International Journal of Food Science and Nutrition
ISSN: 2455-4898, Impact Factor: RJIF 5.14
www.foodsciencejournal.com
Volume 2; Issue 1; January 2017; Page No. 72-76

Simultaneous saccharification and fermentation in the production of Kunun zaki (A Nigerian nonalcoholic beverage) by selected Lactic acid bacteria

[1] Egbere OJ, [2] Idoko BO, [3] Danladi MMA, [4] Yakubu Dabot, [5] Soom Solomon

[1, 2] Microbiology Department, University of Jos, Nigeria
[3] Department of Microbiology, Plateau State Univerity Bokkos, Nigeria
[4] Department of Science Technology, Plateau State Polytechnic, Barkin Ladi, Plateau State, Nigeria
[5] Department of Biological Sciences, University of Mkar, Benue State, Nigeria

Abstract
Kunun-zaki" is a highly relished traditional, non-alcoholic beverage of about 80 million Nigerians that is prepared from pre-gelatinized cereal starch and left to ferment by chance microorganisms. This study was aimed at using selected lactic acid bacteria with dual biochemical roles of hydrolyzing sorghum starch and also converting sugars to lactic acid and other flavour-enhancing compounds in kunun-zaki. Fourty random samples of hawked kunun zaki in Jos metropolis, Nigeria were screened for prevalence of lactic acid bacteria using De -Mans, Rogosa Sharpe Agar (MRSA) as medium and other conventional standard bacteriological procedures. The bacterial isolates were subjected to secondary screening for amylolytic activities measured by the degree of cleared zones of starch hydrolysis by each isolates grown on starch agar. The amylolytic lactic acid bacteria (ALAB) were then used as trial starter cultures in the production of kunun-zaki at the laboratory phase. Control experiment (using malted rice as enzyme source and chance micro flora) was also set up at 35^0C. Glucose content, pH, Titratable acidity and sensory properties were analyzed at regular intervals during the laboratory production of kunun-zaki in order to assess the saccharification and acid production efficacies of the organisms. Twelve lactic acid bacteria, namely; *Lactobacillus plantarum, L. bulgaricus, L. helveticus, L. delbrueckii, L. casei, L. brevis, L. fermentum, L. pointis, Lactococcus lactis, Leuconostoc mesenteroides* and *Streptococcus* sp were isolated from samples of hawked kunun zaki. Three of the lactic acid bacteria, *L. fermentum, L. brevis and L. acidophilus* were found to exhibit high simultaneous saccharification and co-fermentation of kunun zaki with *L. fermentum* being the most efficacious. The optimized fermentation without the use of a cereal based starch- hydrolyzing enzyme (malted rice) will reduce time and cost of industrial production of kunun zaki.

Keywords: kunun-zaki, lactic acid bacteria, amylolytic lactic acid bacteria, saccharification

1. Introduction
'Kunu' is a Hausa compound word for all kinds of gruels or beverages with specifications such as 'zaki' (sweet) 'gyada' (groundnut), 'tsamiya' (tamarind) normally attached to the word to denote the ingredient source or its sensory attribute (Egbere *et al.*, 2008) [3, 4]. Hence, kunun-zaki, means sweet kunu. Kunun-zaki is actually a non-alcoholic, non-carbonated sweetened and free-flowing gruel prepared traditionally and which has been left to ferment spontaneously by chance microorganisms. It is consumed predominantly in Northern Nigeria as a thirst-quencher, an appetizer and/or an energy booster by all classes of the society. Studies by Gaffa *et al.* (2002) [5, 6] has shown that kunun-zaki is the most preferred beverage, with about 73% of the sampled population consuming it daily and (26%) occasionally in Gombe and Bauchi States of Nigeria. In recent times, street hawking and consumption of kunun-zaki is fast spreading to southern parts of Nigeria, probably because it is cheaper and more satisfying than the carbonated beverage to the low income earners in the society. Although, kunun-zaki is produced all year round, its peak production period is within the dry season (October to April).

The major ingredients used for kunun-zaki production include sorghum, millet and maize which are used singly or in combination of any two of the cereals. Spices like ginger and cloves as well as sweetening agents such as malted rice and sweet potato are also used as secondary ingredients (Adeyemi and Umar, 1994) [1]. Information on the elevation of the nutritional value of Kunun- zaki by feeding trial experiment on rats is rare. However, Awogbenga *et al.* (1999) [2] studied the glycemic effect of Kunu-zaki in blood glucose and observed that Kunun- zaki has lower glycemic index (44%) as compared to the high energy drink (glucose as standard (100%) and this may be beneficial to diabetics.

