

Cassava Retting Water: An Alternative Source for Industrial Cellulase (Enzyme)

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ABSTRACT

Cassava fermentation is one of the teaming businesses in almost all the tribes of Nigeria. Among all the methods of cassava processing to food, fermentation is the most used. This produces foul smelling waste water that causes environmental pollution to man and animals. The retted cassava water was checked for cellulase (enzyme) activity to see if it can be a source of cellulase for used in food, paper, textile and other industries. The aim of this work is to seek a way of utilizing the waste water as source of enzymes as this will reduce the importation of these enzymes and make them available always. Cassava tubers were peeled, cut into cylindrical portions of about 3-5 cm and washed. Two hundred grams of the washed tubers were soaked in 5 liters of water and allowed to ret. The retting water was analyzed daily for titratable acidity, cyanide content, *pH*, cellulase activity and the microbial flora were isolated and identified. Results showed that titratable acidity rose from 0.20 to 2.76 mg/g and cyanide content increased from 0.28 to 4.69 mg/ml while *pH* fall from 7.2 – 6.0 tending acidic. Retting started on the 2nd day and complete retting was achieved on the 4th day. β -glucosidase activity rose from 0.05 to 8.0 μ /mol, Filter paper activity increased from 0.06 to 7.5 μ /mol and carboxyl methyl cellulase (CMC) activity increased from 0.05 to 7.7 μ /mol. Ten organisms (*Aspergillus fumigatus*, *Rhizopus stolonifer*, *Bacillus subtilis*, *Candida utilis*, *Citrobacter sp*, *Enterobacter aerogenes*, *Lactobacillus coryneformis*, *Saccharomyces cerevisiae*, *Staphylococcus aureus*, *Streptococcus faecalis*) were isolated from the retting water. Daily increase in the enzyme activities showed that cassava retting when done in a large scale can yield large quantity of enzymes. This will reduce the importation of industrial enzymes and reduce the environmental pollution caused by the waste water.

KEYWORDS: Cassava fermentation, enzyme activity, cellulase, fufu production, pollution

INTRODUCTION

Cassava, (*Manihot esculenta* Crantz), is a perennial crop with a tuberous edible root. It is a good famine relief crop that is eaten by all classes of people. The crop had become widespread and has assumed its current importance as food since the 20th century. It is cultivated through vegetative propagation throughout the lowland tropics for its starchy edible roots (Oyewole, 2002; Ganiyu, 2005). It can be cultivated in an ordinary farmland alone or in combination with

other crops like maize, yam, beans etc. When mature, the roots of cassava plant develop into starchy tubers that can be processed into food.

In Nigeria, these tubers are processed using different methods to obtain different types of food products. It can be cooked, sliced, soaked overnight in clean water, washed and eaten raw or dried as tapioca. It can also be processed into *garri*, *fufu*, *ketonte*, *chickwangu*, etc (Okafor, 1998).

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Retting of fresh cassava tubers for 3-5 days yield wet fufu mash which can be dried into fufu powders (Umeh, *et al.*, 2014). Fufu is a product of cassava obtained after retting fresh cassava tubers for 3-5 days (Sobowale *et al.* 2007; Obadina *et al.* 2006; Umeh and Odibo, 2013). It is a popular food consumed in the Eastern part of Nigeria especially by the Igbos. Fufu production had recently assumed the mode of occupation to many rural dwellers in most parts of this country. The single most important method of cassava processing is by fermentation. This reduces the toxic and poisonous cyanogenic glucosides of the raw fresh tubers (Okafor *et al.*, 1998). It also makes the starch composition of cassava roots available and suitable for consumption as a major food component in the form of boiled paste, flour and granules in the many different dishes prepared according to cultural differences (Essers 1986; Rosling *et al.*, 1993; Okafor *et al.*, 1998; Umeh and Odibo, 2013 a and b).

Cassava fermentation is always accompanied by foul odour waste water which constitutes nuisance to the environment when discarded. Environmental pollution is a major characteristic of fufu producing plants. This work therefore looks into cellulase activities of the waste water and suggests the possible work on it as a source of industrial cellulase. If the enzymes are extracted and purified, it will go a long way to reduce the importation of enzymes by industries which is expensive and making the products of these industries costly. The enzymes will also be available at all times.

