Croatian Journal of Food Science and Technology
journal homepage: www.ptfos.unios.hr/cjfst/

Original scientific paper

DOI: 10.17508/CJFST.2021.13.1.15

Evaluation of lactic acid bacteria viability and anti-diarrhoeagenic *Escherichia coli* activities of non-alcoholic fermented beverage '*Kunu*'

Ayomide F. Sowemimo, Abiola O. Obisesan, Funmilola A. Ayeni*

University of Ibadan, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Ibadan, Nigeria

ARTICLE INFO

Article history:
Received: September 11, 2020
Accepted: January 11, 2021

Keywords:
cereal
Kunu
antimicrobial
diarrhoea
storage temperature

ABSTRACT

Kunu is a non-alcoholic fermented cereal beverage consumed primarily as a refreshing drink. This study investigated the effects of storage conditions on viability of Lactic Acid Bacteria (LAB) in *kunu* and the antibacterial effects of *Kunu* against diarrhoea caused by *Escherichia coli* strains. *Kunu* was prepared according to local traditional method. Viability counts of LAB in *kunu* stored at two different conditions, cold (4 °C average) and room temperature (26 °C average), were evaluated. Isolated LAB from *kunu* were identified by partial sequencing of 16S rRNA gene. Five pathotypes of diarrhoea caused by *E. coli* strains were co-cultured with *kunu* to evaluate its antimicrobial activities. Viable LAB count in *kunu* ranged from 5.0 x 10^9 to 1.0 x 10^{11} cfu/mL. *Pediococcus pentosaceus*, *Lactobacillus plantarum* and *Leuconostoc pseudomesenteroides* were identified from *kunu*. There is a drastic decrease (2-5 log reduction) in *E. coli* strains co-cultured with *kunu*. The observed high viable counts of beneficial LAB in *kunu* with its antimicrobial activities against diarrhoeaogenic *E. coli* strains indicates that *kunu* is not just a refreshing drink, but it also has antimicrobial potential against diarrhoea caused by *E. coli*.

Introduction

Cereal grains are one of the most consumed sources of foods especially in developing countries due to their availability, and their low cost compared to carbonated drinks. Cereals such as maize, sorghum, rice etc., are used as fermented foods after they have been milled and mixed into starch mush which is subsequently prepared into solid foods (pap, *Ogi, Garri* etc.) or local drinks (*Burukutu*, *Kunu* etc.). Fermented foods are made up of beneficial live bacteria and have gone through a process during which bacteria convert the sugar into lactic and acetic acid. Lactic acid bacteria (LAB) are involved in fermentation of foods and this helps to improve the taste, provide health benefit as well as preserve the food (Bamidele et al., 2013, Temba et al., 2016, Afolayan et al., 2017).

Kunu is an indigenous fermented beverage in Nigeria. It is made from cereal grains (millet, maize, sorghum or rice) single or mixed, which could be supplemented with additives such as tiger nut, ginger, cloves, pepper, and sweet potato (Ezekiel et al., 2018). Millet, sorghum, maize and rice were used for *kunu* production in decreasing order of preference, while sorghum and millet were the most common combination in a ratio of 1:2 w/w (Gaffa et al., 2002). It has a cool soothing taste and it is very popular in Nigeria. A survey carried out in 21 local government area of Bauchi and Gombe States, in the north-eastern part of Nigeria, revealed that 73% respondents consumed *Kunu* daily, irrespective of their social status (Gaffa et al., 2002). *Kunu* has also been ascertained to be safe for consumption in terms of mycotoxin contents, if the combination of white sorghum millet, ginger, cloves, and sweet potato were used (Ezekiel et al., 2018).