Although a number of pioneering researchers have embarked on various aspects of this beverage including its microbiology (Onuorah, *et al.* 1987) [11]; Egbere, 1988) nutritive value (Inatimi, *et al.* 1987), improvements in its production (Gaffa and Ayo, 2002) [5, 6], use of selected trial starter organisms (Egbere *et al.*, 2008) [3, 4], the production technology of the beverages is still crude, unhygienic and requiring long hours of fermentation by chance microorganisms.

Three technological steps involved in the production of kunun-zaki include pregelatinization, saccharification and fermentation. Pregelatinization process of kunun -zaki- based starch is a physical, heat-induced process in which the locals expose the starchy paste to mild heat-heating at about 65^0C to enhance swelling or gelling of the starch. This process enhances the breaking down of intermolecular bonds of starch molecules

in the presence of water and heat and thereby allowing absorption of water by the hydrogen bonds of the broken starch granules. During heating, water is first absorbed in the amorphous space of starch, which leads to a swelling phenomenon. This process is crucial to the next enzymatic, hydrolytic phase.

The enzymatic breakdown or hydrolysis of the starch paste of kunun- zaki by amylolytic enzymes is traditionally done by the introduction of ground malted rice into the slurry. In view of the difficulty and the cost of obtaining malted rice for this purpose, amylolytic lactic acid bacteria were envisaged for use in replacement of malted rice. This process is preparatory to the final but most important conversion process, fermentation. The fermentation process of kunun- zaki involves the conversion of the sugars from the cereal starch via glycolysis, Embdem Mayerhoofs' pathways and other possible biochemical routes into acids, aldehydes, ketones and traces of alcohols by the fermenting microorganisms. The present study was therefore, aimed at achieving dual purposes of using one or more lactic acid bacteria as starter organism(s) to carry out the two major conversion processes (saccharification and fermentation concurrently) of starch slurry to obtain the traditional kunun-zaki. This is hoped to optimize the industrial production and safety (freedom from possibly mycotoxin –elaborating fungi during the malting process of rice traditionally used for saccharification) and ultimately reduce production time and cost.

2. Materials and Methods

2.1 Collection of samples

Wholesome grains of red variety of guinea corn (Sorghum bicolour) were obtained from Kwarafa market in Jos metropolis and used for the study. Fifty samples of kunun- zaki were purchased from hawkers in five different locations of Jos metropolis in 250 milliliter capacity sterile bottles and taken to the laboratory for analysis. The sampling locations included those of the University of Jos/Angwan rogo/Bauchi road, Tudun wada Terminus market, Faringada and Gada biu respectively.

2.2. Preparation of samples

2.2.1 Media and media preparation: Nutrient agar and De Man's Rogosa Sharpe agar (MRSA) were prepared according to manufacturers guidelines and kept for use for the general culturing of bacteria and lactic acid bacteria respectively.

2.2.2. Extraction of Sorghum Starch from Sorghum grains

Starch was prepared from *Sorghum bicolor* grains according to the method described by Takeda *et al.*, (2002). Two thousand (2000) grams of the sorghum grains were washed in sterile water, steeped in 600ml of sterile water (containing 5% sodium metabisulphite for decontaminating the grains of indigenous micro flora) for 12 hours to soften the kernels. They were washed in sterile water in three rinses of sterile water (in order to remove residual sulphite), drained in a wicker basket and then wet- milled using a sterile blender (Kenwood Model No. Bs 450). The resulting paste was made into thin slurry by adding 400ml of sterile water and then filtered through two layered muslin cloth by squeezing with hands worn with sterile hand gloves. The filtrate was centrifuged at 5,000 rpm for 15 minutes to obtain pure starch in a cakey sediment form. The supernatant was then decanted leaving the hardened starch sediment.

The recovered starch cake was pulverized and dried in the oven set at 60^0C for 8 hours to give fine Sorghum starch powder. The starch was then packed in sterile air-tight bottles, labeled and kept for further use as basic ingredient for in starch hydrolysis test and for the production of kunun- zaki.

