Original Research Article

Enzymatic potentials of microorganisms associated with cassava retting

ABSTRACT

The enzymatic potentials of microorganisms isolated from retting cassava were evaluated for the purpose of pectinase, cellulase and amylase activities. Cassava tubers of 12 months old were collected from 3 different sources: Ahieke, Ndoru and Umuariga in Abia State. They were hand peeled, cut into cylinders, washed, submerged into water and allowed to ret. After retting a dilution of the retted tubers were inoculated into different media plates: De-Man Rogosa Sharp agar, Sabouraud Dextrose agar, Nutrient agar, Mannitol salt agar and MacConkey agar and incubated at 30^0C for 3-5days. 35 isolates was identified in the retting cassava samples which are bacteria 25(71.43%) and fungi 10(28.57%). The bacterial isolates identified include *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Lactobacillus* spp and *Proteus* spp while fungi isolated were *Aspergillus niger*, *Rhodotorula* spp and *Saccharomyces cerevisiae*. The total viable counts of the isolates increased as the retting hours increased. *Lactobacillus* spp and *Saccharomyces cerevisiae* dominated the later stage of the fermentation. *Bacillus subtilis* has the highest percentage occurrence 8(22.9%) while *Rhodotorula* spp has the lowest percentage occurrence 2(5.7%). The production of amylase enzyme was recorded with all the isolates with the exception of *Staphylococcus aureus*.. Production of cellulase and pectinase enzymes was selective among the isolates. Results from this study shows that enzymes like amylase, pectinase and cellulase from microorganisms played an important role in retting of cassava tubers.

Key words: Cassava tubers, enzymes, fermentation, microorganisms, retting

1.0 INTRODUCTION

Cassava (*Manihot esculenta crantz*) is a potential shrub with an edible starchy root, which grows in the tropical and sub-tropical areas of the world [1]. It is one of the staple foods consumed in Africa and other parts of the world. 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 [2]. The tuber consists of 64-87% starch depending on the stage of the growth or maturity of the tuber but very limited quantities of protein, fats, vitamins, and minerals [3]. The roots contain considerable quantities of anti-nutrients factors, cyanogenic glucoside. The cyanogenic potential of cassava is by far the single factor that adversely constraints the use of cassava as food and feed for animals. This is as a result of the toxic effect of cyanide on humans and animals that rely on cassava as food. Cassava has bitter and sweet varieties. The presence of cyanogenic compounds which predominates in bitter varieties and processes to reduce them were recently reviewed by [4].

Different processing techniques are used to reduce cassava toxicity and selected antinutrients such as, boiling, drying, steaming, baking, frying, soaking, fermentation, steam distillation, etc. Fermentation is the common method of cassava processing and through it, cassava can be processed into different food products such as *Fufu, garri, Lafun, chikwangue*, etc. Cassava retting (fermentation) is a technique involving long soaking of cassava roots in water to affect the breakdown of tissues. Retting is one of the simplest and lactic acid fermentation process for the processing of cassava tubers into various African staple foods. It simply involves steeping of cassava roots in water until they soften. However, this takes about three to four days under optimal condition. In other conditions retting may take considerable longer for example, tubers older than 24months or during the colder seasons of the year. During the consequent fermentation, roots are softened by the activities of microorganisms producing various enzyme, the endogenous cyanogenic glycosides (linamarin and lotaustralin) are subsequently hydrolyzed to glucose and cyanohydrins, which easily break down to ketone and hydrogen cyanide (HCN) [5] and characteristic flavour developed through a pH decrease and organic acid production [6]. The fermentation process (retting) is characterized by the activities of certain microorganisms which produces enzyme such as pectinases, amylase, etc resulting in the breakdown of cassava tissues. The presence of unspecified microorganisms complicates the control of the fermentation process and lead to the production of objectionable odours [5]. This research work aimed at determining the enzymatic potentials of microorganisms associated with cassava retting.

2.0 MATERIALS AND METHODS

2.1 Sample Collection

Cassava tubers of 12months old were collected from Umuariga, Ndoru and Ahiaeke Markets in Umuahia, Abia State and taken to the laboratory for analyses.

2.2 Sample processing and retting Procedure

The cassava tubers were cleaned, hand peeled and cut into pieces. They were completely submerged in 1000 ml Glass beaker containing tap water and allowed to ferment at ambient temperature of 30 ± 2^0C until retting (softening) occurred. The extent of retting of the cassava tubers was determined manually by feeling the degree of softness of the tubers with hand covered with a sterile disposal hand-glove [7].

