SOME MICROBIOLOGICAL STUDIES ON CASSAVA FLOUR

BY

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DECLARATION

This is to certify that the work now submitted for the Master of Science degree (Microbiology) of Ahmadu Bello University was carried out by me at the laboratories of the Department of Microbiology and Biochemistry, Ahmadu Bello University, Zaria, under the supervision of Dr. R. N. Okagbue.

This thesis has not been part of any presentation for any other qualification. However, the work of other investigators have been acknowledged and referred to in this presentation.

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CERTIFICATION

This thesis entitled "Some Microbiological Studies on Cassava Flour" by Vivian Ngozi Yobe meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literatary presentation.

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DEDICATION

This work is dedicated to my dear and loving parents, sister and brothers.

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Many people contributed in various ways to the completion of this work.

First of all, my most sincere gratitude goes to my supervisor, Dr. R. N. Okagbue without whom this work would not have been completed. He was so patient and understanding throughout the different stages of this study. His encouragement, suggestions and good supervision led to the successful completion of this work.

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ABSTRACT

A total of 105 samples of cassava flour obtained from parts of Nigeria were analysed for total aerobic bacteria, aerobic spore formers, lactose ana non-lactose fermenting Enterobacteriaceae, xerophillc and hydrophilic moulds and yeasts. The flour samples were observed to be highly contaminated by the various groups of microorganisms enumerated, The range of counts obtained for the different groups of microorganims were aerobic plate count ($1.1x10^4$ -2.8x10 cfu/g), aerobic spore count ($<1.3x11^3$ -$2.9x10^6$ cfu/g), lactose fermenters ($<1.0x10^3$ -$6.6x10^6$ cfu/g), non-lactose fermenters ($<1.0x10^3$-$3.8x10^5$ cfu/g), xerophilic moulds ($<1.0x10^2$-$1.6x10^5$ cfu/g), hydrophilic moulds ($<1.0x10^2$-$6.4x10^5$ cfu/g), yeasts ($1.0x10^2$-$1.9x10^6$ cfu/g).

The fungal isolates from the cassava flour samples were identified. The predominant generation of moulds were Aspergillus and Penicillium. Sixty percent of the total number of samples were contaminated by A. flavus which was the most predominant species of Aspergillus. The second most predominant fungus, A. niger, was isolated from 43% of the total number of samples.

PeniciIlium spp. occurred in 48% of all the samples examined. Other species of fungi isolated from cassava flour were Sporcndonema sebi, Curvularia lunata, Aureobasidium pullulans (a yeast-like mould), Rhiiopus stolonifer, R. oryzae, pigmented and non-pigmented yeasts.

Thirteen out of 60 A. flavus strains isolated from cassava flour produced aflatoxins in yeast extract broth medium containing 20% sucrose. Ten of the aflatoxigenic strains produced aflatoxins B1, B2, G1, G2 while three produced aflatoxins B1 and B2 only. The amount of aflatoxins produced were relatively high and ranged from 3.0-14.2 ug/ml (B1,) 0.9-11.9 Ug/ml (B_2), 0-12.5 ug/ml (G1) and 0-9.2 ug/ml (G2). Aflatoxin producing strains of A. flavus were isolated from cassava flour samples obtained from different geographical areas-of Nigeria. The results are discussed in relation to possible sources of microbial cohtamination of cassava flour and the need for improvement in overall sanitation during production and sale- of the product.

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CHAPTER I

INTRODUCTION.

The tuber of the cassava plant (Manihot spp) constitutes an important source of food in many tropical regions of the world. Nigeria produces 9% of the total world production of cassava (F.A.O, 1980) and in 1965-75 period alone the mean annual production was over 8 tons (Nwasike, 1984). Although one variety (the sweet type) of cassava can be eaten raw or cooked due to its relatively low cyanide content, the bulk of the harvest is usually processed to yield a variety of fermented foods namely, gari, kpokpogari, fufu, lafun and cassava flour (Ekandem, 1961). To date, most of the cassava flour consumed in Nigeria is produced at the village level. The production involves peeling and cutting tubers into small-sized pieces or chips which are subsequently washed and dried in the sun. The dry chips are milled from time to time by pounding with mortar and pestle or in local milling machines and the flour is sold in open-air markets. There is ample opportunity for microbial contamination during all the stages involved in production and sale of cassava flour.

Ekandem (1961) has described extensively the traditional uses of cassava flour in Nigerian foods.

Briefly, it is primarily used as the main meal (in gelled form) in conjunction with local soups, or as supplements to various other local diets. In addition, the flour has been recommended as a supplement to wheat flour in bread-making (Jones and Akinrele, 1976).

In parts of the world were wheat flour constitutes the main type of flour used in the food industry, considerable information is available on the microbiology of the product. The information has led to official microbiological specifications for the product, to improved methods of processing and sanitation in flour mills and to the use of antimicrobial agents in flour to prolong its shelf-life and guarantee consumer protection. Nigerian factories produce considerable amounts of wheat flour (used in bread-making) from imported wheat and the Standard Organisation of Nigeria (S.O.N., 1980) has specified standards for the product. The organisation recognises the need to safe-guard wheat flour from microorganisms especially rope - forming bacteria and from mycotoxin contamination. Unfortunately, little or no work has been done on the microbiology of local flours including cassava flour, despite the fact that the traditional methods of processing and sale expose them to the danger of

microbial contamination. Since cassava flour has many current uses and is of potential use in baking, there is a need to study the nature and levels of its microflora. In Ghana where the method of production is similar to that of Nigeria, Clerk and Caurie (1968) reported frequent isolation of certain moulds from cassava chips used in flour making and studied some biochemical effects of the moulds. The moulds were Cladosporium herbarum, Sporendonema sebi, Aspergillus and Penicillium species. Recently, Okagbue (1986) reported a preliminary survey of the microbial quality of a variety of flours including cassava flour in Zaria markets. However, only one sample of each type of flour was analysed. The purpose of this study is to conduct a more detailed study of the microbiology of cassava flour prepared and sold in various parts of Nigeria.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Types and Uses of Flours

Flour is a powdery semi-processed food material produced by drying, grinding and sifting grain or certain agricultural products such as tubers. Since it is a dehydrated product, it is generally stable microbiologically. If the moisture is 12% or less no growth of microorganisms takes place (Hesseltine and Graves, 1966).

Wheat flour is the most common type of flour world wide. James and Smith (1948) stated that it is the basic food for a large part of the world's population. It is used in canned foods especially baby foods, and in making bread, cakes, biscuits and other bakery products. In the U.S.A. it is the major raw ingredient in non sterile products like refrigerated canned biscuits, dinner rolls and pizza doughs (Graves and Hesseltine, 1965).

Nigeria has a wide variety of flours. According to Okagbue (1986) they include wheat

flour (usually produced in modern factories) from imported wheat and other types of flours milled at the village level from tuber crops, cereals and legumes. Thus, we have yam and cassava flours milled from root tubers; guinea corn, maize, millet and rice flours etc. milled from cereals; and cowpea, soya bean and bean flours etc. milled from legumes. The flours constitute a significant part of the local staple diets in various parts of Nigeria. Since this study is concerned with cassava flour, various aspects of the product will be reviewed.

2.2: Cassava as Food.

Nomenclature

The plant is called cassava in English - speaking countries of Africa; manioc, manihot, madioca, and yuca in Latin America; tapioca in Asia; and manioc in Francophone countries. Several specific names are applied to cassava. The name *Manihot utilisima* is commonly used, in addition to *M. dulcis*, *M. aipi* and *M. palmata*. These names differentiate between cultivars with different contents of cyanogenic glucoside in the roots, but this distinction is taxonomically

invalid and Crantz's epithet M. esculenta is generally accepted as the correct one (Jennings, 1970). Thus, cassava is botanically called Manihot esculenta.

Production and Distribution

Manihot esculenta Crantz is widely distributed in tropical countries throughout South and Central America, Africa and Asia (Ingram, 1972). Nestel (1973) reported that cassava is produced in more than eighty countries but two-thirds of world production takes place in only five of them namely, Brazil, Indonesia, Zaire, Nigeria and India. Between 1979-81, world production of cassava was about 120 million tons annually. Nigeria, Tanzania and Zaire are the major African cassava producers. Average yields for Nigeria between 1979-81 was 9.2 tons/hectare with a production of 12.40 million tons (FAO, 1981). Tanzania and Zaire followed with a yield of less than 7 tons/hectare and a production of less than 13 million tons (FAO, 1981).

Cassava is generally grown as a subsistence crop. It is particularly valued because of its drought tolerance, its ability to grow in poor soils and its relative resistance to weeds and insects. It can be left in the ground without harvesting for a lengthy period of time (Nestel, 1973; Ekandem, 1961). None of the crop harvested is used as planting material. Furthermore, it is not season bound and can therefore be planted and harvested at any period of the year (Ekandem, 1961). Cassava production in terms of calories per unit land area, per unit of time, appears to be significantly higher than that of other staple food crops (Nestel, 1973).

Nutritional aspects

Both the roots and the leaves of cassava are eaten as food. About 80% of cassava root is edible and it contains 62% water, 35% carbohydrate, 1% protein, 0.3% fat and 1.0% mineral matter which is rich in calcium (13-33 mg/100g). The roots are also rich in ascorbic acid (30-43 mg/100g), and contain nutritionally significant amounts of thiamine (0.06 mg/100g),

riboflavin (0.03mg/100g) and niacin (0.6mg/100g) (Jennings, 1970). The contents of proteins and vitamins are reduced during processing in order to remove hydrogen cyanide, and hence the value of cassava foods is influenced by the methods of preparation. The root proteins are deficient in certain amino acids notably those containing sulphur (Jennings, 1970). Cassava leaves and shoots are consumed in considerable quantities in some parts of Africa. They are richer than the roots in both quantity and quality of protein. Cassava leaves contain 17.8-34.8% of protein by dry weight (Jennings, 1970). Gondwe (1974) reports that in some parts of Malawi, cassava tuber which has been processed to cassava flour is eaten with the very young leaves of cassava cooked as vegetable called "mayani" or "cigwada" and that such people obtain a complete meal from cassava alone. In Nigeria, cassava flour is usually eaten with meat or fish soups to offset the low protein content (Ekandem, 1961). A survey of southern Nigeria, shows that 25 - 56% of the dietary calories came from cassava. In other cassava producing countries, a lot of the calorie intake also comes from cassava (Nestel, 1973). Ekandem (1961)

states that cassava produces the highest return of calories per acre of any of the Nigerian food crops.

Toxicity

In spite of the growing popularity of cassava as a source of dietary calories, the unprocessed tuber is toxic and the danger of eating it (unprocessed tuber) is well known in the tropics (Gondwe, 1974).

The toxicity is caused by the presence of the cyanogenic glucoside linamarin and smaller amounts of the closely related lotaustralin. These substances hydrolyse under the influence of the endogenous enzyme linamarase to liberate hydrogen cyanide (HCN) (Coursey, 1973). In the bloodstream, the cyanide is converted to thiocyanate, a sulphur-containing compound, by the enzyme rhodanase. The compound is later excreted in the urine. The compound plays its toxic role by using the body sulphur in detoxification thereby increasing the body's demand for sulphur containing amino acids or by interferring with the iodine uptake of the thyroid, resulting in goitre (Cock, 1985).

The wide variety of traditional food preparation techniques used for processing cassava in different parts of the world reduce the cyanide content by liberation of the HCN either by solution in water or by volatisation. These processes involve maceration, soaking, boiling, roasting or fermentation of the cassava tubers or a combination of these processes (Coursey, 1973). According to Gondwe (1974), during the traditional process of making cassava flour, a large fraction of the HCN is degraded, giving a product which is safe to eat. Also cooking the flour further reduces the cyanide level. Cock (1985) suggested that the use of iodine probably as iodized oil and a consumption of sufficient animal or fish protein will help in reducing the toxic effect of cyanide. However, an ideally safe sample of cassava is one which by every reliable method for detecting cyanide, has been shown to contain no cyanide at all. This is because, it has been shown that a constant intake of even low cyanide levels over a long period of time can eventually result in poisoning which is manifested by neurological abnormalities (Gondwe, 1974).

In Nigeria, there are two types of cassava, namely:- the bitter and the sweet varieties. The sweet types are said to contain a less amount of hydrogen cyanide than the bitter varieties and for this reason the sweet varieties are usually eaten raw in some parts of the country. However, recent studies have shown that some of the sweet varieties contain more hydrogen cyanide than the bitter variety (Nestel, 1973).

2.3: Non Food Uses of Cassava

Apart from food, cassava can be used in various other ways. They are used as animal feed in some countries. Cassava is a common raw material in starch production. Cassava starch has been used in the industry, for paper making, as a lubricant in oil wells and in the textile industry. It is also used as the substrate for the production of dextrins which are used in glues. Alcohol was produced from cassava in Brazil and Australia between the two world wars. Its use declined with the availability of cheap supplies of petroleum product, but there has been renewed interest in producing alcohol from cassava as the price of crude oil has risen (Cock, 1985).

2.4: Microbial Rotting of Cassava Tubers

Ekundayo and Daniel (1973) showed that cassava tubers cannot be stored for long periods before processing due to microbial rotting of the tubers. Tissue deterioration is due partly to rots caused by Lasiodiplodia theobromae, Trichoderma harzianum, Cylindrocarpon candidum, Aspergillus niger and A. flavus. These microorganisms enter the cassava tubers through wounds, bruises and natural openings. It was also observed that high relative humidities favoured rot development of the tubers. Thus high relative humidities of between 50 - 100% favoured rot development by Lasiodiplodia theobromae and pycnidia were formed in the presence of light. However, Benomyl (a systemic fungicide) was observed to control the soft rot of cassava tubers.

2.5: Preparation of Cassava Based Foods

Owing to the perishability of the tubers, cassava is usually preserved in the processed form. The processing also serves to reduce the cyanide content. As mentioned earlier, some varieties can be eaten raw. In northern Nigeria,

cassava is more often eaten raw or as a boiled or baked vegetable while in the south of the country, cassava is mostly eaten in the processed form. Cassava is usually processed into gari, kpokpo - gari, fufu, and cassava flour (Ekandem, 1961). The processing of cassava into food usually involves some or all of the following processes: Peeling of the tubers, fermentation, sundrying, soaking, roasting and milling.

Various aspects of the technology and microbiology of the fermentations involved in the production of cassava based foods have been described (Odunfa, 1985). More detailed review of cassava flour processing in Nigeria will now be presented.

2.6: Cassava Flour.

Production of cassava flour in Nigeria:

To date, cassava flour production takes place mainly at the village level. In the process, cassava tubers are first peeled, cut into pieces and washed. The small pieces or chips are then

sun-dried after which they are milled into flour.

Okagbue (1986) stated that microbial contamination of locally produced flours takes place at various stages during production. Thus, the contamination of cassava flour takes place mostly during its production. Human hands and unsanitized knives are used during the peeling and cutting of the cassava tubers. The cassava chips which are washed (usually with non-chlorinated water) are sun-dried. The sun-drying is done in the open-air for varying lengths of time. During the milling process, the same machine which is not usually disinfected before or between operations, is often used for different batches of the same or of different products. The flours are subject to further contamination during sale in open-air markets and during manual examination of the quality of the product by customers.

In Nigeria, the chief source of cassava flour (which is called "Lafun" in Yoruba) is the Oyo area. This is because Oyo is situated in the drier part of the rain forest belt with many rock outcrops which are suitable for sun-drying the chips (Ekandem, 1961). Other areas where

cassava flour is produced include Okene area and some parts of Benue and Plateau regions of Nigeria. It is noteworthy that in the Eastern parts of Nigeria, cassava which is an important staple food is processed mainly as "fufu" or "gari" and not as flour.