2.3. Cultivation and Enumeration of bacteria from kunun-zaki samples: Samples of the kunun -zaki were serially diluted up to the 6^{th} dilution factor according to standard microbiological procedures as described by Cheesbrough (2000) [12] and used for the determination of Total aerobic plate count and lactic acid bacteria count respectively. Cultures in Nutrient agar were incubated aerobically while those in MRSA were cultured anaerobically in Nitrogen gas pack, all at 35 ^{0}C for 24 to 48 hours. At the end of the incubation period a digital colony counter was used in counting the bacterial loads in each plate and the numbers determined appropriately.

2.4. Isolation of bacteria from kunun -zaki samples: The bacterial cultures grown in Nutrient agar and MRSA were repeatedly sub -cultured on the basis differences on the morphology, cultural characteristics, gram reactions and biochemical characteristics of the individual colonies observed on the plates following the methods of Buchanan and Gibbons (1974) and Cheesbrough (2002) [12].

2.5. Screening for amylolytic potentials of Lactic acid bacteria.

All the Lactic acid bacterial isolates were screened for amylase production following the procedures of Sun *et al.*, (2010) [14]. The isolates were inoculated by both pour plating and straking methods separately onto sterile solidified MRS agar fortified with 2 percent soluble starch and the plates were incubated 37ºC for 24 hours. Thereafter, the plates were flooded with Lugol's iodine solution for the detection of starch hydrolysis. Zones of clearance around the colonies were indicative of amylase activity and were measured and recorded as measure of amylolytic activity of each organism. Inability of organisms to turn the blue black colouration of the medium or produce zones of clearance around any colony of organisms is indicative of lack of amylase in the bacterium.

2.6. Preparation of test starter cultures

Only three of the most effective amylolytic actic acid bacteria (ALAB) were used for the saccharification and fermentation studies of kunun zaki. The ALAB cultures were prepared by picking a loop full of each colony of the of the pure isolates into 10 ml of each prepared sterile MRS broth and culturing them in the incubator set at 37^0C for 24 hours. The standardization of the organisms was done by comparing the turbidity of the individual broth culture with 0.5 McFarland standards which is equivalent to $0.5x10^8$cfu/ml (Cheesbrough, 2002) [12].

2.7 Controlled saccharification and fermentation of starch paste into Kunun- zaki

Starch prepared from *Sorghum bicolor* as described above was used in the controlled fermentation of kunun- zaki with the aid of amylase-producing lactic acid bacteria. A weight of 110g of prepared sorghum starch was dissolved in sterile 650ml of sterile tap water and used as the fermenting slurry. While prepared dried malted rice powder (1 gram) was added to the slurry meant for the Control as source of hydrolyzing amylase, malted rice

was not added to the experimental set ups. The slurry for the experimental set ups was divided into three equal portions in 250ml conical flasks. introduced into the water bath set at 35^0C together with 10ml of each of the test inocula of bacterial culture broths (namely, *Lactobacillus fermentum, L. lactis* and *L. acidophilus* respectively) isolated from already made kunun-zaki. Glucose content and pH of the fermenting mixtures were determined on hourly intervals. The results were then recorded.

2.8 Determination of Diastatic/Amylolytic Enzyme Activity of the Malted Grains

The diastatic or the amylolytic enzyme activity of the crude enzyme extracts obtained from the various malted grains was determined following Safarik's method as described by Oguntimein (1993). The procedure was as follows: Ten milligram per milliliter (10 mg/ml) of starch solution was prepared and 2 ml of it was pipetted into a test tube. Then 2 drops of 1% iodine solution was added to the test tube. Thereafter, 2ml of the enzyme producing organism in MRS broth was pipetted out and added into the mixture, mixed properly, and incubated in the water bath set at 45^0C for 30 minutes. The optical density (O.D) of the mixture was determined at 600 nm against a blank (a mixture of starch solution of each sample and enzyme that underwent the same procedure but without addition of iodine).
One unit of enzyme activity is defined as the amount of enzyme that produces change of 0.01D optical density in one minute under assay conditions. The procedure was repeated for the determination of the diastatic activity of purified, standard or regular brewery enzyme (α- amylase obtained from Jos International Brewery, Jos, Nigeria).

