Bacteriological Quality of Kunu Drink Sold In Bayelsa State Nigeria and the Pathogenic Potential of Some Isolates.

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ABSTRACT

Introduction: Kunu or kununzaki is a beverage drink made from grains such as millet, sorghum and maize or other combinations. It is a non-alcoholic beverage marketed in public places such as offices, markets, schools, motor parks and used in festivities such as weddings, birthday celebration, naming ceremonies etc. The high bacterial content of kunu calls for investigation.
Aim: The aim of this research is to isolate, identify bacterial contaminants in kunu and determine enterotoxin producing abilities of some isolates.
Methodology: A total of 150 bottles of kunu were purchased, 50 each from Yenagoa, Sagbama and Ogbia respctvely. Each bottle of kunu was properly mixed by gentle inversion several times and 1mL of the kunu was pipetted and added to 9mL sterile peptone water. Subsequent serial dilution was made to 10^5. Then 0.1mL was placed on agar media in duplicate. The plates were incubated at 37°C for 18-24 hours and examined for growth.
Results: The bacteria isolated from Kunu were *S. aureus* 150 (27.8%), *E. coli* 150 (27.8%), *Bacillus sp.* 150 (27.8%) and *Staphylococci sp*. 90 (16.7%) respectively. Out of the *S. aureus* isolated, 25 (16.6%) produced enterotoxin and *E. coli* isolated, 19 (13%) produced enterotoxin respectively.
Conclusions: The contamination of kunu occurs during processing, packaging and by vendors. Improved personal hygiene of the producers, environment and proper preservation methods will reduces microbial proliferation and spoilage of kunu. The consumption of kunu is of public health interest.

Keywords: Bacteria, Contamination, Kunu, Enterotoxin, Strains

1. INTRODUCTION

Kunu or kununzaki is a beverage drink made from grains such as millet, sorghum and maize or their combinations. It is a popular drink in northern parts of Nigeria. Kunu made from sorghum is milky light-brown in colour, while that made from maize is whitish in colour [1,2].
The grain seeds used for the production of kunu drink were allowed to germinate while steeped in water for few days and after which blended with sweet potatoes and ginger or pepper to form a smooth paste. The paste is divided into two, one part is placed in a vessel and boiled water is added to it to form a thick mixture. The unheated half is added to the previous and stirred to give a thick mixture. The mixture is left for a day or two for the grain husk to settle. The husk and other sediments are filtered out of the mixture and the filtrate is boiled for consumption.
Kunu is a non-alcoholic beverage marketed in several public places such as offices, markets, schools, motor parks and a very common consumed beverage in ocasions such as weddings, naming ceremonies, birthday celebrations, burials etc.[3] Kunu is an appetizer,

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food complement and refresher to quench thirst [4,5,6] The proximate analysis of kunu was determined and the content includes; protein 2.31 – 3.63%, fats 3.35 – 3.65%, ash content 1.16 – 1.21% and carbohydrates 82.92 – 83.55% [2]

There are varieties of Kunu depending on the feed stock used for processing, they are; kunuzaki, kunugyada, kunusamiya, kunubaule, kununjiko and kunugayamba [1,7] Out of these, kununzaki is most widely produced and consumed [8,9] Some of the microorganisms involved in the fermentation of kunu were *Lactobacilli, Lactococcus, Enterococcus, Streptococcus, Penicillium* and *Sacharomyces* sp.[10] The high bacterial content of kunu may be an indicator of poor hygiene, poor quality cereals and water used in preparation and packaging processes [11] The bacteria isolated from kunu were *E. coli* 33.3%, *S. aureus* 26.7%, *Streptococcus sp*. 23.3%, *Pseudomonas sp*. 10% and *Bacillus sp*. 6.7% [11]. In a study to determine the microbiological quality of kunu in Yenagoa, Bayelsa State, the bacteria isolated were *E. coli, Enterobacter sp, Bacillus sp, Salmonella sp, Micrococcus and Streptococcus sp.* It was noted that most of the bacteria isolated were of public health importance and they were introduced during processing and handling due to poor hygiene [12] I n another research investigating microbial quality of locally produced kunu in Calabar the bacteria isolated were *Bacillus sp*. 15%, *E. coli* 15%, *Salmonella sp*. 12.5%, *Streptococcus sp.*10%, *Pseudomonas sp.* 7.5%, *Proteus sp.* 7.5%, *Lacobacillus* 22.5% and *S. aureus* 10% respectively [13]. The microbiological qualities of kunu sold in Calaber were below acceptable standard and unfit for human consumption soughtIt was noted that the contamination of kunu could come from different source, the bacteria isolated may present health risk to consumers [14]. Bacteria isolated from kunu might be associated with food spoilage, food infections and poisons [15]. Similar organisms were isolated by other researches and they attributed the contamination of kunu to processing and handling.The processing and handling of kunu should be improved for consumers wellbeing [16]. Kunuzaki contamination with pathogenic bacteria is of public health importance and might cause diverse food related illnesses and infection to consumers [17]. The aim of this work is to determine the bacteriological quality of kunu sold in Bayelsa, identy isloted bacteria and the enterotoxin producing strains of some isolates.

