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Full Length Research Paper

Investigation into Microbiological and Chemical Characteristics of Cassava Flour in Nigeria.

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An investigation was conducted on nine samples of cassava flour samples obtained from six different states locations in Nigeria. Studies were carried on the physicochemical, chemical and microbiological assessment of the various samples. Studies on the moisture, pH, starch, ash, protein, and sugar were in the range of 9.78 – 10.66%, 6.4 – 7.8, 68.49 – 71.04%, 1.85 – 2.85%, 2.10 – 3.44% and 3.64 – 5.12% respectively. The bacteria isolates were identified as *Flavobacterium* sp., *Micrococcus leteus*, *Bacillus subtilis*, *Bacillus polymyxa*, *Bacillus cereus*, and *Escherichia coli*. The fungi isolates were also identified as *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporium* and *Rhizopus* sp. The significance of the strains as possible contaminants in cassava flour is discussed in line with our findings.

Keywords: Cassava flour, Physiochemical, Microbiological, Bacteria, Fungi.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) belongs to the family Euphorbiaceae and is grown almost entirely within the tropics (Amoa - Awua, 1996). The cassava plant is a perennial shrub grown for its edible tubers. It is thought to have originated in Tropical America and to have been introduced to Africa by the Portuguese around the 16th Century. Currently, it is widely cultivated and the major staple crop in most sub – Sahara countries (FAO, 1991). Cassava accounts for over half of the root tuber crops grown in African countries. In 1989, “62 million tonnes of cassava produced, represented 42% of the world, cassava output” (FAO, 1991). Cassava is one of major food products produced in Nigeria, Brazil, Zaire and Thailand which together produced over two – third of the world output (Bokanga, 1996). Despite its low protein content (1 – 3%), cassava is however an excellent source of carbohydrate in the form of starch (80 – 90% of root parenchyma by dry weight). Cassava is rich in thiamine, riboflavin and niacin (Wheatley *et al.*, 1994; Amoa – Awua, 1996). Cassava is the most important food in terms of dietary carbohydrate and many households eat cassava daily in various forms (Kormawa and Akoroda, 2003). Cassava is the seventh most important crop in the world and it constitutes a staple food for 800 million people (Hahn and Keyster, 1985). Cassava is grown almost entirely within the tropics (including the arid and semi – arid tropics). One of the major ways by which cassava can be presented is by processing the tuber into flour. However the processing method employed for flour formation varies for different processors.

In Nigeria, it grows well even in the far North including the Southern and Northern parts of Sudan savannah

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vegetative zones (Oyenuga, 1967). Now cassava is in more than 39 countries in Africa including Nigeria, Benin, Kenya, Tanzania, Uganda, Ghana, Zimbabwe, Republic Democratic of Congo and Mosambique (Oyewole, 2002).Cassava edible portion is 38 percent dry matter. Cassava contains vitamin C only in the vitamin group (Oyenuga 1967) and the vitamin is greatly affected during processing. The concentration of cyanogenic glucosides in cassava tubers increases from the center of the tuber outwards and the hydrogen cyanide (HCN) content of the peel is substantially higher than that of the flesh. Most cassava is consumed raw or as a snack when the plant is still young. Recently, the crop has become tremendously important industrially for production of livestock feed, starch, textile, industrial alcohol and for the manufacture of cassava flour, macaroni variety of food and beverage (FAO, 1991). Traditionally, cassava roots are processed into different products according to local customs and preferences (Hahn, 1987).

Booth (1976), reported that the microbial deterioration of cassava was due to the activities of complex fungi, such as *Phythium, Mucor, Rhizophus* and *Penicillium* as well as some bacteria, *Bacillus* and *Xanthomonas* spp. During processing the cell structural integrity is usually lost, and the cyanogenic glucoside come in contact with limarase thus initiating the formation of Hydrogen Cyanide. Since food processing usually include heating, the HCN produced is likely to evaporate completely (Bokanga, 1996). Various Nigeria daily newspapers reported in 1989 that cassava consumption had introduced acute intoxication while diseases of endermic and epidemic proportion have been reported in scientific literature.

