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MICROORGANISMS ASSOCIATED WITH SOME CASSAVA (Manihot esculenta, Crantz) PRODUCTS

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ABSTRACT

Sweet variety of grated cassava, cassava starch and unfermented cassava flour were analysed for aerobic spore formers, lactic acid bacteria, enterococci, lactose, non-lactose fermenting enterobacteriaceae, yeasts and moulds. The process of production from harvesting to milling was completed in 24hours to ensure good quality products. The population of microorganisms were as follows aerobic spore count: $8.0X10^4$ cfulg, $5.0x10^5$ cfulg and 4.0×10^3 cfulg; Lactic acid bacteria: $2.4x10^5$ cfulg, 1.5×10^5 cfulg; Lactose fermenters (1.4×10^5 cfulg), 2.2×10^6 cfulg and 3.9×10^4 cfulg and non-lactose fermenters (90×10^4 cfulg; 1.8×10^6 cfulg and 5.2×10^3 cfulg respectively. The bacterial isolates were identified as *Escherichia coli*, *Salmonella* sp. *Leuconostoc mesenteroides*, *Shigella flexneri*, *Staphylococcus aureus* and *Pseudomonas* sp while the fungal isolates include *Mucor mucedo*, *Aspergillus niger*, *A, flavus*, *A, fumigatus*, *Rhizopus stolonifer*, *Penicillium* sp, *Neurospora sp*, *Candida* sp *Trichoderma viridae* and *Fusarium moniliforme*.

INTRODUCTION

Cassava, Manihot esculenta, Crantz is a dicotyledonous perennial plant belonging to the botanical family Euphorbiaceae. It is commonly known as cassava, manioc, mandioca, tapioca and thea. The total amount of cyanogenic glycosides in the roots is used to place the numerous cultivars into two major groups - the 'bitter' and 'sweet' varieties. Most cassava produced in African is used as human food. It can be boiled, roasted, pounded, grated and made into different dishes straight from the field whereas high cyanide variety requires 3 to 5 days of processing to reduce the HCN content. Cassava starch is used in the production of paper and textile and as monosodium glutamate (MSG), an important flavoring agent in Asian cooking. In the textile industry, it is also used for warp sizing, cloth and felt finishing (IITA, 1990). In Nigeria, fresh sweet cassava tubers are grated and made into different dishes such as cassava balls (fried grated sweet cassava); cassava 'mbile' (grated sweet cassava in vegetable soup; cassava 'ekoki' (grated cassava steamed and pounded) and cassava 'beiju' (roasted grated cassava) (Adeyinka, 1988). Cassava flour is being partially substituted for wheat flour in Africa, thus providing income to resource poor farmers and saving foreign exchange for national governments. Two major flour types exist; those derived from unfermented dried cassava roots and those obtained from fermented cassava roots. Flours from unfermented cassava roots are more common in areas where sweet cassava dominate (Wheatley *et al;* 1994).

Much information has been made available on other products of cassava such as *"lafun"*, *"garri"* and cassava tubers in the field, at harvest and during storage. Cassava starch, flour and grated cassava have a range of uses, there is a need to study the nature and levels of their microflora. The present investigation reports on this by evaluating the presence of indicator microorganisms and other potential food pathogens in the samples.

MATERIAL AND METHODS

COLLECTION OF SAMPLE

Fresh cassava tubers (the sweet variety) were obtained within Akure metropolis of Ondo State. The tubers were between 7-12 months old. The variety of cassava was identified at the Crop Production Department of the Federal University of Technology Akure.

PROCESSING

Cassava starch: Fresh tubers were peeled, washed and grated aseptically. The grated cassava was mixed with water an then filtered settling of the starch was allowed to take place until the suspension was a clear liquid. Settling of the starch was allowed to take place until the suspension was a clear liquid. De-watering was carried out in a clean bag and the sample dried in the oven at 100°c for 24 hours to allow the removal of all moisture present (Onabolu, *et al.*, 1998).