Clerk and Caurie (1968) reported that flour obtained from dry uncooked cassava tubers is one of the major staple foods in tropical Africa while Cock (1985) described cassava flour as an extremely cheap source of calories.

Cassava flour is eaten in the form of "fufu" which is prepared by putting some quantity of cassava flour into boiling water and stirring until the mixture thickens into brown "fufu" known as "Elubo lafun" in Yoruba. Cassava flour is also used in thickening soups and for making cakes cooked with palm oil and pepper, or thin biscuits. In northern Nigeria, the flour is mixed with cowpea flour and used for making "dan wake" cakes or mixed with millet flour to make "waina" cakes. It is also

mixed with guinea corn flour to make "Tuwo" (guinea corn fufu) (Ekandem, 1961). In other countries, cassava flour is also eaten mostly as a thick sticky mass or porridge similar to the "fufu" eaten in Nigeria. This thick paste is called "funge" in Angola, "Vou-vou" in Cameroun, "luku" in Zaire and "nshima" in Zambia. A mixture of cassava and millet flours is used to make the staple foodstuff of Uganda. In south India and south East Asia, cassava flour is sometimes mixed with rice before cooking. It is also used in making some traditional Indian foods such as "chappatis", "uppuma", "idlies" and "dosas" (Lancaster et al, 1982). A new product called Tapioca macaroni has been developed in India by adding a small quantity of specially prepared groundnut meal and wheat semolina to cassava flour. The mixture is processed, cooked and eaten in the same way as food grains. The product has about 10% protein and is nearly twice as nutritious as rice (Grace, 1971). Also, it is used in making cakes, cookies, spaghetti, noodles, confectioneries etc in some countries (Cock, 1985).

Cassava flour in bread making:

Many studies have shown that cassava flour or starch can readily be incorporated into bread at different levels with little decline in quality. Thus, it has been suggested that cassava flour should be partially substituted for wheat flour in bread making (Grace, 1971; Jones and Akinrele, 1976; Ciacco and D'Appolonia, 1978).

In most developing countries, the demand for bread is increasing rapidly. Only few of these countries can grow wheat on a large scale and so most of them import wheat. This strains the foreign exchange earnings of the developing countries and discourages the use of locally produced substitutes (Cock, 1985). James *et al.* (1973) reports that in the developing countries, unmilled wheat imports stood at 353,295 tons at a cost of ₦20,655,528 in 1971. At the same period, 36,675 tons of milled wheat flour were imported at a cost of ₦3,156,688 The total cost of these two items in 1971 was, therefore, ₦23,812,216. When compared with the figures for 1970 when the importation of unmilled

and milled wheat gave a total cost of ₦15,958, 518, it will be seen that the cost of importing wheat and wheat flour rose to 50% between 1970-71. Thus, the importance of substituting some of our local flours for wheat flour cannot be over emphasized.

Until recently, it was believed that good quality bread could not be made with more than 10% by weight of cassava flour. However, studies begun in 1967 have demonstrated that much larger proportions of cassava flour can be used if the gluten of wheat is supplemented by other chemical binderies. Good quality bread of superior nutritional quality has been made with composite flours made up of 75% wheat flour, 20% cassava flour and 5% soya flour (Jones and Akinrele, 1976). James et al (1975) also reported that composite flour containing 70% wheat flour, 25% cassava starch, and 5% full fat soya flour was successfully used in producing bread and other baked products such as biscuits, chin-chin and doughnuts.

Raised bread has been made using no wheat flour at all. Its appearance is satisfactory and its nutritional quality is better than that

of ordinary wheat bread (Jones and Akinrele, 1976). However, it still has to be subjected to tests of general consumer acceptability. Protein could be provided by various legumes and oil seeds including groundnuts, cottonseed, chick peas and cow peas (Jones and Akinrele, 1976). Grace (1971) stated that the protein quality of cassava-soya and cassava-peanut breads was higher than that of common wheat bread.

If a reasonable admixture of cassava flour were permitted in Nigeria, the country could reduce imports of wheat, Jones and Akinrele (1976) reported that if 10% of wheat flour had been replaced by cassava flour in 1974, Nigeria would have reduced imports by 8 million dollars or 5 million pounds according to FAO estimation of imports. Thus, with the high cost of importation of wheat and wheat flour and increase in the consumption of bread and other baked products due to increased urbanization and greater reliance on purchased food stuffs, the partial replacement of wheat flour by cassava flour is necessary.

2.7 Microbiology of Flours

General

As was mentioned earlier, wheat flour is the commonest type of flour used in the food industry in the world. Thus, most of the studies that have been done on the microbiology of flours have focused on wheat flours. Hesseltine and Graves (1966) reviewed several studies conducted on the microbiology of flours (mainly wheat flour) in the earlier decades. In general, flours contain a microbial population consisting of fungi, bacteria and yeasts whose numbers vary greatly. The bacterial count is usually greater than the mould counts. However, as long as the moisture content of flour is 12% or less, no growth of microorganisms occurs. As flour with a low moisture content is kept in storage, the number of viable microorganisms decreases fairly rapidly (Hesseltine and Graves, 1966).

Graves et al (1967) stated that most of the microbiological studies on American and Canadian wheats and flours were made during the first four decades of the century, when

spoilage of breads by rope-producing bacteria was common. The studies led to the use of the antimicrobial agents calcium and sodium propionates in flour which reduced the bacterial content of flour and made ropiness in bread rare. The studies also led to improved sanitation in flour mills.

The presence of unusually high numbers of microorganisms in flour can affect its quality adversly. Mehrotra and Basu (1975) reported that microorganisms can reduce the quality of flours by various biochemical changes and by an increase in their fat acidity values. Spoilage in flour or spoilage in any food prepared with the flour can result from growth and activity of microorganisms. Furthermore, microorganisms growing on flour can produce toxic metabolites like mycotoxins while others can cause food infections and intoxications through other types of toxins. Some microorganisms are also indicators of poor levels of sanitation practice during production.

Microbial contamination of wheat flour takes place from the air. The microorganisms

contaminate the cereal grains in the field and during harvesting the seed is exposed to more dust and consequently to additional numbers of microorganisms. Further contamination takes place within the mills (Hesseltine and Graves, 1966).

Wheat flour

Although the general information given earlier in this section of the thesis is based on studies conducted mainly on wheat flour, more specific microbiological studies on the product will now be reviewed.

Studies have concerned themselves mainly with estimating the levels of certain groups of microorganisms in wheat flour and relating them with the levels in the wheat from which the flour was milled. Thus, Graves et al (1967) who studied the bacterial and actinomycete flora of wheat and wheat flour from eleven representative flour mills in parts of U.S.A. enumerated faecal streptococci, total aerobic bacteria, psychrophiles, salmonellae, coagulase-positive staphylococci, catalase-negative bacteria, thermophilic spores [illegible]

and actinomycetes. The total and differential counts were low. Counts for total aerobic bacteria ranged from $2.2x10^2$-$2.0x10^2$/g in flour and $1.5x10^4$-$6.6x10^5$/g in wheat. Aerobic plate counts were observed to be consistently lower in the flours than in the parent grains. Actinomycete counts ranged from 0-$5.3x10^3$/g in flour and 0-$3.0x10^2$/g in wheat. Tests for salmonellae and coagulase-positve staphylococci were negative in the samples examined. Of the seven samples examined for psychophiles, only one contained a detectable level of this group of organisms. Faecal streptococci were detected in less than half of the samples and in very low numbers. Thermophilic spore counts were also low. After the identification of bacteria in the study, the most frequently encountered and widely distributed organism in wheat belonged to the genus Flavobacterium while in flour the dominant bacteria was Laracolobactrum aerogenoides. For the Actinomycetes isolated from wheat and flour, Streptomyces albus predominated. Other genera of bacteria isolated from wheat and flour include Bacillus spp., Micrococcus spp. Aerobacter spp., Alcaligenes spp., Pseudomonas spp. It was observed in the study that in

comparison with earlier studies on microbiology of flours, there was a decrease in the bacterial content of commercial flours which was due to improved handling of wheat, better milling practices and the use of modern milling equipment. Lehrotra and Basu (1975) studied the microorganisms associated with cereal grains and their milling fractions in India. The microorganisms enumerated include total aerobic bacteria, psychrophiles, mesophiles, thermophiles, hydrophilic fungi, actinomycetes, osmophilic moulds and bacteria Some of the counts obtained from wheat flour are psychrophilic bacteria 0-$3.6x10^4$/g, mesophilic bacteria $6.0x10^2$-$1.1x10^5$/g, thermophilic bacteria 0-$6.5x10^3$/g, hydrophilic fungi $4.0x10^2$-$5.3x10^3$/g, xerophilic fungi $3.0x10^2$-$7.0x10^3$/g, Actinomycetes 0-$1.5x10^3$/g. It was observed in the study that the microbial contamination was very high. The bacterial counts from both wheat grains and their milling fractions were higher than those of fungi which were also higher than those for actinomycetes. Mesophilic and osmophilic bacteria were present in both the grains and their milling fractions. Thermophiles were more frequently encountered in the fractions than

in the grains. Mesophilic and osmophilic fungi were also present in most of the fractions and grains. Flour microflora was observed to fluctuate from place to place and season to season due to the uncontrolled environmental conditions and storage practices in India. Also bacterial counts formed the major part of the microflora of both wheat grains and its milled products. The types of bacteria isolated belonged mostly to Achromobacter spp., Bacillus spp., Flavobacterium spp, and Micrococcus spp. Of the actinomycetes only Streptomyces was isolated. The fungi isolated were Aspergillus spp, Penicillium spp. some mucorales and Fungi Imperfecti.

Graves and Hesseltine (1965) studied fungi in flour and refrigerated dough products from 19 flour samples representing 11 flour mills in different parts of U.S.A. The fungal counts ranged from 85-8.1×10^5/g in flour, 0-2.4×10^5/g in spoiled doughnuts, 43-57/g in fresh dough nuts, 15-2.2×10^3/g in spoiled dinner rolls, 0-57/g in fresh dinner rolls etc. Except for a few Fungi Imperfecti and mucorales, most of the species isolated from the flours belonged to the genera Aspergillus and Penicillium. Not only did they belong to these genera but to specific groups in each.

In the genus Aspergillus representatives of the A. candidus, A. glaucus, A. flavus-oryzae and A. versicolor groups were the only ones present. In Penicillium, the species commonly encountered were P. cyclopium, P. citrinum and P. urticae. The remaining species were scattered among various genera. The mould most frequently encountered in both flour and doughs was A. candidus. In the dough samples, Aspergillus spp were far more common than the Penicillium species whereas in the flour samples, the reverse was the case. It was observed that the fungal flora of the doughs was a reflection of the flour microflora and that improved handling of wheat and better milling practices were effecting a gradual reduction in fungal counts in commercial flours.

A total of 60 flour samples obtained from 52 mills and 55 wheat samples were examined for yeasts in different parts of U. S. A. by Kurtzman et al. (1970). Yeasts were isolated from more samples of wheat grain than the flour. Cryptococcus albidus was the most commonly isolated yeast from wheat grains but none was present in the flours. A difference of colony

colour ranging from white to faintly pink was observed for the various isolates. The species isolated from wheat were Cryptococcus laurentii, Rhodotorula glutinis, Pichia fermentans and Pichia burtonii while those isolated from flour were Hansenula anomala, Pichia farinosa, P. burtonii, Saccharomyces microellipsodes and Candida spp. The yeast-like mould Aureobasidium pullulans was common to both wheat and flour.

Microbiology of cassava flour and other local flours

As indicated by Okagbue (1986), Nigeria has a wide variety of flours but little or no work has been done on their microbiology and on other aspects of thier safety. It is noteworthy that yam flour was one of various food items destroyed for their unwolesomeness by the Food and Drug Inspectorate in 1977 (Baptist, 1984). An attempt was made by Okagbue (1986) to survey microorganisms associated with flours obtained from Zaria markets. One sample each from eight types of flour, namely:- cassava, yam, guinea corn, millet, cowpea, maize, rice and wheat flours were examined. The groups of microorganisms used as indicators of the microbial quality of the different flours were aerobic bacteria,

aerobic spore formers, lactose and non-lactose fermenters (Enterobacteriaceae), xerophilic and hydrophilic moulds and yeasts,

The counts obtained from cassava flour for the different groups of microorganisms were, aerobic plate count (APC) $7.0x10^7$ cfu/g, aerobic spore count (ASC) $3.6x10^7$ cfu/g, coliform counts $2.0x10^5$ cfu/g, non-lactose fermenters $1.0x10^6$ cfu/g, xerophilic mould counts $1.0x10^3$ cfu/g, hydrophilic mould count $3.0x10^5$ cfu/g. Yeasts were not isolated. For the other types of local flours studied the ranges of counts were APC $4.2x10^4$-$1.2x10^8$ cfu/g, ASC $3.0x10^3$-$3.6x10^7$ cfu/g, coliform count $4.0x10^2$-$8.0x10^6$ cfu/g, non-lactose fermenters $3.0x10^2$-$4.0x10^6$ cfu/g, xerophilic moulds $1.2x10^2$-$1.9x10^3$ cfu/g, hydrophilic moulds $1.0x10^3$-$8.0x10^6$ cfu/g and yeasts $1.5x10^5$ cfu/g. In this study, Okagbue (1986) observed that counts obtained for the different groups of microorganisms were high when compared with those of wheat flour studied in advanced countries. It was also observed that microbial contamination took place at the various stages of production and during sale in open markets. The variation

observed in the microbial counts were suggested to be due to different levels of sanitation practiced by different processors and retailers, to possible differences in the duration of exposure of the flours in open-air markets and also to the different moisture contents of the flour samples. It was suggested that further studies would be required to determine whether toxigenic fungal species such as *A. flavus* occur in local flours. that a more extensive survey of each type of flour would be needed before microbial limits can be specified for consumer protection and for monitoring sanitation practices in flour mills. In the cited survey, specific organisms in flour were not identified. However, the report of Clerk and Caurie (1968) in Ghana where the method of preparation of cassava flour is similar to that of Nigeria has shed some light on the types of fungi which could be found in cassava flour, namely:- *Cladosporium herbarum*, *Sporendonema sebi*, *Aspergillus spp.* and *Penicillium spp.* Some biochemical effects of the moulds on pieces of peeled cassava root contaminated by some of the moulds during commercial preparation of cassava flour were also determined.

They reported that Aspergillus flavipes, A. japonicus, A. niger and A. ochraceus could cause considerable biochemical changes in the cassava tuber. A. japonicus and A. niger caused an extensive loss of dry weight (more than 70%)in 20 days and shifted the initial pH from 6.3 to 4.3 and 4.9 respectively. A. flavipes, A. flavus and A. ochraceus caused moderate dry weight losses (less than 44%)and shifted the pH of the tubers from pH 6.3 to 8.2, 8.0 and 8.1 respectively. Except A. flavipes, the other moulds reduced the total nitrogen content of the cassava tubers. Also, the amino acid and soluble carbohydrate content of the tubers were altered to various extents both quantitatively and qualitatively by the moulds. The biochemical changes in the cassava tubers meant for the production of cassava flour would bring about cassava flour with varying qualities. Apart from these two studies, there seems to be no other microbiological study on cassava flour or other local flours in Nigeria.

2.0 Some Important Considerations on Studying Microbiology of Flours.

General

In studying the microflora of flour, its

dry nature as well as its heterogenous flora have to be taken into consideration. In addition to using media to isolate common bacteria and fungi, specific physiological groups, for example, xerophiles, acid producers, thermophilic spore formers must be considered.

