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Research paper

Amylase Activity during Retting of Cassava Tubers for Wet Fufu Mash Production.

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ABSTRACT

This study looks into the possibility of utilizing cassava waste water as a source of industrial amylase. Traditional retting method was used to ret the tubers in the laboratory. Amylase activity of the retting water was checked daily and found to increase daily from 2.75µ/mol on the first day to 9.48µ/mol on the fourth day when retting was complete. Microorganisms in the retting water were isolated daily and a total of eleven organisms (*Candida tropicalis, Aspergillus sp, Staphylococcus aureus, Staphylococcus epidermidis, Enterobacter aerogenes*, *Lactobacillus coryneformis, Citrobacter aerogenes, Rhizopus stolonifer, Bacillus subtilis, Saccharomyces cerevisiae and Klebsiella aerogenes*) were isolated. The ability of these organisms to produce amylase invitro was checked using an appropriate solid media. All the organisms were able to produce amylase except *Staphylococcus epidermidis, Enterobacter aerogenes and Citrobacter aerogenes.* 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 in our environment, the waste water can be utilized as a source of industrial amylase.

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

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 [1,2]. It was estimated 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 [3]. Cassava tubers cannot be consumed without processing due to the presence of poisonous cyanogenic glycosides present in the roots. Also harvested tubers cannot be stored after harvest without processing due to their high water content. The only method of cassava processing is by fermentation and through this process it can be processed into fufu, garri, lafun and other food products.

Fufu, commonly called “akpu or utala” by the local Igbo dialect, is a food product of cassava obtained after fermenting fresh cassava tubers for 3-5 days [4] and it is a popular food consumed in the Eastern parts of Nigeria especially by the Igbos. Fufu serves as hunger sustaining food for most rural areas where some villagers 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 [5]. This retting usually produces foul smelling waste water. In the areas where fufu is produced, environmental pollution is always the

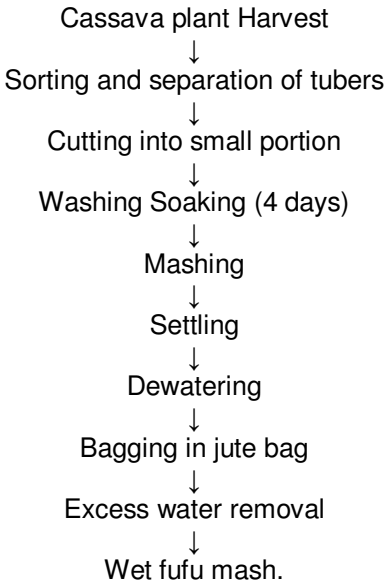
order of the day. Flies zoom around transmitting diseases as well as contaminating the food product and other items around. Those that are retting the tubers close to rivers often pour the waste water into the river thereby contaminating the river and rendering it unfit for survival of aquatic animals. 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 and considered it as a good source of industrial amylase.

MATERIALS AND METHOD

Source of cassava used: A local variety of cassava, (*Manihot esculenta* Crantz), called 'Onuanwuru' and identified as TMS 30555 by the Anambra state Agricultural Development Program (ADP) was cultivated in the farm at the premises of the Nnamdi Azikiwe University, Awka. The cassava tubers were allowed to mature for up to one year and used between the ages of 12 to 18 months. The tubers were transported to the laboratory immediately after harvest. All the reagents and chemicals used for the study were obtained from the Microbiology Department and are of analytical grade.

Laboratory fermentation and preparation of wet fufu: The method of wet fufu production [2,4] were modified to ret the cassava tubers to obtain wet fufu in the Microbiology laboratory at Nnamdi Azikiwe University.

Figure 1: Flow chart for the laboratory preparation of the fufu sample.



Cassava tubers were harvested in the farm and taken to the laboratory. Traditional method of cassava fermentation was used to ret the cassava tubers in the laboratory. The tubers were peeled, cut into small cylindrical shapes and washed. They were packed in a plastic bucket with lid and the water added to cover the tubers. The tubers were retted for four days and the retting monitored daily. Retting ability of the tubers, microbial flora in the retting water and amylase activity of the retting water were monitored daily for the four days when retting was complete. Ability of the isolates to produce the enzyme amylase enzyme was also checked.

Assay for Amylase activity: Amylase activity of the retting water was determined using the method described below [6]. One milliliter of the cassava retting water was pipette 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°C for 30 minutes. Reducing sugars were checked by adding two milliliters of Dinitrosalicylic acid (DNS) reagent to each of the tubes. The mixtures were boiled for 5 minutes and then cooled under running tap water. Thereafter, 2 ml of cold distilled water was added to each of the tubes and the content of the tubes were allowed to stabilize for about 5 minutes under room temperature. The absorbance of the contents of the tubes were determined using a Jenway 6405 UV/V Spectrophotometer set at 540 nm after zeroing the spectrophotometer with reagent blank.

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 [2,4] .

Microbiological analysis: Identification of the bacterial isolates was carried out using the methods stipulated in the Bergey's Manual of Systemic Bacteriology [7,8]. Fungal isolates were identified using the method of Pitt and Hocking [9].

Ability of the isolates to yield the amylase: Ability of the isolates to yield the enzyme, amylase, was determined [10]. Each of the isolated organisms was cultured in starch medium containing 2 g 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.

RESULTS AND DISCUSSION

The only method of presenting cassava food products suitable for consumption is by fermentative processing. It is believed that microorganisms and their enzymes ferment the tubers and render them useful [11].