MATERIALS AND METHODS

Source of cassava and materials used

Cassava, (*Manihot esculenta* Crantz), variety identified as TMS 30555 by Anambra State Agricultural Development Programme (ADP) was cultivated at the Nnamdi Azikiwe University, Awka, premises. The mature tubers (10 months old) were harvested and immediately carried to the laboratory for processing. Other materials and reagents used for the work were obtained from the laboratory of the Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka.

Methods

Laboratory fermentation of the wet fufu

The laboratory method of wet fufu production by Obadina *et al.* (2006) was used to ret the tubers to obtain wet fufu mash. The tubers were selected and accompanied soil washed off. They were peeled and cut into small cylindrical portions. About 200 g of the cut tubers were washed with clean water, and steeped in 5 liters of clean water in a 6 liter capacity bucket for 96 hours. Daily titratable acidity, cyanide content, *pH*, the retting water and the retting ability of the

tubers were checked. Also different cellulase activities (β - glucosidase, filter paper and carboxyl methyl cellulase activities) in the retting water were determined and microbial floras in the retting water were isolated and identified. The figure below is a flow chart for the laboratory production of wet fufu mash as used in the work.

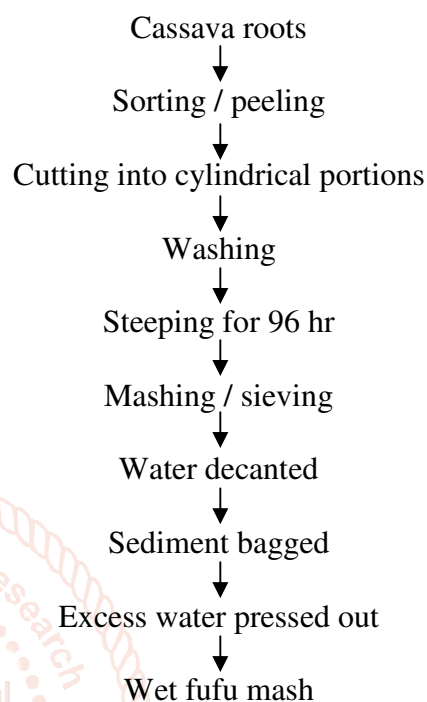


Fig. 1: Laboratory method of wet fufu mash production

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).

Determination of the Total Cyanide content of the samples

The Grignard test as used by Okafor *et al.*, (1998) was used. Standard cyanide curve was first prepared. Twenty milliliters of the filtered waste water was pipetted into a 100 ml flask and Ten milliliters of alkaline sodium picrate solution was added in the flask and mixed. Ten milliliters of the mixture was transferred in a test tube. The tube was incubated in a water bath set at 94°C for 5 minutes and allowed to cool at room temperature. Absorbance of the mixture was read from a Jenway 6405UV/visual spectrophotometer at 540 nm after using distilled water to zero the spectrophotometer. The absorbance was the average of two readings. Then the concentration of potassium cyanide in the sample was calculated from the standard cyanide curve.

Determination of the Titratable acidity

The titratable acidity was determined using the method described by Ogiehor and Ikenebomeh

(2005). This was done by titrating 0.1M sodium hydroxide (NaOH) against the sample using 2 drops of phenolphthalein as indicator. The titratable acidity was calculated as mg of lactic acid in the sample.

Cellulase activity in the retting water

The cellulase enzyme activities checked include; β -Glucosidase, filter paper and carboxyl methyl cellulase activities.

1. β -Glucosidase assay: The method of Bernfold (1955) as used by Umeh (2011) was used. A sample (0.5 ml) of the retting water was transferred into sterile test tubes in racks and labeled. Equal volume of 0.05M citrate buffer of pH 4.5 was added, followed by 0.5 ml of 1% salicin solution. The mixtures were incubated in a water bath set at 40°C for one hour after which 1ml of DNS reagent was added in the tube. The content of the tube was mixed and boiled for 5 minutes in a water bath and allowed to cool at room temperature. Five milliliters of distilled water was added into the tube and allowed to stabilize for 5 minutes. The absorbance of the resulting solution was measured using a Jenway 6405 UV/visual spectrophotometer set at 540nm. Enzyme activity was measured as the number of μ mol of reducing sugar (glucose) released per minute per ml of the test solution. The process was repeated twice and the result taken was the average of two readings.