2.3 Microbial Enumeration

Ten grams of the retted cassava tubers samples were collected for microbial enumeration at 24hours intervals until retting was completed. The collected sample was homogenized using sterile laboratory mortar aseptically and 1 g of the homogenized sample was serially in peptone water. 0.1 ml aliquot of suitable dilution was inoculated on De-Man Rogosa Sharp agar (MRS) for the isolation of lactic acid bacteria; Sabouraud Dextrose Agar (SDA) for the isolation of fungi; Nutrient agar for isolation and enumeration of heterotrophic bacteria; Mannitol Salt agar (MSA) for the isolation of *Staphylococcus aureus*, and MacConkey agar for the isolation of coliforms respectively in triplicates [8]. The media plates for isolation of bacteria were incubated at 35 ^{o}C for 48 hrs while the fungal culture plates were incubated at 22 ^{o}C for 5 days. After incubation, the plates were examined culturally and later sub-cultured to obtain pure cultures. The pure cultures were stored in media slants.

The bacterial cultures were later Gram stained and subjected to biochemical and sugar fermentation tests for identification.

2.4 Identification of fungal isolates

A drop of lactophenol cotton blue was placed on a clean glass slide. Using a sterile wire loop, a small portion of the colony was cut from the culture and placed to the drop of lactophenol cotton blue. The preparation was covered with a cover slip and pressed gently. It was gently heated to remove air bubbles and to spread the fungus evenly throughout the preparation. It was then examined under the microscope using x10 and x40 objectives [9].

2.5 Determination of Activities of Microbial Enzymes during Cassava Retting

The method of [10] was adopted. Overnight culture of each isolate was harvested and introduced in 10ml freshly prepared nutrient broth and was incubated at 30 ^{o}C for 24 h. The broth was centrifuged at 150 rpm for 10min. The supernatant which contained the enzyme was withdrawn and kept at 4 ^{o}C in a refrigerator for further analysis.

2.6 Screening for enzyme production

2.6.1 Screening for production of Amylase enzyme

Qualitative determination of amylase production was carried out using well cut assay with some modifications. The agar plates were supplemented with 1% starch. After agar solidification, 10 mm diameter well was cut out aseptically using cork borer. The well was filled with the culture filter (100µL) and incubated for 24 hrs at 50 ^{o}C. After incubation, the agar was overlaid with 1% iodine solution and the hydrolytic zone around the well (clear zone) was measured. The negative control was maintained by adding sterile water in a separate well [11].

2.6.2 Production of Cellulase enzyme

The production of Cellulases enzyme using screening medium contains 1% (w/v) carboxymethyl cellulose (CMC) by plate assay was performed using agar plate fortified with 1% (w/v) Carboxymethyl cellulose (CMC). After solidification of Agar, the wells were cut aseptically by cork borer puncher for 10mm diameter and the culture filtrate was poured to the well then the plates and incubated for 24 hrs at 37 ºC. To visualize the hydrolysis zone, the plates were flooded with 0.1% Congo red solution and washed with 1 M NaCl. The formation of a clear zone of hydrolysis indicated cellulose degradation. The ratio of the clear zone diameter to colony diameter was measured in order to select for the highest cellulase producer. The largest ratio was assumed to contain the highest activity [12].

2.6.3 Screening of Isolates for the Pectinase Activity

The isolates were screened for pectinase activity using Pectinase screening agar medium (PSAM). The medium was sterilized and poured in a petri dish and allowed to gel. After, wells were cut aseptically by cork borer punch of 10mm diameter and the culture filtrate was poured then the plate was incubated at 30°C for 24 hours to 2 weeks. At the end of the incubation period, the plates were flooded with 50 mM Potassium iodide-iodine solution. A clear halo zone around the colonies indicates the ability of an isolate to produce pectinase [13].

3.0 RESULTS

Table 1 shows the bacterial isolates from retting cassava samples and they include *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Lactobacillus* spp, and *Proteus* spp.

Table 2 shows the fungal isolates recovered from retting cassava tubers. They include two moulds: *Aspergillus niger* and *Rhodotorula* spp and a yeast: *Saccharomyces cerevisiae.*

Table 3 shows the total viable count of isolates from retting cassava samples. The total heterotrophic plate count was in the range: 1.94 x 10^6cfu/ml - 2.52 x 10^6cfu/ml while the total coliform plate count was in the range: 2.014 x 10^6cfu/ml - 7.47 x 10^5cfu/ml. The total lactic acid bacteria plate count was from 1.712 x 10^6cfu/ml to 2.897 x 10^6cfu/ml while the staphylococcal plate count was in the range: 2.131 x 10^6cfu/ml to 7.76 x 10^5cfu/ml. The total fungal plate count was in the range: 1.823 x 10^6cfu/ml to 2.808 x 10^6cfu/ml.