Use of antibiotics

Some antibiotics like tetracycline and cycloheximide are used to inhibit the growth of bacteria and fungi respectively. Graves et al (1967) enumerated the total bacterial population with plate count agar containing 100 p.p.m cycloheximide to inhibit fungal growth. Mehrotra and Basu (1975) enumerated total fungal counts by inoculating diluted samples on Czapek's solution agar containing 30 p.p.m of tetracycline hydrocholoride to inhibit bacterial growth.

Detection of actinomycetes

Actinomycete populations were determined by inoculating diluted samples onto plates of plate count agar containing 100 p.p.m cycloheximide. Colonies were counted after 14 days at 28°C.

Although the antibiotic inhibited the growth of moulds and yeasts, bacteria were unihibited. However, after 2 weeks, the sporulating actinomycete colonies were easily distinguished from the dried, degenerate bacterial colonies. (Graves et al, 1967).

Detection of xerophiles

Xerophiles are isolated in media containing high concentrations of salt or sucrose. Thus, Flannigan and Hui (1976) isolated xerophiles on malt salt agar containing 10% (w/v) Nacl. The plates were incubated at 25°C for seven days while Mehrotra and Basu (1975) isolated xerophilic fungi on czapek's agar containing 20% sucrose.

Detection of thermophilic spore formers

To isolate thermophilic spore formers, diluted samples are usually preheated to destroy the vegetative cells. James and Smith (1948) enumerated thermophilic spores by pre-heating diluted samples at 90°C for 30 min. to destroy vegetative organisms. The diluted samples were then inoculated onto brom-cresol purple

dextrose tryptone agar and incubated at 55°C for 48 hr.

Detection of acid producing bacteria

James and Smith (1948) isolated acid producing bacteria on brom-cresol-purple dextrose tryptone agar at 25°C for six days.

Detection of psychrophiles

Psychrophiles are usually isolated by incubating at very low temperatures. Graves et al (1967) enumerated psychrophilic bacteria by inoculating diluted samples into plate count agar containing cycloheximide and by incubating at 5°C for 10 days.

Detection of anaerobic thermophilic spores

James and Smith (1948) isolated anaerobic thermophilic spores by first pre-heating 0.01 dilution at 90°C for 30 min. Then 2ml of the diluted sample was added to each of ten tubes of medium containing Bacto-liver broth. These were incubated at 55°C for 72hrs. Acid production in one tube was accepted as evidence of the presence of at least one spore. A count of the

tubes showing acid production multiplied by five represented the number of anaerobic thermophilic spores per gram of flour.

Detection of spores of rope bacteria

Each of 0.01 dilution was pre-heated at 90°C for 30 min. One ml of this was added to each of ten tubes containing nutrient broth. These were incubated at 37°C for 72hrs. The presence of a characteristic pellicle was accepted as evidence of at least one spore. A count of the tubes showing pellicle formation multiplied by ten represented the number of spores of rope bacteria per gram of flour (James and Smith, 1948).

CHAPTER 3

MATERIALS AND METHODS.

3.1: Collection of Samples

A total of 105 samples of cassava flour were collected for microbiological analysis, from eight different towns in Nigeria, namely, Kaduna (20 samples), Zaria (27 samples), Okene (24 samples), Jos (14 samples), Lagos (10 samples), Ilorin (4 samples), Port-Harcourt (5 samples) and Owerri (1 sample). The paucity of the samples obtained from the last two towns was due to the fact that cassava was not usually eaten in the form of cassava flour in those areas; consequently, it was not possible to purchase enough flour. In every town, the samples were purchased from different sellers in the open-air market visited. The samples were collected in fresh polythene bags and were brought to Zaria within 2 days after purchase. They were kept at room temperature in the laboratory for a maximum of two days before analysis.

3.2: Isolation and Enumeration of Microorganisms from Cassava Flour Samples

Each sample was thoroughly mixed with a sterilised spoon. Ten grams of samples and 10g of sterile sand (prepared by soaking sand in concentrated

hydrochloric acid for 1hr, washing six times with distilled water, drying and sterilizing in the hot air oven for 1½ hrs), were weighed out on a sterilized aluminium foil. They were transferred to 90 ml sterile 0.1% peptone water in a medicine bottle (110 total volume) and shaken vigorously by hand. Serial dilutions were prepared up to 10^{-6} and 0.1 ml of suspension was spread by means of sterile bent glass rod on duplicate agar plates which had been pre-dried at 40-50°C for 10 minutes.

Nutrient agar (oxoid) was used for determination of total aerobic counts. Dilutions of 10^{-5} and 10^{-6} were plated and incubated at 30°C for 48 hrs. For the quantitative determination of Enterobacteriaceae, the method used was that described by Oblinger et al (1982). In this method, 0.1ml of the sample was inoculated by pour plate method into 15 ml of MacConkey agar (oxoid) containing 1g of glucose. The medium was left to solidify and it was over layered with another 10ml of the medium. Dilutions of 10^{-4} and 10^{-5} were plated out and incubated at 30°C for 5 days. Colonies of lactose fermenters were pink or red in colour while those of non-lactose fermenters were colourless. The population of spore formers was determined by plating out on nutrient agar

plates, dilutions of 10^{-1} and 10^{-2} which had been pre-heated at 80°C for 10 mins. and cooled for 30 mins. The plates were incubated at 30°C for 48 hours. Littmans medium as described by Smith (1969) was used for enumeration of mesophilic moulds and yeasts. Initially, when commercial ox-gall, a component of the medium was not available, fresh bile from the abattoir was used. Nine ml of the fresh ox-bile was used to prepare 100ml of Littmens medium (Oxoid Manual, 1982).

The medium containing the fresh bile was observed to be as good in quantitative isolation of moulds as the commercial ox-gall which was used when it became available. Tetracycline was added to the autoclaved medium to give a final concentration of 30 ug/ml, to inhibit the growth of bacteria. Incubations were made at room temperature (approximately 26°C) and yeasts and moulds were counted after three and five days respectively. For the enumeration of xerophilic fungi (members of the Aspergillus glaucus group) dilutions of samples were plated for 6 days on Malt Salt Agar (MSA) containing 8% Nacl (Christensen, 1946).

Fifteen ml amounts of each autoclaved medium was poured into glass Petri dishes (85 mm diameter) and allowed to set. In all cases, viable counts of

appropriate groups of microorganisms were expressed as colony forming units per gram (cfu/g) of sample. The moulds and yeasts isolated from Littmans medium and from MSA were put on slants of the same media, allowed to grow for one to two days at room temperature and stored in the refrigerator for identification.

3.3: Identification of Moulds

The mould isolates were identified by reference to Smith (1969) and Gilman (1957). Sometimes it was necessary to subculture isolates on to czapek dox agar containing 20% sucrose or on PDA before identification. Isolates identified to be Aspergillus flavus were confirmed by inoculating them on to Aspergillus Differential Medium (ADM) of Bothast and Fennel (1974). Each of the isolates tentatively classified as A. flavus was spot inoculated onto the centre of a plate of ADM and incubated for five days at 28°C. Production of an orange pigment on the reverse side of the colony confirmed the identity of the isolate (Bothast and Fennel, 1974).

3.4: Determination of Aflatoxin Producing Potential of A. flavus Isolates

Aflatoxin producing potential of the A. flavus isolates was detected by using a modified form of the

the Aflatoxin Producing Ability medium (APA) described by Hara et al (1974). The original APA medium is a modified Czapek's solution agar containing corn steep liquor, $HgCl_2$ and $(NH_4)H_2PO_4$ instead of $NaNO_3$. Since commercial corn steep liquor was not available for this work, an attempt was made to device a substitute as follows: Batches of 500g of corn were steeped in 2.5 litres of water for 24 and 48 hrs. The liquor (obtained at 24 or 48 hr) was then boiled in a water bath for one hour filtered and used in preparing a modified APA medium. The pH of the medium was adjusted to 5.5 with a pH meter (PYE unicam 292 MK2). Millet and guinea corn were also used in place of corn to prepare millet steep liquor and guinea corn steep liquor respectively. These were also used to prepare corresponding APA media. After preparing the medium, the confirmed A. flavus strains were spot inoculated onto plates containing the APA medium and the plates were incubated in the dark at 28°C for a maximum period of 10 days. From the fifth through the tenth day of incubation, each inoculated plate was observed under the ultra violet lamp (Hanovia lamps R-52) for fluorescence characteristic of toxigenic A. flavus

isolates (Hara et al., 1974). Only the plates containing toxigenic isolates produced blue fluorescence in the agar surrounding the colonies while those containing non toxic isolates did not. The intensity of the fluorescence was determined subjectively.

3.5: Cultivation of Toxigenic A. flavus Isolates for Aflatoxin Production:

This was carried out in a medium containing 2% yeast extract and 20% sucrose (Lin and Dianese, 1976). Fifty ml amounts of the medium were dispensed into 250 ml Erlenmeyer flasks and autoclaved. For each isolate, spores taken (with a sterilized inoculating loop) from a four to six day old PDA plate were used to prepare an aqueous suspension dense enough to obscure an arbitrarily drawn black line on a piece of paper. One ml of the suspension was then inoculated into each of five flasks which were incubated in the dark at room temperature (approximately 26°C) under stationary conditions for eight days.

3.6: Extraction of Aflatoxin from Culture Filtrates:

The mycelial mat was filtered off from the culture medium by removing the mycelial mat with a sterile pair of forceps after the stated

culti-ation period and filtering with filter paper. Fifty ml of the culture filtrates were then extracted with chloroform in a Gallenkamp orbit environ shaker (No 1H 460) for 24 hrs. The mixture was poured into a separating funnel and the chloroform extracts collected were concentrated to dryness in a rotary flask evaporator (Buchler Instr. Inc. No B97311). The concentrate was then stored in a dark bottle and kept in the freezer.

3.7: Preparation of Chromatoplates:

Thin layer chromatoplates (TLC) were prepared as reported by Robertson et al. (1967). Sixty grams of Kieselgel G (Type 60) was put into a 250ml stoppered Erlenmeyer flask containing 100ml of distilled water and shaken vigorously for about two to three minutes. The slurry formed was then used to coat six 20 x 20 x 0.3 cm glass plates arranged on a shanddon "unoplan leveller". The spreader which was adjusted to give a uniform thickness of 0.5 mm was used in coating the plates which were then allowed to dry for one hour in a dust free atmosphere. The plates were activated for one hour in a hot air oven before use.

3.8 Separation of crude aflatoxins.

A modified thin layer chromatographic procedure described by de Iongh et al (1962, 1964) was employed in the chromatography of crude aflatoxins. The crude concentrate of aflatoxins obtained earlier was re-dissovled in 2ml of chloroform and then streaked on the TLC plates prepared as described earlier, the plates were developed with chloroform and acetone (in the ratio of 90:10 v/v respectively). in unlined, unequilibrated, multi-sheet chromatographic tank. About 250 mls of the solvent mixture was used. When the solvent front was about 4 cm to the top, the plates were removed from the tank and left in a dark place to dry. Fluorescent bands corresponding to aflatoxins, B_1, B_2, G_1 and G_2 were observed when the plates were viewed in ultra-violet light. The bands were marked and scraped off and the aflatoxins were eluted from the kieselgel G with 2% v/v methanol in chloroform. The eluate was concentrated in a rotary flash evaporator and this was again run on kieselgel G coated thin layer plates with the same proportion of chloroform and acetone as the developing agent. The aflatoxin were again recovered from Kieselgel G scrapings by eluting with 2% methanol in chloroform. The eluate was re-dissolved in 2ml

of chloroform after being concentrated to dryness in a rotary flask evaporator.

3.9: Quantitative Estimation of Aflatoxins

This was done by the spectrophotometric method of Rodricks and Stoloff (1970). The concentrations of solutions of the different aflatoxin solutions which were made up to 5mls with chloroform were determined by measuring absorbance (A) with a spectrophotometer (Cecil instruments CE 505) at 360nm against chloroform as the standard.

The concentration of each type of aflatoxin was then calculated with the following formula (Rodricks and Stoloff, 1970):

$$\text{Concentration of aflatoxin ug/ml} = \frac{A \times M.W \times 1000 \times C.F}{E}$$

Where,

M.W = Molecular weight

C.F = Correction factor (obtained from Calibration of the instrument)

E = Molar absorptivity

A = Optical density

The molecular weight and the molar absorptivities were taken from the table provided by Rodricks and Stoloff (1970) and shown below.

Table 1: Molecular weights and absorptivity of the aflatoxins (Rodricks and Stoloff, 1970):

Aflatoxin	Molecular weight	Molar absorptivity
B_1	312	19800
B_2	314	20900
G_1	328	17100
G_2	330	18200

CHAPTER 4

RESULTS

Tables 2 and 3 show the results of a survey of the microbiological quality of samples of cassava flour obtained from various towns in Nigeria. The means and the ranges of the counts of the groups of bacteria determined in the study are shown in Table 2. The mean of the aerobic plate counts (APC) of the samples from Lagos was the lowest ($4.7x10^5$ cfu/g). This was followed by the mean counts from Kaduna, Zaria, Ilorin, and Jos which were of intermediate category while the highest category of means of APC was recorded in samples from Okene ($1.1x10^7$ cfu/g) and Port - Harcourt ($1.0x10^7$ cfu/g). The lone sample from Owerri had a relatively high aerobic plate count ($1.0x10^7$) cfu/g). In contrast, the narrowest range ($7.4x10^6$-$1.3x10^7$cfu/g) was recorded in Port - Harcourt samples. The widest category of the ranges were obtained from Ilorin, Okene and Zaria samples ($6.7x10^4$-$1.8x10^7$, $6.7x10^4$-$2.7x10^7$ and $1.1x10^4$-$2.8x10^7$ cfu/g respectively) while those from Kaduna, Jos and Lagos could be grouped into an intermediate category. In general, the range of the APC's recorded in this study for all the samples (105) was $1.1x10^4$-$2.8x10^7$ cfu/g. In the

aerobic spore counts (ASC) the lowest mean ($1.7x10^4$ cfu/g) was from the Lagos samples. The highest category of the means was recorded from Port-Harcourt ($1.9x10^6$ cfu/g), Jos ($1.7x10^6$ cfu/g) and Okene ($1.1x10^6$ cfu/g). An intermediate category of the means of the ASC was recorded from the samples obtained from Zaria, Kaduna and Ilorin. The narrowest range of ASC ($1.3x10^6$-$2.5x10^6$ cfu/g) was obtained from Port-Harcourt samples. The flour samples from Okene, Jos and Ilorin had an intermediate category of ranges of ASC, while the widest category of ranges were from the samples obtained from Zaria, Kaduna and Lagos ($1.7x10^3$-$1.9x10^6$, $1.9x10^3$-$1.0x10^6$ and $1.3x10^3$-$1.0x10^6$ cfu/g respectively.) The mean counts from the lone sample from Owerri samples was $1.5x10^6$ cfu/g. On the whole, the range of the ASC for all the samples was ($1.3x10^3$-$2.9x10^5$ cfu/g). Lactose fermenters were also isolated from the cassava flourssamples analysed. The means of the counts of the lactose fermenters obtained from Lagos, Port-Harcourt, and Ilorin samples ($2.1x10^4$, $2.7x10^4$ and $2.2x10^4$ cfu/g respectively) were generally the lowest. The next category comprised the means from the samples from Kaduna ($3.5x\ 10^4$ cfu/g) and Jos ($3.2x10^4$ cfu/g) while the highest category were from Zaria and Okene ($2.7x10^5$ and $3.0x10^5$

cfu/g respectively). The narrowest range of counts of lactose fermenters were recorded from Ilorin and Port-Harcourt samples ($1.1x10^4$-$4.3x10^4$, and $1.3x10^4$-$3.9x10^4$ cfu/g respectively). The widest ranges, on the other hand, were from Zaria, Kaduna and Okene ($1.0x10^3$-$6.6x10^6$, $7.0x10^3$-$8.7x10^4$ and $7.0x10^3$-$3.3x10^6$ cfu/g respectively) while in between the two categories of ranges are those from Jos ($3.0x10^3$-$5.3x10^4$ cfu/g) and Lagos ($8.0x10^3$-$3.7x10^4$ cfu/g) samples. The range for lactose fermenters for all the samples was $1.0x10^3$-$6.6x10^6$ cfu/g. The last group of bacteria enumerated in this study were the non-lactose fermenters. For this group of bacteria, the highest mean count ($5.2x10^4$ cfu/g) was obtained from Zaria samples. A second category of mean counts were from flour samples purchased from Jos, Okene, Lagos and Ilorin. The samples with the lowest category of means for counts of non lactose fermenters, were from Kaduna ($9.8x10^3$ cfu/g). The lone sample from Owerri also had a relatively low number of non-lactose fermenters ($8.0x10^3$ cfu/g). The widest category of ranges ($1.0x10^3$-$3.8x10^5$ cfu/g) was from Zaria samples. The narrowest ranges of non-lactose fermenters were from Jos, Lagos, Port-Harcourt and Ilorin samples ($9.0x10^3$-$5.7x10^4$, $3.0x10^3$-$5.8x10^4$, $5.0x10^3$-$4.3x10^4$

and $8.0x10^3$-$3.0x10^4$ cfu/g respectively). The second category of ranges of non-lactose fermenters were from Kaduna and Okene samples ($1.0x10^3$-$4.9x10^4$, $7.0x10^3$-$5.1x10^4$ cfu/g respectively). The range of non-lactose fermenters for all the samples combined was $1.0x10^3$-$3.8x10^5$ cfu/g).