2. Filter paper assay

Strips of filter paper (0.2g) were added into a sterile test tube in the rack. One milliliter of the sample was pipetted in it and 0.5 ml of 0.5M citrate buffer of pH 4.8 was added. The mixture was treated as in the β -glucosidase activity above.

3. Assay for Carboxyl methyl cellulase activity

A sample (0.5ml) of the retting water was added into sterile test tubes in racks. 0.5 ml of 1% carboxyl methyl cellulose (CMC) solution was added. Also

0.5ml of 0.5M citrate buffer of pH 4.8 was added in the tube and the resulting solution treated as the β -glucosidase activity above. Cellulase activity was measured in terms of international units defined as the number of μ mol of reducing sugar released per minute, per ml of the test solution (Lakshmikan, 1990).

Identification and characterization of the microbes

The pour plate method of Collee and Miles (1998) was used to isolate the microorganisms in the retting water. Identification and characterization of the bacterial isolates was carried out as stipulated by Cheesbrough (2000). The method described by Barnett *et al.* (2000) was used to characterize the 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.

Results

From the study, it was observed that retting started on the 2nd day and was complete on the 4th day. Analysis of the retting water showed that pH increased from 7.2-6.0, titratable acidity rose from 0.20-2.76 mg/g and cyanide content rose from 0.28 - 4.69 mg/ml Table 1. Cellulase activities (β -glucosidase, filter paper and carboxyl methyl cellulase [CMC]) checked was all positive reaching 8.0, 7.5 and 7.7 μ /mol respectively (Table 1). Ten organisms (*Aspergillus fumigatus*, *Rhizopus sp*, *Bacillus subtilis*, *Candida utilis*, *Citrobacter sp*, *Enterobacter aerogenes*, *Lactobacillus coryneformis*, *Saccharomyces cerevisiae*, *Staphylococcus aureus*, and *Streptococcus faecalis*) were isolated (Table 2). Table 2 showed the morphological and biochemical characteristics of the bacterial isolates, Table 3 and 4 depict the morphological and biochemical characteristics of the yeast and mould isolates.

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

Day's	pH	Titratable acidity (mg/g of lactic acid)	Cyanide content (mg/ml)	Retting ability	β -glucosidase (μ /mol)	FP (μ /mol)	CMC (μ /mol)
1	7.2	0.20	0.28	-	0.05	0.06	0.05
2	6.7	0.41	1.22	+	2.5	2.3	2.3
3	6.3	1.56	2.48	++	5.5	5.8	5.0
4	6.0	2.76	4.69	+++	8.0	7.5	7.7

Key:

FP: Filter paper activity

CMC: Carboxyl methyl cellulose activity

- No retting

+ Slight retting

++ Moderate retting

+++ Complete retting

Table 2: Morphological and biochemical characteristics of the bacterial isolates

S/ No	Colony morphology	Gram stain	Spore test	Motility	Urase	Catalase	Citrate rate	M R	V P	Indole	H ₂ S test	Gelatin	KC	Coagulase	Gluconose	Lactose	Maltose	Sucrose	Manitol	Probable organisms	
1	Cream, smooth raised, and circular	+ve cocci in clusters	-	-	-	+ve	+ve	-	-	-	-	-	-	+	A	-	-	-	-	<i>Staphylococcus aureus</i>	
2	Smooth mucoid and circular	-ve short rods, small capsules	-	+	-	+	+	-	+	-	-	-	+	-	A	A	-	A	-	<i>Enterobacter aerogenes</i>	
3	Smooth mucoid convex with distinct edges	-ve short rods in chains & singles	-	+	-	+	+	+	-	-	+	-	+	+	-	AG	A	A	A	<i>Citrobacter sp</i>	
4	Cream, rough opaque, circular	+ve long rods in chains	+	-	-	+	+	+	-	-	-	-	+	-	AG	-	-	-	-	<i>Bacillus subtilis</i>	
5	Discoïd colony	+ve cocci in chains	-	-	-	- confirmed by growth in bile salt and haemolysis on blood agar										<i>Streptococcus faecalis</i>					
6	Gray to White on TJA	+ve long rods in chains & singles	+	-	-	- confirmed on growth on Tomato juice agar										nd	nd	nd	nd	nd	<i>Lactobacillus coryneformis</i>