Due to the variations in its processing method, the need to study the microbiological and chemical characteristics becomes very necessary in order to ensure safety of the product sample for consumption. This type of study has however received little attention by researchers in cassava flour research. It is therefore on the basis of ensuring a wider range for the utilization of cassava by its processing into flour that the project was aimed at achieving the following objectives;

1. To study the physicochemical parameters of some samples of cassava flour obtained from different locations in Nigeria.
2. To isolate, characterize and identify the microbial isolates from the flour samples.
3. To speculate on the significance of the various isolated strains and hence profers solution for best practices for cassava preservation.

MATERIALS AND METHODS

Collection of Samples: A total of nine (9) samples of cassava flour were collected from six (6) different states in Nigerias as shown in Table 1 below.

Isolation of Microorganisms from Samples.

One gram (1g) each of cassava flour sample was weighed separately inside sterile heat resistant glass tube. Ten milliliters of sterile water was added to each cassava flour sample as to diluents. A homogenizer was used to ensure for proper mixing together before preparing serial dilution of each sample into various sterilized screw – cap test tubes up to 10^{-10} dilution. From an appropriate dilution of 10^{-2} to 10^{-5}, 1m of each suspension was then plated on each of the media. The diluted sample 1ml each was spread on to prepared Nutrient Agar (oxoid) by the pour plate method in order to determine the total viable counts. Incubation of the plates was carried out at 37^0C for 24hrs – 48hrs (Harrigan and McCance, 1982). The diluted samples, (10^{-2} – 10^{-5}) were also plated onto the Potato Dextrose Agar (Oxoid) with lactic acid (2.5ml% in litre) for inhibition of bacteria, by pour plate method. The plates were then incubated at 30^0C for 24hrs for yeast isolates and 4 – 5 days for moulds (Harrigan and McCance, 1982). After incubation the organisms were enumerated and purified by successive streaking on fresh agar plates to get pure cultures. Pure culture slants were stored in the refrigerator at 4^0C.

Morphological and Biochemical Tests of Bacteria Isolates.

The various micro – organisms were subjected to morphological and biochemical tests for their identification according to the combined specification of Beech *et al,* (1994) and Wheatley *et al*, (1994). Biochemical tests carried out on the isolates were as follow:

Gram's Reaction Test

The bacteria isolated were stained according to the method of Tortora *et al.,* (1994) as follows. A thin smear of the isolate was prepared on a clean and grease free glass slide, air dried and heat fixed and stained with crystal violet for 60 seconds and then rinsed with tap water. The smear was covered with Lugols' iodine solution for 60 seconds and washed off under gentle running tap water. The smear was decolourized using alcohol after which it was washed under tap water and counter stained with carbon fuchsin for 30 seconds rinsed in tap water and blotted dry with a piece of filter paper. The stained cells were later examined under the oil immersion objective of the microscope using magnification x100 (Olutiola, et al., 1991).

Catalase Test

A few drops of freshly prepared 3% hydrogen peroxide were added onto the specimen prepared slide. The

Table 1. Cassava flour samples analyzed and place of collection.

SAMPLE CODE	*Place of collection*	*States*
AVL	Albakem Nig. Ent. Sango	Ogun
EF	Achisa Musa Muhammed Nig. Enterprises	Kano
FIR	Federal Institute of Industrial Research Oshodi	Lagos
FM	Flour Mill Industry Apapa – Lagos	Lagos
HLI	Hillinson Nig. Enterprises Ilorin	Kwara
MAL	Ministry of Agriculture Oko – Oba, Lagos	Lagos
RS	Real processing unit Ife – Ibadan Road	Oyo
UPS	Ultimate Processing Section, Shagamu Rd., Ikorodu	Lagos
YNE	Yasima Nig. Enterprises CBU IFON Road. Ifon.	Osun

All the cassava flour samples were collected aseptically in sterile laboratory polythene bags and were transported immediately to International Institute of Tropical of Agriculture (IITA) laboratory at Ibadan, Oyo state, Nigeria for immediate analysis.

production of catalase enzyme was detected by the evolution of a gas as a white foam (Olutiola *et al*., 1991).

