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Use of different Additives in Retting Cassava tubers for Fufu production

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ABSTRACT: *Over – matured cassava tubers (above 18 months), which do not ret easily, were retted in this study to produce wet fufu mash using different additives of nail, potash and fresh leaves of Jatropha curcas. Four different samples of wet fufu mash were produced with the additives as nail –A, potash – B, Jatropha curcas leaves – C and no additive –D. The effects of these additives on the retting ability of the tubers, pH, titratable acidity, cyanide content and microbial counts (yeast, mould, heterotrophic bacteria and lactic acid bacteria) in the retting system were monitored daily. Observations showed that retting was complete in 3 days in the samples with additives while the control, without additive, did not ret in 3 days. The samples with additives yielded wet fufu mash with low levels of cyanide (0.05, 0.03 and 0.03 mg/g respectively) while the control yielded wet fufu mash with up to 0.8mg/g cyanide content. Wet fufu mash weighing 3.3, 3.5, 3.5 and 1.5 kg for samples A, B, C and D, respectively were obtained from the retting system. All other parameters investigated followed an increasing trend as retting progressed with least microbial count seen in the sample without additive. In conclusion, the use of additives helps in the retting of over - matured tubers which do not easily ret when harvested. Therefore, to encourage effective retting of these tubers, the use of potash or Jatropha curcas fresh leaves as additives is recommended.*

KEY WORDS: *Fufu production, cassava retting, retting additives, Jatropha curcas*

I. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the staple food crops consumed in Africa. It grows well in Nigeria and many regions of the tropics, where it serves as one of the basic food sources for about 200 – 300 million people and some animals. Almost all the farmers in Nigeria intercrop cassava in their farm because it withstands drought, pest and disease attack more than other crops. Nigeria is among the world's largest cassava producers (Sobowale *et al*., 2007) and processes it into many food products like garri, fufu, elubo, etc.

Harvested cassava tubers cannot be stored without processing. Physiological deterioration occurs in cassava roots 2-5 days after harvest followed by microbial deterioration (Achi and Akomas, 2006). The single most important method of cassava processing is by fermentation. Fermentation is one of the oldest and most economical methods of processing and preserving cassava tubers and converting them to food products.

Cassava plants in the farm when mature are not harvested at the same time. Some farmers leave their farm for 18 to 24 months before harvest so as to preserve the tubers. These tubers harvested late (over – matured), do not ret easily as to achieve complete retting in 5 days and more often than not used in the production of garri, another cassava fermented product. To ret these tubers to wet fufu mash, some additives (nails, potash and *Jatropha curcas* fresh leaves) are added locally to necessitate retting. They are added separately in the fermenting system to enable the tubers ret completely within the stipulated period of 4-5 days. There has been no study on the effect of these additives on the retting tubers. This study was therefore carried out to investigate the effects of these additives on some physicochemical parameters and microbial flora of the retting cassava tubers.

II. MATERIALS AND METHODS

A local variety of cassava identified as TMS 30555, by the Anambra State Agricultural Development Programme (ADP) was cultivated at the Nnamdi Azikiwe University, Awka premises. The cassava plant was allowed to overstay in the soil for up to 22 months before use. The tubers were transported to the laboratory immediately after harvest for the study. Potash and nails were bought from a local market. *Jatropha curcas* leaves were obtained from a farm at the University premises and identified at the Department of Botany of the University. Chemicals and reagents used were obtained from the Microbiology Laboratory and were of analytical grade.

Laboratory method of fufu production

The traditional soaking method of wet fufu mash production was used to produce wet fufu mash in the laboratory using the additives (nail, potash and *Jatropha curcas* fresh leaves) (Fig 1). The additives were each sterilized in an appropriate condition before use. The tubers after harvest were peeled, washed and cut into cylindrical portions of almost equal sizes. They were divided into 4 groups of 3 kg each. Each group was transferred into a plastic bucket and labeled A –D. Two pieces of concrete nails (each 3inches in length) were added in bucket A, 0.5g of Potash was added in bucket B, three medium sized leaves of *Jatropha curcas* were added in bucket C and bucket D was without any additive and served as control. Four liters of clean water was added in each bucket and the lid closed. The retting water and tubers were monitored daily for their pH, microbial count, retting ability, titratable acidity and cyanide content. After retting the tubers were mashed with clean water, allowed to settle and excess water decanted. The fufu was transferred in jute bags and excess water pressed out. The quantity of wet fufu mash produced was measured using a weighing balance to know the yield per sample. Also the cyanide contents of the wet fufu produced was checked.

III. ANALYTICAL METHODS

Determination of the pH and retting ability

The pH of the samples was determined using Hanna model pH meter already standardized to pH of 7. 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 which was disposed after checking one sample.

