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Original Research Article

Production of Amylase Using Different Retting Methods during Retting of Cassava Tubers

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Abstract

This study looks into the possibility of using waste cassava retting water as a source of industrial amylase. It also compares the different amylase activity levels using four different retting methods. Different sizes of fresh cassava tubers were retted for fufu production. The sizes include unpeeled whole tubers (UPWT), peeled whole tubers (PWT), peeled sliced tubers (PST) and peeled grated tubers (PGT). Two retting methods were considered, the traditional method, in which the tubers were retted with tap water and the modified method, in which the tubers were retted in aseptic condition using sterile water and starter cultures. In the traditional method, amylase activity of the retting water increased daily reaching the peak of 2.75 µ /mol for UPWT, 4.53 µ /mol for the PWT, 4.60 µ /mol for PST, and 3.66 µ /mol for PGT on the fourth day. Microorganisms in the retting water were isolated daily and a total of nine organisms (*Candida tropicalis*, *Aspergillus sp*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Lactobacillus coryneformis*, *Citrobacter aerogenes*, *Rhizopus stolonifer,* Saccharomyces *cerevisiae and Klebsiella aerogenes*) were isolated. Five organisms were able to ret the tubers and yield considerable amount of amylase. These organisms were used as starter cultures to ret the peeled sliced tubers. They yield a greater amylase activity with the sample for *Lactobacillus coryneformis* yielding the highest amount of *up to* 7.34 µ /mol. Since cassava processing to fufu is usually accompanied with the production of stinking smelling waste water which constitute nuisance to humans, animals and aquatic life, the waste water can be utilized as a source of industrial amylase to save our environment.

Keywords: Amylase activity, Cassava tubers, fufu, industrial amylase, waste water

INTRODUCTION

Cassava, *Manihot esculenta* Crantz, is a perennial shrub with an edible starchy root, which grows in the tropical and sub-tropical areas of the world (Burrell, 2003). It is one of the staple foods consumed in Africa and other parts of the world. It was estimated (IITA) that the crop provides about 40% of all the calories consumed in Africa and ranks second only to cereal grains as the chief source of energy in Nigerian diet (Umeh et al., 2007).

Cassava plants grow 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. Nigeria is among the world's largest cassava producers and processes it into many food products like garri, fufu, elubo, tapioca etc (Okafor et al., 1998).

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. Production of these food products were normally accompanied with stinking waste water.

Fufu, commonly called “akpu or utala’’ by the local Igbo dialect, is a product of cassava obtained after retting or fermenting cassava fresh tubers for 3-5 days and It is a popular food consumed in the Eastern part of Nigeria especially by the Igbos. Fufu serves as hunger sustaining food for most rural areas where some villages of Nigeria consume it as breakfast and supper.

To get wet fufu, fresh cassava tubers are peeled, reduced in size, washed and retted in fresh clean water for 3 – 5 days. The main reason for the retting of the tubers is to soften it and eliminate or reduce drastically the toxic and poisonous constituents (cyanogenic glycosides) of the raw fresh cassava (Okafor et al., 1998). This retting or fermentation usually produces foul smelling waste water. In the areas where fufu is produced, environmental pollution is always the order of the day. Flies zoom around transmitting diseases as well as contaminating the food product. Those that are retting the tubers close to rivers often pour the waste water into the river thereby contaminating the river. This constitutes a great hazard to man and aquatic life using the river.

There is therefore great need to recycle these waste waters and use them in some reasonable ways. This study therefore looks into the amylase activity of the waste water. If the amylase is isolated and characterized, it can be a source of industrial amylase.

MATERIALS AND METHODS

Source of cassava used

A local variety of cassava, (*Manihot esculenta* Crantz), called ‘Onuanwuru’ and identified as TMS 30555 by the Enugu State Agricultural Development Agency (ADA) was cultivated in the farm at Reserve Forest in Ogurugu, Enugu State. The cassava tubers used for the research were allowed to stay up to one year and used between the ages of 12 to 18 months. Other chemicals and reagents used were obtained from the Departmental laboratory and were of analytical grade.

