Evaluation of Micro-Organisms in Cassava Flour (Alebo) and its Effect on Human Health

S. Danladi A., Mohammed & A.A. Galadima
Department of Basic Science,
College of Agriculture P. M. B. 33 Lafia,
Nasarawa State,
Nigeria.
Corresponding author email: Sanidanladi2016@gmail.com

Abstract
This work focused on the isolation and evaluation of micro-organisms that ferment peeled raw cassava for the purpose of determining their health effects on people that feed on it commonly known as (Alebo) in Hausa Language. Three (3) samples were randomly collected from traditionally processing sites of cassava flour in Wamba, Lafia Local Government and Shabu Development Area of Nasarawa State to identify certain micro-organisms that may be presence during processing and their health effect on humans. The result obtained from Shabu recorded the highest number of microbial counts population, followed by Lafia while Wamba recorded the least microbial counts.The results of the analysis reveals the presence of micro-organinsms; staphylocooccus aureus, e.coli, proteus spp and saccharomyces cerevisiae. People who consume much of the flour in the area of this study may suffer from gastrointestinal, hematologic, gastroenteritis, urinary tract infections and neonatal meningitis diseases. The finding also shows that the flour can cause diarrhea and staph infections in humans. Modern methods of processing of the flour should be used than the traditional way in order to reduce the effects of the micro-organisms on human health.

Key words: *flour, micro-organism, infection*

INTRODUCTION

Cassava (*Manihot esculantants*) is a root tuber crops that is widely cultivated in the tropical region of the world (Arontupin and Iyayi 2001; Oboh and Akindahunsi, 2003). As shrubby perennial plants that grow to a height of 6 – 8 ft, it is usually propagated by planting short section of the stems (D'hair, 1995; Oboh, 2005). Suffix to say that different cultivars of cassava abound worldwide. Cassava matures at different rates. However, certain varieties contained large amount of cyanogenic glycosides (Linamerin and cotaustralin) which can hydrolyzed to hydrocyanic acid (HCN) by their endogenous enzymes (linamarase) when the plant tissue is damaged during harvesting, processing or any other mechanical method (Oboh and Akindahunsi, 2003). The protein content of cassava products can be increased by addition of proteins to the deficient food in a way that will not alter the organoleptical qualities of the original food (Oboh, 2005). Also, through controlled fermentation, microflora could be made in large numbers in the mash (Raimbault, 1998; Oboh et al, 2000; Oboh and Akindahunsi, 2003), thus increasing the protein content of cassava product.

Oboh (2005) identified two important wastes that are generated during the processing of cassava tubers to include cassava peels and cassava liquid squealed out of the mash, the bioconversion of the cassava wastes have been documented. (Antia and Mbongo, 1994; Okafor, 1998; Raimbault, 1998; Tweyon et al, 2002; Oboh, 2005) Said that the waste water contains heavy loads of micro-

organisms, lactic acid, lysineamylase capable of hydrolyzing the glycosides. Nigeria is the world largest producer of cassava and it constitutes the most economic sources of starch (Daramola and Osanyinlusi, 2006). During the processing of cassava tubers into various products, liquid waste water generated was reported to cause serious havoc to vegetation, houses and brings about infection too. This is no doubt having being causing serious environmental pollution as a result of its indiscriminate discharge. The liquid squeezed out of fermented cassava flour is use as animal feeds in some places (Okafor, 1998; Oboh and Akindahunsi, 2003). This work is aimed at evaluating the presence of those micro-organisms that are presence in cassava flour during its processing and to ascertain the health effects on humans as a result of too much consumption of the flour as cheap source of food and remedy.

METERIALS AND METHODS

(a) Collection and preparation of the sample for analysis

With the permission of the women processing the cassava flour; three (3) samples of the wet and sun drying cassava flour were randomly collected in Wamba and Lafia Local Governments and Shabu development Area of Nasarawa State, Nigeria. The harvested tubers were washed and soaked in 20L of water for 3 - 4 days at room temperature to allow fermentation to occur which enhances the removal of cyanide content. After 3-4 days; it was removed, re-washed and rinsed to enhance further reduction of the cyanide content as well to give the flour a bright white colour. Pounding was done to break the fermented tubers into tiny particles. It is then removed and placed in baskets and allowed to stand for about 3-4 hours for dehydration (to remove the remaining water content from the fermented and pounded particles). The mashed particles are spread on clean flour for sun dried for about 2-3 days and grinded into cassava flour. The collected samples were stored in polythene bags and were taken to Chemistry Laboratory, University of Jos, Plateau State, Nigeria for microbial analysis of the flour.