The means and ranges of the counts of the groups of fungi determined in this study are shown in Table 3. The highest category of the means of the counts for xerophilic fungi were recorded from samples from Zaria ($2.0x10^4$ cfu/g) and Okene ($1.0x10^4$ cfu/g). The lowest mean was from Lagos samples ($6.7x10^2$ cfu/g) while the intermediate category of the means were obtained from Kaduna, Jos, Port-Harcourt and Ilorin samples ($1.1x10^3$, $2.1x10^3$, $1.3x10^3$ and $1.0x10^3$ cfu/g respectively). The lone sample from Owerri had a xerophilic count of $3.5x10^3$ cfu/g. The narrowest group of ranges of xerophilic mould counts were from Kaduna, Port-Harcourt, Lagos and Ilorin ($1.1x10^2$-$8.6x10^3$, $6.0x10^2$-$3.3x10^3$, $1.0x10^2$-$2.7x10^3$ and $6.0x10^2$-$5.0x10^3$ cfu/g respectively) while the widest category of ranges were from the samples obtained from Zaria, Okene and Jos ($2.0x10^2$-$4.2x10^5$, $1.0x10^2$-$1.6x10^5$ and $1.0x10^2$-$2.0x10^4$ cfu/g respectively). Xerophilic

and $8.0x10^3$-$3.0x10^4$ cfu/g respectively). The second category of ranges of non-lactose fermenters were from Kaduna and Okene samples ($1.0x10^3$-$4.9x10^4$, $7.0x10^3$-$5.1x10^4$ cfu/g respectively). The range of non-lactose fermenters for all the samples combined was $1.0x10^3$-$3.8x10^5$ cfu/g).

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yeasts were detected only in samples from Zaria, Okene and Jos. The lowest mean of xerophilic yeast count was obtained from Okene samples ($1.2x10^3$ cfu/g). The means of the counts of xerophilic yeasts from Zaria and Jos were higher ($3.7x10^4$ and $1.9x10^4$ cfu/g respectively). The narrowest range of xerophilic yeasts was from Okene samples ($1.2x10^3$-$1.6x10^4$ cfu/g) while the widest ranges were from Zaria and Jos ($2.6x10^3$-$1.0x10^6$ and $1.0x10^2$-$2.7x10^5$ cfu/g respectively). The highest category of mean counts for hydrophilic moulds were obtained from Okene and Zaria samples ($3.7x10^4$, and $1.0x10^4$ cfu/g respectively). This was followed by means counts for samples obtained from Kaduna ($1.4x10^3$ cfu/g) and Lagos ($1.1x10^3$ cfu/g). Thelowest mean counts were from Ilorin, Port-Harcourt and Jos samples ($6.8x10^2$, $8.6x10^2$ and $2.3x10^2$ cfu/g respectively). The narrowest category of ranges were obtained from Ilorin and Port-Harcourt samples ($4.0x10^2$-$1.0x10^3$ and $4.0x10^2$-$1.6x10^3$ cfu/g respectively) The next category of ranges were obtained from Laogs, Jos and Kaduna samples ($3.0x10^2$-$2.5x10^3$, $2.0x10^2$-$1.0x10^3$ and $1.0x10^2$-$8.9x10^3$ cfu/g respectively, while the widest category of ranges was found in the samples obtained from Okene ($1.0x10^2$-$6.4x10^5$ cfu/g) and Zaria ($1.0x10^2$-$1.4x10^5$ cfu/g). The last group of fungi enumerated from the flour samples in this

study were the hydrophilic yeasts. Flour samples from Okene yielded the highest mean counts of hydrophilic yeasts ($1.4x10^5$ cfu/g). The lowest recorded means of the hydrophilic yeast counts were recorded from samples purchased from Ilorin and Lagos ($1.3x10^2$ and $5.6x10^2$ cfu/g respectively), while the recorded mean counts of an intermediate category were obtained from samples purchased from Jos, Kaduna and Zaria ($5.8x10^4$, $5.7x10^4$ and $7.8x10^4$ cfu/g respectively). No hydrophilic yeasts were detected in samples obtained from Port-Harcourt and from the lone sample from Owerri. The narrowest range of hydrophilic yeasts ($<6.0x10^2$-$3.8x10^3$ cfu/g) was obtained from Lagos samples. The samples from Okene and Kaduna yielded an intermediate category of ranges of hydrophilic yeasts ($<4.4x10^3$-$1.2x10^6$, $<1.9x10^3$-$1.0x10^6$ cfu/g respectively) while the widest range of hydrophilic yeasts ($<6.8x10^2$-$1.9x10^6$ cfu/g) was from Zaria samples.

Tables 4a to 4h show the numbers of cassava flour samples contaminated with various fungi, the viable counts and the ranges of the counts of the fungi as determined on potato dextrose agar (PDA) and malt salt agar (MSA) for the various areas sampled. Table 4a shows the results from the Zaria samples. Fifteen species of moulds, Penicillium

spp, Mycelia sterilia and yeasts were isolated and the majority of them belonged to the genera Aspergillus and Penicillium. Based on the highest number of samples positive for each organism irrespective of the medium of isolation, more than half of the flour samples contained Aspergillus flavus (15 samples) and Penicillium spp (18 samples). One-third of the samples yielded A. niger (nine samples) and Rhizopus stolonifer (nine samples) while A. candidus, A. versicolor, A. wentii, A. oryzae and Mycelia sterilia occurred in less than one-third of the samples analysed. Apart from A. fumigatus which occurred in only four (approximately one-seventh) of the samples, other moulds were isolated from only a few samples and include Eurotium amstelodami, E. repens, Rhizopus oryzae, Sporendonema sebi, Curvularia lunata and Aureobasidium pullulans. Non-pigmented yeasts were isolated from about one-quarter of the total number of samples obtained from Zaria. No pigmented yeasts were isolated in any of the Zaria samples.

Some of the moulds, identified in this study appeared to show preference to either of the two media used. For example, E. amstelodami, A. versicolor, S. sebi and C. lunata which occurred in only two, five, one and one samples respectively were only

isolated on MSA. On the other hand, A. oryzae, R. oryzae and Aureobasidium pullulans were isolated from five, three, one, samples respectively and only on PDA. In some cases, both media (PDA and MSA) appeared to be equally suitable for isolating certain fungi though a slight preference for one medium could be observed for example, A. flavus, A. clavatus, A. niger, A. wentii, R. stolonifer, M. sterilia and E. repens seemed to be more preferentially isolated on PDA or MSA as the case may be. A. fumigatus seemed to be equally capable of growing on the two media, thus the numbers of samples positive for the organisms on the two media were equal. It is also noteworthy that the Penicillium spp and A. candidus seemed to have a marked preference for MSA. Thus for the former (Penicillium spp), the number of samples positive on MSA was 18 as opposed to six on PDA, and for the latter, five samples were positive on MSA as opposed to one on PDA. Also the yeasts isolated from Zaria samples showed some preference for PDA. They were isolated from seven samples on PDA but only from three samples on MSA.

The range of viable propagules of the fungi also differed on the two media used. On PDA, with the exception of those isolates detected on lone

samples, the range of viable propagules could be grouped into two categories. The narrowest ranges of viable propagules included M. sterilia ($1.0x10^2$-$2.0x10^2$ cfu/g), R. oryzae ($1.0x10^2$-$5.0x10^2$ cfu/g), Penicillium spp ($1.0x10^2$-$1.8x10^3$ cfu/g) and A. wentii ($1.0x10^2$-$2.0x10^2$ cfu/g) while the category of moulds with the widest range on PDA were R. stolonifer, A. oryzae, A. niger, A. fumigatus, A. flavus and A. clavatus. Yeasts also had a very wide range of viable propagules ($3.0x10^2$-$1.2x10^6$ cfu/g) and it was wider than the ranges of the moulds. The isolates from lone samples on PDA were observed to have high counts e.g. E. repens ($1.4x10^5$ cfu/g), A. candidus ($1.0x10^4$ cfu/g), and Aureobasidium pullulans ($1.9x10^6$). A. pullulans had very high counts almost similar to that of the yeasts. Also, with the exception of the isolates from lone samples, the moulds isolated on MSA fall into three categories of ranges which did not necessarily reflect the ranges of viable propagules on PDA. The narrowest ranges of viable propagules on MSA were recorded for R. stolonifer, A. versicolor A. niger, A. flavus, A. candidus, A. repens, E. amstelodami and M. sterilia. The range of A. fumigatus ($9.0x10^2$-$5.0x10^4$ cfu/g) was of the intermediate category

while the widest ranges were recorded for A. wentii and Penicillium spp ($1.0x10^2$-$4.5x10^5$, $1.0x10^2$-$7.7x10^5$ cfu/g respectively). Yeasts again had a range of viable propagules wider than that of the moulds ($2.6x10^3$-$1.0x10^6$ cfu/g) on MSA. The counts for the isolates from lone samples on MSA are A. clavatus ($3.4x10^5$ cfu/g), S. sebi ($3.0x10^2$ cfu/g) and C. lunata ($3.0x10^2$ cfu/g). For those isolates that were isolated from both PDA and MSA, it was observed in some cases that the range of viable propagules on MSA was narrower than the range on PDA e.g. A. flavus, A. niger, R. stolonifer and yeasts. For the A. flavus and the A. niger, more samples were positive on MSA than on PDA, while the reverse was true for R. stolonifer and yeasts. In constrast, some of the fungi e.g A. wentii, Penicillium spp and M. sterilia had wider ranges of counts on MSA than on PDA even though they were isolated from a greater number of samples on MSA. The number of samples positive for A. fumigatus as well as the ranges of counts on both MSA and PDA were similar.

MSA detected yeasts from a lower number of samples than PDA, that probably explains at least partially, the lower range of propagules which was detected on the same medium (MSA).

Twelve species of moulds, Penicillium spp, Mycelia sterilia and yeasts were isolated from Kaduna samples. As was the case with the Zaria samples, the majority of the moulds belonged to the genus Aspergillus together with the Pencillium spp. Based on the highest number of samples positive for each organism irrespective of the medium of isolation, more than half of the flour samples contained Aspergillus flavus (12 samples), Penicillium spp (14 samples) and pigmented yeasts (19 samples). More than one-third contained A. niger (eight samples) and non-pigmented yeasts (nine samples) while one-quarter of the flour samples contained A. fumigatus. The other moulds namely, Eurotium amstelodami, E. repens, A. candidus, A. clavatus, A. oryzae, A. versicolor, A. wentii, Rhyzopus oryzae and M. sterilia were isolated from only a few samples. It is noteworthy that the pigmented yeasts were isolated from 19 out of the 20 samples from Kaduna. Some of the moulds and the yeasts were isolated either on MSA or PDA. For examples, E. repens, A. oryzae, A. wentii, R. oryzae, and the yeasts were isolated only on PDA while A. candidus, A. clavatus and A. versicolor were isolated only on MSA. It is noteworthy that all the yeasts were isolated only on PDA. Both

media appeared to be equally suitable for isolating certain moulds. MSA seemed to be markedly superior to PDA for detecting A. flavus and Penicillium spp while the latter medium appeared to be slightly better for A. fumigatus and A. niger.

The range of viable propagules differed on the two media used. On PDA, with the exception of those isolates detected on lone samples, the narrowest group of ranges of counts were recorded for E. repens ($2.0x10^2$-$3.0x10^2$ cfu/g), A. wentii ($1.0x10^2$-$3.0x10^2$ cfu/g), Penicillium spp ($3.0x10^2$-$7.0x10^2$ cfu/g) and M. sterilia ($1.0x10^2$-$2.0x10^2$ cfu/g). The intermediate group of ranges were for A. niger ($1.0x10^2$-$2.4x10^2$ cfu/g), A. oryzae ($1.0x10^2$-$1.5x10^3$ cfu/g) and the non-pigmented yeasts ($1.1x10^3$-$1.0x10^4$ cfu/g) while the widest groups of ranges were for A. flavus ($1.0x10^2$-$1.0x10^4$ cfu/g), A. fumigatus ($1.0x10^2$-$1.0x10^4$ cfu/g) and the pigmented yeasts $1.0x10^3$-$1.0x10^6$ cfu/g). In the latter category, the ranges of the yeast counts was markedly wider than those of the moulds. E. amstelodami and R. oryzae which occurred in lone samples as determined on PDA, had relatively low counts ($1.0x10^2$ and $1.0x10^2$ cfu/g respectively). Again, with the exception of the isolates from lone samples, the narrowest

range of viable propaqules detected on MSA were for A. clavatus ($1.0x10^2$-$4.0x10^2$ cfu/g) and A. fumigatus ($1.0x10^2$-$2.0x10^2$ cfu/g). The widest ranges were $1.0x10^2$-$2.1x10^3$ cfu/g; $1.0x10^2$-$3.5x10^3$ cfu/g and $1.0x10^2$-$2.1x10^3$ cfu/g for A. flavus, A. niger and Penicillium spp. respectively. E. amstelodami, A. candidus, A. versicolor and M. sterilia were each detected only once on MSA and the counts were relatively low ($2.0x10^2$-$1.0x10^3$ viable propagules per gram). Among the fungi which were isolated from both FDA and MSA, some had relatively narrower ranges of viable propagules on MSA than on PDA. This was true of A. flavus and A. fumigatus which were isolated from greater numbers of samples on the different media. In contrast, the Penicillium spp. had a wider range of counts on MSA which also detected them from a greater number of samples. The ranges of counts of A. niger were similar on both media used in this study.

Thirteen species of moulds, Penicillium spp, Mycelia sterilia and yeasts were isolated from Okene samples (Table 4c). The majority of moulds belonged to the genera Aspergillus

and Penicillium. Based on the highest number of samples positive for each organism irrespective of the medium of isolation, more than half of the flour samples contained A. flavus (19 samples), A. niger (13 samples), Rhizopus stolonifer (16 samples), A. clavatus (10 samples), A. wentii (11 samples) and the non-pigmented yeasts were isolated from more than one-third of the samples. A. candidus (6 samples), A. versicolor (7 samples), Penicillium spp (6 samples) and the non-pigmented yeasts occurred in about one-quarter of the samples while Eurotium amstelodami, E. repens, A. fumigatus, A. oryzae, Rhizopus oryzae, Curvularia lunata and M. sterilia were isolated from only a few samples from Okene.