2. MATERIAL AND METHODS

2.1 Study Area

The study was conducted in Bayelsa State, Nigeria. The samples of Zobo drink were purchased from the three (3) senatorial district headquarters of Bayelsa, namely; Yenagoa (the capital), Sagbama and Ogbia town. Bayelsa state was carved out of River State in 1996. Bayelsa is located in latitude 4°〖15〗^' North, latitude 5°23' South and longitude 5°22' west and longitude 6°45' east. It is bound by Delta State on the North, River State on the East and Atlantic Ocean on the West and South. Bayelsa has the largest wetland in West African sub-region. It has a population of about 1.7 million people.

2.2 Collection of Samples

The organism used for the experiment is *P. aeruginosa.* It was molecularly identified at Lahor Research Laboratories, Benin, Edo State, Nigeria. The organism was stored in 50% glycerol and kept at -20°C.

2.3 Bacteriological Examination of Samples

Each sample of kunu was gently mixed by inversion several times and 1mL of the sample (neat) was added to 9ml of sterile peptone water (sterilized by autoclaving at 121° C for 15 minutes). Subsequent serial dilutions were made up to 105 and 0.mL of the last dilution

(105) was dropped on already prepared and dried agar plates in duplicates (nutrient, MacConkey and salmonella/shigella). These was spread evenly on agar media with aid of sterile glass rod (sterilized by dipping in absolute alcohol and flaming in bunsen flame).

The inoculated plates were allowed to dry and incubated at 37° C for 18 - 24 hours before examining for growth.

2.4 Test for Bacterial Load in stored Kunu

A set of freshly prepared kunu were kept at room temperature and another in the refrigerator at about 4° C after the initial determination of the bacteria counts in CFU/mL. The counts from the preserved kunu at room and refrigeration temperature were determined on the second and third day respectively.

2.5 Identification of Isolated Bacteria

The bacteria isolated were identified using morphology, cultural, Gram's stain reaction, chemical and biochemical reactions such as citrate, VP, Methyl red, indole, catalase, coagulase and carbohydrate fermentation etc.

2.6 Detection of Enterotoxin Producing *E. coli* from Kunu

PROTM 0157 KIT detects enterotoxin producing E. coli. The Hardy Diagnostics PROTM 0157 KIT provides a rapid latex agglutination method to detect E. coli serogroup 0157 antigen from colonies isolated in the laboratory. These were E. coli producing verotoxin (VT-producing pathogen). Hardy diagnostic E. coli PROTM 0157 Kit contains due latex particles coated with an antiserum against E. coli 0157 antigen. When the coated latex particle is mixed with fresh colonies of E. coli serotype 0157, the bacteria will bind to the antiserum, causing the latex particles to visibly agglutinate, which is indicative of positive result.

2.6.1 Procedure

The reagents were allowed to attain room temperature for about 20 minutes prior to use. Then a drop of sterile saline (Cat. no.K59) was dropped in the circle on the test card and overnight cultures of E. coli were emulsified by mixing it with the saline on the test card. The Latex Reagents were mixed by inverting the tubes several times, prior to use. One (1) drop of E. coli PRO™ O157 Latex Reagent were dispensed onto the test circle on the test card. The Latex Reagent and the organism suspension were then mixed with the wooden applicator provided, using the complete area of the circle. A new stick was used for each reagent. Then the entire card was gently hand-rocked, allowing the mixture to flow slowly over the ring area for up to 2 minutes. Under normal lighting conditions, agglutination (strong clumping) of the latex particles were examined. All organisms yielding a positive agglutination reaction were retested with the Negative Control Latex Reagent.