(b) Isolation and Identification of Microorganisms

The associated microorganisms of the samples were isolated using serial dilution pour plate of Akerele, 1990. In this experiment, 0.1 mL of dilutions x 105 and x 102 of the samples were inoculated on nutrient agar (NA) and potato dextrose agar (FDA) for bacteria and fungi, respectively. The inoculated media were incubated at 37°C for 24hrs for bacteria and at 30°C for 3-5 days for fungi. Discrete colonies that developed on the plates were counted and recorded as colony forming unit per milliliter (cfu mL^{-1}). The pure cultures of bacteria obtained by sub culturing were identified using the methods of Holt (1994), while the fungal isolates were identified by Barnett and Hunter (1972).

(c) The screening of Microbial Isolates for Amylase and Cellulase

The method described by Cowan and Steel (1990) was used for the screening of microbial isolates for the production of amylase. This involved the incorporation of the potato starch into nutrient agar. The mixture sterilized, cooled, inoculated with the isolates and incubated. The plates were then flooded with Lugol's iodine solution for starch hydrolysis which indicated the production of amylase. A modified method of Ogundero (1982) was employed for the production of cellulase. The basal medium-carboxymethylcellulose (CMC) consisting of Mg 504.7 H_20, 0.75 g; $NaNO_3$, 2.5 g; KH_2P0_4, 1.75 g; $CaCl_2.H_20$, 2g; CMC, 10g as carbon source, agar-agar and 1000 mL distilled water. The mixture warmed, autoclaved and poured into already sterilized Petri dishes. The bacterial isolates were streaked on the plates according to the standard method and incubated at 37°C for 48 hrs, while the fungal isolates were inoculated on the plates by the method

of Arotupin and Akinyosoye (2001) and incubated at 30°C for 3-5 days. Formation halo of zone around the colony after flooding with 0.l% congo red solution and washed with 0.1 N NaCI confirmed the production of cellulase.

RESULTS AND DISCUSSION

The results of the microbial analysis were presented in the respective tables. The data analysis shows that all the cassava flour (Alebo) samples contain arrays of micro-organisms. These include; staphylococcus aureus, e. coli, proteus spp, lactobacillus spp and saccharomyces cerevisiae. That of Lafia local government area contains staphylococcus aereus, lactobacillus spp and Saccharomyces cerevisiae. Based on the findings, the result agreed with the documented research results of (Antia and Mbongo 1994; Okafor 1998; Raimbault, 1998; Tweyongyere and Katongole, 2003; Oboh 2005). Table 1 present the total microbial counts from each of the sample. Shabu recorded the highest number of microbial count population, followed by Lafia while Wamba recorded the least microbial counts. Frequency of the occurrence of the microbial isolates is presented in table 3. The high microbial counts may be due to the lack of efficient control measures in the discharge of the waste water into the environment (Arotupin, 2007). Staphylococcus aureus bacterium caused staph infections in humans, according to Mayo Clinic, 2013. Although these bacteria are usually harmless and are commonly found in the surface of the skin. Mayo Clinic reported that they can prevail over the body's natural protection, once the skin is damaged or injured to produce infection. The infections caused by staphylococcus aureus range from superficial skin infection to life threatening ones. Skin infections are the most common effects of staphylococcus aureus, according to (Medlineplus 2009). These infections can start as a simple crusting of the skin known as impetigo. Other skin infections caused by staphylococcus aureus are folliculitis, an inflammation of a hair follicle, furuncle a small abscesses affecting the skin and sub cutaneous tissues, and carbuncle a collection of furuncle. Some of these skin infections usually go away without treatment; they can develop into septicemia, which is a life threatening systemic condition. This shows that cassava flour can caused skin diseases in humans.

People eating contaminated cassava flour develop symptoms within one to six hours. This research work agreed with Denish's result which was produced in1982 and published in 1984 and the work of Amy, 2002 which both says Staphyloccus aureus caused stomach and skin diseases in humans. Toxic shock syndrome caused by staphylococcus is life threatening,. Toxic shock syndrome include redness of the skin, fever and low blood pressure, it usually involves organs and systems, such as gastrointestinal, muscular, renal, hematologic and nervous systems. Virulent strain of E. coli can caused gastroenteritis, urinary tract infection, and neonatal meningitis. In rear cases virulent strains are also responsible for laecmolyticurinic Syndrome (HUS), peritonitis, mastitis, septicemia and gram-negative pheumonia (Girish, 2009). It was also discovered that some bacteria like lactobacillus found in cassava flour are useful to human beings. Lactobacillus are friendly bacteria that normally live in human digestive, urinary, and genital systems or organs without causing any disease, lactobacillus is also in some fermented foods like yogurt and dietary supplements and do not cause any harmful effect on the consumers.