Methods

Laboratory retting of the tubers

The traditional method of wet fufu production was modified to ret cassava tubers (Umeh and Odibo, 2013a; Umeh and Odibo, 2013b) in the Department of Applied Microbiology and Brewing laboratory at Nnamdi Azikiwe University to obtain wet fufu. The retting water was analyzed daily. Below is the modified flow chart used for the retting of the tubers in the laboratory. (Figure 1)

The traditional method and modified method were used to ret the tubers in the laboratory. Four different sizes were retted. In all the methods, 10kg of fresh cassava tubers were retted using 5 liters of water

In the traditional method, the cassava tubers after harvest were washed, the head and tail portions were cut off, leaving the tubers unpeeled whole (UPWT) measuring about 5- 7 cm long. The peeled whole tubers (PWT) were peeled and washed, the head and tail portions were cut off, measuring also about 5- 7 cm long. For the peeled sliced tubers, they were peeled and sliced into smaller cylindrical portions measuring about 2 – 4 cm long (PST) and the peeled grated tubers (PGT) were peeled washed and grated into pulp using a manual Corona grinding machine. Retting was done in plastic buckets with lid for four days.

Microbial flora in the retting water was isolated daily and their ability to ret the tubers was tested. Those that ret the tubers were tested for their ability to yield amylase on solid media. Those that were able to ret the tubers and produce amylase were used as starter cultures to ret the peeled sliced tubers using the modified method (since the PST yield the highest amount of amylase in the traditional method).

In the modified method, sterility was observed. The tubers were peeled, sliced (2-4cm) washed in sterile water, then with 70% ethanol and rewashed with sterile water to reduce drastically their microbial load. They were socked in separate sterile air tight jars using sterile water. The different isolates were aseptically inoculated in their corresponding jars and allowed to ret. The amylase activity in their retting water was checked daily.

Assay for Amylase activity

Amylase activity was determined using the method of (Alli et al., 1998). One milliliter of each sample of the cassava retting water was pipetted into sets of clean test tubes arranged in racks. One milliliter of starch solution in 0.2M phosphate buffer of pH 6.9 was added in each of the tubes. The tubes were incubated in a water bath set at 30^{o}C for 30minutes. Reducing sugars were checked by adding two milliliters of the dinitrosalicylic acid (DNS) reagent to each of the tubes. The mixtures were boiled for 5 minutes and then cooled under running tap water. Thereafter 5 ml of cold distilled water was added to each of the tubes and the content of the tubes are allowed to stabilize for about 5 minutes at room temperature. The absorbance of the contents of the tubes was determined using a Jenway 6405 UV/V Spectrophotometer set at 540nm after zeroing the spectrophotometer with the reagent blank.

One unit of amylase activity was taken as the amount of enzyme in 1ml of crude enzyme that produced 1.0mg