2.7 Enterotoxicity Testing For *S. auerus*

The Prolex™ Staph Latex Kit provides a rapid platform for the identification of Staphylococcal isolates particularly S. aureus that produce enterotoxin. The Prolex™ Staph Latex Kit utilizes blue polystyrene latex particles that have been sensitized with fibrinogen and IgG

2.7.1 Procedure

The test kit was removed from the refrigerator 20 minutes prior to use and the latex reagents were allowed to attain room temperature. The latex reagent was re-suspended by inverting the dropper bottle several times. This was followed by dispensing 1 drop of Staph Test Latex Reagent into a circle on the test card. A sterile loop was used to transfer two colonies of the test isolate into the circle. The reagent and colonies were mixed and spread to cover the entire area of the circle and this was rocked gently on the card allowing the mixture to flow slowly over the entire test ring area. Agglutination was observed for 20 seconds. Negative Control Latex Reagent was included as quality control. Strong agglutination within 20 seconds with the Staph Test Latex Reagent indicates positive result.

3. RESULTS

3.1

A total of 150 samples of kunu were examined for the presence of bacteria. The result obtained showed that from Yenaguoa S. aureus was 50 (27.8%), E. coli 50 (27.8%), Bacillus sp. 50 (27.8%), and Streptococcus sp. 30 (16.7%) respectively. From kunu bought from Sagbama, S. aureus were 50 (27.0%), E. coli 50 (28.6%), Bacillus sp. 50 (27.0%) and Streptococcus sp. 35 (18.9%) respectively. Kunu purchased from Ogbia town had S. aureus 50 (28.6%), E. coli 50 (28.6%), Bacillus sp. 50 (28.6%) and Streptococcus sp. 25 (14.3%) respectively. The overall percentage occurrences of isolated bacteria were S. aureus 150 (27.8%), E. coli 150 (27.8%), Bacillus sp. 150 (27.8%) and Streptococcus sp. 90 (16.9%) respectively shown of Table 1.

Table 1. Percentage Occurrence of Bacteria Isolated from Kunu Drink

Location	*S. aureus*	*E. coli*	*Bacillus sp.*	*Streptococcus sp.*	**Total**
Yenegoa	50 (27.8)	50 (27.8)	50 (27.8)	30 (16.7)	185 (33.3)
Sagbama	50 (27.8)	50 (27.8)	50 (27.8)	35 (27.8)	185 (34.6)
Ogbia	50 (27.8)	50 (27.8)	50 (27.8)	25 (14.3)	175 (32.4)
Total	150 (27.8)	150 (27.8)	150 (27.8)	90 (16.7)	540

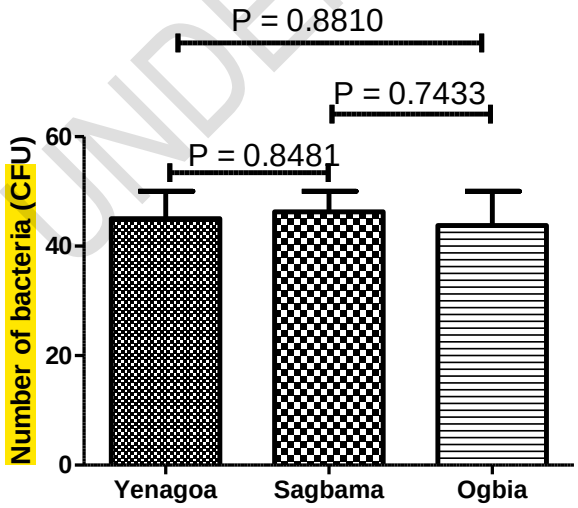

Fig Comparison of the bacteria isolated from different locations

4.2 Enterotoxin Producing *S. aureus* and *E. coli* from Kunu

A total of 50 S. aureus were isolated from kunu in Yenegoa out of which 7 (14%) were enterotoxin producing strains and 50 S. aureus were isolated from Sagbama, 10 (20%) produced enterotoxin, while in Ogbia, Town 50 S. aureus were isolated, 8 (16%) were positive for enterotoxin production respectively. Overall total of S. aureus that produsedenterotoxin were 25 (16.6%) as shown in Table 2.