Methy – red Voges proskauer (MRVP)

The cultures inoculated into 10ml sterile MRVP medium dispensed in test tubes were incubated at 30^0C for 72hrs. After incubation the cultures were divided into two portions of 5ml and 1ml respectively. To the 5ml portion, five drops of 0.4% methyl red solution were added. To the 1ml portion was added 0.5ml of 6% ethanolic solution of alpha – naphtol and 0.5ml 6% potassium hydroxide. The tubes were observed for colour reaction (Olutiola *et al.,* 1991).

Sugar fermentation

The sugar solutions were 1% of glucose, galactose, maltose, arabinose, fructose and xylose. The medium used was 1% peptone water, glucose and fructose solutions were sterilized at 115^0C for 10minutes and the rest at 121^0C for one minute. Phenol red 0.1% was used as the indicator for acid production. Ten millilitre portion of the medium was dispensed into test tube containing inverted Durham tubes to detect the production of gas by the isolates.

Identification of Mould Isolates

The mould isolates were identified by initial sub – culturing as pure cultures. Smear was made with a sterile inoculating loop with lactophenol cotton blue reagent on a clean grease free slide. A cover slip was applied and further examined under the oil immersion objectives of the light microscope at magnification X40. The main characters employed in the identification of moulds are summarized as follows: *Hyphae*: septate or non septate. *Mycelium*: coloured or non – coloured. Types of asexual spores, nature of spores, presence of special structures such as stolon, rhizoids, and foot cells.

Chemical analysis of Samples.

Moisture Content Determination

The moisture content of the sample was determined using AOAC (1990) method. Two grams was weighed into already – weighed clean drying crucible. The crucible with the sample were then placed in a well – ventilated oven (Fisher Scientific Isotemp oven, by Fisher Scientific Co. USA, model 65SF) maintained at 105^0 C for 16 –18 hours the drying crucible were then transferred into a desiccators to cool after the final weights were taken.

Calculation involved:

Percentage moisture content (MC)
Percentage dry matter = 100 – MC
Where:
W1 = weight of sample before drying.
W2 = weight of can with sample after drying.
S = Weight of sample

Ash Content Determination

This was carried out using AOAC (1984) method. 2g of the samples was weighed into a crucible dish, which had been ignited and weighed. The crucible dish was then placed on a hot plate inside the fume cupboard to char the organic matter, while the remaining residue (inorganic matter) was

later transferred into the muffle furnace, (Fisher Scientific Isotemp Muffle by Fisher Scientist Co. USA, model 186A) maintained at 600^0C for 6 hours to completely ash the sample. The crucible dishes were then transferred into a dessiccator to cool and they were weighed thereafter. The percentage ash content was calculated as follows:
% Ash content =
Where W1 weight of crucible.
W2 = weight crucible + sample before ashing.
W3 = weight of crucible + sample after ashing

Protein Determination

This was carried out using AOAC (1990) method. 0.20g of cassava sample was weigh into the digesting tube individually and 4ml each of concentrated tetraoxosulphate IV acid (H_2S0_4) and hydrogen peroxide (H202) were added. One (1) table of Kheldal catalyst was added and the sample was digested on the digestion block 370C for three (3) hours.

The tube was then filled up with distilled water to the mark and then covered with aluminium foil and was mixed well. One millilitre of the digest was pipetted into 25 millilitres volumetric flask. Three drops each of polyvinyl alcohol solution and mineral stabilizer were added and filled up to the 25millilitre mark with distilled water. Then one millilitre of Nessler reagent was added. The mixture was then poured into the Hach Spectrophometer to determine the concentration of nitrogen at 460nm.

Calculation:
% Nitrogen =
% Protein = Nitrogen x 6.25
Carbohydrate Determination

The carbohydrate content was determined using Dubois et al., (1956) method. The sample (0.2g) was weighed into a centrifuge tube. One millilitre of 100% ethanol, two millilitres of distilled water and 10mls of hot ethanol were added. Then vertex the mixture and centrifuge for 10minutes at 2000rpm. The supernatant was then decanted into another centrifuge tube (this is the sugar portion of the sample). The sediment is the starch portion of the sample.

