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Characterization of Bacteria Isolates from Fermented Cassava Steeping Water

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Abstract

Cassava is a tuber crop mainly cultivated in Africa countries. The presence of unwanted microorganisms can complicate the control of the fermentation process, which can lead to the production of objectionable odors in steeping water. Microbial and physical analyses during *fufu* production were performed on the steeping water. Bacteria count decreased with an increase in the fermentation time. The highest bacterial count of 6.6 × 10^3 CFU/mL, while the least count of 2.2 × 10^3 CFU/ml was recorded, respectively. The bacteria isolated *include Bacillus subtilis, Pseudomonas species, Lactobacillus fermentum, Proteus mirabilis*, and *Klebsiella* sp. The pH value of 3.32 was recorded on day four, while the temperature was constant during the fermentation process. A high value of 45.2 mg/L total solids was recorded on day four, while the least value of 15.0 mg/L was obtained on day one. The effect of fermenting microorganisms under a controlled environment reduces the foul odor usually perceived during *fufu* production. Hence, the survival of these microorganisms at low pH can inhibit the growth of unwanted microorganisms, thus contributing to the acceptability of the cassava product, i.e. *fufu*.

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Keyword
Cassava; fermentation process; food contamination; steeping water; starter culture

Introduction

Cassava (*Manihot esculenta*) is a tuber crop serving as a cheap source of carbohydrates and other nutrients in the diet of the teeming population in Africa and Asia, which provides energy for about 500 million people (Achi & Akomas, 2006). Bamidele *et al.* (2015) posited cassava as a supplementary staple food to more than 200 million Africans aside from its uses as livestock feeds. Nigeria is ranked first and largest producer of cassava but with less export compared to Thailand (Otekunrin & Sawicka, 2019). Cassava production and processing are usually concentrated in the hands of numerous smallholder farmers located primarily in the South and Central regions of Nigeria. Cassava tuber consists of 64 - 87% starch depending on the growth stage or at maturity, but with low protein, fats, vitamins, and minerals (Aloys & Hui Ming, 2006). Cassava tubers are composed of starch depending on the growth stage or at maturity and the starch content compared to other starchy carriers (Lindeboom *et al*., 2004).

Adequate processing of cassava tubers by traditional means has yielded to a variety of edible products, such as *fufu*, '*Akpu'*, *Lafun*, *Garri*, *Abacha,* and *Tapioca*. Fermentation is one of the oldest and most important traditional food processing and preservation techniques. Employing this technique enhances the nutrient content and reduces the anti-nutrient contents in cassava (Oyetayo, 2006).

In Nigeria, cassava has been processed into many fermented and unfermented products in many ways. Some of the fermented products include cassava flour (*lafun),* which is produced by drying and milling fermented cassava tubers, cassava flakes (*garri),* which is produced by grating, soaking, fermenting, and roasting cassava mash. Other products include fermented cassava slurry used to produce "*fufu*". The product of interest of this research is *fufu*, a fermented cassava mash, which comes as a wet mash or a dry powder (Adegbehingbe *et al*., 2019) and is most commonly consumed in the Eastern and Southern regions of Nigeria. Fermentation of cassava involves the steeping of cassava roots in water for 3 to 4 days, which softened the root to disintegrate the tissue structures in contact with linamarin which is located in the cell walls by the action of linamarase (Adeleke & Olaniyi, 2018). This enzyme hydrolyses linamarin to glucose and cyanohydrins and subsequently breaks down to ketone and HCN (Aloys & Hui-Ming, 2006). Traditionally, African fermented foods and products, for instance, *garri* and *fufu* can be obtained from a series of operational procedures, which include grating, dewatering, fermenting, and roasting and these processes generate waste among which is steeping water, wastewater, and solid waste. However, the focus of this study is on steeping water.

About one-third of the cassava tubers harvested in Nigeria are utilized domestically for *fufu* production (Essers *et al*., 1995). It is bulky, easily contaminated by microorganisms, and cannot be stored for long due to its high water content, thus susceptible to easy spoilage. The inefficient method of preservation of wet cassava mash can be linked to the microbial growth that causes undesirable odor, and sometimes total spoilage. Nigeria has a large, sustainable, and expanding market for cassava *fufu*, with a population of over 180 million people (Ezedinma *et al*., 2006). In Nigeria, the consumption pattern varies according to ecological zones as *Garri*, a roasted granule is widely accepted in both rural and urban areas. Interestingly, it can be consumed without any additives or with a variety of additives, such as sugar, groundnut, fish, meat, and stew (Graffham *et al*., 2019).