Lactobacillus is also used for treating and preventing diarrhea including some infectious diseases such as pot-viral diarrhea in children and traveller's diarrhea. It is also used to prevent diarrhea assisted by using antibiotics. Some people use lactobacillus for general digestion problems, irritable bowel syndrome (IBS), colic in babies, crohn's diseases, inflammation of the colon, and a serious gut problem called necrotizing enterocolitis (NEC) in babies born prematurely (University of Maryland Medical Center, 2013). Saccharomyces cerevisiae is a species of yeast. It is perhaps the most useful yeast, having been instrumental to wine making, baking and brewing since ancient

times. It is believe that it was originally isolated from the skin of grapes, one can see the yeast as a component of the thin white film on the skin of some dork coloured fruits such as palms, it exist among the waxes of the cuticle. Saccharomyces cerevisiae is currently the only yeast cell that is known to have Berkeley body presents which are involve in particular secretory pathway (Cabral *et al*, 2003).

CONCLUSSION

Cassava is a root tuber crop widely cultivated and used as food in the tropical region of the world. Many people depend on it as a cheap source of food for their consumption. However, it is discovered to be contaminated with micro-organisms during its processing to cassava flour. The result of the research work indicates that eating too much quantity of the flour can results to certain illness or infections that can affects human health.

RECOMMENDATIONS

The Nasarawa State government and other relevant authorities should look into the method of locally processed cassava flour and improve the standard of it.

Government and non-Governmental organization should provide modern processing machineries and equipments for cassava flour for better processing sites in Nasarawa State.

During fermentation of cassava, the water should be change regularly and properly dried in a clean environment to prevent or reduce the effects of micro-organisms that can affect the consumers.

The general public should be sensitized and be aware of the dangers of eating cassava flour that may be contaminated with micro-organisms.

Table 1: Colony count (cfu lml) dilution factor 10^6

sample	**Nutrient agar (n.a)**	**MaCconkey agar (MCA)**	**Mrs agar**	**Salmonella shigella agar (ssa)**
Wamba	1.56×10^6	1.0×10^6	4.0×10^6	1.3×10^6
Lafia	2.51×10^6	6.3×10^6	3.4×10^6	3.4×10^6
Shabu	6.0×10^6	2.0×10^6	6.5×10^6	1.84×10^6

Cfu = colony forming unit

Table 2: Biochemicals analysis of micro-organism present in cassava flour (alebo).

Sample	Catalase	Cugulase	Indole	Oxidase	Urease	Citrate	Motility	Glucose	Lactose	Sucrose	Triple sugar ion agar			Slope	butter	gas	H_2S
Wamba	+	+	+	-	+	+	+	+	+	+	Y	Y	T		-		
Lafia	+	+	-	-	-	-	-	+	+	+	Y	Y	-		-		
Shabu	+	+	-	-	+	-	-	+	-	-	R	-	-		-		

R = red, Y = yellow catalase + cuagulase positive (+) = stagphylococusaereusindole, positive (+) = E. coli, oxidase negative = pseudomonas spp, urease positive (+) = proteus spp, citrate positive (+) = entersbacteriae, TSI agar slant = salmonella spp, E.coli (y-y) (H_2S)

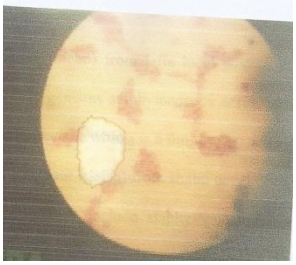
Table 3: Frequency of occurrence of the microbial isolates

Bacteria	**Wamba**	**Lafia**	**Shabu**
Staphylococcus aureus	+	+	+
E. coli	+	-	-
Proteus spp	+	-	+
Lactobacillus spp	+	+	-
Fungi			
Saccharomyces cerevisiae	+	+	+

+ = present, - = absent

Fig. 1: Micro organisms present in the cassava flour Samples

Wamba sample



Lafia sample

Shabu sample

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