3. Results and Discussion

3.1. Number and Frequency of occurrence of lactic acid bacteria associated with kunun zaki in Jos metropolis Nigeria

The various results of the study are shown in Tables and a Figure below. The results of the 50 samples of kunun zaki collected, 10 each from the five sampling locations of Jos metropolis (Table 1) indicate that the Total aerobic plate count (TAPC), Lactic acid bacterial count(LABC) and pH of kunun zaki sold in Jos metropolis were not significantly different among the locations at 5 % level of probability. The results agreed with those of Wonnang *et al.* (2002) [15] for kunun zaki sold in Jos metropolis. However, the acidity of the samples were lower than those obtained for kunun zaki sampled in Makurdi and Maiduguri by Egbere *et al.* (2008) [3, 4].
The results showing frequencies of occurrence of the Lactic acid bacteria in kunun zaki sampled in Jos metropolis showed that out of the 11 isolates of the Lactic acid bacteria, *Lactobacillus plantarum, L. fermentum, L. bulgaricus* and *L. acidophilus* were predominant in occurrence over and above the other isolates. The results confirm those of Egbere and co-investigators in 2008 for samples of kunun zaki in Makurdi and Maiduguri. Some of these lactic acid have long been found in association with cereal based fermentations in Africa (Hesseltine *et al.*,1980) [7, 9] and confirming the earlier assertions by Egbere *et al*. 2008a [3, 4] that kunun zaki is a lactic acid based fermented food. The use of known starter lactic acid bacteria as starter organisms in the production kunun zaki will make the beverage a promising health food for Nigerians who relish and consume it on a daily basis.

Table 1: The Total aerobic plate, Lactic acid counts of Kunun zaki samples sold in Jos metropolis

Location	Mean pH (n=10)	Mean TAPC ($X10^6$cfu/ml) n=10	Mean LABC ($x10^6$ cfu/ml (n=10)
Unijos/Angwan-rogo/Bauchi Road	4.10	4.37	1.08
Tudun wada	4.18	4.14	1.5
Terminus market	4.24	6.23	1.8
Faringada	4.17	7.37	2.10
Gada biu	3.95	7.00	1.86
Grand Mean	4. 13	5.86	1.67

Key
LABC= Lactic acid bacterial count no= number of samples
MPN =Most Probable Number

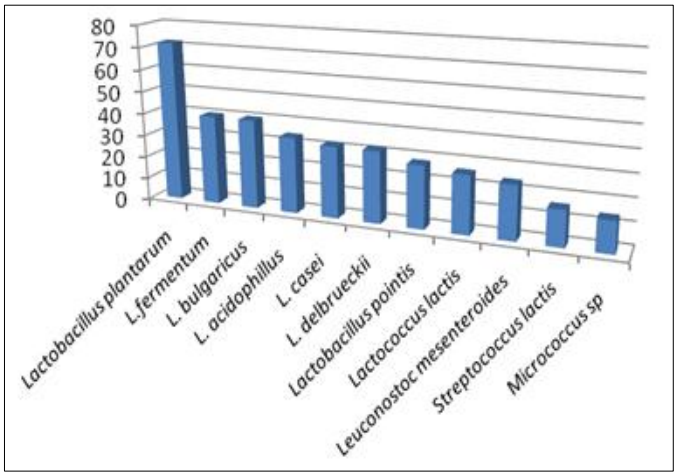

Fig 1: Percentage occurrence of Lactic acid bacteria associated with locally produced "kunun-zaki" in Jos Metropolis

Table 2: The Abilities of the Species of the Lactic acid bacterial isolates associated with Kunun-zaki Production to Produce Amylase.

Bacterial isolate	Amylase production	Zones Clearance (mm)	
L. fermentum	+*	3.0	3.2
L. plantarum	- *	-	-
L. brevis	+	2.8	2.7
L acidophilus	+	2.0	2.0
L casei	+	1.8	1.9
L delbrueckii	+	0.5	0.8
L pointis	+	1.5	1.5
Lactococcus lactis	-	-	-
Leuconostoc mesenteroides	-	-	-
Streptococcus lactis	-	-	-
Micrococcus sp	-	-	-
		-	-

*+= Positive
- = Negative

Table 3: Starch Hydrolysis Time with Respect to Concentration of Starch using 1% Inoculum Enzyme Sources (LAB*)

LAB	Concentration (%)(w/v) of starch (Y)	Hydrolyzing time (minutes) (X)
Lactobacillus fermentum	0.0 (control)	0
	1.0	3
	2.5	7
	5.0	14
*Regr:Y= -1.008+3.245X * r = 0.9976	10.0	32
Lactobacillus brevis	0.0	0
	1.0	5
	2.5	11
	5.0	26
Regr:Y= -0.195+4.961X r = 0.9984	10.0	49
Lactobacillus acidophilus	0.0	0
	1.0	3
	2.5	8
	5.0	15
Regr:Y= -0.617+3.323X r = 0.9987	10.0	33
Control (malted rice)	0.0	0
	1.0	4
	2.5	9
	5.0	19
*Regr: Y= -2.303 + 4.228X r = 0.9812	10.0	42

