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Low Cyanide-High Protein Dry Fufu Powder Processed from Cassava using Starter Culture Isolated from the Fermenting Mash

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ABSTRACT

Fufu, a processed food from cassava (*Manihot esculenta)* tubers is one of the staple foods consumed in Nigeria. Some methods of processing allow it to harbor high cyanide content in form of Hydrogen Cyanide (HCN). Traditionally processed fufu are obtained as wet mash after fermentation. This research looks into processing dry fufu powder with little or no cyanide content as an improvement on fufu production. Traditional method of wet *fufu* production was employed to produce wet fufu mash. Low cyanide-high- protein dry fufu powder was produced from the wet mash. Microbial flora in the retting water were isolated, characterized and identified as *Bacillus subtilis, Staphylococcus aureus, Klebsiella aerogenes, Enterobacter aerogenes, Pseudomonas aeruginosa, Escherichia coli, Lactobacillus coryneformis, Candida tropicalis, Saccharomyces cerevisiae, Aspergillus sp and Rhizopus sp.* The isolates were tested for their ability to ret the tubers and four isolates retted the tubers completely. Two organisms (*Lactobacillus coryneformis* and *Saccharomyces cerevisiae*) were used as starter cultures to ret the tubers as single cultures and mixed cultures to produce wet fufu samples F1 (*Lactobacillus coryneformis* alone), F2 (*Saccharomyces cerevisiae* alone), and F3 (mixture of *Lactobacillus coryneformis* and *Saccharomyces cerevisiae*). The three fufu mashes produced were each dried in the oven at 65^{o}C for 72 hours. They were crushed to powder and sieved, and assessed for their protein and cyanide contents. The percentage crude protein contents were 9.43%, 9.75% and 10.20% respectively and their cyanide contents were 0.028, 0.020 and 0.017mg/g respectively. The samples were aseptically packaged in cellophane bags. They lasted for six months without loosing their organoleptic qualities. Sensory evaluation of the samples accepts all the samples with most preference to F3. Therefore to enable long time storage of fufu with low cyanide and improved protein contents, starter cultures can be used to ferment the tubers and the wet mash aseptically oven-dried, crushed and sieved to fufu powder.

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INTRODUCTION

Fufu is a fermented white, soft cassava mash which can be as a wet mash or dry powder (Okoro, 2007). It is commonly consumed in the eastern and south-south zones of Nigeria. Traditional method of producing wet *fufu* mash and their improved versions still involve peeling, cutting (optional), soaking for 3-5 days, macerating and sieving, then settling and decanting excess water (Okpokiri *et al*., 1984; Obadina *et al*., 2006). Wet *fufu* mash can be stored in mesh bags and transported to other townships, but it can easily get contaminated. Further fermentation of the wet fufu mash can also occur resulting to the growth of moulds and other spoilage organisms which may be harmful to humans and animals. All these cause spoilage of the product within a short time. Wet *fufu* mash when cooked and pounded into dough also cannot stay up to 10 days without spoilage. Microbial contaminations affect this product thereby affecting its storage.

Fufu is a very good source of carbohydrate (about 98%) and energy but very low in protein (about

2.0%). Therefore, there is a great need to improve the storage life and protein content of *fufu* because of the need to produce food rich in protein to feed the increasing population of the world (Umeh and Odibo, 2013a). Conventional protein sources such as fish, meat, eggs, etc are expensive and cannot be afforded by low income earners and peasant farmers. Since the bulk of diets of these poor people consist mainly of carbohydrate foods that are low in protein, protein-deficiency diseases abound. High consumption of low protein foods result in high sugar concentration in the blood, which can cause coronary disease and even abnormal growth in children resulting to kwashiorkor (Fagbemi and Ijah, 2005).

Since *fufu* is widely consumed in Nigeria both by the poor and the elite, there is great need to produce *fufu* that can be stored for a longer time with low cyanide and high protein contents. Wet fufu mash is heavy to carry due to its water content. It is obtained by submerged fermentation of cassava tubers which can be done at home or near the river bank.

Cassava (*Manihot esculenta* Crantz) is grown widely in Nigeria and in many regions of the tropics. It serves as one of the basic food sources for about 200-300 million people (FAO, 1991). Nigeria produced about 33 million tons of cassava in 1999 and since then she is among the world's largest producers of cassava (Sobowale, *et al*., 2007; Umeh and Odibo, 2013).