Lactobacilli are the dominant genus in *kunu* (Ezekiel et al., 2018, Oguntoyinbo and Narbad, 2012). There have been current interests in *kunu* as a medicinal food (drink), because it contains naturally occurring LAB with their metabolites that could be beneficial against toxigenic *Aspergillus flavus* (Olonisakin et al., 2017). Diarrhoea is often acquired by the consumption of

*Corresponding author E-mail: funmiyeni@yahoo.co.uk

water or food contaminated with faeces, or directly/indirectly from an individual who is a carrier (infected). Illness, as a result of diarrhoea, is of great public health concern as it is a major cause of morbidity and mortality of infants and some adults residing in sub-saharan Africa, and other countries (Mokomane et al., 2018). Factors prompting the high occurrence of diarrhoea in the affected countries could include: inadequate water supply, bad sanitation and hygiene, poor water supply and insufficient education (Gomes et al., 2016). *Escherichia coli* is a broad versatile bacterium, which explains its commensal and pathogenic potential in human host. The impact of diarrhoeagenic *E. coli* is significant as it is amongst the leading causes of gastrointestinal disorders (Frank et al., 2011).

The frequent use of antibiotics for diseases such as dysentery, diarrhoea and other gastrointestinal infections, might compromise the potential use of such drug in future due to resistance development. Hence, there is a need for alternatives, which include the exploration of the use of fermented foods. Also, the cost of new generation antibiotics (which are more effective) is an hinderance to its constant usage, because some developing communities might not be able to afford them, whereas the cost of *kunu* and other fermented foods are affordable. There has been a report of LAB isolated from *kunu* being effective against toxigenic *Aspergillus flavus* (Olonisakin et al., 2017), but no study is available on antibacterial properties of *kunu* against diarrhoeagenic *E. coli*. This study is designed to evaluate the viability of LAB present in *kunu* at different storage conditions, to identify the LAB present in *kunu* and also to assess the antimicrobial ability of *kunu* against different pathotypes of diarrhoeagenic *E. coli* strains.

Materials and methods

Bacterial strains

Five different pathotypes of diarrhoeagenic *E. coli* strains were obtained from the molecular laboratory unit of Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Nigeria. Three strains; Enterotoxigenic *E. coli* (ETEC) (LLD21A), Enteroaggregative *E. coli* (EAEC) (LLD25D) and Enteroinvasive *E. coli* (EIEC) (LLD21E) were obtained from cases of diarrhoea in children in a hospital in Ibadan, while Enteropathogenic *E. coli* (EPEC) (LLH78D) and Shiga-toxin producing *E. coli* (STEC) (LLH74B) were obtained from healthy children in a community in Ibadan, Nigeria.

Preparation of kunu

Kunu was prepared according to the local traditional method in order to get the physical conditions and constituents as close as possible to the locally produced *kunu*. In summary, sorghum grains and sweet potatoes were obtained from Bodija market, Ibadan and sifted. Sweet potatoes are commonly used in *kunu* production as flavour enhancer and for the reduction of viscosity. The sorghum grains were then soaked in water for a period of 48 h to allow fermentation. However, the sweet potatoes were soaked for 6 h before they were both wet milled and sieved. Two-third of the filtrate was boiled until it gelatinized, while one-third of the filtrate was left uncooked and added to the altered prepared portion. The preparation was sieved, bottled and kept for 18 hours for spontaneous fermentation process. The *kunu* was thereafter aseptically dispensed into two different bottles, one kept at room temperature (26 °C average), while the other one was kept in the refrigerator (4 °C average). Informal acceptability assessment of the stored kunu at two storage conditions were done daily by local consumers (3-5) for a period of four days to determine their personal acceptability of the stored *kunu* at the room temperature and refrigerated conditions.