Table 4 shows the succession of the microbial isolates during the cassava retting. All the bacterial and fungal isolates were found in the retting medium at the beginning of the fermentation. However, at the end of the fermentation, only *Lactobacillus* spp and *Saccharomyces cerevisiae* were recovered from the samples.

Table 5 shows the percentage occurrence of microbial isolates with a total of 25 bacterial and 10 fungal isolates from the retting procedure. *B. subtilis* has the highest occurrence 8 (22.9%) while *Rhodotorula* spp had the lowest occurrence (5.7%).

Table 6 shows the enzymatic potential of isolates from retting cassava samples. All the isolates except *Staphylococcus aureus* exhibited amylase activity with diameter of clear zones ranging between 8.5 - 13.5 mm while cellulase activity was exhibited by all the isolates except *Staphylococcus aureus*, *Lactobacillus* spp and *Proteus* spp with cleared zones of 8.5 - 12.5mm. Only *Rhodotorula* spp, *Escherichia coli*, *Staphylococcus aureus* and *Proteus* spp did not exhibit pectinase activity. The diameter of cleared zones ranged from 8.0 - 13.0 mm.

Table 1: Identification and characterize of bacterial Isolate from retting cassava samples

Colony feature	**Gram reaction**	**Cell arrangeme**	**Catalase**	**Spore stain**	**Oxidase**	**Coagulase**	**Indole**	**Citrate**	**Motility**	**Glucose**	**Lactose**	**Mannitol**	**Sucrose**	**Probable isolates**
White colour	Gram +	Short rod	+	+	+	-	-	+	+	**A**	**A**	**A**	**A**	*Bacillus subtilis*
Pink pigment	Gram –	Short rods	+	-	-	-	+	-	+	**A**	**AG**	**NAG**	**A**	*Escherichia coli*
Golden yellow	Gram +	Cocci group	+	-	-	+	-	+	-	**AG**	**AG**	**AG**	**AG**	*Staphylococcus aurues*
Creamy white	Gram +	**Long rod**	-	-	-	-	-	-	-	**AG**	**AG**	**AG**	**AG**	*Lactobacillus* spp
Cream mucoid	Gram -	**Short rod**	+	-	-	-	+	-	+	**AG**	**AG**	**AG**	**AG**	*Proteus* spp

Key: + = positive, - = Negative, A = acid production, AG = Acid and gas production, NAG = No acid and gas production

Table 2: Identification and characterization of Fungal isolates

Cultural Characteristic	Morphological Characteristic	Identification
Dark- brown mycelium	Conidiophores long and Septate hyphae Irregularly branched conidiophores	*Aspergillums niger*
Red-pink colours	Biseriates the vesicles were spherical to enlongated budding yeast like cells	*Rhodotorula* spp
Small white to creamy Circular convex colonies With thick surface	Actively budding yeast form pseudo mycelium	*Saccharomyces cerevisiae*