Titratable acidity content of the samples

The titratable acidity was determined using the method of AOAC (1990).

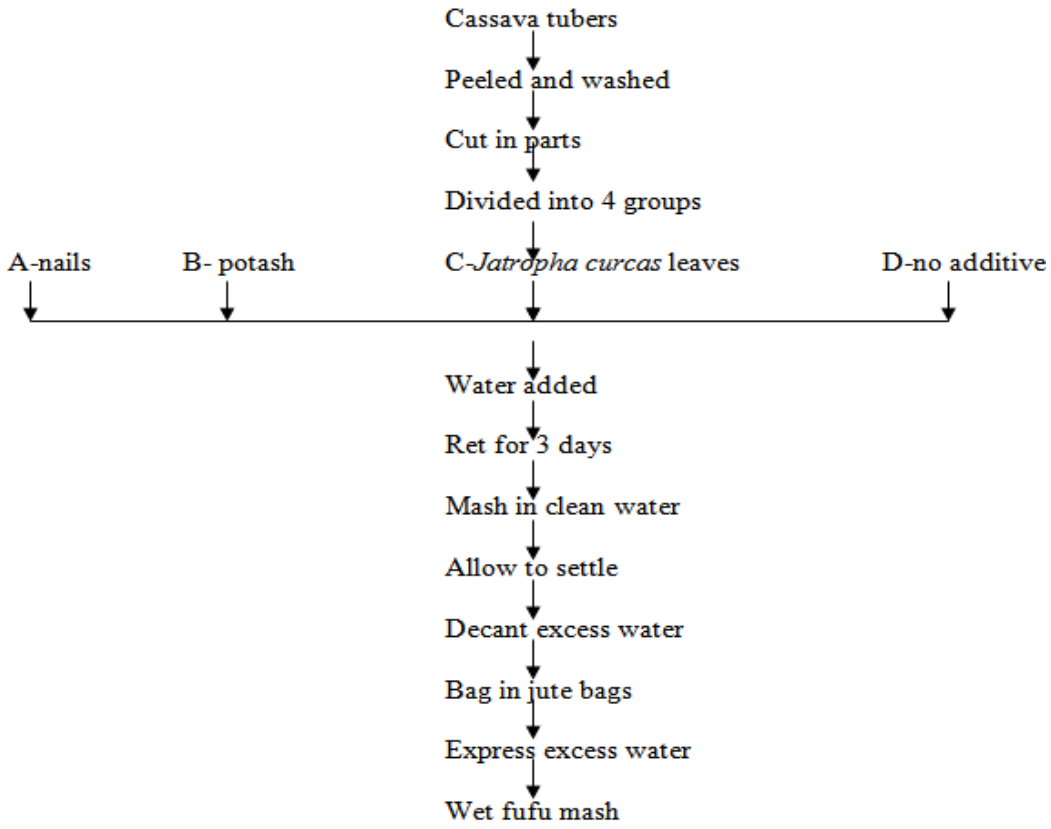

Fig 1: Flow chart for the production of wet fufu mash in the laboratory

Determination of the total cyanide content of the samples

The Grignard test used by Okafor *et al*. (1998) was used. A potassium cyanide standard curve was first prepared. Twenty milliliters of the filtered cassava retting water was pipetted into a 100 ml conical flask and 10 ml of alkaline sodium picrate solution was added 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 540nm after

using distilled water to zero the spectrophotometer. The absorbance was the average of three readings. The readings were plotted and the cyanide concentration calculated from the standard curve.

Viable count of the microbial flora in the retting water

The pour plate method of Collee and Miles (1989) as used by Umeh and Odibo (2013a and b) was used to determine the viable counts of the organisms in the retting water using appropriate culture media. Serial 10 - fold dilutions of the four sets of the retting water were made and 10^6 dilutions were cultured. From these dilutions, 0.1 ml was plated by pour plate method on nutrient agar containing nystatin to eliminate fungi; Sabouraud's dextrose agar and malt yeast extract agar each containing 0.25g/100 ml of chloramphenicol, to eliminate bacteria. The plates were incubated at room temperature, 28 ± 2^oC, 24 hours for the nutrient agar plates and 48 hours for the Sabouraud's dextrose agar and malt yeast extract agar plates. Also tomato juice agar plates were prepared for the lactic acid bacteria. These plates were incubated at room temperature for 48 hours but under micro-aerophilic condition. The colonies that developed were counted and the average number of the duplicate count on the plates was used to estimate the number of the organisms.