Table 2. Percentage Occurrence of Enterotoxin Producing *S. aureus* and *E. coli* from Kunu

Location	S. aureus	Number positive	E. coli	Number positive
Yenegoa	50	7 (14)	50	7 (14)
Sagbama	50	10 (20)	50	5 (10)
Ogbia	50	8 (16)	50	7 (14)
Total	150	25 (16.6)	150	19 (13)

Numbesr in parentheses =percentages
Numbers in parentheses = percentages

Table3. Bacterial Counts from Preserved Refrigerated and Non-Refrigerated Kunu (CFU/mL)

Temperature	0 Hr	24 Hrs	48 Hrs
Refrigeration	7.48×10^5	8.40×10^5	8.72×10^5
Room	7.48×10^5	11.96×10^5	13×10^5

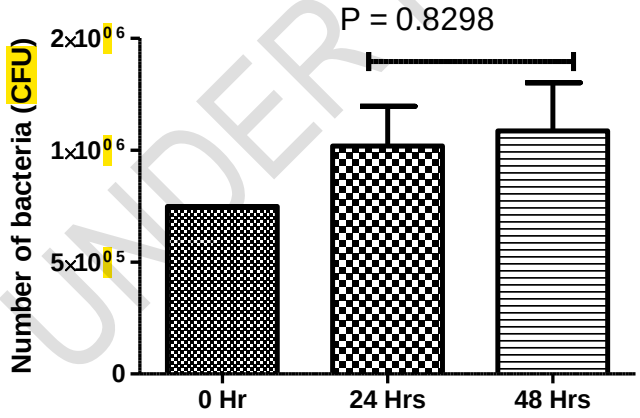

Fig. Comparison of bacterial counts from 0 Hr, 24Hrs and 48Hrs.

S/No	Colour	Surface	Edge	Translucency	Texture	Gram Rxn	Size	Shape	Motility	Methyl Red	Voges Proskauer	Oxidase	H2S Production	Indole	Coagulase	Catalase	Citrate	Urease	Starch Hydrolysis	Glucose	Lactose	Sucrose	Maltose	Galactose	Mannitol	Arabinose	Oxidative	Fementative	Bacteria
1	M	R	E	C	D	+	Md	Rd	+	-	+	-	-	-	-	+	-	-	+	A	A	A	A	A	±	-	+	+	***Bacillus sp.***
2	Cr	S	E	C	Mt	-	Md	Rd	+	-	-	-	-	+	-	+	-	-	-	AG	AG	AG	AG	AG	AG	AG	+	AG	***E coli***
3	Cr	S	E	C	Nt		Md	Co	-	-	-	-	-	-	-	-	N	N	N	+	+	-	+	-	-	-	+	+	***Streptococcus sp.***
4	Cr	S	E	C	Mt	+	Md	Co	-	+	+	-	-	-	-	+	N	N	-	A	A	A	-	A	A	-	+	+	***S. aureus***

4. DISCUSSION

The bacteria isolated from kunu drinks in this study were *Staphylococcus sp, E. coli, Bacillus sp.* and *Streptococcus sp*. The contamination after the boiling process (post CCP contamination) might be responsible for the presence bacteria in kunu. Similar bacteria were isolated from kunu by other researchers in other parts of Nigeria such as *Bacillus sp. Salmonella sp. Micrococcus, Staphylococcus aureus* and *Streptococcus sp*.[13], *E. coli, S. aureus, Salmonella sp* and *Shigella sp* in Maiduguri [18], In Kaduna, *Lactobacillus, Bacillus sp.* and *E. coli* [19], while some reported *E. coli, S. aureus*, *Streptococcus pyogenes* [20]. Other researchers had *S. aureus* 4(10%), *Lactobacillus* 9(22.5%), *Proteus sp.* 3(7.5%), *Streptococcus sp.* 4(10%), *Pseudmonas sp.* 3(7.5%), *Bacillus sp.* 6(15%) and *E. coli* 6(15%) respectively [13]. Most researchers had comparable bacteria in Port Harcourt [7], Oyo and Lagos [1,2], Kano [21], Maiduguri [18], Jalingo [3] in Calabar cross River State [5]. The poor microbial quality of kunu produced locally were because of poor hygiene and poor environmental conditions under which kunu were produced. Health hazard may be associated with consumer of locally produced kunu.

