Antibacterial Activity of Lactic Acid Bacteria Isolated From Fermented Cereal Products Against Organisms Implicated in Gastrointestinal Tract Infections

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Abstract: The present study evaluates the in-vitro antibacterial activities of lactic acid bacteria (LAB) isolated from two fermented cereal products – *ogi* and *kunun-zaki*, against organisms implicated in gastrointestinal tract infections. A total of fifteen (15) LAB strains with 5 each from white maize *ogi*, yellow maize *ogi*, and *kunun-zaki* were isolated and characterized. The LAB strains were tested against 5 clinical pathogens. The highest zone of inhibition against *Shigella* (20.0mm) was shown by *Lactobacillus helveticus* YM0007 and *L. casei* YM2434 bothfrom yellow maize *ogi* while the lowest zone of 1.0mm was observed in *L. lactis* KN49 from *kunun-zaki*. Against *Salmonella typhi*, the highest zone of inhibition of 22.5mm was shown by *L. fermentum* WM4825 from white maize while the lowest zone of 1.0mm was observed in *L. plantarum* KN56. *L. brevis* WM4832 had the highest zone of 25.0mm against *Escherichia coli* while the lowest zone of 1.0mm was observed in *L. lactis* KN49 and *L. brevis* KN46. The highest zone of inhibition (20.0mm) against *Enterococcus faecalis* was observed in *L. helveticus* YM0007 while the lowest zone of 5.0mm was shown by *L. casei* KN39. Against *Klebsiella* sp., the highest zone of inhibition of 27.5mm was shown by *L. casei* WM4826 while the lowest zone of 4.0mm was observed in *L. casei* KN39. The highest production of lactic acid was observed in *L. bulgaricus* KN46. *L. plantarum* KN56 produced the highest amount of hydrogen peroxide while the highest amount of diacetyl was produced by *L. lactis* KN49. This study suggests that lactic acid bacteria found in *ogi* and *kunun-zaki* could be useful in ameliorating gastrointestinal tract infections.

Keywords: Antibacterial activity, Gastrointestinal tract infections, *Kunun-zaki*, Lactic acid bacteria, *Ogi*.

Introduction

Lactic acid bacteria (LAB) are industrially important group of microorganisms employed in food fermentation and are well known for their health and nutritional benefits (Caplice and Fitzgerald, 1999; Calderon *et al.*, 2001). There is an increased interest in the use of LAB in food preservation because of their safe association with human fermented foods and stability (Dike and Sanni, 2010). They are commonly found in foods and feeds and they are accepted as generally regarded as safe (GRAS) product for human consumption (Caplice and Fitzgerald, 1999).

Gastrointestinal tract (GIT) infections are diseases that affect the digestive tract as a result of ingestion of infectious bacterial organisms. Infectious bacteria such as *Escherichia coli, Salmonella and Shigella* are among the most common sources of gastrointestinal tract infections (Cynthia, 2013). GIT infections cause nausea with or without vomiting, diarrhoea and other gastrointestinal symptoms. They can be treated with antibiotics and nutritional support as well as fluids. Left untreated, they may lead to severe dehydration and electrolyte imbalances which can result to shock or coma and may be life-threatening (Cynthia, 2013).

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The resistance of bacterial pathogens to antimicrobials to which they were susceptible to has been on the increase in the recent times (Oli *et al*., 2012). Assessing effective drugs is not easy for the poor populace of the developing nations. This necessitates the search for affordable alternative therapy to antimicrobial substances (Aderiye and David, 2013).

Ogi and *Kunun-zaki* are the most common lactic acid fermented foods popularly consumed in Nigeria. They are fermented products of maize, sorghum, and millet, either separately or in combination. Advantages of these fermented products include enhanced nutritional value, digestibility, therapeutic benefits, and safety against pathogens (Oranusi, 2003). Osuntoki and Korie, 2010 reported several beneficial activities of lactic acid bacteria which include immunomodulatory, anti-allergic, antimicrobial, anti-hypertensive and anti-tumourigenic. Another supporting use of LAB fermentation to prevent diarrhoeal diseases is based on the fact that they modify the composition of intestinal microorganisms thereby acting as deterrents for pathogenic enteric bacteria (Olukoya *et al*., 2011).