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

Table 3: Morphological characteristics of the yeast isolates

S/ no	Culture characteristics	Cell morphology	Glucose	Maltose	Lactose	Galactose	Sucrose	Dextrose	Manitol	Gluconose	Maltose	Lactose	Galactose	Sucrose	Dextrose	Manitol	Probable organisms
1	Cream, white Smooth & flat	Budding cells with Pseudohyphae	-	-	-	-	+	+	-	-	+	-	-	+	+	-	<i>Candida utilis</i>
2	Smooth Cream White to tan, hairy	Budding cells	+	+	-	-	+	-	+	+	+	-	-	+	-	-	<i>Saccharomyces cerevisiae</i>

Table 4: Morphological characteristics of the mould isolate

Young culture morphology	Old culture morphology	Microscopy	Texture	Days	Probable organisms
Whitish with Yellow reverse	Blue-green to dark green	Double branching septate hyphae short conidiophores	Powdery and velvety	3-4	<i>Aspergillus fumigatus</i>
Dense grayish cottony	Green to brown to black filling the plate	Oval non-septate hyphae with sporangiophores	Fluffy and cottony	2-3	<i>Rhizopus stolonifer</i>

Discussion

The fermentation of cassava roots allows softening of the tubers for further processing and the reduction of the potentially toxic cyanogenic glucosides present in

the roots (El – Tinay *et al.*, 1984; Oyewole, 2002). Retting of the tubers in this work was complete in 4 days. This was in conformity with the findings of other researchers (Okafor *et al.* 1998; Oyewole, 2002;

Obadina *et al.* 2006) who postulated the fufu can be produced after retting fresh cassava tubers for 3-5 days.

The *pH* of the retting water decreased as the fermentation days increased, tending towards acidity. The finding did not agree with the report of Fagbemi and Ijah (2005) in which the *pH* of the surrounding water was decreasing as retting days increased. Titratable acidity of the retting water and the cyanide content were increasing daily. These showed that the soaked tubers were releasing their acidic and cyanide content inside the retting water thereby detoxifying the tubers. This is in line with the findings of Fagbemi and Ijah (2005) who observed that cassava fermentation to fufu yields retting water high in titratable acidity and cyanide content with a corresponding reduces titratable acidity on the tubers.

Ten organisms (*Aspergillus fumigatus*, *Rhizopus stolonifer*, *Bacillus subtilis*, *Candida utilis*, *Citrobacter sp*, *Enterobacter aerogenes*, *Lactobacillus coryneformis*, *Saccharomyces cerevisiae*, *Staphylococcus aureus* and *Streptococcus faecalis*) were isolated from the retting system. Most of these organisms had been reported by many researchers (Oyewole, 1990; Oyewole, 2002; Oboh, 2005; Achi and Akomas, 2006; Obadina *et al.*, 2006 and Sobowale *et al.*, 2007). Traditional method of fermentation which involves uncontrolled retting of the tubers was used in the work. This is the reason for the isolated organisms in the system. Uncontrolled retting method although the simplest involves a complex microbial processes as seen in Tables 2, 3 and 4. The number and types of organisms in a fermenting system may result in variations in the quality of fufu produced (Daeschel *et al.*, 1987; Ogumbawo *et al.*, 2004). Lactic acid bacteria which are known to utilize sugars to form mixed acids were among the isolated organisms. Their involvement in fermentation had been reported by Adegoke *et al.* (1993) and Sobowale *et al.* (2007).

There is a noticeable cellulase activity in the retting. The three cellulase activities were increasing as retting increased. Little or no activity was recorded when no retting was observed. Since retting helps in softening the tubers, it makes room for the microorganisms in the retting system to release their cellulase in to the retting water.

In conclusion, cassava waste water after retting the tubers for fufu production possesses the three different cellulase (β -glucosidase, filter paper and CMC) activities. These enzymes can be harvested from the retting water and purified for use in our industries to minimize their importation and risk of isolating them from pathogenic organisms.

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