Cassava has been variously used in the production of different types of food in Africa, such as *garri, fufu, lafun, abacha, tapioca* etc. It is normally processed before consumption due to the presence of toxic cyanogenic glycosides present in the fresh roots. The only most single method of processing is by fermentation which helps in the detoxification, preservation and modification of the food product (Oyewole, 1991). The fermentation process can be classified into solid state (without soaking, as in *garri*) and submerged fermentation (soaking in water, as in *fufu*) (Oyewole, 2002).

Materials and methods

Source of cassava tubers, chemicals and reagents: Cassava tubers of the TMS 30555 specie were harvested from the farm at the Nnamdi Azikiwe University Awka premises and immediately transported to the laboratory. Chemicals and reagents used are obtained from the Applied Microbiology and Brewing Laboratory of the institution and are of analytical grade.

Method of *fufu* production:
The method of wet *fufu* mash production by Umeh and Odibo (2013a) was used to produce wet *fufu* mash in the laboratory. The tubers after harvest were peeled, cut into cylindrical portions (4-7 cm long) and washed with tap water. Three kg (3 kg) of the peeled cut tubers were soaked in 5liters of water for 4 days using plastic bucket with lid. The retting water and tubers were monitored daily for retting ability and the presence of microbial flora. The isolated organisms were identified and characterized. Their ability to singly ret the tubers and produce an acceptable wet *fufu* mash was tested. Four organisms were able to ret the tubers and produce acceptable wet fufu mash. The four organisms that retted the tubers and produced acceptable wet fufu mash were tested for their ability to grow in cassava medium within 28 – 48 hours. Two organisms that grow faster in the cassava medium were used as starter cultures to ret the tubers as single cultures and as mixed cultures aseptically.

At the end of the four days, the retted tubers were washed, mashed in clean water and sieved through a fine mesh to remove the fibers and the vascular bundles. The mixture was allowed to settle and excess water decanted. The wet *fufu* mash is then transferred into a clean jute bag and the remaining water pressed out.

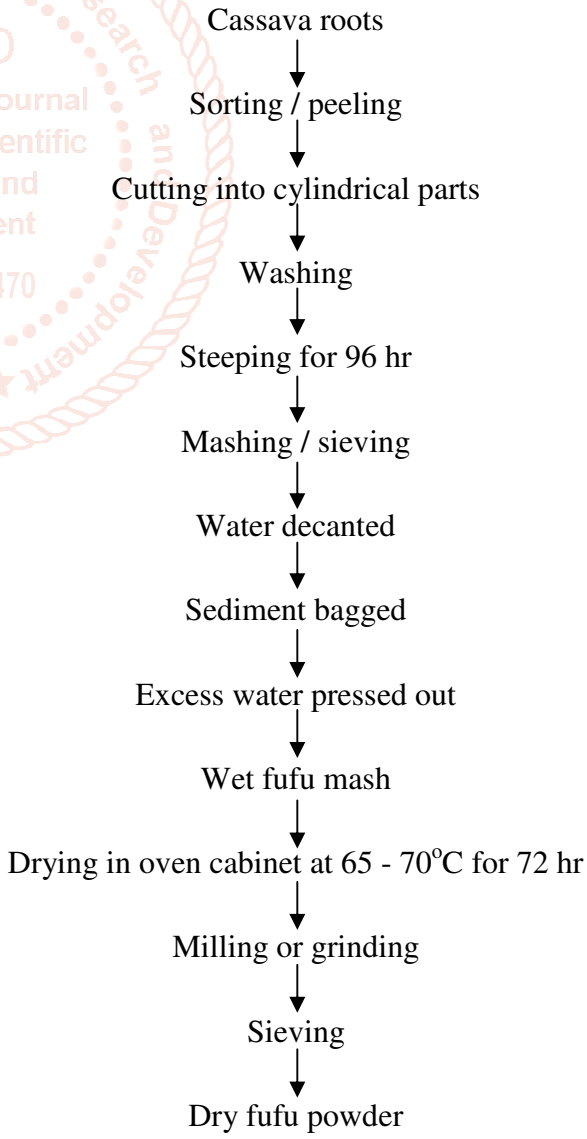

Figure 1: Method of fufu powder production

Method of analysis:

Determination of the retting ability of the tubers

The retting ability of the tubers was determined manually by feeling the degree of softness of the tubers with hand covered with a sterile disposable hand glove (Umeh and Odibo, 2013b).