Therefore, lactic acid bacteria act as barrier against organisms that are non-acid tolerant and their fermented food products can help control diarrhoeal diseases in children (Oyewole and Isah, 2012).

Materials and Methods

Collection of Samples and Indicator Organisms: White and yellow maize (for *Ogi*) and millet (for *Kunun-zaki*) grains were purchased at Bodija market, Ibadan, Nigeria and brought to the laboratory for immediate processing. The gastrointestinal tract pathogens used as indicator organisms were obtained from the Medical Microbiology Department, University College Hospital, Ibadan, Oyo State.

Laboratory Preparation of *Ogi* And *Kunun-Zaki*: The method described by Odunfa and Adeyeye (1985) was used for the preparation of *Ogi* while *Kunun-zaki* was prepared in the laboratory using the method described by Oluwajoba *et al.* (2014).

determination of PH and Total Titratable Acidity (TTA) of Fermenting *Ogi* and *Kunun-Zaki*: Samples were aseptically drawn every 24 hours for pH determination during the fermentation using a Humboldt pH meter (Humboldt H4382 model). The electrode of the pH meter was dipped in buffer solutions of pH 4 and 9 respectively to standardize the pH meter. Total titratable acidity was determined as described by AOAC, (1990).

Isolation and Characterization of Microorganisms: MRS agar was used for the isolation of lactic acid bacteria. The isolates were Gram stained to observe the gram reaction and cell morphology. Biochemical tests including catalase, indole, oxidase, citrate, and nitrate reduction were done using standard procedures (Olutiola *et al*., 2000). Sugar fermentation profile of the isolates was determined using API 50CH strips and API 50CHL medium (API System, Bio-Merieux, France).

Antibacterial Activity of Lab Isolates Against Clinical Pathogens: Five LAB isolates each was selected from each substrate for use against the clinical pathogenic organisms using dual agar overlay method described by Aween *et al.* (2012). The LAB isolates were spot inoculated on MRS agar plates and grown at 30^0C for 24 hours in anaerobic jars. The plates were overlaid with 15ml of nutrient agar containing the clinical isolates. After 24h of aerobic incubation at 30^0C, the diameter of zones of inhibition was measured. The tests were done in duplicate and the mean was taken.

Determination of Antimicrobials Produced by The Lab Isolates: The fifteen selected organisms were grown in MRS broth for 48h and samples were taken for the determination of lactic acid, hydrogen peroxide and diacetyl production using methods described by AOAC (1990).

Results

The pH changes during the fermentation of white maize *ogi* and yellow maize *ogi* are represented by Figure 1. The pH of white maize *ogi* dropped from 4.7 observed at the start of fermentation to 4.1 at the end of the 72-hour fermentation. Similarly, in yellow maize *ogi*, the pH was 4.9 at the start of fermentation and this dropped to 4.2 at the end of the fermentation period (Figure 1). The total titratable acidity (TTA) in white maize *ogi* increased from 1.03g/L at the start of fermentation to 1.77g/L at the end of fermentation. In yellow maize *ogi*, the increase in TTA was from 0.81g/L to 1.67g/L at the start and end of the fermentation respectively (Figure 2).

The morphological and biochemical characteristics of the isolates showed that all the isolates were Gram positive with rod shape. All the isolates were catalase negative and non-motile. They were negative for indole, oxidase, citrate, nitrate reduction and starch hydrolysis. The sugar fermentation profile of the isolates was used for the identification of the isolates using APILAB software (Table 1). The isolates were identified as *L. fermentum* (1), *L. casei* (4), *L.lactis* (2), *L. bulgaricus* (4), *L. helveticus* (2), and *L. plantarum* (2).