Identification and characterization of the microbial flora from the retting water

The identification of the bacterial isolates was carried out as stipulated by Krieg and Holts (1984) while the method of Barnett *et al.* (1990) was used to identify the fungal isolates.

IV. RESULTS

Additives of nail, potash and *Jatropha curcas* leaves were used to ret over – matured cassava tubers to produce wet fufu mash. Table1 presents the daily pH, titratable acidity, cyanide content of the retting water and the retting ability of the tubers. The pH of the retting water decreased (tending towards acidic) as retting progressed while titratable acidity and cyanide content increased. Retting ability of the tubers increased with increase in retting days and complete retting occurred in three days in all the samples with additives. Table 2 shows the daily microbial count in the retting water. The microbial counts also increased daily as retting was achieved. Sample D had the highest lactic acid bacterial count on the first day and lowest count on the third day. It also had the lowest heterotrophic bacterial count on the third day. Table 3 and 4 present the morphological and biochemical characteristics of the bacterial and fungal isolates respectively. Six bacterial isolates (*Bacillus sp*, *Enterobacter sp*, *Escherichia coli*, *Pseudomonas sp*, *Staphylococcus aureus* and *Lactobacillus sp*) and three fungal isolates (*Candida sp*, *Saccharomyces sp* and *Aspergillus sp)* were obtained. Heterotrophic bacteria are most predominant while lactic acid bacteria had the least count. Table 6 shows the weight and cyanide content of the fufu mash obtained from each sample. The samples with additives retted completely yielding larger amount of wet fufu mash.

Table 1: Daily pH, titratable acidity, cyanide content of the retting water and retting ability of the tubers

Samples	Days	pH	Titratable acidity (mg/g of lactic acid)	Cyanide content (mg/ml)	Retting ability
A	1	6.2	0.20	0.28	+
	2	5.7	0.41	1.22	+ +
	3	5.1	1.56	2.48	+ + +
B	1	8.4	nd	0.26	+
	2	7.8	0.02	1.25	+ +
	3	7.0	0.28	2.47	+ + +
C	1	6.0	0.21	0.28	+
	2	5.9	0.43	1.22	+ +
	3	5.2	1.57	2.46	+ + +
D	1	6.6	0.02	0.27	-
	2	6.0	0.41	0.88	-
	3	5.5	0.88	0.97	+

Key: nd – not determinable
- no retting
+ incomplete retting
+ + incomplete retting
+ + + complete retting

Table2: Daily microbial count of the retting water

Samples	Days	Yeast Count ($x10^6$cfu/ml)	Mould count ($x10^6$cfu/ml)	Heterotrophic bacterial count ($x10^6$cfu/ml)	LAB count ($x10^6$cfu/ml)
A	1	1.6	1.2	5.6	1.0
	2	3.2	4.0	6.9	3.3
	3	5.4	5.6	7.6	5.8
B	1	1.5	1.2	5.6	1.2
	2	3.8	4.2	5.9	3.7
	3	5.4	5.8	6.8	5.6
C	1	1.6	1.3	5.5	1.2
	2	3.7	4.0	7.0	3.6
	3	5.4	5.5	7.6	5.8
D	1	1.6	1.2	5.4	1.3
	2	3.5	4.0	5.9	3.6
	3	4.2	4.9	6.5	4.6

Key: LAB - Lactic acid bacteria

Table 3: Quantity and cyanide content of wet fufu mash obtained from each sample

Sample	Quantity (kg)	Cyanide content (mg/g)
A	3.3	0.05
B	3.5	0.03
C	3.5	0.03
D	1.5	0.80

Table 4: Morphological and biochemical characteristics of the bacterial isolates

Iso-lates	Colony morphology	Gram stain	Spore test	Moti-lity	Urea-se	Cata-lase	Cit-rate	MR	VP	Ind-ole	H_2S	Gela-tine	KCN	Coag-ulase	Glu-cose	Lact-ose	Mal-tose	Suc-rose	Man-itol	Probable organisms
1	cream, rough, opaque & circular	+ve long rods in chains	+	+	-	+	+	+	-	-	-	-	-	-	AG	-	-	-	-	*Bacillus sp*
2	Smooth mucoid circular	-ve short rods, small capsules present	-	+	-	+	+	-	+	-	-	-	+	-	A	A	-	A	-	*Enterobact-ter sp*
3	Cream white, non viscous flat	-ve short rods	-	-	-	-	-	-	-	+	-	-	-	-	A	A	-	A	A	*Escherich-ia coli*
4	Colonies Blue to dirty Green & Convex	- ve rods	-	+	-	+	+	-	-	-	-	-	+	-	AG	-	-	-	-	*Pseudom-onas sp*
5	Cream Smooth raised circular	+ve cocci in clusters	-	-	-	+	+	-	-	-	-	-	-	+	A	-	-	-	-	*Staphyloc-occus aureus*
6	Gray to white on TJA	+ve long rods in chains & singles	+	-	-	-	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	*Lactob-acillus sp*