The presence of unwanted microorganisms complicates the control of the fermentation process, thus leading to the production of objectionable odors in steeping water (Omar *et al*., 2000). Therefore, to provide basic information on the microorganism present in the fermenting substrates, there is a need to understand the type of the fermenting microbes in the steeping process to ensure improvement in the quality of cassava and cassava products. Therefore, this study was designed to isolate, enumerate, and identify microorganisms present in cassava steeping water and also determine the physical properties of the steeping water.

Materials and Methods

Sample collection and processing

This study was carried out in February 2017. Cassava tubers were obtained from a cassava processing site in Ikeji-Arakeji inside sterile plastic bags and then transported to the

Department of Microbiology, Joseph Ayo Babalola University, Osun State, Nigeria for further processing, such as washing, peeling, cutting, and re-washed with sterile distilled water.

Fermentation and microbial analysis

Cassava tubers were fermented for four days inside fermenting vessels containing sterile distilled water at room temperature. Microbial analyses were performed daily by pipetting 1 mL of the sample stock solution and then serially diluted up to the appropriate dilutions. One milliliter from the dilutions, 10^{-2} and 10^{-4} was pipetted, gently dispensed into Petri dishes, and then pour plated using Nutrient Agar (NA), *Salmonella Shigella* agar (SSA), Mannitol salt agar (MSA), MacConkey agar (MA) and Eosin Methylene Blue agar (EMB). The Petri plates were incubated at 37^{o}C for 24 hours to determine bacterial growth and morphological details. Pure cultures were obtained by repeated streaking of the bacterial inoculum on the fresh bacteriological media and stored on slants inside the refrigerator for further use. Furthermore, the pure culture was subjected to various morphological and biochemical characterization tests, such as catalase, oxidase, and indole, hydrogen sulfide, coagulase, and sugar fermentation, to determine the identity of the bacteria isolates (Adeleke *et al*., 2017).

Physical properties

The physical properties of distilled water were used in the soaking of the cassava were carried out in the laboratory. The physical parameters determined were pH, temperature, total dissolved solids, total suspended solids, and total solid according to the modified method of and Afuye & Mogaji (2015) and Adegbehingbe *et al.* (2019).

Statistical analysis

Data were reported as average of triplicate determinations and analysed using Analysis of Variance (ANOVA) on SPSS. Duncan's multiple tests at 5% level significance were used to determine the significant differences among the samples.

Results and Discussion

Total bacteria count

The population of bacteria isolated from the cassava steeping water indicated a reduction from day one to the last day of the fermentation process. The bacterial count ranged from 2.2×10^3 CFU/mL to 6.6×10^3 CFU/mL. Day one had the highest bacterial count of 6.6×10^3 CFU/mL while the least bacterial count of 2.2×10^3 CFU/mL was recorded on day four (Table 1). Cassava is usually processed into various products through the fermentation process to increase the shelf life, easy packaging, and transportation for economic purposes (Aro, 2008). Processing of cassava by submerged state fermentation techniques by traditional methods and beyond specified fermentation time usually produce mash, which contains a foul odor resulting from the uncontrolled fermentation and storage techniques (Oyewole & Odunfa, 1988). During the process of retting of cassava, a decrease in the total viable count of aerobic mesophiles was observed and this can be linked to the increase in acidity of the fermentation medium (Adegbehingbe *et al*., 2019). Also, an increase in acidity of the medium can cause a decrease in the growth of pathogenic-like microorganisms (Ojo *et al*., 2019).

Nine bacterial isolates were identified based on colonial and cellular morphological characterization. The identifiable bacterial isolates include, *Klebsiella pneumonia*, *Lactobacillus species*, *Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas* spp, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Table 2).