Processing of the wet fufu mash into dry fufu powder

The resulting wet *fufu* mash was spread and dried in an oven at 65^{o}C for three days, ground, sieved and packaged in airtight cellophane bags.

All the fufu powder samples produced were tested for total cyanide and protein content and analyzed organoleptically for colour, taste, texture and general acceptability and the results obtained confirmed statistically.

Determination of the Total Cyanide content of the samples

The Grignard test used by Okafor *et al*., (1998) was used. Standard cyanide curve was first prepared. One gram of the dry fufu powder was dissolved in 100 ml of water and allowed to settle. Twenty milliliters of the filtrate was pipetted into a 100 ml flask. Ten milliliters of alkaline sodium picrate solution were added in the flask and mixed. Ten milliliters of the mixture was transferred in a test tube. The tubes were incubated in a water bath set at 94^{o}C for 5 minutes and allowed to cool at room temperature. Absorbance of the mixtures was read from a Jenway 6405UV/V Spectrophotometer at 540 nm after using distilled water to zero the spectrophotometer. The absorbance was the average of two readings. Then the concentrations of potassium cyanide in the sample were calculated from the standard cyanide curve.

Identification and characterization of the bacterial isolates

The pour plate method as described by Collee and Miles (1989) was used to determine the microbial counts in the retting water. Identification of the bacterial isolates was carried out as stipulated by Cheesbrough, 2000. Pure isolates of the were stored on slant and preserved in a refrigerator at 4^{o}C. The bacterial isolates on slant were sub cultured on fresh nutrient agar plates by streaking and incubated at room temperature (28 $^{\pm}$ 2^{o}C). Their morphological characteristics were observed after 24 and 48 hours. The lactic acid bacteria were sub-cultured on fresh tomato juice agar plates, incubated anaerobically and examined after 48 hours and 72 hours. Features such as colour, smell, edge elevation and size of the colony were examined.

Identification of fungal isolates

The morphological and biochemical tests were used to identify the yeasts while morphology and colour matching, (using the colour Atlas) were used to identify the moulds. The methods used were as described by Barnett *et al.* (1990).

Estimation of protein content

Crude protein content of the four different fufu powders produced were determined by the micro Kjeldahl method of Pearson (1976) and calculated using a protein conversion factor of 6.25.

Sensory evaluation of the fufu flour

The method of Fagbemi and Ijah (2005) was used to prepare the fufu dough. Ninety gram of the different fufu powders were stirred in 150 ml of boiling water. The fufu dough was allowed to cook for 20 minutes with intermittent stirring. After cooking the different doughs were evaluated for colour, taste, texture, and general acceptability by 10 panelists who are conversant with the organoleptic qualities of fufu–dough. The scores were analyzed statistically using the Kruskal – Wallis test.

Results

The daily changes in the microbial counts of the retting water were presented in Table 1. On the zero day only the heterotrophic bacteria were present in the retting water. Subsequently the total microbial counts increased with increase in retting days. Table 2 showed the morphology and biochemical properties of the yeast isolated. Table 3 presents the morphology and biochemical properties of two moulds isolated. While Table 4 showed the morphological and biochemical characteristics of the isolated bacterial. Ability of the different isolates to ret the tubers as well as their growth in cassava medium was as shown in Table 5. Only four isolates were able to ret the tubers completely and two were able to grow quickly in a mineral salt medium containing cassava water as a carbon source within 24-48 hours. Table 6 showed the result of the crude protein and cyanide content of the dry fufu powder. The sample produced using the mixed culture gave fufu powder sample with highest crude protein content and lowest cyanide content. Table 7 presents the statistical analysis of the organoleptic qualities of the samples with the F3 most preferred.