Table 2 describes the antibacterial activity of the LAB isolates against five clinical gastrointestinal pathogens. The highest zone of inhibition against *Shigella* (20.0mm) was shown by *Lactobacillus helveticus* YM0007 and *L. casei* YM2434 both from yellow maize *ogi* while the lowest zone of 1.0mm was observed in *L. lactis* KN49 from *kunun-zaki*. *L. casei* YM0017 had no zone of inhibition. Against *Salmonella typhi*, the highest zone of inhibition of 22.5mm was shown by *L. fermentum* WM4825 from white maize while the lowest zone of 1.0mm was observed in *L. plantarum* KN56. No zone of inhibition was observed for *L. bulgaricus* YM0002 against *S. typhi*. *L. brevis* WM4832 had the highest zone of 25.0mm against *Escherichia coli* while the lowest zone of 1.0mm was observed in *L. brevis* KN46 and *L. lactis* KN49. *L. casei* YM0017 and *L. plantarum* YM0022 had no zone of inhibition against *E. coli*. The highest zone of inhibition (20.0mm) against *Enterococcus faecalis* was observed in *L. helveticus* YM0007 while the lowest zone of 5.0mm was shown by *L. casei* KN39. No zone of inhibition was observed in *L. casei* WM4826, *L. bulgaricus* WM4832, and *L. casei* YM0017 against *E. faecalis*. Against *Klebsiella* sp., the highest zone of inhibition of 27.5mm was shown by *L. casei* WM4826 while the lowest zone of 4.0mm was observed in *L. casei* KN39. *L. bulgaricus* WM4832, *L. casei* YM0017, and *L. lactis* KN49 had no zone of inhibition against *Klebsiella* sp.

The highest production of 3.20g/L of lactic acid was observed in *L. bulgaricus* KN46 while the lowest value of 1.9g/L was observed in *L. casei* WM4826. *L. plantarum* KN56 produced the highest

amount of 0.007g/L of hydrogen peroxide and the lowest value of 0.003 was shown in *L. fermentum* WM4825. The highest amount of diacetyl (0.9g/L) was produced by *L. lactis* KN49 while the lowest value of 0.4g/L was observed in *L. bulgaricus* WM4832 (Table 3).

Discussion and Conclusion

Lactic acid bacteria were isolated and characterised from two fermented cereal products (*ogi* and *kunun-zaki*). The presence of lactic acid bacteria in these cereals had earlier been reported (Halm *et al*., 1993; Wakil *et al*., 2004). The LAB isolated in this study exhibited the ability to produce antimicrobial substances which are known to be active against pathogenic microorganisms. Several studies have shown that pathogens such as enterotoxigenic *E. coli, Shigella flexneri, Salmonella typhimurium and Bacillus cereus* are adversely affected when present in traditional fermented food (Kunene *et al.*, 2000; Obadina *et al.*, 2006). Some of the antimicrobial properties exhibited by these fermented foods may be as a result of the low pH of the food as well as metabolites produced by microorganisms such as LAB involved in the fermentation. Lactic acid bacteria are known to produce antimicrobial substances mainly in the form of organic acids and metabolites (Obadina *et al.*, 2006).

Lactic acid bacteria arising as a result of fermentation of indigenous foods were isolated and used against clinical pathogens in which they vary in their sensitivity to the different LAB isolated. It has been demonstrated from this study that *Escherichia coli*, *Shigella* and *Salmonella typhi* were all susceptible to all the isolates used against them. *Klebsiella sp.* and *E. faecalis* however varied in their susceptibility. The fact that some indicator organisms were resistant to the LAB isolates does not necessarily mean that the isolates cannot inhibit the clinical pathogens. Relative abilities of the clinical pathogens to survive the acidic conditions of fermented food products may occur through certain mechanisms (Ogunshe *et al*., 2007). The indicator organism can acquire enhanced resistance to certain environmental conditions probably due to pre-exposure of bacterial cells to such or similar food environmental conditions (Olsen *et al*., 1995).

In conclusion, results from this study suggest that lactic acid bacteria hold a great potential for use as starter cultures for production of *ogi* and *kunun-zaki* which could be consumed in the case of gastrointestinal tract infections.