Among the bacteria isolated, Staphylococci were the most prevalent organism. Staphylococcus is normal inhabitant of the human body which can be found on the skin, mouth, nostril, hands, various surfaces etc. these were possible sources from where Staphylococci sp. can contaminate kunu during processing and packaging. Bacteria might be present in storage containers, sieve used to filter the finished product and contamination from handlers. The percentage of *S. aureus* was 21.7% [11],while in this study *S. aureus* were 26.7%. The prevalence of *E. coli* was 27.8% from this study but others *E. coli* as 33.3% [11] and *E. coli* as 5.0% [5]. *E. coli* is the most prevalent aerobic bacteria in human and mammal faeces. Contamination by *E. coli* might be by faecal contamination, contaminated water, handlers, processing and packing. *Bacillus sp.* were 27.8% occurrence. *Bacillus sp.* are geophilic and the spores are found in the soil, dust etc. The contamination of grains and spices by *Bacillus sp*. and their spores from soil and dust are likely. The percentage of *Bacillus sp.* isolated other workers were 23.3% [5], and 7.6% [12]. Spores may survive during boiling at about 100°C (the only Critical Control Point (CCP) in the processes of kunu production) and germinate to re-contaminate kunu. Kunu should be preserved under refrigeration temperature at 4° C and or pasteurized to reduce the microbial load and to increase the shelf life [21,5].

It was noted that local drinks such as kunu may act as vehicle for the transmission of zoonotic and bacterial infections such as staphylococosis, salmonellosis, shigellosis, tuberculosis, listerosis etc. (Umaru et al., 2014)[3]. Kunu as a beverage sold in public places such as markets, schools, offices etc. is patronized because of the cheap price compared to other soft drinks and is served in occasions such as weddings, naming ceremonies, birthday celebrations for economic reasons and public acceptability. The consumption of contaminated kunu drink may result in outbreak of food borne illness. Preparation of kunu in environment with poor sanitary conditions predisposes the preparation and packaging processes to contamination and exposes the consumers to health risk.

The isolation of 13% of *E. coli* and 17% of *S. aureus* capable of producing enterotoxin indicates that the consumption of kunu is of public health concern. The production of enterotoxin in bacteria is mostly associated with gastrointestinal disturbances and/ or food borne illnesses.

Conclusions: The production kunu as non alcoholic beverage for public consumption should be regulated by appropriate regulatory agencies to reduce the risk of consumer infection. Producers should be made to have fair knowledge of food preservation and food sanitation.The isolation of 13% of *E. coli* and 17% of *S. aureus* capable of producing

enterotoxin indicates that the consumption of locally produced kunu is a public health concern.

REFERENCES

1. Amusa NA, Odunbaku OA (2009) Microbiological and Nutritional quality of hawked kunu (Sorghum based non-alcoholic beverage widely consumed in Nigeria. Pakistan Journal of Nutrition 8(1): 20-25.

2. Amusa NA, Odumbako O, Ashaya A (2009) Microbiological and nutritional quality of hawked Kunu widely consumed in Nigeria. Journal of Food Microbiology 12: 1-11.

3. Umaru, G. A., Tukur, I. S., Akensire, U. A., Adamu, Z., Bello, O. A., Shawulu, A. H. B., Sunkani, J. B., Adamu,S. G. and Adamu, N. B. 2014. Micro flora of ofkunu-zaki and sobo drinks in relation to public health in Jalingo metropolis, North-Eastern Nigeria. International Journal of Food Research, 1: 16-21.

4. Adelekan O. A., Alamu, A. E., Ngozi, U. A., Yetunde, O. A., and Dosa, A.S. (2013). Nutritional, Microbiological and Sensory Characteristics of Malted Soy-Kunu Zaki: An Improved Traditional Beverage. Advance in Microbiology, (3), 389-1637.

5. Mbachu, A. E., Etok, C. A., Agu, K. C., Okafor, O. I., Awah, N. S., Chidi-Onuorah, L. C., Ekweme, V. C., Okpala, J., Ogbue, M. O., and Ikele, M. O., (2014). Microbial Quality of Kunu Drink Sold in Calabar. Journal of Global Bioscience, 3(2), 511-513.