Some of the moulds were detected better by either of the two media used. For example, E. amstelodami, E. repens and A. candidus which occurred in only one or six samples were only isolated on MSA. On the contrary, A. oryzae, C. lunata and the pigmented yeasts were isolated only on PDA. The two media appeared to be suitable for isolating some of the fungi and only slight preference for one of the media

could be observed. For example, A. fumigatus, A. wentii and Penicillium spp were detected on both media but greater numbers of samples were positive for the organisms on MSA than on PDA, while A. niger, R. oryzae, M. sterilia and the non-pigmented yeasts were detected better by PDA. In contrast, MSA was markedly selective for A. clavatus, A. flavus, A. versicolor and R. stolonifer than PDA.

Differences were also observed in the ranges of viable propagules of the fungi isolated in the two media in this study. On PDA, with the exception of those isolates detected only once, the range of viable propagules could be grouped into three categories. The category with the narrowest range of counts included the A. fumigatus, ($5.0x10^2$-$6.0x10^2$ cfu/g), Penicillium spp ($4.0x10^2$-$7.0x10^2$ cfu/g), R. oryzae ($1.0x10^2$-$1.2x10^3$ cfu/g) and M. sterilia ($3.0x10^2$-$1.8x10^3$ cfu/g). The category with the intermediate ranges of counts contained R. stolonifer ($1.0x10^2$-$3.0x10^4$cfu/g), pigmented yeasts ($1.3x10^3$-$6.1x10^5$ cfu/g) and non-pigmented yeasts ($3.8x10^3$-$7.8x10^5$ cfu/g) while the widest ranges of counts were for A. flavus,

A. niger, and A. wentii ($2.0x10^2$-$2.0x10^5$, $1.0x10^2$-$1.3x10^5$ and $2.0x10^2$-$2.0x10^5$ cfu/g respectively). The fungi isolated from lone samples on PDA were A. clavatus, A. oryzae, A. versicolor and C. lunata ($1.2x10^3$, $5.0x10^2$, $8.0x10^2$ and $3.3x10^3$ cfu/g respectively). On MSA, the range of viable propagules of the fungi could also be grouped into three categories which do not necessarily reflect those of PDA. The narrowest ranges were recorded for A. versicolor and the non-pigmented yeasts ($1.0x10^2$-$3.0x10^2$ and $1.0x10^4$-$1.7x10^4$ cfu/g respectively). The intermediate category of ranges were taken by R. stolonifer, A. wentii, A. niger, A. fumigatus and A. clavatus. The widest ranges were for Penicillium spp, A. flavus and A. candidus ($2.0x10^2$-$2.1x10^5$, $1.0x10^2$-$5.0x10^4$, $1.0x10^2$-$5.0x10^4$ cfu/g respectively). The counts of the isolates from lone samples on MSA were E. amstelodami ($3.0x10^2$ cfu/g), E. repens ($7.0x10^2$ cfu/g) and M. sterilia ($1.0x10^2$ cfu/g). For those fungi that were isolated from both PDA and MSA, it was observed in some cases that the range of viable propagules on MSA was narrower than on PDA e.g. A. flavus, A. niger, A. wentii, R. stolonifer and the non-

pigmented yeasts. While A. flavus, A. wemtii, and R. stolonifer were isolated from a higher number of samples on MSA than on PDA, A. wentii and the non-pigmented yeasts were isolated from a higher number of samples on PDA. In contrast, some of the ranges of viable propagules on MSA were wider than those of their counterparts on PDA, e.g. A. fumigatus and Penicillium spp and as were observed for samples from Zaria and Kaduna, these isolates were detected on greater number of samples by MSA than by PDA.

Seven species of moulds, Penicillium spp. and yeasts were isolated from Lagos samples. The majority of moulds belonged to the genera Aspergillus and Penicillium. Based on the highest number of samples positive for each organisms irrespective of the medium of isolation, more than half of the flour samples contained A. clavatus (5 samples), A. flavus (8 samples) and Rhizopus stolonifer (6 samples). About one-third of the samples contained Penicillium spp. (4 samples) R. oryzes (4 samples) and the non-pigmented yeasts (3 samples). The other moulds e.g.

Eurotium amstelodami, A. candidus, A. niger and the pigmented yeasts were isolated from just few of the samples.

Some of the moulds identified in this study, appeared to show preference to either of the two media used (PDA and MSA). Thus E. amstelodami and A. candidus which occurred in two and one samples respectively were isolated from only MSA while R. oryzae and both the pigmented and non-pigmented yeasts were isolated from four, two and three samples respectively on PDA only. Both media appeared to be equally suitable for isolating certain moulds but a slight preference for one medium was observed e.g. A. flavus showed a slight preference for PDA while Penicillium spp. showed a slight preference for MSA. However, A. clavatus showed a marked preference for PDA while R. stolonifer showed a marked preference for MSA.

With the exception of those isolates detected on lone samples, the ranges of viable propagules on PDA could be grouped into three categories. The narrowest category of ranges were for R. stolonifer, A. niger, Penicillium spp. and pigmented yeasts. ($1.0x10^2$-$3.0x10^2$,

$7.0x10^2$-$1.3x10^3$, $2.0x10^2$-$1.0x10^3$ and $4.0x10^2$-$2.1x10^3$ cfu/g respectively) while the non-pigmented yeasts had the widest range of [illegible] viable propagules on PDA ($2.0x10^2$-$1.2x10^5$cfu/g). The ranges fell into two categories in MSA. The narrowest category of ranges was for E. amstelodami, A. flavus, and Penicillium spp ($1.0x10^2$-$2.0x10^2$, $1.0x10^2$-$4.0x10^2$ and $1.0x10^2$-$2.0x10^2$ cfu/g respectively). R. stolonifer had the widest range of $1.0x10^2$-$2.7x10^5$ cfu/g on MSA.

The counts of the isolates from lone samples on MSA were A. candidus ($1.0x10^2$cfu/g), A. clavatus ($2.0x10^2$ cfu/g) and A. niger ($2.0x10^2$ cfu/g). Here again, the ranges of viable propagules on PDA did not reflect the ranges of viable propagules on MSA. For those organisms that were isolated from both media, A. flavus and Penicillium spp. had a narrower range on MSA than on PDA. A. flavus was isolated from a higher number of samples on PDA while Penicillium spp was isolated from a higher number of samples on MSA. However, R. stolonifer had a wider range of viable propagules on MSA

than on PDA and it was isolated from a higher number of samples on MSA. A. niger was isolated from equal number of samples on both media and the counts were almost similar.

Table 4e shows results from flour samples purchased from Jos. Seven species of moulds, Penicillium spp. and yeasts were isolated from the samples. The majority of moulds isolated belonged to the genera Aspergillus and Penicillium. Based on the highest number of samples positive for each organism irrespective of the medium of isolation, more than half of the samples contained the non-pigmented yeasts (11 samples). One-third of the samples contained Rhizopus stolonifer (5 samples) and A. niger (5 samples). About one-quarter of the samples contained Penicillium spp. (4 samples) and Eurotium amstelodami (3 samples). A. flavus, A. fumigatus, A. wentii and Curvularia lunata were isolated from just a few of the flour samples. No pigmented yeasts were isolated from any of the samples.

Some of the moulds appeared to show preference to either of the two media (PDA and

MSA) used. Thus, E. amstelodami and A. wentii were isolated from three, and two samples respectively on MSA only, while A. fumigatus, A. niger and C. lunata were isolated from one, five and one samples respectively on PDA only. In cases were both media appeared to be equally suitable for isolating some of the fungi, a slight preference for one medium could be observed. For example, A. flavus and R. stolonifer seemed to be more preferentially isolated on PDA. However, Penicillium spp showed a marked preference for MSA thus it had four samples positive on MSA and only one positive on PDA. In contrast, the non-pigmented yeasts showed a marked preference for PDA with 11 samples positive on PDA as opposed to six positive on MSA.

The range of viable propagules differed on the two media used. On PDA, with the exception of those isolates detected on lone samples, the range of viable propagules could be grouped into two categories. The range of the narrowest group of categories were recorded for A. niger and R. stolonifer ($1.0x10^2$-$4.0x10^2$, $1.0x10^2$-

$3.0x10^2$ cfu/g respectively) while the widest category of ranges was for the non-pigmented yeasts ($5.0x10^2$-$5.5x10^5$ cfu/g). The isolates from lone samples were A. flavus ($1.0x10^2$ cfu/g) A. fumigatus ($2.0x10^2$ cfu/g), Penicillium spp. ($4.0x10^2$ cfu/g) and C. lunata ($1.5x10^3$ cfu/g). On MSA, the fungi isolated fell into three categories. The narrowest ranges fo viable propagules on MSA were for A. wentii and Penicillium spp. ($3.0x10^2$-$4.0x10^2$, and $1.0x10^2$-$7.0x10^2$ cfu/g respectively) while the widest category were recorded for E. amstelodami, A. flavus, R. stolonifer and the non-pigmented yeasts. The range of viable propagules for R. stolonifer was narrower on PDA than on MSA and the isolate was from a higher number of isolates on MSA while the range for the non-pigmented yeasts were wider on PDA than on MSA and it occurred on a higher number of samples on PDA.

Five species of moulds, Penicillium spp. and Mycelia sterilia were isolated from flour samples purchased from Ilorin (Table 4f). The majority of moulds belonged to the genera

Aspergillus and Penicillium. No yeasts were isolated from Ilorin samples. Based on the highest number of samples positive for each organism irrespective of the medium of isolation, all the flour samples contained A. flavus and A. niger. More than half of the samples contained Eurotium amstelodami (three samples) and A. oryzae (three samples). Exactly half of the samples contained Penicillium spp (two samples) while M. sterilia and A. fumigatus occurred in one quarter of the samples.

Some of the moulds were also detected better by either of the two media used. For example, E. amstelodami, which occurred in three samples were isolated from MSA only. On the contrary, A. fumigatus and A. oryzae were isolated from one and three samples respectively on PDA only. However, A. flavus and M. sterilia were isolated from equal number of samples on both media and did not show preference for any of the two media. A niger seemed to be more preferentially isolated on PDA. Thus, the number of samples positive for the organism was four on PDA and only two on MSA. Penicillium spp. also seemed to prefer the

PDA medium thus on PDA the number of positive samples for the organism was two while the number of positive samples on MSA was one.

On PDA, with the exception of those isolates detected on lone samples, the range of viable propagules were narrow. These were for A. niger and Penicillium spp ($2.0x10^2$-$5.0x10^2$, $1.0x10^2$-$2.0x10^2$ cfu/g respectively). The counts for the other moulds isolated from the flour samples were A. flavus, A. fumigatus A. oryzae and M. sterilia ($1.0x10^2$, $3.0x10^2$, $1.0x10^2$, $1.0x10^2$ cfu/g respectively). On MSA also only one category of ranges of viable counts were obtained. The narrow ranges were recorded for E. amstelodami, A. flavus and A. niger ($1.0x10^2$-$3.0x10^2$, $1.0x10^2$-$2.0x10^2$ and $2.0x10^2$-$3.0x10^2$ cfu/g respectively). The isolates from lone samples had the following counts on MSA, Pencillium spp ($4.0x10^2$ cfu/g) and M. sterilia ($1.0x10^2$ cfu/g). For those isolates that were isolated on both media, the counts and ranges were almost similar e.g, A. niger. M. sterilia, A. flavus and Penicillium spp.

Eight species of moulds and Penicillium spp. were isolated from samples purchased from Port-Harcourt. (Table 4g). The majority of moulds were of the genera Aspergillus and Penicillium. No yeasts were isolated. Based on the highest number of samples positive for each organism irrespective of the medium of isolation, more than half of the samples contained A. niger (3 samples) and Penicillium spp. (3 samples). Two-fifth of the samples contained A. clavatus, A. flavus, Rhizopus oryzae and R. stolonifer while one-fifth contained Curvularia lunata, A. versicolor and A. fumigatus. Some of the moulds isolated in this study appeared to show preference to either of the two media used. For example, A. clavatus and Penicillium spp. which occurred in two and three samples respectively were isolated on only MSA while A. fumigatus, A. niger, A. versicolor, R. oryzae and C. lunata were isolated only on PDA. On the contrary, A. flavus and R. stolonifer were isolated on both media but they both seemed to be detected slightly better on MSA than on PDA. Thus the number of positive samples for the two organisms were two on MSA as opposed to one on PDA.

On PDA, with the exception of those isolates detected on lone samples, the narrow range of viable propagules obtained fell into just one category. The ranges for this category were recorded for A. niger and R. oryzae ($4.0x10^2$-$6.0x10^2$, and $2.0x10^2$-$3.0x10^2$ cfu/g respectively). The counts for the isolates obtained from lone samples on PDA were A. flavus, A. fumigatus, A. versicolor, R. stolonifer and C. lunata ($1.0x10^2$, $1.1x10^3$, $1.0x10^2$, $3.0x10^2$ and $3.0x10^2$ cfu/g respectively). On MSA, the ranges of viable propagules falls into two categories. The narrowest category of ranges were for A. flavus, Penicillium spp and R. stolonifer ($2.0x10^2$-$3.0x10^2$, $3.0x10^2$-$6.0x10^2$ and $2.0x10^2$-$4.0x10^2$ cfu/g respectively) while the widest range was for A. clavatus ($1.3x10^2$-$6.0x10^3$ cfu/g).

Table 3h shows results for the lone sample from Owerri. Five species of moulds were isolated from it and the majority of them belonged to the genus Aspergillus. All the moulds were isolated on PDA while only three were isolated on MSA. Eurotium repens and A. flavus showed a preference

for PDA. For those isolates that were isolated on both media, though they occurred in equal number of positive samples on both media, the number obtained on MSA were higher than those obtained on PDA. The moulds that were isolated on PDA and their counts were E. repens, A. flavus, A. niger, A. wentii and Rhizopus stolonifer ($2.0x10^2$, $8.0x10^2$, $1.0x10^2$, $2.0x10^2$ and $1.0x10^2$ cfu/g respectively). Those that were isolated on MSA were A. niger, A. wentii and R. stolonifer ($9.0x10^2$, $7.0x10^2$ and $9.0x10^2$ cfu/g respectively).