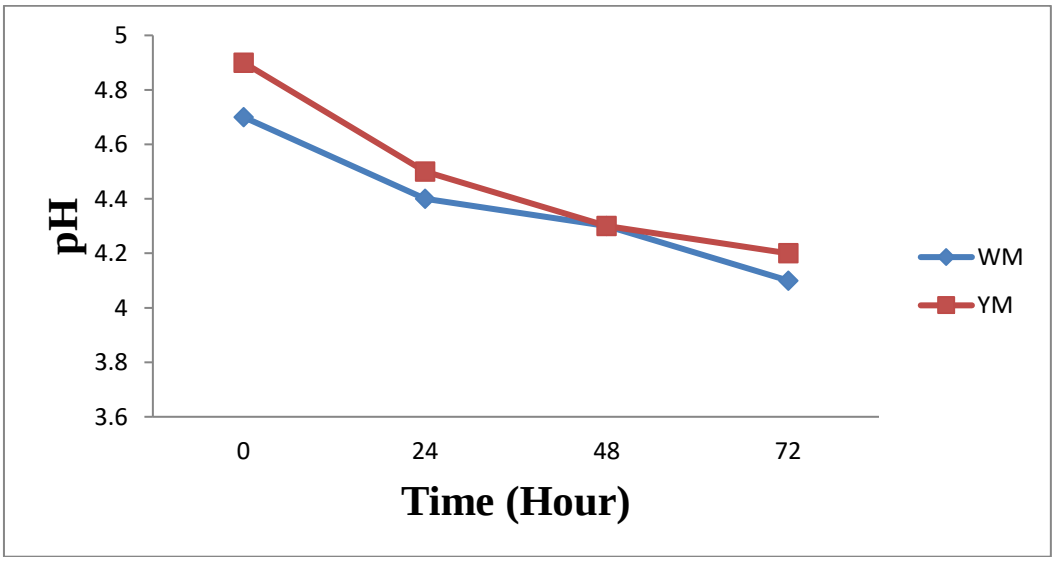

Fig 1: pH changes during fermentation of white maize (WM) *ogi* and yellow maize (YM) *ogi*

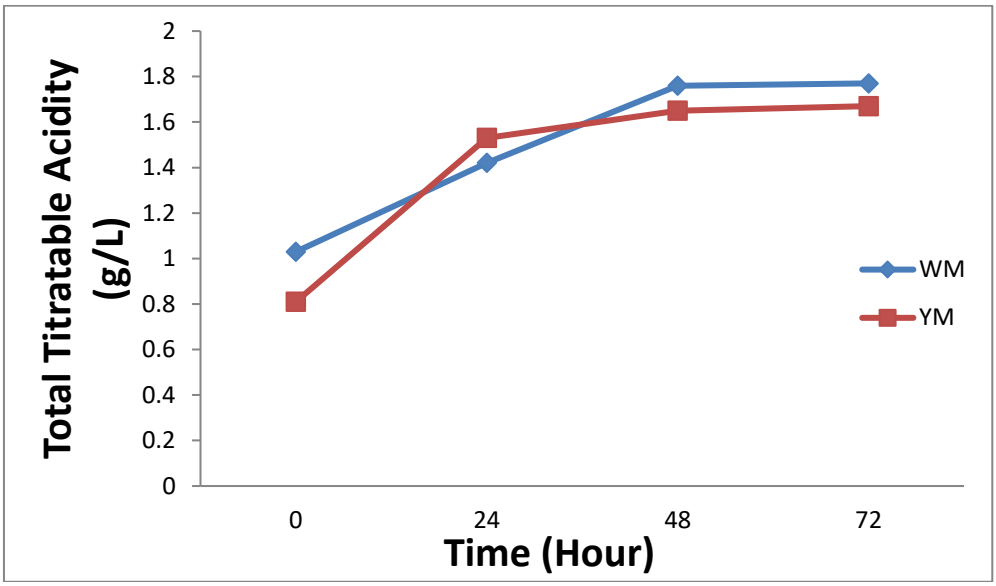

Fig 2: Changes in total titratable acidity during fermentation of white maize (WM) *ogi* and yellow maize (YM) *ogi*

Table 1: Morphology, biochemical characteristics, and sugar fermentation pattern of the LAB isolates from *ogi* and *kunun-zaki*

LAB Isolates	Biochemical characteristics									Sugar fermentation								Probable identity
	Gram reaction	Morphology	Motility	Catalase	Indole	Oxidase	Citrate utilization	Nitrate reduction	Starch hydrolysis	Glucose	Mannitol	Lactose	Maltose	Sucrose	Sorbitol	Dulcitol	Fructose	
WM4825	+	R	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	*Lactobacillus fermentum*
WM4826	+	R	-	-	-	-	-	-	-	+	-	-	+	-	-	-	+	*L. casei*
WM4831	+	R	-	-	-	-	-	-	-	-	+	-	+	-	-	+	+	*L. lactis*
WM4832	+	R	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	*L. bulgaricus*
WM4835	+	R	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	*L. bulgaricus*
YM0002	+	R	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	*L. bulgaricus*
YM0007	+	R	-	-	-	-	-	-	-	+	-	+	+	-	+	-	+	*L. helveticus*
YM0017	+	R	-	-	-	-	-	-	-	+	-	-	+	-	-	-	+	*L. casei*
YM0022	+	R	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	*L.plantarum*
YM2434	+	R	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	*L.casei*
KN16	+	R	-	-	-	-	-	-	-	+	-	+	+	-	+	-	+	*L.helveticus*
KN39	+	R	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	*L.casei*
KN46	+	R	-	-	-	-	-	-	-	+	-	+	-	-	+	+	+	*L.bulgarlcus*
KN49	+	R	-	-	-	-	-	-	-	+	-	+	+	-	+	-	+	*L.onlactis*
KN56	+	R	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	*L.plantarum*