Table 3: Total viable count

Retting Interval (Hrs)	Sample sources	Colony Forming Units (cfu/ml)				
	Ahieke	**THPC**	**TCPC**	**TSPC**	**TFPC**	**TLABC**
24hrs	1	1.235 x10^6	1.165 x 10^6	2.345 x 10^6	2.01 x10^6	2.01 x 10^6
	2	1.845 x 10^6	1.825x 10^6	2.49 x10^6	1.295 x 10^6	1.295 x 10^6
	3	1.165 x 10^6	1.45 x10^6	1.69 x 10^6	1.45 x 10^6	1.45 x10^6
	4	2.24 x10^6	2.005 x 10^6	2.555 x 10^6	1.67 x10^6	1.67x 10^6
	5	1.6 x10^6	1.83 x10^6	2.185 x 10^6	1.275 x 10^6	1.275 x 10^6
	Mean value	**1.054 x10^6**	**1.617 x10^6**	**1.655 x10^6**	**2.253 x10^6**	**1.54 x10^6**
	Ndoru 1	1.86 x10^6	2.595 x 10^6	1.8 x10^6	2.845 x 10^6	1.61 x10^6
	2	3.115 x 10^6	2.705 x 10^6	2.235 x 10^6	2.325 x 10^6	1.79 x 10^6
	3	1.275 x 10^6	2.21 x10^6	2.26 x10^6	2.415 x 10^6	1.5 x10^6
	4	1.685 x 10^6	2.875 x 10^6	2.555 x 10^6	2.13 x10^6	2.295 x 10^6
	5	3.155 x 10^6	1.935 x 10^6	3.17 x10^6	1.235 x 10^6	1.29 x10^6
	Mean value	**1.697 x10^6**	**2.218 x10^6**	**2.464 x10^6**	**2.404 x10^6**	**2.19 x10^6**
	Umuariga 1	1.36 x10^6	1.635 x 10^6	1.78 x 10^6	1.495x 10^6	2.5 x10^6
	2	1.93 x 10^6	2.155 x 10^6	1.355x 10^6	1.215 x 10^6	2.06 x 10^6
	3	2.95 x 10^6	2.76 x10^6	2.15 x10^6	2.34 x 10^6	1.915 x 10^6
	4	1.725 x 10^6	1.485 x 10^6	1.3 x10^6	2.15 x10^6	1.78 x10^6
	5	1.96 x10^6	1.58 x 10^6	2.106x 10^6	1.505 x 10^6	1.245 x 10^6
	Mean value	**1.9 x10^6**	**1.985 x10^6**	**1.923 x10^6**	**1.738 x10^6**	**1.741 x10^6**

Key: THPC = Total Heterotrophic plate count, TCPC = Total coliform plate count, TSPC = Total staphylococcal plate count, TFPC = Total fungal plate count, TLABC = Total lactic acid bacteria plate count

48hrs	Ahieke	1	1.55 x 10^{6}	9.0 x 10^{6}	1.2 x 10^{6}	1.395 x 10^{6}	2.25 x 10^{6}
		2	2.335 x 10^{6}	1.225 x 10^{6}	1.23 x 10^{6}	2.15 x 10^{6}	1.79 x 10^{6}
		3	1.75 x10^{6}	1.6 x 10^{6}	1.455 x 10^{6}	2.885 x 10^{6}	2.055 x 10^{6}
		4	2.6 x 10^{6}	1.24 x 10^{6}	1.775 x 10^{6}	1.735 x 10^{6}	2.65 x 10^{6}
		5	2.05 x 10^{6}	1.225 x 10^{6}	1.285 x 10^{6}	2.605 x 10^{6}	2.755 x 10^{6}
	Mean value		**2.057 x10^{6}**	**1.238 x10^{6}**	**1.389 x10^{6}**	**2.154 x10^{6}**	**2.3 x10^{6}**
	Ndoru	1	2.285 x 10^{6}	1.455 x 10^{6}	1.325 x 10^{6}	3.005x 10^{6}	1.96 x 10^{6}
		2	2.95 x10^{6}	1.055 x 10^{6}	1.39 x 10^{6}	2.6 x 10^{6}	2.015 x 10^{6}
		3	1.715 x 10^{6}	2.2 x 10^{6}	8.85 x 10^{6}	2.845 x 10^{6}	1.835 x 10^{6}
		4	1.965 x 10^{6}	8.25x 10^{6}	1.33 x 10^{6}	2.315 x 10^{6}	2.725 x 0^{6}
		5	3.15 x10^{6}	1.16 x 10^{6}	1.21 x 10^{6}	1.785 x 10^{6}	1.95 x 10^{6}
	Mean value		**2.413 x10^{6}**	**1.339 x10^{6}**	**1.228 x10^{6}**	**2.51 x10^{6}**	**2.097 x 10^{6}**
	Umuariga	1	1.574 x 10^{6}	9.25 x10^{6}	1.07 x 10^{6}	1.89 x 10^{6}	2.825 x 10^{6}
		2	2.5 x10^{6}	1.165 x 10^{6}	1.45 x 10^{6}	1.455 x 10^{6}	2.45 x 10^{6}
		3	3.13 x 10^{6}	1.455 x 10^{6}	7.8 x 10^{6}	2.62 x 10^{6}	2.55 x 10^{6}
		4	2.005 x 10^{6}	1.05 x 10^{6}	1.025 x 10^{6}	2.39 x 10^{6}	2.385 x 10^{6}
		5	2.4 x 10^{6}	1.46 x10^{6}	1.21 x 10^{6}	2.33 x 10^{6}	2.05 x 10^{6}
	Mean value		**2.322 x10^{6}**	**1.211 x10^{6}**	**1.107 x10^{6}**	**2.138 x10^{6}**	**2.452 x10^{6}**