Sugar determination

Distilled water (9ml) was added to the supernatant and vortexed). Then 0.2ml was pipetted into a test tube and 0.8ml of distilled water, 0.5ml of phenol and 2.5ml of conc. H_2S0_4 were added and vortexed. The sample was then allowed to cool and the absorbance read in a spectrophotometer (Milton Ray Company USA model spectronic 601) already standardized at 490mm wavelength.

Calculation:
% Sugar =
Abs. = Absorbance (abs)
Dilution factor = 5
Volume = 20
Slope = 0.0055
Intercept = 0.0044

Starch determination

Approximately 7.5ml perchloric acid was added to the starch portion and allowed to stand for 1 hour. To this was added 1.7ml of distilled water then vortexed 0.05ml of the solution was pipetted into a test tube. Distilled water (0.95ml), 0.5ml of phenol and 2.5ml of H_2S0_4 were added and vortexed. The sample was checked using spectrophotometer, made by Milton Ray Company USA model Spectronic 601 already standardized at 490nm wavelength.

Calculation:
% Starch =
Abs. = Absorbance (abs)
Dilution factor = 20
Dilution = 25
Slope = 0.0055
Intercept = 0.0044
% Crude fibre

RESULTS AND DISCUSSION

A total of nine samples of cassava flour were collected. All the nine samples were off white colour. The moisture content ranged from 9.6% (sample HL1) to 10.6% (for sample FM). The pH values obtained ranged between 6.1 and 7.8. The samples AVL, HLI, UPS and YNE had pH values above the neutral pH (7) while samples EF, FIR, FM, MAL and RS had values below the neutral pH value(7) and were therefore slightly acidic in nature. For the starch, highest percentage was obtained 70.4% for MAL sample while AVL has the least value of 68.5%. The percentage of ash analysed for the nine samples has the highest value of 2.85 for the AVL and the lowest value of 1.85 for HL1. The protein analysed, the highest value was obtained for the RS has the highest values of 3.44% and the lowest value for the AVL has the value of 2.10%. (Table1).The value for the sugar obtained ranged from 5.21% for RS, which was the highest value while the least value 3.56% was obtained for sample VIR (Table 2).

The group of bacteria and fungi observed in this study confirm microbial contamination of cassava flour samples obtained from different locations. A total of six (6) bacteria

Table 2. Physico-Chemical Analysis of Cassava Flour Samples

SAMPLE Code	*Colour*	*MOISTURE Content (%)*	*pH*	*STARCH (%)*	*ASH (%)*	*PROTEIN (%)*	*SUGAR (%)*
AVL	Off White	10.08	7.4	68.49	2.85	2.10	4.13
EF	Off White	9.78	6.9	69.66	1.92	2.17	4.41
FIR	Off White	9.80	6.1	70.10	2.12	2.40	3.56
FM	Off White	10.66	6.2	69.44	2.07	2.35	3.89
HLI	Off White	9.66	7.1	71.04	1.85	2.35	3.90
MAL	Off White	9.84	6.4	70.40	2.15	2.15	4.42
RS	Off White	10.32	6.5	70.18	1.91	3.44	5.21
UPS	Off White	10.54	7.8	69.61	2.19	2.44	4.04
YNE	Off White	9.91	7.6	69.31	2.14	2.22	3.64