Table 2 : Viable counts of some groups of bacteria in cassava flour

Origin of sample	Total no of samples analysed	Aerobic plate count (cfu/g) Mean	Range	Aerobic spore count (cfu/g) Mean	Range	Enterobacteriaceae Lactose fermenters (cfu/g) Mean	Range	Enterobacteriaceae Non-lactose fermenters (cfu/g) Mean	Range
Zaria	27	5.1×10^{6}	1.1×10^{4}–2.8×10^{7}	4.5×10^{5}	1.7×10^{3}–1.9×10^{6}	2.7×10^{5}	<1.0×10^{3}–6.6×10^{6}	5.2×10^{4}	<1.0×10^{3}–3.8×10^{5}
Kaduna	20	2.8×10^{6}	2.3×10^{4}–6.5×10^{6}	1.4×10^{5}	1.9×10^{3}–1.0×10^{6}	3.5×10^{4}	<7.0×10^{3}–8.7×10^{4}	9.0×10^{3}	<1.0×10^{3}–4.9×10^{4}
Okene	24	1.1×10^{7}	6.7×10^{4}–2.7×10^{7}	1.1×10^{6}	1.0×10^{4}–2.7×10^{6}	3.0×10^{5}	<7.0×10^{3}–3.3×10^{6}	2.4×10^{4}	<7.0×10^{3}–5.1×10^{4}
Jos	14	8.0×10^{6}	1.2×10^{5}–2.5×10^{5}	1.8×10^{6}	2.1×10^{4}–2.9×10^{6}	3.2×10^{4}	3.0×10^{3}–5.3×10^{4}	2.7×10^{4}	9.0×10^{3}–5.7×10^{4}
Lagos	10	4.7×10^{5}	2.7×10^{4}–4.2×10^{6}	1.7×10^{4}	1.3×10^{3}–1.0×10^{6}	2.1×10^{4}	8.0×10^{3}–3.7×10^{4}	1.0×10^{4}	3.0×10^{3}–5.8×10^{4}
Port-Harcourt	5	1.0×10^{7}	7.4×10^{6}–1.3×10^{7}	1.9×10^{6}	1.3×10^{6}–2.5×10^{6}	2.7×10^{4}	1.3×10^{4}–3.9×10^{4}	1.8×10^{4}	5.0×10^{3}–4.3×10^{4}
Owerri	1	1.0×10^{7}*	NA	1.5×10^{6}*	NA	1.2×10^{4}*	NA	2.0×10^{3}*	NA
Ilorin	4	7.2×10^{6}	6.7×10^{4}–1.8×10^{7}	8.3×10^{5}	2.0×10^{4}–2.2×10^{6}	2.2×10^{4}	1.1×10^{4}–4.3×10^{4}	1.4×10^{4}	8.0×10^{3}–3.0×10^{4}

* Count recorded for the lone sample

NA = Not applicable.

Table 3: Viable counts of some groups of fungi in cassava flour

Origin of Sample	Total no of samples analysed	Xerophilic fungi (cfu/g)				Hydrophilic fungi (cfu/g)			
		moulds		yeasts		moulds		yeasts	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
Zaria	27	$2.0x10^4$	$2.0x10^2$-$4.2x10^5$	$3.7x10^4$	$<2.6x10^3$-$1.0x10^6$	$1.0x10^4$	$<1.0x10^2$-$1.4x10^5$	$7.8x10^4$	$<5.8x10^2$-$1.9x10^6$
Kaduna	20	$1.1x10^3$	$1.1x10^2$-$8.6x10^3$	ND	ND	$1.4x10^3$	$<1.0x10^2$-$8.0x10^3$	$5.7x10^4$	$<1.9x10^3$-$1.0x10^6$
Okene	24	$1.0x10^4$	$1.0x10^2$-$1.6x10^5$	$1.3x10^3$	$<1.2x10^3$-$1.6x10^4$	$3.7x10^4$	$<1.0x10^2$-$6.4x10^5$	$1.4x10^5$	$<4.4x10^3$-$1.2x10^6$
Jos	14	$2.1x10^3$	$<1.0x10^2$-$2.0x10^4$	$1.9x10^4$	$<1.0x10^2$-$2.7x10^5$	$2.3x10^2$	$<2.0x10^2$-$1.0x10^3$	$5.8x10^4$	$<1.5x10^3$-$7.8x10^5$
Lagos	10	$6.7x10^2$	$1.0x10^2$-$2.7x10^3$	ND	ND	$1.1x10^3$	$<3.0x10^2$-$2.6x10^3$	$5.6x10^2$	$<6.0x10^2$-$3.8x10^3$
Fort Harcourt	5	$1.3x10^3$	$6.0x10^2$-$3.3x10^3$	ND	ND	$8.6x10^2$	$4.0x10^2$-$1.6x10^3$	ND	ND
Owerri	1	$3.5x10^3$*	NA	ND	NA	$5.2x10^3$*	NA	ND	NA
Ilorin	4	$1.0x10^3$	$6.0x10^2$-$2.0x10^3$	ND	ND	$6.8x10^2$	$4.0x10^2$-$1.0x10^3$	$1.3x10^2$	$<5.0x10^2$

* Count from the lone sample

ND Not detected

NA Not applicable

Table 4a: Distribution of fungi in samples of cassava flour obtained from markets in Zaria,

	Total no of flour samples examined	No of positive samples determined on PDA	Range of no of viable propagules on PDA(cfu/g)	No of positive samples determined on MSA	Range of no of viable propagules on MSA(cfu/g)
Botryotrichum amstelodami	27	-		2	$5.0x10^2$-$9.0x10^2$
B. piluliferum	27	1	$1.4x10^5$	3	$3.0x10^2$-$6.0x10^2$
Aspergillus candidus	27	1	$1.0x10^4$	5	$1.0x10^2$-$5.0x10^2$
A. clavatus	27	2	$1.0x10^2$-$1.0x10^4$	1	$5.4x10^5$
A. flavus	27	13	$1.0x10^2$-$1.0x10^4$	15	$1.0x10^2$-$8.0x10^2$
A. fumigatus	27	4	$1.0x10^2$-$1.0x10^4$	4	$9.0x10^2$-$5.0x10^4$
A. niger	27	8	$1.0x10^2$-$3.0x10^4$	9	$1.0x10^2$-$6.0x10^2$
A. oryzae	27	5	$1.0x10^2$-$1.0x10^4$	-	-
A. versicolor	27	-	-	5	$1.0x10^2$-$2.0x10^2$
A. ventii	27	4	$1.0x10^2$-$2.0x10^2$	7	$1.0x10^2$-$4.9x10^5$
Penicillium spp.	27	6	$1.0x10^2$-$1.0x10^3$	18	$1.0x10^2$-$7.7x10^5$
Rhizopus oryzae	27	3	$1.0x10^2$-$5.0x10^2$	-	-
R. stolonifer	27	9	$1.0x10^2$-$1.0x10^4$	6	$2.0x10^2$-$6.0x10^2$
Sporendonema sebi	27	-	-	1	$3.0x10^2$
Curvularia lunata	27			1	$3.0x10^2$-
Aureobasidium pullulans	27	1	$1.9x10^6$	-	-
Mycelia sterilia	27	3	$1.0x10^2$-$2.0x10^2$	5	$1.0x10^2$-$1.6x10^3$
Pink yeasts	27	-	-	-	-
Other yeasts	27	7	$3.0x10^2$-$1.2x10^6$	3	$2.0x10^2$-$1.0x10^6$

Table 4b: Distribution of fungi in samples of cassava flour obtained from markets in Kaduna

Fungi	Total no of flour samples examined	no of positive samples determined on PDA	Range of no of vaible progagules on PDA(cfu/g)	No of positive samples determined on MSA	Range of no of viable progagules on MSA (cfu/g)
Eurotium amstelodami	20	1	$1.0x10^2$	1	$2.0x10^2$
E. repens	20	2	$2.0x10^2$-$3.0x10^2$	-	-
Aspergillus candidus	20	-	-	1	$9.0x10^2$
A. clavatus	20	-	-	2	$1.0x10^2$-$4.0x10^2$
A. flavus	20	9	$1.0x10^2$-$1.0x10^4$	12	$1.0x10^2$-$2.1x10^3$
A. fumigatus	20	5	$1.0x10^2$-$1.0x10^4$	4	$1.0x10^2$-$2.0x10^2$
A. niger	20	8	$1.0x10^2$-$2.4x10^3$	6	$1.0x10^2$-$3.5x10^3$
A. oryzae	20	4	$1.0x10^2$-$1.5x10^3$	-	-
A. versicolor	20	-	-	4	$1.0x10^3$
A. ventii	20	2	$1.0x10^2$-$3.0x10^2$	-	-
Penicillium spp	20	2	$3.0x10^2$-$7.0x10^2$	14	$1.0x10^2$-$2.1x10^3$
Rhizopus oryzae	20	1	$1.0x10^2$	-	-
R. stolonifer	20	-	-	-	-
Mycelia sterila	20	2	$1.0x10^2$-$2.0x10^2$	1	$2.0x10^2$
Pink yeasts	20	19	$1.9x10^3$-$1.0x10^6$	-	-
Other yeasts	20	9	$1.1x10^3$-$1.0x10^4$	-	-

Table 4c: Distribution of fungi in samples of cassava flour obtained from markets in Ogbomoso

Fungi	Total no of flour samples examined	No of positive samples determined on PDA	Range of no of viable propagules on PDA (cfu/g)	No of positive samples determined on MSA	Range of no of propagules on MSA (cfu/g)
Eurotium amstelodami	24	-	-	1	$3.0x10^2$
E. repens	24	-	-	1	$1.1x10^3$
Aspergillus candidus	24	-	-	6	$1.0x10^2$-$5.0x10^4$
A. clavatus	24	1	$1.2x10^3$	10	$2.0x10^2$-$1.9x10^3$
A. flavus	24	13	$2.0x10^2$-$2.0x10^5$	19	$1.0x10^2$-$5.0x10^4$
A. fumigatus	24	2	$5.0x10^2$-$6.0x10^2$	4	$2.0x10^2$-$1.3x10^3$
A. niger	24	13	$1.0x10^2$-$1.3x10^5$	10	$1.0x10^2$-$1.6x10^3$
A. oryzae	24	1	$5.0x10^2$	-	-
A. versicolor	24	1	$8.0x10^2$	7	$1.0x10^2$-$3.0x10^2$
A. wentii	24	9	$2.0x10^2$-$2.0x10^5$	11	$1.0x10^2$-$1.5x10^3$
Penicillium spp	24	3	$4.0x10^2$-$7.0x10^2$	6	$2.0x10^2$-$2.1x10^3$
Rhizopus oryzae	24	3	$1.0x10^2$-$1.2x10^3$	1	$7.0x10^2$
R. stolonifer	24	7	$1.0x10^2$-$3.0x10^4$	16	$1.0x10^2$-$2.3x10^3$
Curvularia lunata	24	1	$3.3x10^3$	-	-
Mycelia sterilia	24	2	$8.0x10^2$-$1.8x10^3$	1	$1.0x10^2$
Pink yeasts	24	6	$1.3x10^3$-$6.1x10^5$	-	-
Other yeasts	24	8	$3.8x10^3$-$7.8x10^5$	5	$1.0x10^4$-$1.7x$

Table 4d: Distribution of fungi in samples of cassava flour obtained from markets in Lagos

Fungi	Total no of flour samples examined	No of positive samples determined on PDA	Range of no of viable propagules on PDA(cfu/g)	No of positive samples determined on MSA	Range of no of viable propagules on MSA(cfu/g)
Eurotium amstelodami	10	-	-	2	$1.0x10^2$-$2.0x10^2$
A. candidus	10	-	-	1	$1.0x10^2$
A. elevatus	10	5	$5.0x10^2$-$3.0x10^4$	1	$2.0x10^2$
A. flavus	10	8	$1.0x10^2$-$1.0x10^4$	7	$1.0x10^2$-$4.0x10^2$
A. niger	10	2	$7.0x10^2$-$1.3x10^3$	2	$2.0x10^2$
Penicillium sup	10	2	$2.0x10^2$-$1.0x10^3$	4	$1.0x10^2$-$2.0x10^2$
Rhizopus oryzae	10	4	$1.0x10^2$-$3.0x10^4$	-	-
R. stolonifer	10	2	$1.0x10^2$-$3.0x10^2$	6	$1.0x10^2$-$2.7x10^5$
Pink yeasts	10	2	$4.0x10^2$-$2.1x10^3$	-	-
Other yeasts	10	2	$2.0x10^2$-$1.2x10^5$	-	-

Table 4a: Distribution of fungi in samples of cassava flour obtained from markets in Jos

Fungi	Total no of flour samples examined	No of positive samples determined on PDA	Range of no of viable propagules on PDA (cfu/g)	No of positive samples determined on MSA	Range of no of viable propagules on MSA (cfu/g)
Eurotium amstelodami	14	-	-	3	$1.0x10^2$-$1.0x10^4$
Aspergillus flavus	14	1	$1.0x10^2$	2	$7.0x10^2$-$1.0x10^4$
A. fumigatus	14	1	$2.0x10^2$	-	-
A. niger	14	5	$1.0x10^2$-$4.0x10^2$	-	-
A. wentii	14	-	-	2	$3.0x10^2$-$4.0x10^2$
Penicillium spp	14	1	$4.0x10^2$	4	$1.0x10^2$-$7.0x10^2$
Rhizopus stolonifer	14	3	$1.0x10^2$-$3.0x10^2$	5	$1.0x10^2$-$1.0x10^4$
Curvularia lunata	14	1	$1.5x10^3$	-	-
Pink yeasts	14	-	-	-	-
Other yeasts	14	11	$5.0x10^2$-$5.5x10^5$	6	$1.0x10^3$-$1.6x10^5$

Table 4f: Distribution of fungi in samples of cassava flour obtained from markets in Ilorin

Fungi	Total no of flour samples examined	No of positive samples determined on PDA	Range of no of viable propagules on PDA (cfu/g)	No of positive samples determined on MSA	Range of no of viable propagules on MSA(cfu/g)
Eurotium amstelodami	4	-	-	3	$1.0x10^2$-$3.0x10^2$
A. flavus	4	4	$1.0x10^2$	4	$1.0x10^2$-$2.0x10^2$
A. fumigatus	4	1	$3.0x10^2$	-	-
A. niger	4	4	$2.0x10^2$-$3.0x10^2$	2	$2.0x10^2$-$3.0x10^2$
A. oryzae	4	3	$1.0x10^2$	-	-
Penillium sp.	4	2	$1.0x10^2$-$2.0x10^2$	1	$4.0x10^2$
Mycelia sterilia	4	1	$1.0x10^2$	1	$1.0x10^2$

Table 4g: Distribution of fungi in samples of cassava flour obtained from markets in Port-Harcourt

Fungi	Total no of flour samples examined	No. of positive samples determined on PDA	Range of no of viable propagules on PDA(cfu/g)	No of positive samples determined on MSA	Range of no of viable propagules on MSA(cfu/g)
A. clavatus	5	-	-	2	$1.3x10^2$-$6.0x10^2$
Aspergillus flavus	5	1	$1.0x10^2$	2	$2.0x10^2$-$3.0x10^2$
A. fumigatus	5	1	$1.1x10^3$	-	-
A. niger	5	3	$4.3x10^2$-$6.0x10^2$	-	-
A. versicolor	5	1	$1.0x10^2$	-	-
Penicillium spp	5	-	-	3	$3.0x10^2$-$6.0x10^4$
Rhizopus oryzae	5	2	$2.0x10^2$-$3.0x10^2$	-	-
R. stolonifer	5	1	$3.0x10^2$	2	$2.0x10^2$-$4.0x10^4$
Curvularia lunata	5	1	$3.0x10^2$	-	-

Table 4n: Distribution of fungi in the lone samples of cassava flour obtained from Owerri

Fungi	Total no of flour samples examined	No of positive samples determined on PDA	Range of no of viable propagules on PDA (cfu/g)	No of positive samples determined on MSA	Range of No of viable propagules on MSA (cfu/g)
Eurotium repens	1	1	$2.0x10^2$	-	-
Aspergillus flavus	1	1	$8.0x10^2$	-	-
A. niger	1	1	$1.0x10^2$	1	$9.0x10^2$
A. wentii	1	1	$2.0x10^2$	1	$7.0x10^2$
Rhizopus stolonifer	1	1	$1.0x10^2$	1	$9.0x10^2$

Table 5: Percentage contamination of the Cassava Flour Samples by fungi

% Frequency of the fungi in Samples.