GRATED CASSAVA Fresh cassava tubers were washed, peeled, washed again and grated finely. Lumps and fibres were removed and the grated sample dewatered in a clean white muslin cloth. The resulting cake was broken into fine granules, ready for analysis. (Onabolu, *et al.*, 1998).

CASSAVA FLOUR Peeling, washing, grating of fresh cassava tubers to obtain cassava mash was carried out. The mash was de-watered by pressing in a clean muslin cloth. Cake breaking and oven drying to obtain dried cassava granules which was later dry milled were done. The resulting high quality unfermented cassava flour was then packed in new polythene bag and kept in a dessicator containing some pellets of $CaCl_2$.

MICROBIAL COUNTS Microbial counts and types were made on selective media after decimal dilution of the samples using sterile 0.1% peptone water by the pour plate method as described below:

TOTAL LACTIC COUNTS These were performed on Tomato Dextrose Agar containing (g/ml/l): Dextrose, 2.0; Yeast extract, 0.6; Tomato juice, 20.0, Peptone, 1.5; Distilled water, 1000.0; Agar, 1.5. (Olutiola *et al.*, 1991). Plates were incubated at 37°C.

COLIFORM COUNTS These made on MacConkey Agar and Eosin Methylene Blue Agar. Plates were incubated at 37°C.

YEAST AND MOULD COUNTS These were made on acidified Malt Extract Agar and Sabouraud Maltose Agar. Plates were incubated at room temperature (26-28°C) for 3 to 5 days.

SPORE-FORMERS COUNTS Dilutions of samples heated to 80°C for 10 minutes, cooled for 30 minutes were plated into Nutrient Agar. Plates were incubated at 37 °C for 48hours.

ENTEROCOCCI COUNTS KF agar was used for the selective isolation of such organism. Plates were incubated at 37 °C.

The colonies which appeared after incubation period were counted as colony – forming units (c.f.u)/g sample. The colony characteristics and cell morphology were observed microscopically after Gram staining. All cultures were identified according to Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). Yeast and mould were identified according to Barnet, 1983.

RESULTS AND DISCUSSION

The groups of bacteria and fungi used in this study to indicate the extent of microbiological contamination of the samples were selected on the basis of their potential as health hazards, as indicators of general levels of sanitation or spoilage agents. A total of (10) bacteria species were isolated. These are: *Staphylococcus aureus, Escherichia coli, Klebsiella aerogenes, Salmonella* sp *Leuconostic mesenteroides*, Lactobacillus acidophilus, *Bacillus subtilis, B. cereus Shigella flexneri and Pseudomonas aeuroginosa.* Table I shows the occurrerice in each of the sample. The isolation of *Salmonella* sp from the samples is an indication of possible health hazards. *Salmonella* spp are known to cause Salmonellosis which is a gastro intestinal infection characterized by elevated temperature, diarrhoea-sometimes sufficient to cause severe dehydration, intestinal pain and perhaps vomiting (Merlin, 1982). *E coli* is not permissible in food sample. Although some strains are normal inhabitant of intestine of man and other animals. *Staphylococcus aureus* is an organism commonly associated with the production of "enterotoxin" during growth in foods. Aerobic spore formers have been implicated in spoilage of bread and starch-based foods and in food intoxication. Thus, they constitute health hazards and are potential spoilage agents *B. cereus* (a spore – forming organism) is widely distributed in nature and in foods. Their spores have ability to withstand high temperature and they produce enterotoxins which may cause food poisoning. The aerobic spore forming bacteria have been enumerated and used to investigate the quality of cassava flour 'Lafun' (Abba – Kareem and Okagbue, 1991). Bacillus spp. can survive and thrive on

various kinds of foods Rama – Raju and Kiran Kumer (1988) isolated *Bacillus* sp. in milk heated above 100°c, thus indicating the ability of their spores to withstand high temperature. The presence of bacteria such as *Lactobacillus* and *Pseudomonas* species are less commonly implicated in food intoxications and food infections but they have been suspected to cause food infections when excessive numbers (about 10^8) of their living cells are present in foods. The relative absence of Enterococci in grated cassava and cassava starch samples in not enough proof to declare the samples safe from enteric pathogens especially when the samples are highly contaminated with lactose and non-lactose fermenting Enterobacteriaceae (Fig.1). Most of the organisms isolated might have been introduced into the food samples from soil and water used during processing. Some are common inhabitants of air and human body. Among the requirements for foods to be of good sanitary quality is that they must be free of hazardous microorganisms or those present should be at a safe low level. The quality of foods is determined by the content of indicator organisms. Indicator organism is any organism whose presence or number serve to indicate the condition or quality of materials. Their presence in foods may indicate that the foods were exposed to conditions favourable for the introduction and growth of pathogenic organisms.