Key: AVL: Cassava Sample obtained from Albakam Nig. Enterprises.
EF: Cassava Flour Sample obtained from Achisa Musa Mohammed Nig. Ent. Kano State
FIR: Cassava Sample obtained from The Federal Institute of Industrial Research, Oshodi, Lagos State
FM: Cassava Flour Sample obtained from Flour Mills, Apapa, Lagos State
HLI: Cassava Flour Sample obtained from Hillinson Nig. Ent. Ilorin, Kwara State
MAL: Cassava Flour Sample obtained from Ministry of Agriculture. Oko-Oba, Lagos State
RS: Cassava Flour Sample obtained from Real Processing unit, Ile-Ife
UPS: Cassava Flour Sample obtained from Ultimate Processing, Ikorodu, Lagos State
YNE: Cassava Flour Sample obtained from Yasina Nig. Enterprises, OSun Sate

species were isolated. These isolates belong to the genera; *Flavobacterium* sp., *Micrococcus leuteus*, *Bacillus subtilis*, *Escherichia coli* and *Bacillus polymyxa*. The isolation of *Escherichia coli and Bacillus cereus* is an indication of possible health hazards associated with these organisms (Olowoyo *et al*., 2001). *Escherichia coli* is an indication of pathogen in food samples although some strains are normal inhabitants of intestine of man and other animals (Frazier and Westhoff, 1994). Aerobic spore formers have been implicated in the spoilage of bread and starch – based foods and in food intoxications. Thus, they constitute health hazards and are potential spoilage agents. *B. cereus* (a spore forming organism) is widely distributed in nature and in foods. Its spores have ability to withstand high temperature and they produce enterotoxins which may cause food poisoning (Midura *et al*., 1990, Olowoyo *et al*., 2001). The aerobic spore forming bacteria have been enumerated and used to investigate the quality of cassava flour "*lafun*" (Abba *et. al*., 1991). *Bacillus* spp can survive and thrive on various kinds of foods. Isolated *Bacillus* spp in milk heated above 100^0C, thus indicating the ability of their spores to withstand high temperature. Most of the organisms isolated might have been introduced into the food samples from soil and water used during processing. Among the requirements for foods to be of good sanitary quality is that they must be free of hazardous microorganisms or those present should be at a safe low level (Frazier and Westhoff, 1994). The quality of foods is determined by the content of indicator organism. Indicator organisms are organisms whose presence or number serve to indicate the condition or quality of materials. Their presence in foods may indicate that foods were exposed to conditions favourable for the introduction and growth of pathogenic organisms as described in Table 3. The occurrence of lactose fermenters such as *Esherichia coli* indicates the presence of coliforms which suggests a degree of contamination with faecal discharges of human and animal (Anon, 1974, Refai, 1979, Uriah and Izuagbe, 1990, Olowoyo *et al*., 2001).

The most predominant moulds isolated in this investigation are *Penicillium* sp, *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* sp, and *Fusarium* sp. As shown in Table 4 below.

Kuku et al., (1984), Abba – Kareem et al .(1991), isolated similar fungi such as *Aspergillus fumigatus*, and *Aspergillus niger* from cassava flour. Species of *Aspergillus* isolated in this work are undesirable in foods. Some strains of *Aspergillus flavus* have been reported to be toxin producers. These toxins are heat – stable and hence when consumed may result in food intoxication. Moulds are potential spoilage agents (Uriah and Izuagbe, 1990). The genus *Penicillium* sp isolated in this study show one of the three major mycotoxins producing fungi (Dicken and Jones, 1981, Olowoyo et al; 2001). The discoloration coupled with the off flavour of the cassava flour is due to the activities of the infecting mould species. Moulds are

Table 3. Biochemical Characteristics of Bacteria Isolated from Cassava Flour Samples

Isolate codes	Colony morphology	Cell characteristics	Caralase	Oxidase	KOH	Coagulase	Oxidation	Fermentation	Motility	Gelatin hydrolysis	Starch Hydrolysis	Casein Hydrolysis	Lactose	Arabinose	Sucrose	Fructose	Galactose	Maltose	Xylose	Glucose	Probable identity
AV L_1	Yellow colonies spreading circular raised entire	Gram negative rods	+	+	+	-	Aerobic -	Anaerobic -	+	+	-	+	-	-	-	+	-	-	+	+	*Flavobacterium* sp
AV L_2	Yellow colonies non-spreading flat and entire	Gram negative rods	+	-	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	+	*Flavobacterium* sp
AV L_3	Yellow colonies spreading circular raised and entire	Gram negative rods	+	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	+	+	*Flavobacterium* sp
EF_1	Creamy, entire, small and flat	Gram positive cocci	+	+	+	-	-	-	-	+	+	+	-	-	-	-	+	-	-	-	*Micrococcus luteus*
EF_2	Creamish white round, small and convex	Gram positive rods	+	-	+	-	+	+	+	+	-	-	+	+	+	+	-	-	-	+	*Bacillus subtilis*
EF_3	White colonies large, flat and serrated	Gram negative (short) rods	+	+	+	-	+	+	+	+	-	-	+	+	+	-	+	+	-	+	*Escherichia coli*
EF_4	Creamy, entire small and flat	Gram positive rods	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Bacillus polymyxia*
FIR_1	Creamish white, round small and convex, spreading	Gram positive rods	+	+	+	-	+	-	+	+	+	+	+	-	+	-	-	+	-	+	*Bacillus subtilis*