*LAB = Lactic Acid Bacteria

Table 4: Glucose Yield with Respect to Fermentation Time using 10% Inoculum as Enzyme Source

Time (Hr)	Control		*Lactobacillus fermentum*		*Lactobacillus brevis*		*L. acidophilus*	
	pH	Glucose (mg/ml)	pH	Glucose	pH	Glucose (mg/ml)	pH	Glucose (mg/ml)
0	6.8	0.40	6.7	0.40	6.7	0.40	6.7	0.40
2	4.9	0.52	5.3	0.54	5.0	0.62	4.2	0.58
4	5.6	0.64	5.8	0.62	5.6	0.82	5.4	0.78
6	5.4	0.70	5.6	0.74	5.4	1.02	5.4	0.96
8	5.2	0.80	5.4	0.86	5.2	1.20	5.0	1.12
10	4.8	0.84	4.4	0.96	4.8	1.36	4.8	1.18
12	4.6	0.90	4.1	1.40	4.4	1.38	4.5	1.30

3.2. Effect of Amylolytic potentials of lactic acid bacteria isolated from kunun zaki

Results on amylolytic potentials of the *Lactobacilli* (Table 2) showed that out of the lactic acid bacteria screened, only *L. fermentum*, *L. brevis*, *L. acidophilus*, *L. case*i, *L. delbrueckii*, and *L. pointis* had amylase producing ability, though to varied degrees as ascertained by the various sizes of zones of clearance produced. The results also revealed that *Lactobacillus fermentum* had the highest zones of clearance (3.2mm) followed by *L. acidophilus* (2.7mm) and L. *brevis (2.0 mm)* respectively. *Lactobacillus delbrueckii* had the least clearance zone (0.8mm). Amylolytic lactic acid bacteria (ALAB) have been reported from different tropical amylaceous fermented foods, prepared mainly from cassava and cereals (e.g., maize and sorghum). Amylolytic strains of *Lactobacillus plantarum* have been isolated from African cassava-based fermented products and the new ALAB species *Lactobacillus manihotivorans* (Morlon-Guyot *et al.*, (1998) was isolated from cassava sour starch fermentations carried out in Colombia. ALAB have also been isolated from cereal-based fermented foods. Olympia *et al.,* (1995) characterized amylolytic strains of *L. plantarum* isolated from burong isda, a fermented food made from fish and rice in Philippines. Amylolytic strains *of Lactobacillus fermentum w*ere isolated for the first time from Benin maize sourdough (ogi and mawè) by Agati *et al.,* (2002). More recently, Sanni *et al*., (2002) described amylolytic strains of *L. plantarum and L. fermentum* strains in various Nigerian traditional amylaceous fermented foods.

The relatively high amylase producing ability of three of the isolates in kunun -zaki samples justified the use of amylolytic lactic acid bacteria for simultaneous fermentation of kunun-zaki as this will circumvent the conventional use of malted rice as starch- hydrolyzing enzyme source by local makers of kunun-zaki.

3.3. Starch Hydrolyzing Time of Amylolytic Lactic Acid Bacteria (ALAB)

The results on the starch hydrolyzing time of the ALAB (Table 3) with respect to starch concentration showed that hydrolyzing time was directly depended on the concentration of the starch solution in all cases of the of the bacterial enzyme sources used: the results corroborating with the zone of clearances of the organisms shown previously in Table 2. This was evidenced by very strong Regression factors (r) of 0.9976, 0.9984 and 0.9984 for the two parameters under consideration. The results indicating a shorter time of 32 minutes to attain extinction point of hydrolysis for *L. fermentum* (as against 33, 42 and 44 minutes for *L. acidophilus, L.brevis* and malted rice (control) respectively) showed that indeed, *L. fermentum* had superior amylolytic potential than the rest of the organisms used. Shorter saccharification times could be increased by use of higher amounts of microbial biomass during production of kunun zaki.