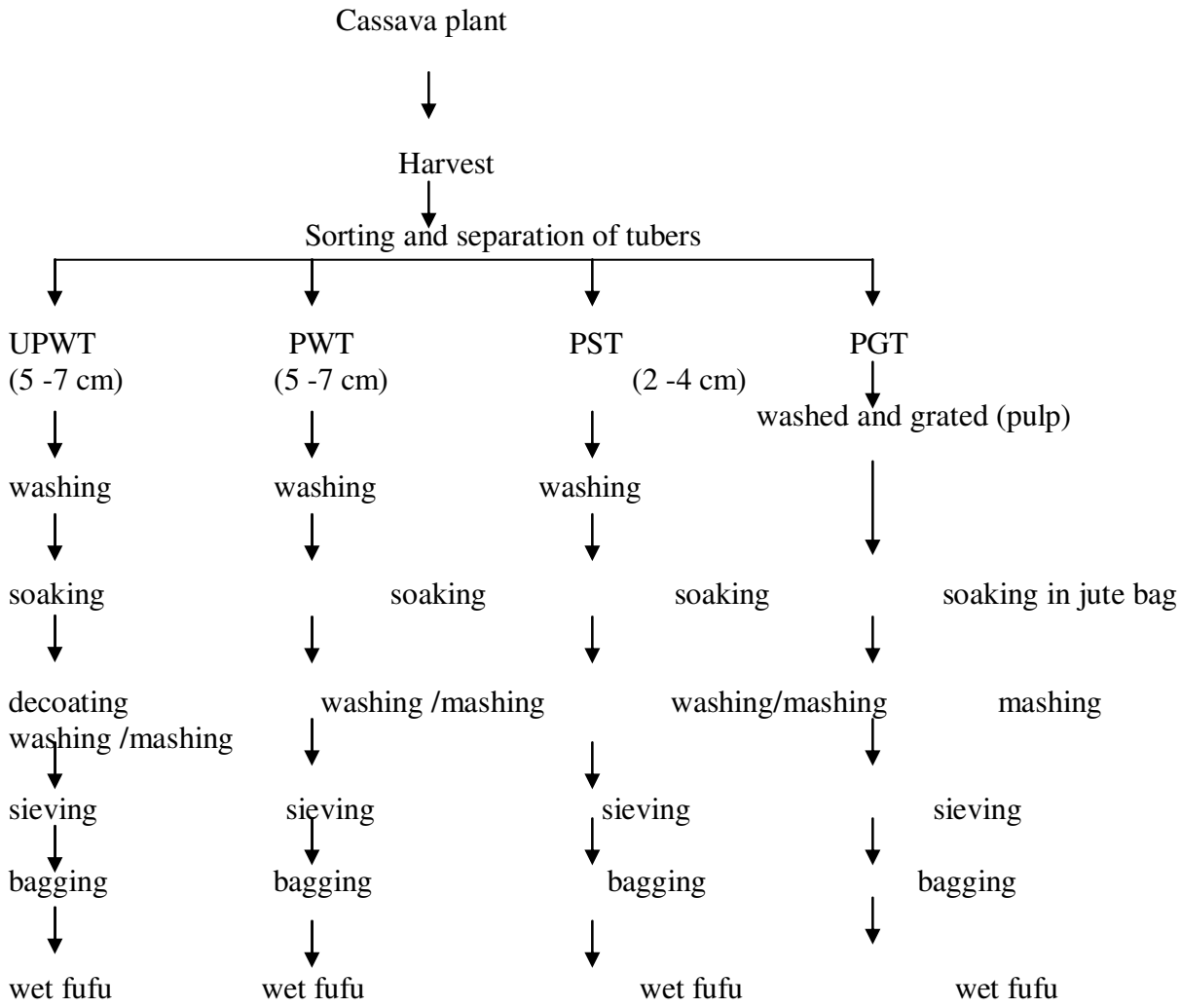

Figure 1. Flow chart for the laboratory preparation of the fufu samples

of the reducing sugar as glucose under the assay condition (Oboh, 2005).

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.

Microbiological analysis

The pour plate method (Collee and Mile, 1998) was used to determine the growth of the organisms in the retting water. The organisms were sub cultured in specific media before identification. Identification of the bacterial isolates was carried out using the methods stipulated in the Bergey's Manual of Systemic Bacteriology (Collee and Mile, 1998). Fungal isolates were characterized and identified (Pitt and Hocking, 1977; Barnett et al., 1990).

Ability of the isolates to yield the amylase

The ability of each isolate to yield the enzyme, amylase was checked (Ajayi and Fagade, 2003). Each of the isolated organisms was cultured in starch medium containing 2g of soluble starch in 100 ml of nutrient agar. The organisms were incubated according to their incubation conditions. At the end of incubation the plates were flooded with iodine solution. Clear zones in the media indicated a positive reaction while blue – black colouration on the plates showed negative reaction.

RESULT AND DISCUSSION

Nine organisms (five bacteria, two yeasts and two moulds) were isolated from the retting water as shown in

Table 1. Morphological and biochemical characteristics of the bacterial isolates

S/No	Colony morphology	Gram stain	Spore test	Motility	Urase	Catalase	Citrate	MR	VP	Indole	H_2S test	Gelatine	KCN	Coagulase	Glucose	Lactose	Maltose	Sucrose	Mannitol	Probable organisms
1	Cream, smooth raised, and circular	+ve cocci in clusters	-	-	-	+ve	+ve	-	-	-	-	-	-	+	A	-		-	-	*Staphylo-coccus aureus*
2	Smooth mucoid and circular	-ve short rods, small capsules	-	+	-	+	+	-	+	-	-	-	+	-	A	A		-	A	-	*Enterobacter*
3	Smooth mucoid convex with distinct edges	-ve short rods in chains & singles	-	+	-	+	+	+	-	-	+	-	+	+	-	AG	A	A	A	-	*Citrobacter aerogenes*
4	Slimy mucoid dry white, yellow when old	-ve short rods in chains & singles	-	-	+	+	+	-	+	-	-	-	+	-	-	AG	A	A	A		*Klebsiella aerogenes*
5	Gray to White on TJA	+ve long rods in chains & singles	+	-	-	-	nd	nd	nd		nd		nd		nd	nd	nd	nd	nd		*Lactobacillus* coryneformis

Key: + = positive, - = negative, A = Acid, AG = Acid & gas ,TJA = Tomato juice agar

Table 2. Morphological characteristics of the yeast isolates

			Sugar fermentation							Sugar assimilation							
S/no	Culture characteristics	Cell morphology	Glucose	Maltose	Lactose	Galactose	Sucrose	Dextrose	Mannitol	Glu	Malt cose	Lact ose	Gala ose	Suc ctose	Dex rose	Mani trose	Probable tol organisms
1	Cream, white Smooth & flat	Oval budding cells with Pseudohyphae	+	+	-	+	+	+	-	+	+	-	+	+	+	-	*Candida tropicalis*
2	Smooth Cream White to tan hairy	Budding cells	+	+	-	-	+	-	+	+	+	-	-	+	-	-	*Saccharomyces cerevisiae*