Table 1: Mean population of bacteria from the steeping water

Days	Mean	CFU/mL x 10^3
1	66	6.6
2	50	5.0
3	30	3.0
4	22	2.2

Key: CFU - Colony-forming unit

Table 2. Characterization and identification of isolates

	Colonial morphology					Staining reactions		Biochemical tests				Sugar fermentation				
Isolate code	Shape	Elevation	Cell shape	Cell arrangement	Colour	Gram staining	Spore staining	Catalase test	Indole production	Coagulase test	H_2S production	Sucrose	Lactose	Glucose	**Citrate utilization**	**Probable isolates**
IA	CR	RD	R	S	CR	+ve	-ve	-ve	- ve	-ve	-ve	-veG	-veG	+veG	+ve	*Lactobacillus fermentum*
IB	CR	RD	SR	C	WH	- ve	-ve	+ ve	- ve	- ve	- ve	+ve	+ve	+ ve	-ve	*Klebsiella pneumoniae*
IC	CR	RD	R	S	CR	- ve	-ve	+ ve	- ve	- ve	- ve	- veG	- veG	-veG	+ve	*Pseudomonas* sp.
ID	CR	F	R	C	WH	- ve	-ve	+ ve	- ve	- ve	- ve	- ve	- ve	+ ve	+ve	*Proteus mirabilis*
IE	IR	F	R	C	CR	+ ve	+ve	+ ve	- ve	- ve	+ ve	+veG	+veG	+veG	+ve	*Bacillus subtilis*
IF	CR	RD	SR	C	WH	- ve	- ve	+ ve	- ve	- ve	-ve	+ ve	+ ve	+ve	-ve	*Klebsiella pneumoniae*
IG	CR	CV	CC	C	LY	+ ve	-ve	+ ve	- ve	+ ve	- ve	+ ve	+ ve	+ ve	-ve	*Staphylococcus aureus*
IH	CR	RD	SR	S	CR	+ ve	-ve	- ve	- ve	- ve	- ve	- veG	- veG	+veG	-ve	*Lactobacillus* sp.
II	CR	RD	R	S	GR	-ve	-ve	+ ve	-ve	- ve	- ve	- ve	- ve	- ve	- ve	*Pseudomonas aeruginosa*

Ezedinma *et al*. (2006) reported the amylolytic activity of *Bacillus subtilis,* which produces an enzyme necessary for the breakdown of starch to sugar needed for the growth of other fermenting microorganisms, including lactic acid bacteria. In the report of Freire *et al*. (2015), the authors reported some members of Enterobacteriaceae during the fermentation of cassava, which is similar to the results obtained in this study. The presence of *Proteus* spp and *Klebsiella* spp could underline microbial activities in the rotting of cassava roots which was consistent with the findings of Achi & Akomas (2006). *Bacillus* spp and *Lactobacillus* spp were detected at the later stage of the retting process due to their persistence and ability to grow in an acidic medium. Obilie *et al*. (2003) and Essers *et al*. (1995) reported the involvement of *Bacillus* species in the textural modification of cassava roots, which cause a softening of the cassava tissues, which is evident in this study.

The presence of *Staphylococcus aureus,* a normal skin microflora in the cassava steeping water may originate from human contact, contamination, poor hygienic conditions, and post-contamination (Olopade *et al*., 2014). Identification of *S. aureus* from diverse fermenting substrates has been reported in many studies (Nout, 1994; Fowoyo & Ogunbanwo, 2017; Anyogu *et al*., 2021). However, the results from this study agree with the findings of Oyetayo (2006), who reported some pathogenic bacteria from fermenting cassava steeping water. *Pseudomonas* spp and *Proteus* spp have also been reported in the fermentation of cassava, due to their enzyme activities in the reduction of cyanide (Izah *et al*., 2018). The *Lactobacillus fermentum* isolated from this study agreed to the findings of Adeleke *et al*. (2017) who reported *L. fermentum* from the fermented cassava peels. The presence of these bacteria can contribute significantly to the fermentation process of cassava wastewater for desirable output.

Table 3 shows the physicochemical properties of steeping water. A pH value of 3.67 was recorded at day one while at day four a pH value of 3.32 was recorded, respectively.