Table 1: Daily changes in the microbial counts of the retting water

Days	Heterotrophic bacterial count x10^{6}cfu/ml	Yeast count x10^{6}cfu/ml	Mould count x10^{6}cfu/ml	Lactic acid bacterial count x10^{6}cfu/ml
0	27.0	nd	nd	nd
1	40.0	30.5	28.5	29.0
2	51.0	40.0	46.0	30.5
3	55.5	46.5	53.0	39.5
4	65.0	50.0	56.0	48.0

Key: nd – not determinable

Table 2: Morphology and biochemical properties of the yeast isolates

			Sugar fermentation					Sugar assimilation					
sno	Culture charact e -ristics	Cell morpholog y	Glu c ose	Ma l tose	Galactos e	Dex tros e	Manito l	Glucos e	Ma l tose	Gala ctos e	Dex tros e	Maniol t	Probable organism
1	Cream white smooth & flat	Oval Budding cells, pseudo -hyphae	+	+	+	+	-	+	+	+	+	-	*Candida tropicalis*
2	Smooth cream white to tan, hairy	Budding cells	+	+	-	-	+	+	+	-	-	-	*Saccharo - myces cerevisiae*

Table 3: Morphological characteristics of the mould isolates

S/no	Young culture morphology	Old culture morphology	Microscopy	Texture	Days	Probable organisms
1	Whitish with yellow reverse	Blue-green to dark-green	Double branching septate hyphae, short conidiophores	Powdery and velvety	3-4	*Aspergillus sp*
3	Dense grayish cottony	Green to brown to black filling the plate	Oval non-septate hyphae with sporangiophores	Fluffy and cottony	2-3	*Rhizopus sp.*

Table 4: Morphological and Biochemical Characteristics of the Bacterial isolates

Sno	Colony morpholog y	Gram stain	Spo re	Mo ti lity	Ur a se	Cat al ase	Cit r ate	M R	V P	In d ole	H_2 S	Gel a tine	KC N	Coa g ulas e	Gl u cos e	La c tos e	M al tos e	Su c ros e	Ma n itol	Probabl e organis ms
1	Slimy mucoid dry, white. Yellow when old	-ve short rods in chains & singles	-	-	+	+	+	-	+	-	-	-	+	-	-	A G	A	A	A	*Klebsiell a aerogene s*
2	Cream, rough, opaque and circular	+ long,ro ds in chains	+	+	-	+	+	+	-	-	-	-	-	-	AG	-	-	-	-	*Bacillus subtilis*
3	Cream,smo oth raised, circular	+ ve coci in clusters	-	-	-	+	+	-	-	-	-	-	-	+	A	-	-	-	-	*Staphylo -coccus aureus*
4	Smooth, mucoid and	-ve short	-	+	-	+	+	-	+	-	-	-	+	-	A	A	-	A	-	*Entero- bacter*

	circular	rods,+ve capsules																	*aerogenes*
5	Blue to dirty green low convex colonies	+ve rods	-	+	-	+	+	-	-	-	-	-	+	-	AG	-	-	-	-	*Pseudomonas aeruginosa*
6	Cream white nonviscous flat colonies	-ve shot rods	-	-	-	-	-	-	-	+	-	-	-	-	A	A	-	A	A	*Escherichia coli*
7	Gray to white on TJA	+ve rods	+	-	-	-														Lactobacillus coryneformis

Key: A = acid
AG = acid and gas
TJA = Tomato juice agar used to grow *Lactobacillus species*

Table 5: Ability of the organisms to ret the tubers and grow in Cassava medium

Organisms	Retting ability	growth in Cassava medium (24 hrs)
Aspergillus sp	-	-
Bacillus subtilis	++	+
Candida tropicalis	++	+
Enterobacter aerogenes	+	-
Escherichia coli	-	-
Klebsiella aerogenes	+	-
Lactobacillus coryneformis	++	++
Saccharomyces cerevisiae	++	++
Staphylococcus aureus	+	-
Rhizopus sp	-	-
Pseudomonas aeruginosa	+	-

Key: - no retting, no growth
+ partial retting and partial growth
+ + complete retting and full growth

Table 6: Percentage crude protein and Cyanide content of the fufu powder

Samples	Crude protein content (%)	Cyanide content(mg/g)
Fo	2.02	0.280
F1	9.43	0.028
F2	9.75	0.020
F3	10.20	0.017

Key: Fo – sample from traditional method
F1 – sample with *Lactobacillus coryneformis* alone
F2 – sample with *Saccharomyces cerevisiae* alone
F3 – sample with mixed culture

Table 7: Statistical analysis of the organoleptic qualities

Parameters	Fo	F1	F2	F3
Colour	4.0	4.3	4.4	4.8
Taste	3.0	4.4	4.8	5.0
Texture	3.3	4.5	4.5	4.8
General acceptability	4.1	4.8	4.6	5.0

Retting inference: 5 – excellent, 4 – very good, 3 – good and 2 – bad

Discussion

Fufu, a fermented cassava food product, widely consumed in Nigeria had suffered deterioration and contamination by microorganisms due to its high moisture content. Most of these organisms accompany the product from the fermentation system, storage containers, surrounding air and even human handling. The product is very bulky and cannot be stored for a long time without spoilage. Also, the food product is very low in protein and sometimes high in cyanide based on the method of fermentation. It is an energy giving food and therefore need to be improved in both storage and nutritive value.