Most foods are regarded as unwholesome when they have a large population of microorganisms, even if the microorganisms have not altered the character of the food. The counts obtained for the different groups of microorganisms from grated cassava, cassava starch and cassava flour were as follows: Aerobic spore count: 8.0×10^4 cfulg, 5.0×10^5 cfulg and 4.0×10^3 cfulg; Lactic acid bacteria: 2.4×10^5 cfulg, 1.5×10^6 cfulg; Lactose fermenters: 1.4×10^5 cfulg, 2.2×10^6 cfulg and 3.9×10^4 cfulg and non-lactose fermenters: 9.0×10^4 cfulg, 1.8×10^6 cfulg and 5.2×10^3 cfulg respectively (Fig. 1) The international microbiological standards recommended limit of bacteria contaminants for foods is less than 10^5 cfulg of food for total bacteria plate counts (Anon, 1974 and Refai, 1979). High viable counts often indicate contaminated raw materials, unsatisfactory sanitation or unsuitable time and temperature condition during production or storage or a combination of these. High counts also foretell the likelihood of spoilage, because most foods contain 10^6 to 10^8 microorganism per gram at the time when decomposition becomes evident. The occurrence of high counts of lactose fermenters indicate the presence of coliforms which suggest the degree of contamination with faecal discharges of humans and animal. The recommended limit for coliforms in foods is 10^1 to 10^2 cfulg (Anon, 1974 and Refai, 1979).

The most predominant mould isolated in this investigation is *Rhizopus stotonifer* (Table 1). Kuku *et al*, (1984) and Abba-Kareem and Okagbue (1991) isolated similar fungi such as *Aspergillus fumigatus, A. flavus* and *A. niger* from cassava flour. Species of *Aspergillus* isolated in this work are undesirable in foods. Some strains of *A flavus* have been reported to be toxin-producing. These toxins are heat-stable and hence when consumed may result in food intoxication. Moulds are potential spoilage agents. The genus *Penicillium* isolated in this study constitutes one of the three major mycotoxins producing fungi (Dicken & Jones, 1981). The discoloration coupled with the off-flavour of the grated cassava sample after three days are due to the activities of the infecting mould species. Moulds are generously endowed with extracellular proteolytic or lipolytic enzymes and so can cause softening of food products. Mould growth also causes off-flavours in foods and change in appearance of food have been related to mould growth (Elmer, 1990). Spores of various species of moulds are heavily suspended in air especially in an untidy and unhygienic environment. These sporulating moulds therefore easily get in contact with foods that are openly displaced in baskets or bowls. The similarity in the types of organisms isolated from the samples and those reported by other workers confirms the fact that some of the microorganisms are indigenous to the products.

It is rather impossible to avoid microbial contamination of foods during harvest and subsequent processing. Therefore the practice of basic sanitary rules in preparing foods should be employed to improve on the hygienic condition of foods. Contamination of food by these pathogens could be eliminated for some by heat treatment. Alternative method to sundrying should be employed to prevent mouldiness of starchy product.