Key: THPC = Total Heterotrophic plate count, TCPC = Total coliform plate count, TSPC = Total Staphylococcal plate count, TFPC = Total fungal plate count, TLABC = Total lactic acid bacteria plate count

72hrs	Ahieke	1	1.85 x 10^{6}	7.2 x10^{5}	6.05 x 10^{5}	1.885 x 10^{6}	2.98 x 10^{6}
		2	2.875 x 10^{6}	6.5 x 10^{5}	7.0 x 10^{5}	2.615 x 10^{6}	2.49 x 10^{6}
		3	2.105 x 10^{6}	6.75 x 10^{5}	9.5 x 10^{5}	3.025 x 10^{6}	2.395 x 10^{6}
		4	2.625 x 10^{6}	7.3 x 10^{5}	8.3 x 10^{5}	2.325 x 10^{6}	3.075 x 10^{6}
		5	2.755 x 10^{6}	6.7 x 10^{5}	8.9 x 10^{5}	2.995 x 10^{6}	3.23 x 10^{6}
	Mean value		**2.442 x10^{6}**	**6.89 x10^{5}**	**7.95 x10^{5}**	**2.569 x10^{6}**	**2.834 x10^{6}**
	Ndoru	1	2.55 x 10^{6}	6.05 x 10^{5}	9.5 x 10^{5}	3.425 x 10^{6}	2.38 x 10^{6}
		2	2.83 x 10^{6}	6.6 x 10^{5}	6.75 x 10^{5}	3.28 x 10^{6}	2.785 x 10^{6}
		3	2.06 x 10^{6}	9.5 x 10^{5}	7.2 x 10^{5}	3.335 x 10^{6}	2.445 x 10^{6}
		4	2.585 x 10^{6}	6.0 x 10^{5}	6.2 x 10^{5}	3.095 x 10^{6}	3.185 x 10^{6}
		5	2.725 x 10^{6}	8.3 x 10^{5}	8.25 x 10^{5}	2.325 x 10^{6}	2.805 x 10^{6}
	Mean value		**2.55 x10^{6}**	**7.29 x10^{5}**	**7.58 x10^{5}**	**3.092 x10^{6}**	**2.72 x10^{6}**
	Umuariga	1	1.855x10^{6}	7.2 x 10^{5}	5.95 x 10^{5}	2.335 x 10^{6}	3.295 x10^{6}
		2	3.175 x 10^{6}	8.25 x 10^{5}	9.0 x 10^{5}	2.055 x 10^{6}	3.555 x 10^{6}
		3	2.725 x 10^{6}	8.1 x 10^{5}	7.05 x 10^{5}	3.6 x 10^{6}	3.22 x 10^{6}
		4	2.55 x 10^{6}	9.2 x 10^{5}	7.15 x 10^{5}	2.88 x 10^{6}	2.94 x 10^{6}
		5	2.555 x 10^{6}	8.5 x 10^{5}	9.65 x 10^{5}	2.95 x 10^{6}	2.68 x 10^{6}
	Mean value		**2.572 x10^{6}**	**8.25 x10^{5}**	**7.76 x10^{5}**	**2.764 x10^{6}**	**3.138 x10^{6}**

Key: THPC = Total Heterotrophic plate count, TCPC = Total coliform plate count, TSPC = Total Staphylococcal plate count, TFPC = Total fungal plate count, TLABC = Total lactic acid bacteria plate count

Table 4: Distribution of isolates from the retting cassava samples

Cassava source and retting interval (hrs)

Isolate	**Ahieke Market**			**Ndoru Market**			**Umuariga**			
	24	48	72	24	48	72	24	48	72	No. positive
				Bacteria						
Bacillus subtilis	+	+	+	+	+	-	+	+	+	8
Escherichia coli	+	+	-	+	-	-	+	-	-	4
Staphylococcus aureus	+	+	-	+	+	-	+	-	-	5
Lactobacillus spp	-	+	+	-	-	+	-	+	+	5
Proteus spp	+	-	-	+	-	-	-	+	-	3
				Fungi						
Rhodotorula spp	-	+	-	-	-	-	-	+	-	2
Saccharomyces cerevisiae	-	-	+	-	+	+	-	+	+	5
Aspergillus niger	-	+	-	-	-	+	-	+	-	3
Total	**4**	**6**	**3**	**4**	**3**	**3**	**3**	**6**	**3**	**35**

Table 5: Percentages occurrence of isolates from the retting cassava samples.