Table 3. Physicochemical properties of the steeping water

Parameters	Days	
	One	Four
pH	3.67	3.32
Temperature (^oC)	26.2	26.2

The temperature was constant. Figure 1 shows the chemical properties of the steeping water. The high total solids value of 45.2 mg/L was obtained at day four while the lowest total dissolved solids of 15.0 mg/L were obtained at day one of the fermentation processes. In this study, the pH of the retting liquor (steep water) was acidic after 24 hours of the fermentation process. This explains the high count of lactic acid bacteria in the later stage of the fermentation, probably due to increased acidity of the medium, which favored the growth of the microorganisms (Adegbehingbe *et al*., 2017b). The decrease in pH recorded throughout the fermentation period may be associated with the fermentation by *Lactobacillus fermentum*. An increase in the acidity and decrease in pH of tuber fermenting medium has been documented in several studies (Oboh, 2006; Olufemi & Murtala, 2015; Adegbehingbe *et al*., 2017a). The results of the effect of temperature on cassava retting showed that the optimum temperature was 26˚C. This is an indication that temperature had a very strong effect on the retting time. Moreover, the container should be covered immediately after soaking the cassava to prevent contamination and allow the chemical reaction to take place. Furthermore, this study showed the chemical parameters of fermented cassava steeping water. The values of total solids were higher in the fermented samples during the fermentation process. A total suspended solids value of 9.28 mg/L in cassava effluents has been reported by (Lawal *et al*., 2018). Proper measures should be taken to treat cassava wastewater for irrigation purposes instead to be indiscriminatingly discharged into the environment.

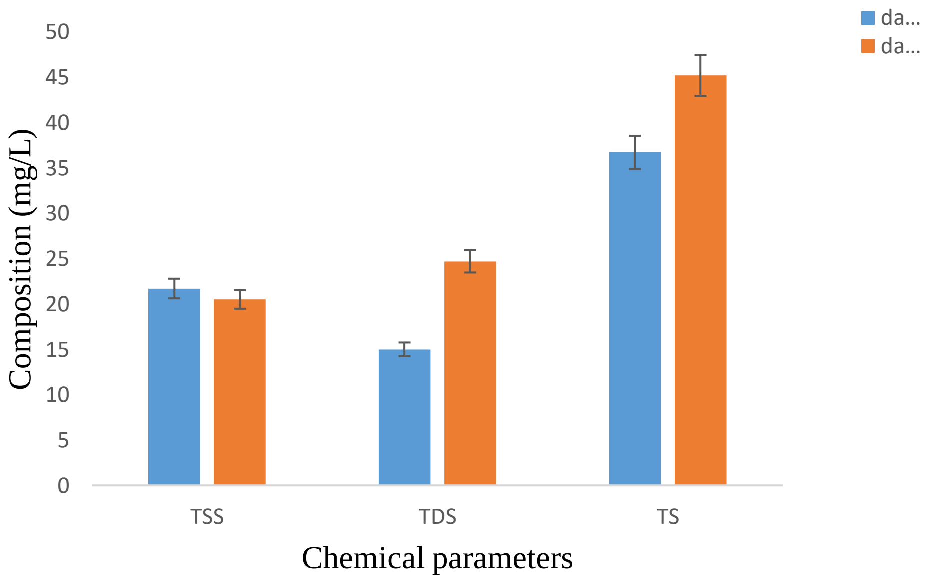

Figure 1. Chemical properties of steeping water. Key: TSS - total suspended solids, TDS - total dissolved solids, TS - Total solids

Conclusions

In conclusion, this study has revealed microorganisms involved in the fermentation of cassava steeping water. Ensuring quality control during *fufu* production by soaking cassava tubers is essential to reduce contaminants from the fermenting substrates. The presence of *Lactobacillus* species in the fermenting medium can be beneficial in softening the cassava tubers and inhibiting the growth of other pathogens which might cause foul odor during *fufu* production. The physical properties of the steeping water reveal the survival rate of the isolated microorganism fermentation medium. Therefore, the microorganisms identified in this study can further be harnessed as a starter in the

Authors' contributions

This work was performed in collaboration with all the authors. Authors BOB and OII conceptualize the study. Author OII performed the laboratory work. Authors BOB, BSA, and AOA managed the literature searches and wrote the first draft of the manuscript. Authors BOB AOA and OII performed the data analysis. Author BOB and BSA revised the drafts. All authors read and approved the final manuscript for publication.

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