TABLE 1: MICROBIAL ISOLATES OF CASSAVA PRODUCTS

Type	Grated Cassava	Cassava starch	Cassava Flour
Bacillus cereus	+	-	+
Bacillus subtilis	+	+	-
Escherichia coli	+	+	+
Klebsiella aerogenes	+	+	+
Lactobacillus acidophilus	+	+	+
Leuconostoc mesenteroides	-	+	-
Pseudomonas aeruginosa	-	-	+
Salmonella sp.	+	+	+
Shigella flexneri	-	+	-
Staphylococcus aureus	-	-	+
Aspergillus flavus	-	+	+
A. fumigatus	+	-	-
A. niger	+	+	+
Candida sp	+	-	-
Fusarium moniliforme	+	+	-
Mucor mucedo	+	+	-
Neurospora sp.	-	+	-
Penicillium sp	+	-	+
Rhizopus stolonifer	+	+	+
Trichoderma viridae	-	-	+

+, Present ; -, Absent.

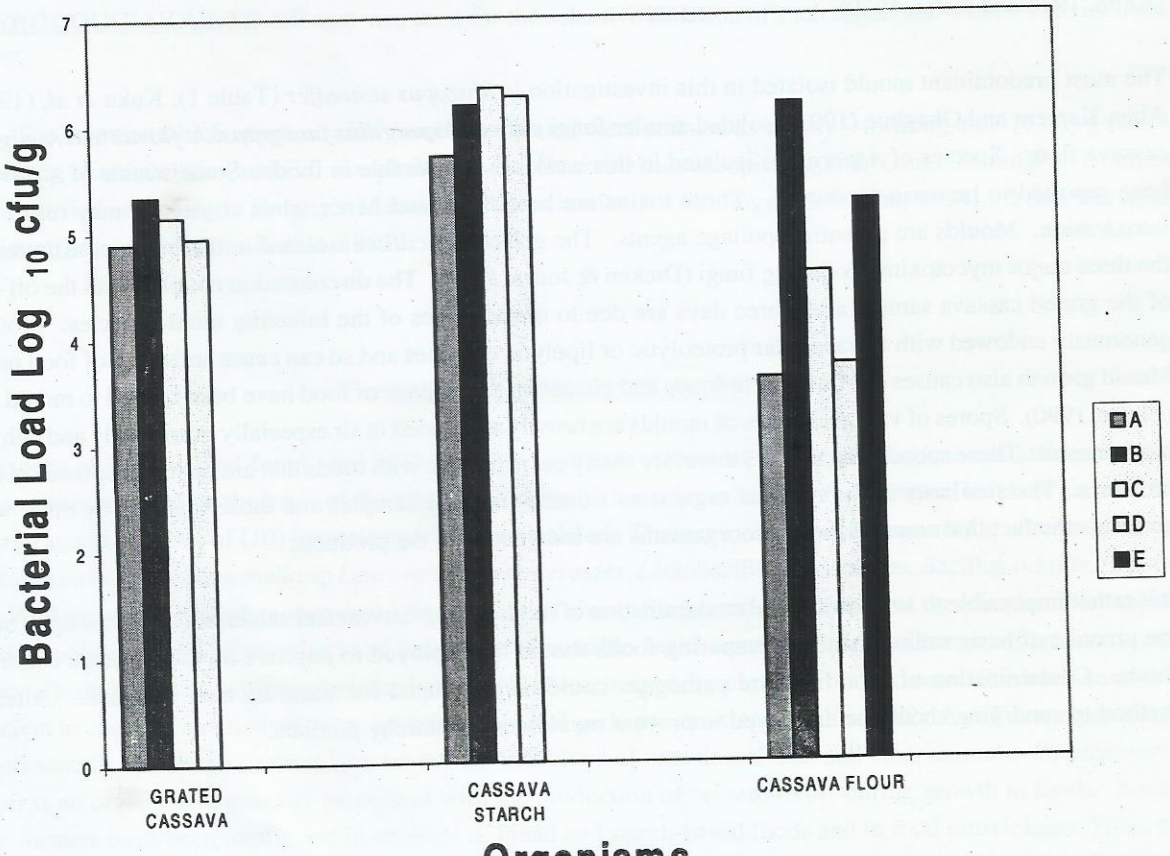

FIG.1: Viable counts of groups of Bacteria in cassava products.

Key : A - Aerobic spore counts, B- lactic counts, C- Lactose fermenters counts, D- Non- lactose fermenters counts, E- Enterococci counts.

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