Isolates	Numbers of Isolate	%
	Bacteria	
Bacillus subtilis	8	22.9
Escherichia coli	4	11.4
Staphylococcus aureus	5	14.3
Lactobacillus spp	5	14.3
Protues spp	3	8.6
	Fungi	
Rhodotorula spp	2	5.7
Saccharomyces cerevisiae	5	14.3
Aspergillus niger	3	8.6
Total	**35**	**100%**

Table 6: Enzymatic Activities of isolates from cassava retting (mm)

Isolates	Amylase	Cellulase	Pectinase
	Bacteria		
Staphylococcus aureus	0.00	0.00	0.00
Bacillus subtilis	13.5	8.5	11.0
Escherichia coli	12.0	10.5	0.00
Lactobacillus spp	12.0	0.00	13.0
Proteus spp	9.0	0.00	0.00
	Fungi		
Rhodotorula spp	8.5	9.0	0.00
Saccharomyces cerevisiae	9.00	12.5	8.0
Aspergillus niger	12.0	12.0	13.0

DISCUSSION

The present research determined the enzymatic potentials of microbes associated with retting of cassava. The predominant microbes in the fermenting medium were lactic acid bacteria which contributed to the acidification of the medium and *Saccharomyces cerevisiae*. [14] reported that *Lactobacillus* spp was the dominant bacterium in the fermenting cassava for lafun production by submerged fermentation. Sugars produced by fermenting medium were suitable substrate for yeast that caused the number of yeast higher than molds. *Escherichia coli* detected at the early stage was characteristics of acid fermentation. The increased acidity of the medium could have resulted in the decreased growth of the species. [15] found that during solid state fermentation of cassava in *attieke*, processing lactic acid bacteria became dominant and contributed to the most acidification of the product. The progressive increase in the frequency of lactic acid bacteria and fungi observed during the retting process (later stage of fermentation) may probably be due to increased acidity which favoured the growth of the microorganisms, although the pH of the retting medium was acidic at 24 h of incubation. The decrease in pH throughout the fermentation period was due to the production of organic acids by associated microorganisms during fermentation.

The cassava roots softness occurred as a result of enzymatic cell wall degradation of cassava tubers. It was established that the cell wall degradation of the cassava tubers resulting to root softening is attributed to the enzyme from both plants and the isolated microorganisms. Some of the isolates were able to produce amylase, pectinase and cellulase enzyme. [7] reported that *Bacillus subtilis* produced amylase enzyme that are necessary for the breakdown of starch to sugar which are needed for the growth of other fermenting microorganisms. According to [16], some yeast and fungi contributed to tissue cassava breakdown by cellulase production. Enzymes from lactic acid bacteria hydrolysis cell wall components partially such as hemicellulose, pectin that destroyed the firm structure of cell. Degradation of cellulose leads to fragmentation and hydrolysis of cell wall and starch granules that facilitate starch granules to leach [17] and decreased starch content.

The presence of high pectinase production indicated the possibility of faster softening of cassava roots. The ability of *Lactobacillus* spp to produce a high degree of zone of inhibition of pectinase enzyme suggests that it is of great important in retting period which therefore will aid in faster retting of cassava roots.

Amylolitic activities of microbial strains in cassava contributes to the breakdown of cassava starch to simple sugar thereby increasing the energy density cassava and providing carbon source for lactic acid bacteria in view of the fact that amylase is a rare trait among lactic acid bacteria isolated from starchy foods [18]. Lactic acid bacteria are most responsible for acidification during cassava retting [19]. It was recorded that among all the isolates it was only *Staphylococcus aureus* that could not produce any of the assayed enzyme. The occurrence of *Staphylococcus aureus* and

Enterobacteriaceae may be as a result of contamination from cassava sample source or water, containers, and utensils used in preparation of the soaking/retting of cassava roots.

CONCLUSION

Among all the enzymes assayed, only amylase was produced by all the isolates except *Staphylococcus aurues*. This shows that amylase played an important role in retting of cassava tubers (fermentation).

5.3 RECOMMENDATIONS

Results from this study have shown that enzymes produced by microorganisms involved in cassava fermentation are the major factors affecting cassava retting. Therefore, further studies should focus on the development of techniques in high production of enzymes for utilization in the cassava based food industries.

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