Fungi	Zaria (27)	Kaduna (20)	Okene (24)	Lagos (10)	Jos (14)	Ilorin (4)	Port-Harcourt (5)	Owerri (7)	Total samples contaminated
Eurotium amstelodami	7.41	5.00	4.17	20.00	21.43	75.00	-	-	11.43
E. repens	11.11	10.00	4.17	-	-	-	-	+	6.67
Aspergillus candidus	18.52	5.00	25.00	10.00	-	-	-	-	12.38
A. clavatus	7.41	10.00	41.67	50.00	-	-	40.00	-	20.00
A. flavus	55.5	60.00	73.17	80.00	14.28	100.00	40.00	+	60.00
A. fumigatus	14.81	25.00	16.67	-	7.14	25.00	20.00	-	15.24
A. niger	33.30	40.00	54.17	20.00	35.17	100.00	60.00	+	42.86
A. oryzae	18.52	20.00	4.17	-	-	75.00	-	-	12.38
A. versicolor	18.52	5.00	29.17	-	-	-	20.00	-	13.33
A. wentii	29.92	10.00	45.83	-	14.28	-	-	+	21.90
Penicillium spp.	66.66	70.00	25.00	40.00	28.57	50.00	60.00	-	48.57
Rhizopus oryzae	11.11	5.00	12.50	40.00	-	-	40.00	-	12.38
R. stolonifer	33.30	-	66.67	60.00	57.71	-	40.00	+	37.14
Sporendonema sebi	3.71	-	-	-	-	-	-	-	0.95
Curvularia lunata	3.71	-	4.17	-	7.17	-	20	-	3.81
Aureobasidium pullulans	3.71	-	-	-	-	-	-	-	0.95
Pink yeasts	-	95.00	25.00	30.00	-	-	-	-	25.71
Other yeasts	25.92	45.00	20.83	30.70	78.57	-	-	-	36.19
Mycelia sterilia	18.52	10.00	8.33	-	-	25.00	-	-	9.52

+ Present in the lone sample.

Table 6: Reactions of Aspergillus flavus Isolates on Aspergillus differential medium (ADM)

Isolate	Reaction on ADM	Isolate	Reaction on ADM
1d	++	51c	++
4a	++	52a	++
5a	++	54c	+
8b	++	55d	++
10h	++	56b	++
11e	++	57d	++
12f	++	59a	++
15b	++	60a	++
18a	+	63a	++
19c	++	65b	++
20b	++	66b	++
21a	++	67c	++
22a	++	69b	++
26e	++	70c	++
27g	++	71b	++
31c	++	74c	++
32j	++	75c	++
33a	++	77a	++
34c	++	79b	++
35b	++	85b	++
37c	++	86a	++
38d	++	87b	++
39b	++	88k	++
40c	++	89a	+
41e	++	90a	++
42b	++	92c	++
43c	++	93c	++
45d	++	102b	++
49d	++	91f	++
50c	++	IAR586	++

++ A sharp and intense pigmentation estimated visually on the reverse of agar plate.

+ very slight intensity of pigmentation observed visually on reverse of agar plate.

Table 7: Effect of concentration of corn steep liquor and time of incubation on development of fluorescence of Aspergillus flavus isolates from cassava flour

	Concentration (v/v) of corn steep liquor								
	5%(v/v)			50% (v/v)			100%(v/v)		
Isolates	7days	8days	9days	7days	8days	9days	7days	8days	9days
1d	-	-	-	-	-	-	-	-	-
4a	-	-	-	-	-	-	-	-	-
5a	-	-	-	-	-	-	-	-	-
8b	-	-	-	-	-	-	-	-	-
10h	-	-	-	-	-	-	-	-	-
11e	-	-	-	-	-	-	-	-	-
12f	-	-	-	-	-	-	-	-	-
15b	-	-	-	-	-	-	-	-	-
18a	-	-	-	-	-	-	-	-	-
19c	-	-	-	-	-	-	-	-	-
20b	-	-	-	-	-	-	-	-	-
21a	-	-	-	-	-	-	-	-	-
22a	-	-	-	-	-	-	-	-	-
26e	-	-	-	-	-	-	-	-	-
27g	-	-	-	-	-	-	-	-	-
31c	-	-	-	-	-	-	-	-	-
32j	-	-	-	-	-	-	-	-	-
32a	-	-	-	-	-	-	-	-	-
34c	-	-	-	-	-	-	-	-	-
35b	-	-	-	-	-	-	-	-	-
37c	-	-	-	-	-	-	-	-	-
38d	-	-	-	-	-	-	-	-	-
39b	-	-	-	-	-	-	-	-	-
40c	-	-	-	-	-	-	-	-	-
41e	-	-	-	-	-	-	-	-	-
42b	-	-	-	-	-	-	-	-	-
43c	-	-	-	-	-	-	-	-	-
45d	-	-	-	-	-	-	-	-	-

Table 7: (Contd)

Isolates	Concentration (v/v) of corn steep liquor 5%(v/v)			50%(v/v)			100% (v/v)		
	7days	8days	9days	7days	8days	9days	7days	8days	9days
49d	-	-	-	-	-	-	-	-	-
50c	-	-	-	-	-	-	-	-	-
51c	-	-	-	-	-	-	-	-	-
52a	-	-	-	-	-	-	-	-	-
54c	+	++	+	+	++	+	+	++	+
55d	-	-	-	-	-	-	-	-	-
56b	-	-	-	-	-	-	-	-	-
57d	-	-	-	-	-	-	-	-	-
59a	++	+++	++	++	+++	++	+++	++++	+++
60a	+	++	+	+	++	+	+	++	+
63a	+++	++++	+++	+++	++++	+++	+++	++++	+++
65b	++	+++	++	++	+++	++	++	+++	++
66b	-	-	-	-	-	-	-	-	-
67c	-	-	-	-	-	-	-	-	-
69b	++	+++	++	++	+++	++	+++	++++	+++
70c	++	+++	++	++*	++*	++*	+++*	++++*	++*
71b	+++	++++	+++	+++*	++++*	+++*	+++*	++++*	+++*
74c	-	-	-	-	-	-	-	-	-
75c	++	++	+	++	+++	+	+++	++++	++
77a	+	++	-	+	++	-	+	+	-
79b	-	-	-	-	+	-	+	+	-
85b	+	++	+	+	++	+	+	++	+
86a	+	++	+	+	++	+	++	+++	++
87b	-	-	-	-	-	-	-	-	-
88k	-	-	-	-	-	-	-	-	-
89a	-	-	-	-	+	-	+	+	+
90a	-	-	-	-	-	-	-	-	-
92c	-	-	-	-	-	-	-	-	-
93c	-	-	-	-	-	-	-	-	-
102b	-	-	-	-	-	-	-	-	-
91f	+	++	+	++	+++	+	++	+++	++
IAR586	++	+++	++	**+++	**+++	**+++*	**+++	[illegible]	[illegible]

+,++,+++ Refers to the relative levels of intensity of fluorescence observed on the agar around the colonies.

- No fluorescence observed

* Fluorescence observed when guinea corn steep liquor instead of corn steep liquor was used in the preparation of the modified APA medium. However, the intensity of the fluorescence was very slight.

** Fluorescence observed when millet steep liquor instead of corn steep liquor was used in the preparation of the modified APA medium. Also, the intensity of the fluorescence was very slight.

Table 8: Production of aflatoxin by A. flavus isolated from cassava flour

Strain	Origin	Concentration of aflatoxins (ug/ml) B1	B2	G1	G2
54C	Lagos	2.99	0.95	ND	ND
59a	Okene	9.69	7.51	7.86	3.81
63a	Okene	8.19	5.86	6.71	1.99
65b	Okene	11.50	8.86	9.21	5.98
69b	Okene	3.31	1.50	2.30	0.60
70c	Okene	13.39	10.37	11.89	8.15
71b	Okene	6.14	3.75	4.47	1.65
75c	Okene	14.18	11.94	12.46	9.25
77a	Okene	6.17	4.66	5.75	1.63
60a	Okene	3.64	0.98	ND	ND
85b	Ilorin	4.73	2.85	3.64	0.76
86a	Ilorin	8.98	6.16	ND	ND
91f	Zaria	5.54	3.00	4.41	0.54
IAR 586	Institute of Agricultural Research	15.76	13.52	14.38	10.88

Medium used was yeast extract-sucrose medium

ND = Non detected.

CHAPTER 5

DISCUSSION

The purpose of the first section of this work was to determine the microbiological quality of samples of cassava flour obtained from different parts of Nigeria. As mentioned earlier, the flour is important as a component of the staple diets of Nigerians and as a potential supplement for wheat flour in the making of bread and other bakery products (Jones and Akinrele, 1976). Okagbue (1986) discussed the factors which could affect the microbiological status of local flours including cassava flour and used certain groups of micro-organisms as indicators of microbiological quality. The groups were aerobic bacteria, aerobic spore formers, lactose and non-lactose fermenting Enterobacteriaceae, xerophilic and hydrophilic moulds and yeasts, and they were selected on the basis of their potential as health hazards, as indicators of general levels of sanitation, or as spoilage agents. In this study, the same groups of microorganisms have been used to indicate the extent of microbiological contamination of cassava flour samples purchased from different parts of Nigeria.

The aerobic plate counts (APC) obtained in this study ranged from 1.1×10^4-2.8×10^7 cfu/g 47.6% of all the samples analysed had more than 5.0×10^6 cfu/g. Graves et al (1967) determined APC's of 2.2×10^2-2.8×10^4/g from wheat flour obtained from 11 representative mills in parts of the U.S.A. Also, Mehrotra and Basu (1975) who surveyed micro-organisms associated with cereals and their milling fractions in India recorded APC counts of 3.1×10^3-2.0×10^5/g. FAO (1979) reported that aerobic plate counts ranging from 2.0×10^4-5.0×10^6/g were common for wheat flours. Since aerobic bacterial counts give an overall picture of the bacterial load of foods, and of the sanitary conditions of their production and handling, the results of this work indicate that sanitation in production of cassava flour in Nigeria is inferior to those of wheat flour in Western countries. This is particularly true when it is considered that 47.6% (not shown in Tables 2 and 3) of the cassava flour samples analysed contained more than 5.0×10^6 cfu/g (the upper limit of APC reported by FAO (1979) It is noteworthy that apart from samples obtained from Lagos (Table 2) counts greater than 5.0×10^6 cfu/g were generally recorded from all parts of the country while in Port-Harcourt, all the samples

had APC's above $5.0x10^6$ cfu/g. In an earlier study, Okagbue (1986) recorded APC of $7.0x10^7$ cfu/g for cassava flour and a range of $4.2x10^4$-$1.2x10^8$ cfu/g for other local flours sold in Zaria.

The aerobic spore count (ASC) was another parameter used to determine the microbiological status of cassava flour. Aerobic spore formers have been implicated in spoilage of bread and starch based foods and in food intoxication (Jay, 1978). According to Rogers (1976) Bacillus subtilis and B. licheniformis which are aerobic spore formers are associated with a type of food spoilage known as ropiness in bread, and B. cereus and B. subtilis produce food poisoning endotoxin in starchy foods. Furthermore, it was shown by Rogers (1976) that spores of Bacillus species from wheat flour could survive baking temperatures of 350-450°F and that other Bacillus spp. could survive cold temperatures. The spores would then germinate and grow in baked or chilled foods stored at room temperatures. If cassava flour is to be supplemented for wheat flour in bread making and in production of other bakery products like chin-chin, doughnuts etc. aerobic spore formers, especially

the species mentioned above could constitute health hazards and potential spoilage agents. In addition, the spores would survive heat treatments used in preparation of "fufu" and other local meals made from cassava flour and would cause problems especially since the foods are usually non-refrigerated in many Nigerian homes. Frazier (1967) stated that samples of wheat flour from retail markets contain about 20-30 Bacillus spores per gram. It is clear that the aerobic spore counts ($1.3x\ 10^3$-$2.9x10^6$ cfu/g) obtained from cassava flour in this study were very high. Although, Okagbue (1986) recorded a high aerobic spore count of $3.6x10^7$ cfu/g for cassava flour and a range of $3.0x10^3$-$1.2x10^7$ cfu/g for other local flours, the present counts for cassava flour are very high when compared with those (5-$2.1x10^2$/10g) recorded by Graves et al (1967) in parts of U.S.A. and those (47-$8.2x10^2$/g) reported by James and Smith (1948) in Canada. Further work would be needed to identify the aerobic spore formers isolated in this work in order to determine the relative levels of rope-forming and potential toxin producers present in cassava flour.

Lactose and non-lactose fermenting Enterobacteriaceae were enumerated from cassava flour in this study. Presence of the lactose fermenters indicates the possible presence of coliforms which are generally undesirable in foods. The presence of coliforms in food is considered to indicate faecal contamination and some bacteria of the coliform group have also been reported to cause food infections (Frazier, 1967). Presence of non-lactose fermenting Enterobacteriacea may suggest the possible presence of enteric pathogens such as Shigella and Salmonella. A few samples out of 20, 24 and 27 obtained from Kaduna, Okene, and Zaria respectively did not contain detectable numbers of Enterobacteriaceae. Based on the total (105) number of samples examined, lactose and non-lactose fermenters were undetectable in 14.3 and 9.5% respectively. The relative absence of these groups of bacteria in some of the samples shows that even with present handling practices, some cassava flour samples could be safe from enteric pathogens.

Very few studies carried out on the microbiology of flours in the Western world, have emphasized the importance of Enterobacteriaceae as microbial indicators of the quality of wheat flour. This may

be due to the way and means in which the flour is used as food in those countries. A reported search for bacteria of the Salmonella group in wheat, flour, scourings, offal and dust from mills in England was made by Hesseltine (1966). The coliform count was over $1.0 \times 10^3/g$ in two of the 115 samples of flour examined. Hesseltine (1968) reported that the infrequent recovery of Escherichia coli indicated that faecal contamination was minimal in the samples of wheat flour examined in parts of U.S.A. Since cassava flour is used in different ways in the food system of Nigeria, it is considered that Enterobacteriacea should be one of the parameters in determining its microbiological status. Lactose and non-lactose fermenters have been seperated in this study but total Enterobacteriaceae could be used as an indicator of sanitary quality of foods (Jay, 1978). Further work would be needed to determine the nature of the enteric bacteria present in cassava flour

Yeasts were also isolated from cassava flour in this work. The role of yeasts in spoilage of foods is well known. James and Smith (1943) stated that yeasts cultured at 25°C could affect fermentation during the leavening process of bread

making. The yeast counts obtained from samples from five brands of Canadian wheat flour ranged from 12-$2.68x10^2$/g. When compared with the yeast counts obtained in this study, it is obvious that some Nigerian cassava flour samples contain high levels of that group of microorganisms. Although some samples were apparently devoid of yeasts, counts of up to $1.9x10^6$ cfu/g were detected in other samples. Since yeasts are mainly hydrophilic, it is not surprising that most of the isolations were made on PDA. Those isolated on MSA (from 11.4% of the cassava flour samples) were relatively few and have been found to grow well on PDA, thus indicating that they were not obligate xerophiles. The relatively high levels of yeasts detected in some samples probably indicate that the cassava flour samples involved had high moisture contents.