Table 3. Continue

FIR$_2$	Creamish white, flat small and convex spreading	Gram positive rods	+	+	+	-	+	-	+	+	+	+	+	-	+	-	-	+	-	+	*Bacillus subtilis*
FIR$_3$	Creamish white, slightly raised, spreading	Gram positive rods	+	+	+	-	+	-	+	+	+	+	+	-	+	-	+	+	+	+	*Bacillus subtilis*
FM$_1$	Creamish, flat and non – spreading	Gram positive rods	+	+	+	-	+	+	-	+	-	-	-	-	+	+	+	+	-	+	*Bacillus cereus*
FM$_2$	Creamish, and non – spreading	Gram positive rods	+	+	+	-	-	-	-	+	+	+	-	-	+	+	+	+	-	+	*Bacillus cereus*
HL$_1$	Creamish, off white, slightly raised and mucoid	Gram positive rods	+	+	+	-	+	-	+	+	+	+	+	-	+	-	-	+	-	+	*Bacillus subtilis*
MA L$_1$	Yellow colonies, spreading, raised and entire	Gram negative rods	+	+	+	-	-	-	+	+	-	+	-	-	-	+	-	-	+	+	*Flavobacterium* sp
MA L$_2$	Yellow colonies, spreading, raised and entire	Gram negative rods	+	+	+	-	-	-	+	+	+	+	+	-	+	-	-	+	-	+	*Flavobacterium* sp
MA L$_3$	Cream, entire small and flat	Gram positive rods	+	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	+	+	*Flavobacterium* sp
UP S$_1$	Yellow colonies raised and spreading	Gram negative rods	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	*Flavobacterium* sp
UP S$_2$	Yellow colonies raised and spreading	Gram negative rods	+	+	+	-	-	-	+	+	-	+	-	-	-	+	-	-	-	+	*Flavobacterium* sp

Table 3. Continue

UP S_3	Yellow colonies raised and spreading	Gram negative rods	+	+	+	-	-	-	+	+	+	+	-	-	-	+	-	-	-	+	*Flavobacterium* sp
UP S_4	Yellow colonies raised and spreading	Gram negative rods	+	+	+	-	-	-	+	+	-	+	-	-	-	+	-	-	-	+	*Flavobacterium* sp
YN E_1	Yellow colonies flat and spreading	Gram negative rods	+	+	+	-	-	-	+	+	-	+	+	+	+	-	-	+	+	+	*Flavobacterium* sp
YN E_2	Yellow colonies flat and spreading	Gram negative rods	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	*Flavobacterium* sp