+: positive reaction; -: negative reaction; R: RodTable 2: Antibacterial activity of LAB isolates against clinical pathogens (Zone of inhibition in mm)

LAB Isolates/ Clinical pathogens	*Shigella*	*Salmonella typhi*	*Escherichia coli*	*Enterococcus faecalis*	*Klebsiella sp*
Lf WM4825	7.5	22.5	7.5	12.5	20.0
Lc WM4826	15.0	11.3	7.5	NZ	27.5
Ll WM4831	11.5	20.5	13.0	10.0	22.5
Lb WM4832	18.0	18.3	25.0	NZ	NZ
Lb WM4835	15.0	21.3	9.8	12.5	21.3
Lb YM0002	7.0	NZ	8.0	6.5	8.0
Lh YM0007	20.0	5.0	15.0	20.0	15.0
Lc YM0017	NZ	6.5	NZ	NZ	NZ
Lp YM0022	14.0	5.0	NZ	10.5	17.0
Lc YM2434	20.0	7.8	7.5	8.8	19.3
Lh KN16	3.0	8.0	11.0	13.0	5.0
Lc KN39	15.0	8.0	16.0	5.0	4.0
Lb KN46	3.0	9.0	1.0	10.0	9.0
Ll KN49	1.0	4.0	1.0	18.0	NZ
Lp KN56	6.0	1.0	17.0	7.0	9.0

Lc: *Lactobacillus casei*, *Lb*: *L. bulgaricus*, *Ll*: *L. lactis*; *Lf*: *L. fermentum*; *Lp*: *L. plantarum*; *Lh*: *L. helveticus*

Table 3: Production of antimicrobials by LAB isolates (g/L)

LAB Isolates/ Antimicrobials	**Lactic acid**	**Hydrogen peroxide**	**Diacetyl**
Lf WM4825	2.60	0.003	0.7
Lc WM4826	1.90	0.005	0.5
Ll WM4831	3.10	0.006	0.6
Lb WM4832	2.30	0.005	0.4
Lb WM4835	2.50	0.005	0.5
Lb YM0002	2.30	0.004	0.8
Lh YM0007	2.00	0.005	0.7
Lc YM0017	2.50	0.006	0.5
Lp YM0022	2.60	0.005	0.4
Lc YM2434	2.50	0.005	0.6
Lh KN16	2.90	0.006	0.6
Lc KN39	2.80	0.006	0.6
Lb KN46	3.20	0.005	0.5
Ll KN49	2.10	0.005	0.9
Lp KN56	2.30	0.007	0.5

Lc: *Lactobacillus casei*, *Lb*: *L. bulgaricus*,*Ll*: *L. lactis*, *Lf*: *L. fermentum*, *Lp*: *L. plantarum*, *Lh*: *L. helveticus*

References

A.O.A.C. (1990). Official Methods of Analysis. 15th Ed., Association of Official Analytical Chemist. Washington DC.

Aderiye, B.I. and David, O.M. (2013). Effects of Fermented Maize Gruel (*OGI*) on the Haemato-biochemical Profile of Wistar Albino Rats Challenged with *Shigella Dysenteriae* JBA 010.*Journal of Food Studies* 2(1): 33-41.

Aween, M.M., Hassan, Z., Muhialdin, B.J., Noor, H.M. and Eljamel, Y.A. (2012). Evaluation on Antibacterial Activity of *Lactobacillus acidophilus* Strains Isolated from Honey.*AmericanJournalofAppliedSciences* 9(6): 807-817.