This work is therefore carried out to find the best method of storing the food product as well as increasing its nutritive value. Starter cultures isolated from the fermenting system were used to ret the tubers and after fermentation the tubers were mashed to produce wet fufu mash. The wet mash was oven dried to produce the dried fufu powder which can be stored for a long time without deterioration and contamination.

Many microbes had been reported to be responsible for cassava fermentation to produce fufu (Okafor *et al*, 1998; Umeh and Odibo2013 a & b). In this work, eleven organisms were isolated; [*Bacillus subtilis, Staphylococcus aureus, Klebsiella aerogenes, Enterobacter aerogenes, Pseudomonas aeruginosa, Escherichia coli, Lactobacillus coryneformis, Candida tropicalis, Saccharomyces cerevisiae, Aspergillus sp and Rhizopus sp.*]; seven bacterial isolates and four fungal isolate (Tables 2, 3 and 4). Microbial counts of these organisms in the retting water increased daily with increase in retting days (Table 1). This is in agreement with the findings of Fagbemi and Ijah, (2005). The increase in counts may be as a result of favorable conditions which enable them to multiply (Fagbemi and Ijah, 2005). The multiplication of coliforms, especially in the early and intermediate days of fermentation is a characteristic of mixed acid fermentations (Davis *et al*., 1980). It was also observed that the fungal and lactic acid bacterial counts were very small on the zero days and is considered as not determinable (nd) (Table 1). Among the eleven isolated organisms, four were able to cause complete retting of the tubers; four caused partial retting while three did not ret the tubers (Table 5). Two of the organisms (*Lactobacillus coryneformis* and *Saccharomyces cerevisiae*) that ret the tubers were able to utilize cassava as a carbon source in 24 - 48 hr while others needed 48 – 72hr to start growth in the cassava medium (Table 5). The two organisms (*Lactobacillus coryneformis* and *Saccharomyces cerevisiae*) were chosen as starter cultures and they provide a high protein content and most acceptable fufu powder. Fagbemi and Ijah (2005) used *Candida utilis* and *Saccharomyces cerevisiae* isolated from 'burukutu' to enrich fufu and got similar results in protein content. Some of the organisms isolated were not able to ret the tubers or caused partial retting. This is in line with the findings of Okpokiri *et al*., (1985) and Okolie *et al*., (1992) that submerged fermentation over four days by traditional method usually produces a mash and retting water which contain a foul odour resulting from uncontrolled fermentation, undesirable organisms and poor storage techniques. These unwanted organisms may result in variations in the quality of the fufu produced (Ogumbawo *et al*., 2004). This the more reason why it is necessary to use starter cultures in retting to eliminate the effect of the unwanted organisms. The starter cultures also help a lot in the mode of reduction of cyanogenic glycosides at various stages of fermentations (Sobowale *et al*., 2007; Umeh and Odibo, 2013 a).

Cyanide content of the retting water was increasing with increase in retting days, while that of the tubers was decreasing. The cyanide content of the tubers during the traditional method decreased from 2.8mg/kg – 0.28mg/kg while that of mixed culture of the starter cultures decreased from 2.8mg/kg – 0.017mg/kg. This supports the report of Fagbemi and Ijah (2005) that there could be a high reduction in cyanide content using starter cultures.

The percentage crude protein content of the fufu powder produced was determined. It was found that the crude protein of F3 was highest (10.2 %), followed by that of F2 and F1 (9.75 % and 9.43 %) respectively, as seen in Table 6. This shows that the use of starter cultures can help a lot in improving the protein content of fufu (Umeh and Odibo, 2013a).

All the 10 panelists preferred the F3 fufu dough in all the tested qualities (Table 7). All the four samples of fufu powder were able to last for up to six months without loosing their qualities and protein content. It is therefore recommended to use the starter cultures in producing wet fufu flour before preserving to dried form. This will help to get a high protein fufu powder that can be stored for a long time, less bulky to carry and easy to be prepared into fufu dough.

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