile petri dish. With the aid of a sterile 2 ml syringe, 0.5 ml of the molten Saboraud Dextrose Agar (SDA) maintained at 45 ^{O}C in a water bath was dispensed on the sterile glass slide. The cover of the petri dish was replaced and the molten agar allowed to gel. Upon gelling, a sterile inoculation needle was used to inoculate the agar bump with a small amount of fungus at the centre of the

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bump. Thereafter, a heat-sterilized coverslip was laid just over the agar bump without pressure. The plates were incubated at room temperature for 3 to 5 days depending on the growth rate of the fungus. When desired growth was observed, few drops of Lactophenol cotton blue stain was dropped at the interface of the developing cultures on the slide and the coverslip so as to preserve the integrity of the culture and allowed to permeate the entire culture before viewing under the microscope. Referencing was done using Fungal Atlases (Barnett and Hunter, 2000; Watanabe, 2002; Ellis *et al*., 2007).

RESULTS

The microorganismsisolated from the sample revealed the presenceof 3 different bacteria and five (5) different species of fungi. The bacteria include *Bacillus sp.Lactobacillus* sp and *Streptococcus sp*., while the fungi include*Aspergillusflavus, Penicilliums*p, *Aspergillus fumigatus, Geotricium sp and Rhizopus stolonifer.*

Table 1 shows the biochemical characteristics of the isolates. The soaked cocoyam tuber gave a pH of 5.9

Table 2 shows the cultural and microscopic features of the mould isolates.

Table 3shows the occurrence of bacteria isolated from the soaked cocoyam flour while the occurrence of the mold isolates from the soaked cocoyam flour is shown in table 4.

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Table 1: Morphological and Biochemical Characteristics

Samples	Colony morphology and characteristics	Cell shape and gram stain	motility	catalase	coagulase	citrate	Oxidase	methyl	indole	v.p	Glucose	Lactose	Sucrose	Probable Organism
A	Large colonies, cream to yellowish on nutrient agar	Gram positive cocci in chains	+	_	_	_	_	_	_	_	AG+	AG+	AG+	*Streptococcus* spp
B	Very flat, smooth, opaque, small white colonies on nutrient agar.	Gram positive rods in short chains	+	+	+	+	_	_	+	_	AG+	AG+	AG+	*Lactobacillus*p
C	Whitish medium sized mucoid colonies on nutrient agar	Gram positive long rods	+	+	_	+	+	_	_	_	AG+	AG-	A+	*Bacillus* sp

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Keys:

\+ Positive

\- Negative

A+ Positive and Acid produced

AG+ positive and produces both Acid and Gas

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Table 2:Cultural and Microscopic features of the fungal isolates

Isolates	Cultural features	Microscopic features	Organism
1	On SDA, colonies showed typical blue-green surface pigmentation with a suede-like surface consisting of adense felt of conidiophores. Texture was powdery and the colour on the reverse side was yellow	Septate hyphae with thin-walled Conidiophore stipes are short, smooth-walled and had conical-shaped terminal vesicles. Conidia were produced in basipetal succession forming long chains and are globose to subglobose	*Aspergillusfumigatus*
2	On SDA, Colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age.	Conidial heads are typically radiate, later splitting to form loose column. Conidiophore stipes are hyaline and coarsely roughened, often more noticeable near the vesicle. Conidia are globose to subglobose.	*Aspergillusflavus*
3	On SDA, Colonies are very fast growing, cottony to fluffy, white to yellow, becoming dark-grey.	Sporangiospores are hyaline, grey or brownish, globose to ellipsoidal, and smooth-walled, and erect, simple or branched, forming large, terminal, globose to spherical, multispored sporangia, without apophyses and with well-developed subtending columellae.	*Rhizopusstolonifer*
4	On SDA, Colonies are usually fast growing, in shades of green, sometimes white.	Conidiophores are hyaline, smooth or rough-walled. Phialides are usually flask shaped, consisting of a cylindrical basal part and a distinct neck. Conidia are in long dry chains, divergent or in columns, are globose, ellipsoidal, cylindrical or fusiform, hyaline or greenish, smooth or rough-walled.	*Penicilliumsp*

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5	Colonies are fast growing, flat, white to cream, dry and finely suede-like with no reverse pigment.	Hyphae are hyaline, septate, branched and break up into chains of hyaline, smooth, one-celled, subglobose to cylindrical arthroconidia.	*Geotriciumsp*

Table 3: **Occurrence of the bacteria isolates in the cocoyam flour**

Samples	***Streptococcus* sp**	***Lactobacillus* sp**	***Bacillus sp***
1	+	+	_
2	_	_	+
3	+	_	+
4	_	+	_
5	+	_	+
6	_	+	+
7	_	+	_
8	+	_	+
9	_	_	_
10	+	_	+

+ = detected.