Key: + = positive
- = negative
A = acid
AG = acid and gas
nd = not determined
TJA = Tomato juice agar

Table 5: Morphological and biochemical properties of the fungal isolates

			Sugar fermentation							Sugar assimilation							
Sno	Culture characteristics	Cell morphology	Glu-cose	Malt-ose	Lact-ose	Galac-tose	Suc-rose	Dext-rose	Mani-tol	Glu-cose	Malt-ose	Lact-ose	Galac-tose	Suc-rose	Dext-rose	Mani-tol	Probable organism
1	Cream white and smooth	Budding cells and pseudo-hyphae	-	-	-	-	+	+	-	+	+	-	-	+	-	-	*Candida sp*
2	Smooth cream white to tan hairy	Budding cells	+	+	-	-	+	-	+	+	+	-	-	+	-	-	*Saccharo-myces sp*
3	Whitish colony when young	Blue-green to dark-green when old	Double branching septate hyphae							Powdery & velvety		3-4 days					*Aspergillus sp*

V. DISCUSSION

The pH of the retting water decreased as retting progressed, tending towards acidity. This is in line with the findings of Fagbemi and Ijah (2005) and Obadina *et al*. (2005). The use of additives affected the rate of pH decrease with different additives having its specific rate. The use of potash showed the least decrease, alkaline initially, changing to neutral. The pH of the sample with potash was the highest (high alkalinity) on the first day

indicating the reaction of potash with the surrounding water to form alkali. From this study it can be seen that cassava tubers can ret in low acidity as well as low alkaline conditions.

The titratable acidity and cyanide content of the retting water increased as retting progressed, indicating that the acidity and cyanide of the tubers were released in the retting water for the tubers to detoxify. This is in agreement with the findings of Umeh and Odibo (2013 a & b) that during fermentation of fresh cassava tubers, the retting water turns acidic with high cyanide while the tubers tend towards alkaline with low cyanide. Also the samples with additives produced fufu samples with little amount of cyanide (0.03 - 0.05 mg/g) while the control yielded fufu sample with 0.8mg/g of cyanide. Using the additives reduced drastically the amount of cyanide in the fufu samples produced because the tubers retted completely. Potash and *Jatropha curcas* leaves produced fufu mash with least cyanide content (0.03mg/g) each and highest quantity of the mash. This indicates complete retting and detoxification of the tubers.

Retting old tubers without additives not only produced little quantity of wet fufu mash but also fufu sample that contains much cyanide. Although the amount of cyanide present in this sample is lower than the recommended level by SON (1985) standards, accumulation of this cyanide with time, (since fufu is a staple food) may result in cyanide toxicity (Okoro, 2007).

The effects of the additives on the retting ability of the tubers are shown in Table 1. The samples (A, B and C) with additives retted completely after 3 days while the control without additive did not ret.

Complete retting of the samples with additives gave large quantity of wet fufu mash greater than 3 kg (though wet) from the samples except the control which yielded about 1.5 kg as seen in (Table 3).

Table 2 presents the daily microbial count (yeast, mould, heterotrophic bacteria and lactic acid bacteria) in the retting water. The microbial counts in all the samples increased daily. This is in line with the findings of Fagbemi and Ijah (2005) and Achi and Akomas (2006). Heterotrophic bacterial count was predominant in all cases with the lactic acid bacterial count being the least. It was also observed that the microbial counts of the sample without additive ranked lowest. This may be that the additives provide a suitable environment for the microorganisms to trive. Metals (nails) are known to act as electrophilic catalysts. They are major growth factors in bioprocessing of molecules. Also the leaves of *Jatropha curcas* contain an antioxidant apigenin (Islam *et al*., 2011). It is possible that the apigenin plays a role in the fermentation of the tubers by removing free oxygen molecules, thereby reducing the oxygen tension of the fermenting tubers. This results in the amelioration of the environment for the growth of lactic acid bacteria and yeasts associated with the retting of the tubers.

All the fufu samples produced were of good quality except that sample A, with nail as additive, produced fufu sample which turned black few days after production. Of the three additives used, potash and the leaves of *Jatropha curcas* are recommended as the best as they gave the highest quantity of fufu with least amount of cyanide.

This study has shown that over-matured cassava tubers which are usually recalcitrant to retting can be retted for fufu production using nails, potash and fresh leaves of *Jatropha curcas* as additives. This will reduce wastage of the tubers or their diversion to other uses.

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