3.4. Simultaneous Fermentation of Kunun zaki by Amylolytic Acid Bacteria (ALAB)

The results on the use of ALAB to enhance both starch hydrolysis and subsequent biocatalysis of the sugar obtained from lactic acid, aldehydes and other desirable metabolites that give characteristics attributes of Kunun-zaki without the use of an amylolytic enzyme (Table 4) showed that the sugar yields of the organisms used corresponded with their rates of starch hydrolysis as discussed above, with *L. fermentum* leading. The rate in the second level of fermentation as indicated by the drop in pH was also faster with *L. fermentum* than with *L. acidophilus* and *L. brevis* respectively. The rates of acid production by the ALAB compares favourably with that of the control (malted rice and chance microbial fermenters as could be seen in both yields of glucose and acid production).

3.5. Conclusion

The concept of Simultaneous saccharification and co-fermentation (SSCF) has been recognized as a feasible option for ethanol production from xylose-rich lignocellulosic materials and has been a focus of biomass energy research in recent times (Itelima *et al.*, 2013) [10]. The rationale is to optimize fermentation without the use of a different starch hydrolyzing enzyme (in the case of kunun-zaki, without the use of malted rice). This will reduce and the time and cost of production of kunun zaki particularly at the industrial scale level.

4. References

1. Adeyemi IA, Umar S. Effect of method of manufacture on quality characteristics of kunun zaki, a millet based beverage. Nig. Food J. 1994; 12:944-947.
2. Awogbenga MD, Ahmadu M, Ozigis AAA. The glycemic effect of Kunun-zaki on blood glucose in non diabetic subjects. Proceedings of the 23rd Annual Conference of Nigerian Institute of Food Science and Technology, 25 – 27th, October, Abuja, Nigeria. 1999, 217-218.
3. Egbere OJ, Onwuliri FC, Oyero SK, Henry UI. Accelerated Fermentation process of kunun-zaki (A Nigerian Non-Alcoholic Beverage). Adv. Sci. Technol. 2008; 2(1):36-41.
4. Egbere OJ, Luka J, Soo JN, Zumbes HI, Chollom P, Henry UI. A Survey of Local Preparation Method and quality of Kunun-zaki in two metropolitan towns of Northern Nigeria. Global Journal of Pure and Applied Science. 2008; 13(4):383-386.
5. Gaffa T, Jideani IA, Nkama I. Traditional production, consumption and storage of kunu: A non alcoholic cereal beverage. Plant Food Hum. Nutr. 2002; 57:73-81.
6. Gaffa T, Jideani IA, Nkama I. Nutrient and sensory qualities of kunun zaki from different saccharification agents. Int. J. Food Sci. Nutr. 2002; 53:109-115.
7. Hesseltine CW, Wang HL. The Importance of Traditional Fermented Foods. Bio Science. 1980; 30:402-404.
8. Inatimi EEB, Abasiekong SF, Chiemaka I. Kunun zaki and tsamiyaBNon-alcoholic beverages prepared from sorghum grains. Chemical analysis for nutrient contents of fresh and aging samples. Nigerian Journal of Biotechnology. 1987; 5:21-23.
9. Hesseltine CW, Wang HL. The Importance of Traditional Fermented Foods. Bio Science. 1980; 30:402-404.
10. Itelima J, Ogbonna A, Pandukur S, Egbere OJ, Salami A. Simultaneous Saccharification and Fermentation of Corn Cobs to Bio-Ethanol by Co-Culture of Aspergillus niger and Saccharomyces cerevisiae. Internal Journal of Environmental Science and Development. 2013; 4(2):239-24
11. Onuorah SI, Adesiyun AA, Adeyeye JO. Survival and multiplication of *Staphylococcus aureus* and *Escherichia coli* in a Nigeria cereal drink (*Kunun zaki*): Effect of spices and temperature. J. Food Agric. 1987; 1:169-173.
12. Cheesbrough M. District Lab. Practice in Tropical Countries (Part 2). Publishers: Gopson Papers Ltd. India. 2000.
13. Sanni A, Morlon-Guyot J, Guyot JP. New efficient amylase-producing strains of *Lactobacillus plantarum* and *L. fermentum* isolated from different Nigerian traditional fermented foods. International Journal of Food Microbiology. 72, 53-6.
14. Sun H, Zhao P, Ge X, Xia Y, Hao Z, Liu J. *et al*. Recent advances in microbial raw starch degrading enzymes. Applied Biochemistry and Biotechnology. 2010; 160:988-1003.
15. Wonnang DI, Amienyo CA, Ekeleme OP, Dazol DJ. Bacteriologial assessment of Kunun zaki: a local Beverage sold in Jos, Plateau State. Journal of Environmental Sciences. 2002; 4:5-7.