During the retting process in this study, eleven organisms were isolated from the retting water. Six bacteria and one lactic acid bacterial isolates as shown in Table 1, two yeast and two mould isolates as presented in Tables 2 and 3 respectively. Ability of each isolate to ret the tubers and produce the enzyme, amylase was as shown in Table 4 and the daily amylase activity of the retting water was recorded in Table 5. All the isolates were able to produce amylase except *Staphylococcus epidermidis, Enterobacter aerogenes* and *Citrobacter aerogenes.* Clear zones in the media used to check for amylase production indicates a positive reaction while blue – black colouration on the plates showed negative reaction. One unit of amylase activity was taken as the amount of enzyme in one milliliter of crude enzyme that produced 1.0 mg of the reducing sugar as glucose under the assay condition [6].

The tubers retted completely in four days. Retting of cassava tubers for fufu production is always accompanied with unwanted foul-odoured waste water. This waste water if not taken care of constitute nuisance to the environment. Flies and other unwanted insects breed in them and if disposed in the water bodies, also constitute hazard to the aquatic life.

Fermentation is an important process in the processing of almost all the cassava food products in Africa. It had been reported to be responsible for product stability, flavour development and cyanide elimination [12, 2]. It also helped to soften and detoxify the tubers. Many researchers had reported the presence of different types of amylase in the fermentation of different plant products [6, 13]. From the above findings, it can be deduced that fermentation of plant products can yield amylase which may be useful in our food industries.

The amylase activity of the retting water increased from 2.75µ/mol the first day to 9.48µ/mol the fourth day. It can be said that the amylase activity of the retting water is the combined function of the organisms and the tubers.

Table 1: Characterization and identification of the bacterial isolates

S/no	Colon morph ology	Gram stain	Spore	Motility	Urase	Cataal se	Cit rate	M R	V. P	Ind ole	H_2S	Gelatin e	KCN	Coagul ase	Glucos e	Lactos e	Maltos e	Sucros e	Minitol	Probable organism
1	Cream, rough, opaque ,& circular	+ ve long, rods in chains	+	+	-	+	+	+	-	-	-	-	-	-	AG	-	-	-	-	Bacillus subtilis
2	Yellow smooth rai-sed circular	+ ve cocci clusters	-	-	+	+	+	-	-	-	-	-	-	-	AG	A	-	+	-	Staphylococcu s epi- dermidis
3	Smooth mucoid and circular	- ve short rods small capsule -s (+)	-	+	-	+	+	-	+	-	-	-	+	-	A	A	-	A	-	Enterobac-ter aerogenes
4	Cream smooth raised circular	+ ve cocci in clusters	-	-	-	+	+	-	-	-	-	-	-	+	A	-	-	-	-	Staphylococcu s aureus
5	Slimy mucoid dry white yellow at old age	- ve short rods chains & singles	-	-	+	+	+	-	+	-	-	-	+	-	-	AG	A	A	A	Klebsiella aerogenes
6	Mucoid smooth convex cir- cular, disti-nct edged	-ve short rode in chains & clusters	-	+	-	+	+	+	-	-	+	-	+	-	AG	A	A	A	-	Citrobacte-r aerogenes
7	Gray to white on TJA	+ve long rods in chains and singly	+	-	-	-	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	Lactoba-cillus corynefor-mis

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

Table 2: Characteristics and morphology of the yeast isolates

		Sugar Fermentation							Sugar Assimilation							
S/No.	**Cell morphology**	**Glucose**	**Maltose**	**Lactose**	**Galactose**	**Sucrose**	**Dextrose**	**Manitol**	**Glucose**	**Maltose**	**Lactose**	**Galactose**	**Sucrose**	**Dextrose**	**Manitol**	**Probable organisms**
1	Budding cells and pseudo-hyphae	+	+	-	+	+	+	-	+	+	-	+	+	+	-	Candida tropicalis
2	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	102 *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 stolonifer*

Table 4: Ability of the isolates to ret the tubers and produce amylase

S/No	Organisms isolated	Retting ability	Amylase production
1	*Candida tropicalis*	+ +	+
2	*Aspergillus fumigatus*	-	+
3	*Staphylococcus aureus*	+	+
4	*Staphylococcus epidermidis*	+	-
5	*Enterobacter aerogenes*	-	-
6	*Lactobacillus coryneformis*	+ +	+
7	*Citrobacter aerogenes*	-	-
8	*Rhizopus stolonifer*	-	+
9	*Bacillus subtilis*	+ +	+
10	*Saccharomyces cerevisiae*	+ +	+
11	*Klebsiella sp*	+ +	+

Key: **Retting ability** (+ + Complete retting; + Partial retting; - no retting). **Amylase production** (+ enzyme production; - no production)

Table 5: Daily amylase activity of the retting water

Days	Amylase activity (µ/mol)
1	2.75
2	3.55
3	6.74
4	9.48

CONCLUSION

In this work, chitin was obtained from a fish scale waste. This biopolymer was used for the removal of Cr (VI) from aqueous solutions. Both Langmuir and Freundlich isotherms were used to successfully describe the adsorption process. The adsorption is exothermic, pH-dependent and maximum adsorption capacity was found at pH 6-8. The negative values of DG and DS implies a decrease in adsorption energy and an increase in the feasibility of the adsorption process at lower temperature. Finally, this work revealed the applicability of a waste material as a potential industrial biomaterial for wastewater treatment.

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