6. Oranusi, S. U., Ogbonna, O. I., and Agusi, E., (2013). Microbial Quality Assessment of Foods Sold in Student Cafeterias. Global Research Journal of Microbiology. 3(1): 1-7.

7. Essien, E., Monago, C. and Edor, E. (2009). Evaluation of the Nutritional and Microbiological Quality of Kunu (A Cereal Based Non-alcoholic Beverage) in Rivers State, Nigeria. The Internet Journal of Nutrition and Wellness. (10):2.

8. Gaffa, T., Jideani, I. A., and Nkama, I., (2002). Traditional Production, Consumption and Storage of Kunu; A Non-alcoholic Cereal Beverage. Plant Food for Human Consumption. African Journal of Food Science, 57(1), 202-81.

9. Ayo, J.A., Onuoha, O.G., Ikuomola, D.S., Esan, Y.O., Ayo, V.A. and Oigiangbe, I.G. (2010).Nutritional Evaluationof Millet-beniseed Composite Based Kunun-zaki. Pakistan Journal of Nutrition 9 (10): 1034-1038.

10. Oyewole, O. A., and Isah, P., (2012). Locally Fermented Foods in Nigeria and their Significance to National Economy: A Review. Journal of Recent Advances in Agriculture. 1(4):92-102.

11. Ikpoh, I. S., Lennox, J. A., Agbo, B. E., Henshaw, E. E., and Udoekong, N. S., (2013). Microbial Quality Assessment of Kunu Beverage Locally Prepared and Hawked in Calabar, Cross River State, Nigeria. Global Journal of Biodiversity Science and Management, 3(1):58-61.

12. Orutugu LA, Izah SC, and Asiebai ER (2015). Microbiological quality of kunu sold in some major markets o Yenagoa Metropolis, Nigeria. Continental Journal of Biomedical Sciences, 9(1) 9 – 15.

13. Etang UE, Ikon GM. Udofia SM, Umo EN, Udo AN, Uyanga FZ and Ohagim PI (2017). Microbiological analyses of kunu drinks locally produced and sold in Calabar, South Nigeria. Journal of Advances in Microbiology, 5(2). 1 – 8.

14. Tiku DR, Antai SP and Asuquo NE (2017). Microbiological and nutritional quality characteristics of kunu drink sold in Calabar Metropolis. International Journal of Science Research and Methodology. 9(1),.221- 235

15. Briade W, Ukagwu N, Lugbe PB, AkienAli AI and Adeleye SA (2018). Chemical properties and microbial profile of kunuzaki, a non alcoholic beverage. Journal of Scientific and Technical Research, 4(1), 1 – 5.

16. Nwache I, Nosano MY, Andefiki U and Nnamdi (2017). Microbiological quality of millet based kunu (gruel) made with aqueous tamarind (Tamarindus indica). AASCIT Journal, 3(3), 14 – 18.

17. Elijah AI, Edem V and Umoh SH (2017). Microbiological quality of kunuzaki sold in Etyenkorin, Kwara State, Nigeria. Nigerian Journal of Agriculture and Food Environment. 13(12), 112 – 116.

18. Ogbonna, I. O., Opobiyi, M. Y., Katuka, B. and Waba, J. T. 2011.Microbial evaluation and proximatecomposition of kunu-zaki, an indigenous fermented food drink consumed predominantly in Northern Nigeria. *Internet Journal of Food Safety,* 13: 93-97.

19. Edward, KC and Ohaegbu, CG (2012). The effect of gingerand garlic on the microbial load and shelf life of Kunun-zaki.*Journal of Applied Pharmaceutical Science*. 02 (05); 150-153.

20. Musa, A.A. and Hamza, A. (2013). Comparative Analysis of Locally Prepared 'KununAya' (Tiger-Nut Milk)Consumed By Students of Kaduna State University, Kaduna-Nigeria. *Science World Journal,* 8 (2): 13 – 18.

21. Bukar, A. Uba, A. and Ayeyi, T. I. (2010). Occurrence of Some Enteropathogenic Bacteria in Some Minimally and Fully Processed Ready to Eat Foods in Kano Metropolis, Nigeria. *African Journal of Food Science.* 4(2), 32-36

DEFINITIONS, ACRONYMS, ABBREVIATIONS

Here is the Definitions section. This is an optional section.

Term: Definition for the term