- = undetected.

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Table 4: **Occurrence of the fungal isolates in the cocoyam flour**

Samples	***Aspergillusfumigatus***	***Aspergillusflavus***	***Rhizopusstolonifer***	***Penicillium* sp**	***Geotricium* sp**
1	+	_	_	_	+
2	_	+	_	+	_
3	+	+	_	_	_
4	_	_	+	_	_
5	+	_	_	+	+
6	_	+	+	+	_
7	+	_	+	_	+
8	+	+	_	+	_
9	+	_	_	_	+
10	+	_	+	+	+

+ = detected.

- = undetected.

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DISCUSSION

Roots and tubers are usually consumed after processing which tends to increase their palatability, digestibility, safety and shelf life. During processing, the nutritive value of the roots and tubers may be adversely affected. This is also true for foods with anti-nutritional factors (Bradbury and Holloway, 1988).

This research has shown that the microorganisms associated with production and fermentation of cocoyam flour in *Eke*-Awka market Awka south Local Government Area, Anambra State, were *Aspergillusfumigatus, Aspergillusflavus, Rhizopusstolonifer, Penecilliumsp and Geotriciumsp* which belong to fungi while *Bacillus sp and streptococcus sp*. belongs to the bacteria family. Deterioration is a major challenge limiting commercial production of cocoyam flour. As soon as the root is uprooted from the ground, it begins a process of postharvest physiological deterioration within the next 24hours. This situation proposes that fresh root cannot be stored longer than 4 weeks because of its high moisture content. The effective utilization of cocoyam root is greatly constrained not only by low protein level but also by the rapid rate of deterioration normally characterized by a blue black discolouration of the root and the streaking of the xylem tissue (Iyer*et al*., 2010).

The occurrence of the microbial isolates as shown in table 3 and 4 shows that *Bacillus*spans*Aspergillusfumigatus* occurred mostly in the entire sample, while *Lactobacillus*sp and *Rhizopusstolonifer* had the least number of occurrences. This work is agreement with Odebunmi, which said that *Aspergillus*sp had the highest number of occurrence while *fusariumsolani* had the least occurance (Odebunmi *et al.*, 2007).

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The results of this study are in agreement with the findings of other researchers (Agu *etal*., 2014) that fungi constitute a menace in storage rots of many agricultural commodities. It was observed that *Aspergillus*sp *and Rhizopusstolonifer* were the most frequently isolated fungus from different spoilt crop tubers in Anambra State.

Therefore, the shelf life of the root is shortened, leading to wastage, poor products yield, economic losses, reduction in market quality and poor commercialization (Van Oirschot*et al*., 2000).The effect of blanching however became more pronounced on the nutritional values. Unblanched fermented samples had more nutritional value than the blanched fermented samples. The mineral content of unblanced fermented sample was higher than that of the blanched sample. Ihekoronye and Ngody (1985) stated that the loss of vitamin and minerals during blanching can be significant and is a function of surface area per mass of the product, degree of maturity of product, type of blanching (hot water or steam) blanching time and method of cooling after blanching (water or air).

The result of this study also revealed that the longer the storage time, the higher the bio-load, pH and moisture content. Therefore, proper storage methods such as dry environment (with very low relative humidity and moisture) is required to prevent the growth and survival of microorganisms associated with tubers especially cocoyam and its produce and the public health concerns (such as food intoxication and illnesses) that may result from toxinsharbours by some of these microorganisms especially microtoxins produced by fungi when they are consumed by the public.

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CONCLUSION

The storage of agricultural raw materials is an essential aspect of food processing that ensures that food remains available even in time of scarcity. Traditional marketing and storage systems have been adapted to avoid tuber perishability. These adaptations include processing the tuber into flour which will elongate the shelf life of the cocoyam. Other means of storage is through sun drying, fermentation. The results of this study have revealed the cocoyam tuber been sold at *Eke*-Awka market were mainly contaminated with *Bacillus sp.*,*Lactobacillus*sp, *Streptococcus sp*, *Aspergillusflavus*, *Penicillium*sp, *Aspergillusfumigatus*, *Geotriciumsp and Rhizopusstolonifer* as this poses a serious threat to deterioration of the tuber and health of consumers.

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