Calderon, M., Loiseau, G. and Guyot, J.P. (2001). Nutritional improvements and simplified cultivation medium to study growth and energetic of a sourdough lactic acid bacterium *Lactobacillus fermentum Ogi* E1 during heterolactic fermentation of starch. *Journal of Applied Microbiology* 90: 1-9.

Caplice, E. and Fitzgerald, G.F. (1999). Food fermentation: role of microorganism in food

production and preservation. *International Journal of Food microbiology* 50: 131-149.

Cynthia, H.E. (2013). Inhibition of food-borne bacterial pathogens by lactic acid bacteria. *Applied Environmental Microbiology* 57: 1683-1688.

Dike, K.S. and Sanni, A.I. (2010). Influence of starter culture of lactic acid bacteria on the shelf life of agidi, an indigenous fermented cereal product.*African Journal of Biotechnology*. 9(46): 7922-7927.

Halm, M., Lillie, A., Srensen, A.K., and Jakobsen, M. (1993). Microbiological and aromatic characteristics of fermented maize dough for *kenkey* production in Ghana. *International Journal of Food Microbiology* 19: 135-143.

Kunene, N.F. Geornaras, I., von Holy, A. and Hastings, J.W. (2000). Characterization and determination of origin of lactic acid bacteria from a sorghum-based fermented food by analysis of soluble proteins and amplified fragment length polymorphism fingerprinting. *Applied and Environmental Microbiology* 66: 1084-1092.

Obadina, A.O., Oyewole, O.B., Sanni, L.O. and Tomlins, K.I. (2006). Bio-preservative activities of *Lactobacillus plantarum* strains in fermenting Casssava "fufu". *African Journal of Biotechnology* 5(8): 620-623.

Odunfa, S.A. and Adeyele, S. (1985). Microbiological changes during the traditional production of *ogi* baba, a West-Africa fermented maize gruel. *Journal of Cereal Science* 3:173-180.

Ogunshe, A.A.O., Omotoso, M.A. and Adeyeye, J.A. (2007). In vitro antimicrobial characteristics of bacteriocin-producing *Lactobacillus* strains from Nigerian indigenous fermented foods. *African Journal of Biotechnology* 6(4): 445-453.

Oli, A.K., Rajeshwari, R.S., Nagaveni, S. and Kelmani, C.R. (2012). Biofilm formation byMultidrug resistant *Enterococcus faecalis* (MDEF) originated from clinical samples. *Journalof Microbiology and Biotechnology Research*, 2(2): 284-288.

Olsen, A., Halm, M. and Jakobsen, M. (1995). The antimicrobial activity of lactic acid bacteria from fermented maize (Kenkey) and their interaction during fermentation. *Journal of Applied Bacteriology* 79: 506-512.

Olukoya, D.K., Ebigwei, S.I., Olasupo, N.A. and Ogunjimi, A.A. (2011). Production of *DogiK*: an Improved Ogi (Nigerian Fermented Weaning Food) with Potentials for Use in Diarrhoea Control. *Journal of Tropical pediatrics* 40(2):108 -113.

Olutiola, P.O., Famurewa, O. and Sonntag, H.G. (2000). An introduction to General Microbiology: A practical approach. Bolbabay publication publishing and media consultant. pp 167-176.

Oluwajoba, S.O., Akinyosoye, F.A. and Oyetayo, V.O. (2014). Antibiotic Susceptibility and Intestinal Epithelial Cell Adhesion Pattern of Homo Fermenting Lactic Acid Bacteria (LAB) Isolated from Kunu-Zaki, a Spontaneously Fermenting Nigerian Cereal Beverage. *British Microbiology Research Journal* 4(12): 1311-1324.

Oranusi, S.U., Umoh, V.J., and Kwaga, J.K.P. (2003). Hazards and critical control points of *Kunun-zaki*, a non-alcoholic beverage in Northern Nigeria. *Food Microbiology* 20: 127-132.

Osuntoki, A. and Korie, I. (2010). Antioxidant Activity of Whey from Fermented Milk, Fermented with *Lactobacillus* Species Isolated from Nigerian Fermented Foods. *Food Technology Biotechnology,* 48(4): 505–511.

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.

Wakil, S.M., Bamgbose, O.O. and Ilo, E.C. (2004). Influence of fermentation time on the microbial profile, sensory attributes and shelf-life of "*kunu-tsamia*" *Advances in Food Sciences* 26(2): 52-55.