Table 1, 2 and 3. The organisms were tested for their ability to ret the tubers and produce amylase enzyme and the result was shown in Table 4. Four organisms (*Candida tropicalis*, *Lactobacillus coryneformis*, *Saccharomyces cerevisiae* and *Klebsiella aerogenes*) were able to ret the tubers completely as well as produce amylase. Amylase activity of the retting water during the traditional method was recorded in Table 5. The unpeeled whole tubers (UPWT) yielded the least amount of amylase while the peeled sliced tubers (PST) yielded the highest amount (2.75µ /mol and 4.6µ /mol respectively). Amylase activity of the retting water using the starter cultures to ret the PST for four days were shown in Table 6. *Saccharomyces cerevisiae* yield the least amount (5.67µ /mol) and *Lactobacillus coryneformis* yield the highest amount 7.34 µ /mol.

Retting of cassava tubers for fufu production is always accompanied with unwanted waste water. This waste water if not taken care of constitute nuisance to the environment. They retting water had been shown to possess amylase activity (Alli et al., 1998). The amylase activity is found to differ when different sizes of tubers are retted (Table 2) and increase the more if starter cultures are used for retting (Table 3). PST showed the highest activity while UPWT showed the least activity (Table 2) on the fourth day. According to previous works, large quantity of enzymes can be extracted from fermenting waste water when high enzyme activity is detected (Alli et al., 1998). Therefore the PST with highest activity was used in the modified method and the starter cultures showed a quite increase in the activities. The starter cultures used showed a wide range increase in the

Table 3. Morphological characteristics of the mould isolates

Sno	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*
2	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. Ability of the isolates to ret the tubers and produce amylase

Sno	Organisms isolated	Retting ability	Amylase production
1	*Staphylococcus aureus*	+	+
2	*Candida tropicalis*	+ +	+
3	*Saccharomyces cerevisiae*	+ +	+
4	*Aspergillus sp*	-	+
5	*Lactobacillus coryneformis*	+ +	+
6	*Enterobacter aerogenes*	-	-
7	*Rhizopus stolonifer*	-	+
8	*Klebsiella aerogenes*	+ +	+
9	*Citrobacter aerogenes*	-	-

Retting ability
Key: + + Complete retting
+ Partial retting
- no retting

Amylase production
+ enzyme production
- no production

Table 5. Amylase activity of the traditional method

Days	UPWT (µ/mol)	PWT (µ /mol)	PST (µ /mol)	PGT (µ /mol)
1	1.73	2.16	2.61	1.26
2	2.14	3.11	3.45	1.84
3	2.55	3.64	4.28	2.71
4	2.75	4.53	4.60	3.66

Table 6. Amylase activity using starter cultures on the fourth day

Organisms	Amylase activity (µ/mol)
Candida tropicalis	5.82
Saccharomyces cerevisiae	5.67
Klebsiella aerogenes	6.11
Lactobacillus coryneformis	7.34

enzyme activity (Table 3). Retting with Lactobacillus coryneformis and Klebsiella aerogenes produced the highest enzyme activity and showed the possibility of isolating much enzyme from their retting water. Using Saccharomyces cerevisiae as starter culture showed the least activity though it exceeds those of the traditional method. It was believed that the amylase activity of the retting water can be the combined function of the organisms and the tubers.

Nine organisms were isolated from the retting water in the four days. Some of these organisms did not ret the tubers while some caused partial retting (Table 1). This calls for the need to use starter cultures to ret cassava tubers. All the starter cultures were able to ret the tubers and yield amylase (Table 3) more than the amount observed in the traditional method. These organisms help to detoxify the tubers and release their amylase in the retting water (Alli et al., 1998). Those organisms that did not ret the tubers and those that caused partial retting may be contaminants. Their entrance in the fermenting system may be through the water, the fermenting buckets or the cassava tubers themselves. Using starter cultures to ret the tubers aseptically will result in the production of a more hygienic wet fufu mash and eliminate drastically the unwanted microorganisms which were observed in the traditional method. The use of starter cultures also yields large amount of amylase which can be explored by our industries as a source of industrial amylase.

Amylase enzymes are very useful in our food and beverage industries. If this amylase from cassava waste water were purified, characterized and analyzed, they can serve as cheap sources of industrial amylase. This will also reduce the rate of environmental pollution which is observed in indiscriminate disposal of the waste water after fermentation.

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