The last group of microorganisms used to determine the microbiological quality of cassava flour were the moulds. Two groups were enumerated, namely the xerophilic and the hydrophilic moulds. The ranges of counts for the xerophilic and hydrophilic moulds were $1.0x10^2$-$4.2x10^5$ and $1.0x10^2$-$6.4x10^5$ cfu/g respectively. Okagbue(1986) reported counts for xerophilic moulds for cassava

flour as $1.9x10^3$ cfu/g and for hydrophilic moulds as $3.0x10^5$ cfu/g. The range of counts for xerophilic moulds for the other types of local flours was $1.2x10^2$-$2.9x10^3$ cfu/g while that for hydrophilic moulds was $1.0x10^3$-$8.0x10^6$ cfu/g. When compared with the counts of xerophilic moulds ($1.1x10^3$-$3.9x10^3$/g) and hydrophilic moulds ($1.0x10^3$-$2.8x10^3$/g) recorded by James and Smith (1948) from five brands of Canadian wheat flours, the mould counts were particularly high. Mehrotra and Basu (1975) also detected hydrophilic moulds ranging from $4.0x10^2$-$5.3x10^3$/g and xerophilic moulds ranging from $3.0x10^2$-$7.0x10^3$/g from flour samples in India. It is well known that, in addition to their potential as spoilage agents, many moulds are mycotoxin producers. Mould growth in flour exposed to damp weather could cause economic losses. Besides, Reiss (1978) had shown that mycotoxins in wheat flour can survive bakery operations, it was shown that the baking temperature (120°C for 30 min) as well as the 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 mycotoxins is used in bread making, with obvious consequences to the health of consumers.

Generally, most of the cassava flour samples were contaminated with the groups of microoganisms enumerated in this study. The origins of the samples do not seem to have any influence on the levels of contamination observed. Okagbue (1986) discussed the sources of microbial contamination of local flours including cassava flour. They include the soil, air, water and poor sanitation practices involved in production and in sale in open-markets. In the case of cassava flour, contaminantsnacquired by the tubers during peeling, cutting and washings, would grow for sometime during sun drying of the cassava chips. The longer the period of drying (especially in damp weather) the more the contamination from the soil and air and the more the growth of microbial contaminants. Clerk and Caurie (1968) observed considerable fungal growth on cassava chips during sun drying in Ghana. Experience shows that a similar phenomenon occurs in Nigeria, and definitely serves to account for the fungi enumerated in this study. Further contamination would occur in milling machines. Thatcher et al (1953) observed that the reason for high contamination of wheat flours by moulds were "pockets" within the milling machinery which served as reservoirs for multiplying moulds in substandard mills. In Nigeria, most cassava

milling occurs at village level and the mills are not well sanitized before or between operations. During sale of the flours in the markets, the containers are usually large pans and the flours are exposed to the open air where further contamination from the air could take place. The variations observed in this study in the microbial counts obtained from the flour samples from each area could be due to the different levels of sanitation practices achieved by different producers and sellers during the production and sale. It is noteworthy that transportation of flour from a place of production to another place could facilitate further contamination. This would be particularly true of cassava flour obtained from Zaria, Kaduna and Jos where cassava is not grown as a major agricultural crop.

In general the results of this study indicate that there is a need to control microbial contamination of cassava flour. This can be achieved by educating village level processors and sellers (who now constitute the great majority) on the need for and methods of improving sanitation during production and sale of the product. Since the present levels of microorganisms were achieved under apparently insanitary conditions of manufacture

and safe, the data could be used for monitoring future sanitation practices at village or factory level. The data could also be useful to government regulatory agencies involved in specifying microbial limits for foods. Although limits would depend on, among other things, the intended use/s of cassava flour, the lower limits of microbial levels recorded in this study indicate that with improved sanitation cassava flour with determined safe levels of microorganism could be produced in Nigeria.

In the second part of this work, the fungi isolated from the cassava flour samples were identified as far as possible. The aim was to determine the predominant genera or species which could be potential spoilage agents and/or mycotoxin producers.

The three main genera of moulds isolated from cassava flour were Aspergillus, Penicillium and and Rhizopus. Among the aspergilli, members of the A. glaucus (group) were few, their perfect stages were commonly observed. It may be that some components of cassava flour induces the formation of the perfect stages which are characterised by cleistothecia. The aspergilli were identified

as Eurotium amstelodami and E. repens (Smith, 1969 and Gilman, 1957). Other aspergilli found in cassava flour were A. candidus, A. clavatus, A. flavus, A. fumigatus, A. niger, A. oryzae, A. versicolor, and A. wentii. The Rhizopus species were identified as R. oryzae and R. stolonifer. Other moulds were Curvularia lunata, Sporendonema sebi, Aureobasidium pullulans and Mycelia sterilia.

Many of the fungi found in cassava flour in this study have been reported in other types of flours. For example, as found in this study, Graves and Hesseltine (1965) reported that Aspergillus, Penicillium and Mucorales were the predominant fungi in samples of wheat flour from parts of U.S.A. However, some differences are noticeable in the species and genera of fungi found in both wheat flour and cassava flour. Out of the eight Aspergillus spp. isolated from wheat flour by Graves and Hesseltine (1965), five were common to both wheat and cassava flours. They are A. amstelodami (which was not found in the perfect stage in wheat flour), A. candidus, A. clavatus, A. flavus and A. versicolor. The following were isolated from wheat flour and were not obtained from cassava flour: A. chevalieri, A. sydowi,

A. terreus. Similarly, of the ten Aspergillus spp isolated from cassava flour, the following were absent from wheat flour: A. repens, A. fumigatus, A. niger, A. oryzae and A. wentii. A wide variety of Penicillium spp. were isolated from wheat flour. Although the Penicillium spp. isolated from cassava flour in this study were not identified to the species level, their gross colonial colour and morphology on the agar plates showed that up to six different species were present. It is also noteworthy that the members of the mucorales which were isolated from wheat flour (Graves and Hesseltine, 1965) were Mucor spp. whereas those isolated from cassava flour are two species of Rhizopus, namely, R. stolonifer and R. oryzae. The reason for the discrepancy (ie difference in types of mucorales) is not known. Interestingly, Rhizopus has also been isolated from flour in the tropics by other workers. Gattani (1951) reported a rich flora of Aspergillus and Rhizopus species in samples of sweet potato and wheat flours in India. The most common species of Aspergillus isolated in that study were A. glaucus, A. candidus and A. niger. As mentioned earlier,

A. niger was isolated from cassava flour in this study but not from wheat flour (Graves and Hesseltine, 1965).

With very few exceptions, the mould flora of cassava flour seems to be independent of the geographical area of Nigeria from which samples were obtained. However, it should be noted that most of the flour is made in the Southern parts and middle belts of Nigeria. Sporendonema sebi and Aureobasidium pullulans were the only uncommon fungi and they were isolated only once from Zaria samples (Table 3). Clerk and Caurie (1968) have also isolated S sebi among other fungi (mainly Penicillium spp. and Aspergillus spp.) from sun-dried cassava chips meant for flour production in Ghana. A. pullulans, a yeast-like mould, was isolated by Kurtzman et al (1970) from both wheat and wheat flour in the U.S.A. It is possible that Graves and Hesseltine (1965) failed to record the yeast-like fungus because the yeasts isolated in their study were not identified. Adeniji (1976) isolated from market samples of gari many fungi similar to

those of the present study and showed that they could cause deterioration of the product (gari). Aspergillus glaucus was not isolated from garri because the media used in the study could not isolate xerophilic fungi.

In general, the fungi isolated from cassava flour in this work were mainly air and soil microorganisms. Aspergillus spp, Penicillium spp, Rhizopus spp, Curvularia lunata and sterile mycelia were among the fungi isolated from the air at Samaru. (Dansfield, 1966). However, the Aspergillus, Penicillium and Rhizopus occurred in very low frequencies in the air while C. lunata had a very highfrequency of occurrence. Ogbonna and Pugh (.983) studied the fungal flora of Nigerian soil and isolated most of the micro-organisms that occurred in cassava flour. The micro-organisms included A. candidus, A. flavus, A. niger, A.oryzae, A. repens, A. versicolor, A. wentii, R. oryzae, Aurecbasidium pullulans, C. lunata and sterile mycelia. In the study, Aspergillus was the most frequently isolated genus from the soil and Pencillium was among the next most frequently isolated genera. When compared to the studies on air

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and soil, the results of this study suggest that the two environments are the main sources of contemination of cassava flour and that the soil is probably of greater signficance.

Yeasts were also isolated from cassava flour. Both non-pigmented and pink yeasts were observed. Kurtzman et al (1970) also observedthat the colony colours of the various yeasts isolated from wheat and wheat flour ranged from white to faintly pink. The presence of appreciable numbers of yeasts in some cassava flour samples indicates that the moisture contents of some of the samples were relatively high. Although the moisture levels were not determined in this work, it has been recommended that moisture content of gari (a Nigerian fermented, dehydrated cassava based food) must be 12% and below to prevent detericration (Adeniji, 1976). Yeasts were not isolated by Adeniji(1976) from gari probably because they did not add any antibiotics to the media they used. Further work will be needed to identify the different types of yeasts that occur in cassava flour since they are potential spoilage agents.

The most frequently isolated and widely distributed fungus in cassava flour samples examined was A. flavus (Table 5). It was isolated from 60% of the total number of samples examined. Except for Jos samples, the fungus occurred in more than 40% of the samples obtained from every town involved in this study. The next most frequently isolated fungus was A. niger which occurred in 43% of the total number of samples. A. wentii had a high frequency of occurrence and was isolated from 22% of all the samples. However, A. candidus which was reported to be the most frequently occurring fungus in wheat flour (Graves and Hesseltine, 1965) was isolated from only 12% of the samples in this study. It is also noteworthy that unlike A. flavus, A. candidus was not isolated from the samples obtained from some areas in this study.

The non-pigmented yeasts were more frequently isolated from cassava flour than the pink yeasts (Table 5). The former category of yeasts were isolated from 36% of the total number of samples whereas the latter occurred in 26%.

Interestingly, the pink yeasts were isolated from 95% of the samples purchased from Kaduna. The reason for this high level of occurrence is not clear.

Some of the moulds isolated from cassava flour in this study are potential spoilage agents, and/or mycotoxin producers. Graves and Hesseltine (1965) compared the mould flora of fresh and spoiled dough samples and concluded that the fungal flora of the doughs was a reflection of the flour microflora. It is interesting that some of the fungi isolated from the spoiled refrigerated dough products like Aspergillus amstelodami, A. candidus, A. fumigatus, A. repens, A. versicolor, A. flavus-oryzae and Penicillium spp. were isolated from cassava flour in this study. Also the following fungi; Rhizopus spp. Aspergillus niger, A. versicolor, A. flavus, A. candidus, Curvulavia lunata, Penicillium spp. and a few others, that were shown to cause the deterioration of gari by Adeniji (1976) were also isolated from cassava flour in tnis study. This shows that some of the fungi isolated from cassava flour havespoilage potential for flour and for

In addition to their ability to cause biochemical changes, some ofthe moulds isolated from cassava flour are potential mycotoxin producers. In this connection, it is well known that Aspergillus flavus and A. versicolor are potential producers of the aflatoxins and sterigmatocystin respectively (Scott et al, 1970). As mentioned earlier, Reiss (1978) has shown that the baking temperature does not degrade aflatoxin B_1 to any reasonable extent. A. wentii which was isolated from 21% of the total number of cassava flour samples examined (Table 4), has also been reported to produce an intracellular toxin by Wu et al (1974). Mycelial extracts of an A. wentii strain were highly toxic when inoculated into chicken embryos or fed to mice. Although the Penicillium spp. were not identified in this study, the genus constitutes one of the three described as major mycotoxin producers by Scott et al (1970). In general, the presence of A. flavus, A. versicolor, A. wentii and Penicillium spp. in cassava flour could pose a health hazard to the consumers.

In view of the well known significance of A. flavus in foods, its widespread distribution as shown in this study and its high frequency of occurrence among the samples, it was studied further. Its hazardous nature in foods may be due to its spores which may also contain

toxins (Hesseltine et al, 1966) or it may be due to aflatoxins released into the food material. However, even under proper environmental conditions, toxigenicity depends on the strain of A. flavus. Thus, there was a need to determine whether the A. flavus isolates from cassava flour were toxigenic.

The identities of the strains of A. flavus isolated in this study were confirmed by inoculating them onto Aspergillus Differential Medium (ADM) (Bothast and Fennel, 1974). All of them were positive on the medium (Table 6). This means that as was observed by Flannigan and Hui (1976) in Britain, the ADM is reliable for identifying A. flavus.

Since commercial corn steep liquor, a component of aflatoxin producing ability (APA) medium devised by Hara et al (1974) for detecting toxigenic A. flavus isolates was not available locally, an attempt was made to produce simulated corn steep liquor (SCSL) as described in 'materials and methods'. The SCSL was then used (at various concentrations) for preparing modified aflatoxin producing ability (MAPA) medium. The effects of the concentrations

of SCSL and time of incubation of inoculated plates (at room temperature of 26°C) on the development of fluorescence around the colony (an indication of aflatoxin producing ability of A. flavus isolates) were then determined. All the concentrations (5, 10 and 100% v/v) of SCSL in the MAPA medium induced production of fluoresence (Table 6) by thirteen A. flavus strains which formed approximately 22% of all the strains (60) tested. The different concentrations did not affect the number of A. flavus strains that had potential for aflatoxin production. At all concentrations of SCSL tested, production of fluorescence was noticed on the seventh day of incubation. The intensity increased on the eight day and then decreased on the ninth day. By the tenth day, no fluorescence was observed. In the medium of Hara et al (1974) fluorescence was also observed from the seventh day of incubation. It increased on the tenth day and decreased by the thirteenth day of incubation. Therefore, the fluorescence produced by the aflatoxin-producing strains of A. flavus lasted for a shorter period of time in the MAPA medium containing the locally prepared

corn steep liquor. Since corn is readily available in Nigeria, the results of this study showed that corn steep liquor suitable for use in testing aflatoxin producing ability of A.flavus isolates can readily be prepared locally in the country. It is also likely that with further experimentations and modifications, samples which can duplicate the patterns of occurrence of fluorescence described for the commercial CSL may be produced locally. In Brazil, Lin and Dianese (1976) have succeeded in substituting coconut extract which was readily available in that country for corn steep liquor in the APA medium. When two other cereals, namely, guinea corn and millet were used in place of corn to prepare the modified APA medium, the results were different. Only very few isolates and the authentic aflatoxin producer (IAR 586) showed very slight fluorescence. It appears that there is a factor in corn (not available in guinea corn or millet)which enhances development of fluorescence by A. flavus strains.

As mentioned earlier, out of a total of sixty A. flavus isolates, thirteen produced

aflatoxins. Ten of the A. flavus strains produced all the known aflatoxins, comprising of aflatoxins B_1, B_2, G_1 and G_2 while three produced only aflatoxins B_1 and B_2. The amount of aflatoxins produced ranged from 3.0-14.2ug/ml (B_1), 0.9-11.9 ug/ml (B_2), 0-12.5ug/ml. (G_1), and 0-9.2 ug/ml (G_2). (Table 7). Although more aflatoxin producing strains of A. flavus were isolated from Okene samples, this could have been merely accidental. The important observation made was that aflatoxin producing strains were isolated from cassava flour samples purchased from different geographical areas in Nigeria. It was also observed that although the cassava flour isolates of A. flavus had relatively lower ability to produce aflatoxin (Table 7) than the potent aflatoxin producing strain obtained from the Institute of Agricultural Research (IAR 586), some of the cassava flour isolates produced relatively high concentrations of aflatoxins.

Generally, the occurrence of aflatoxin-producing strains of A. flavus in cassava flour suggests possible presence of the toxins in the flour marketed in Nigeria. Further work will be needed to confirm this. However, the

speculation is possible because A. flavus can grow during the drying process of cassava chips used in flour production. In addition, Alozie et al (1980) have shown that A. flavus can grow and produce toxin in gari, a cassava product. Okpokiri et al (1984) reported that Aspergillus spp have been implicated in problems of aflatoxicity in cassava root meals in Thailand, Hong Kong, Brazil and India. This study therefore shows that considerable risk of aflatoxicoses exists in the current and potential uses of cassava flour made at village level in Nigeria and suggests a need for control of overall mould contamination of the product.

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