Table 4. Characteristrics of Mould Species Isolated From Cassava Flour

Isolates **Attributes**	1	2	3	4	5
Appearance of culture on medium	Freely branched greenish mycelium	Colony profusely branched which appear brownish black.	White mycelia growth which branched profusely	Greenish mycelia growth	Whitish cream reverse, burgundy and macroconidia
Hypahe Nature of Conidiophores	Septate Septate Perpendicular and branches to the top	Septate Arise by Stolon	Non – Septate Upright Sporangiophore connected by Stalon	Septate Perpendicular and branches to the top.	Septate
Types of Sexual Spores Characteristics of Asexual Spores	Conida Sterigmata arousing from rear	Conida Vesicles bearing chains	Sporangiophore Dark pear shaped Spoagium	Conida Vesicles bearing chains	Borne on monophialides, falcate to straight, apical cell some whatpointed, basal cell foot – shaped usually 3 – septate
Special features:	Absent	Absent	Present	Absent	Absent
Stolon	Absent	Absent	Present	Absent	Absent
Rhizoids	Absent	Absent	Present	Absent	Absent
Foot cells					
Microscopic Spores	Round light	Brownish black conidia in chain	Small globular sporagio spores	Conisdia radiates from the Vesicles	-----
Probable identify	*Penicillum* Sp.	*Aspergillus* niger.	*Rhizsopus* Spp.	*Aspergillus* flavus	*Fusarium spp*

Table 5. Various Moulds Isolated on each Cassava Flour Samples

SAMPLE CODE	PROBABLE ISOLATES
AVL	*Fusarium oxysporum, Aspergillus niger, Aspergillus flavus, Penicillium* spp
EF	*Aspergillus niger*
FIR	*Penicillium* spp*, Aspergillus flavus*
FM	*Penicillium, Aspergillus niger, Aspergillus flavus spp, Rhizopus* spp
HLI	*Rhizopus* spp*, Aspergillus niger*
MAL	*Penicillium* spp*, Aspergillus niger, flavus*
RS	*Aspergillus niger, Penicillus* spp*, Fusarium oxysporium; Aspergillus flavus*
UPS	*Aspergillus niger, Aspergillus flavus*
YNE	*Aspergillus niger, Aspergillus flavus*

generously endowed with extracellular proteolytic or lipolytic enzymes and so can cause softening of food products (Frazier and Westhoff, 1994, Olowoyo, et al., 2001). Moulds growth also causes off flavours in foods and change in appearance of food have been related to mould growth (Elmer,1990). Spores of various species of moulds are heavily suspended in air especially in an untidy and unhygienic environment. These sporulating moulds therefore easily get in contact with foods that are openly displaced in baskets or bowls. The mould isolates from the various locations were identified as shown in Table 5 above.

The similarity in the types of organisms isolated from the samples and those reported by other workers confirms the fact that some of the microorganisms are indigenous to cassava flour (Abba – Kareem *et al*. 1990). Alozie *et al* (1980), have shown that *Aspergillus flavus* can grow and produce toxins in *gari*, a cassava product. Furthermore, according to Olowoyo, et al., 2001 the baking temperature (120^0C for 30 mins) as well as low amounts of volatile acids in bread do not degrade aflatoxin B_1 to any reasonable extent. A similar phenomenon can occur if cassava flour containing aflatoxins is used in bread making, with obvious consequence to the health of the consumers. Studies on the moisture content as presented in Table 2 show that all the samples had various values that were very low with the lowest value of being 9.66% and the highest of 10.54%. The significance of low moisture contents in foods cannot be overemphasized as they help to enhance the shelve life of food samples and prevent rapid spoilage by microorganisms (Uriah and Izuagbe, 1990).

CONCLUSION

Studies carried out revealed the presence of pathogenic microorganisms which include bacteria species of *Flavobacterium* sp, *Escherichia coli*, *Bacillus cereus, B. substilis*, *B.polymyxa* and *Mixcrococus luteus*. Also pathogenic mould species which include *Penicillium* sp, *Aseprgillus niger*, *A. flavus*, *Rhizopus* sp. and *Fusarium* sp. were isolated from cassava flour. The presence of these pathogenic microorganisms is thus an indication of microbial contaminations with an incessant possibility hazards on the health of man. It is rather impossible to avoid microbial contamination of foods during harvest and subsequent processing. Therefore, the practice of basic sanitary rules in preparing foods should be employed to improve on the hygienic condition of foods. Contamination of flour samples by these pathogens could be eliminated by some heat treatment and observing the necessary basic sanitary rules of hygiene. Also, the use of sterilized processing equipment should be given adequate attention as this will go a long way at alleviating problem associated with the presence of these contaminating microorganisms.

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