Evaluation of viable lactic acid bacteria in kunu

Isolation of viable LAB from *kunu* was done according to the modified method of Olonisakin et al. (2017). In summary, on preparation of *kunu* at zero-hour (before fermentation), 1 mL of *kunu* sample at 0 h was serially diluted, plated out on de Mann Rogosa Sharpe (MRS) agar (Oxoid, UK) and incubated under micro-aerophilic conditions at 37 for 48 h. The LAB that grew on the agar plates were counted after assessment of their morphological and physical characteristics on MRS agar. After the evaluation of LAB viability in *kunu* at 0 h, the *kunu* was allowed to ferment for 18 h and aseptically dispensed into two different bottles, one kept at room temperature (26 °C average), while the other one was kept in the refrigerator (4 °C average). This was done to ascertain possible longevity of *kunu* before spoilage and viability of LAB in *kunu* at different storage conditions. This was repeated daily for four days for both samples.

Isolation and identification of lactic acid bacteria in kunu

Fresh *kunu* was prepared and allowed to ferment naturally for 18 h at room temperature (26 °C average), then different dilutions were inoculated into MRS agar (Oxoid, UK) using pour plate technique. The plates were incubated at micro-aerophilic conditions for 48 h. Pure colonies were obtained and gram positive, catalase

negative organisms were selected for further identification (Oloniosakin et al., 2017).
Identification of LAB was done by partial sequencing of 16S rRNA gene. The DNA of all selected organisms was extracted using Accu® Prep genomic DNA extraction kit (Bioneer, South Korean), according to the manufacturer`s instructions. The extracted DNA was used as template in PCR targeted at 16S rRNA gene usingtheprimers:27F (AGAGTTTGATCMTGGCTCAG) and 1389R (ACGGGCGGTGTGTACAAG) with the following PCR conditions: 1 cycle of 95 °C for 4 min, followed with 25 cycles of 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min 30 s and finally, 1 cycle of 7 min at 72 °C (Pinloche et al., 2013). The PCR products were visualised on gel electrophoresis and sequenced using standard procedures. The sequences for each strain were compared with GenBank database using the Basic Local Alignment Search Tool (BLAST) program for the identification of the isolates. The sequences were deposited in GenBank with accession numbers MW264996 -MW265000.

Co-culture experiment

An examination of interference of *kunu* with the growth of diarrhoeagenic *E. coli* pathotypes was done by co-incubating individual *E. coli* strains (ETEC LLD21A, EAEC LLD25D, EIEC LLD21E, EPEC LLH78D and STEC LLH74B) with *kunu* drink in comparison to *E. coli* strains in normal saline (control) by using a modified method previously described by Ojo et al. (2017). In summary, a 24 h nutrient broth of each strain of *E. coli* was prepared and 1 mL was inoculated into 99 mL of undiluted *Kunu*. A control was also set up simultaneously by inoculating 1 mL of *E. coli* into 99 mL normal saline. One milimeter of the test and control samples were appropriately diluted, plated out on MacConkey agar (Oxoid, UK) plates and incubated for 24 h aerobically, after which the viable cells were counted in order to get initial viability of the *E. coli* strains at time zero in test and control samples. The remaining mixture of *kunu* and *E. coli* (99 mL) for test and control samples were incubated for 24 h after which 1 mL of each sample was appropriately diluted, plated out on MacConkey agar (Oxoid, UK) and incubated for 24 h aerobically. The viable cells were counted in order to get final viability counts of the *E. coli* strains after 24 h incubation in test and control samples.

Results

At room temperature, the cfu/mL of LAB in *kunu* ranged between 5.0 x 10^9 to 8.0 x 10^{10} for 3 days, whereas at refrigerated condition the cfu/ml ranged between 1.10 x10^{10} to 2.0 x 10^{11} for 4 days (Fig. 1). An informal consumer acceptability of *kunu* kept at both conditions (room and refrigerated) was evaluated by local consumers and the consensus was that the good taste and aroma were maintained for three days. However, on the 4th day, it was no longer consumable and it gave off a strong acidic odour. *Kunu* kept at refrigerated conditions had good taste and aromafor four days. However, the viability counts greatly declined, so, experiment was discontinued (Table 1).
Identification of LAB isolates were further confirmed by partial sequencing of their respective 16S rRNA genes. The identified organisms are 3 strains of *Pediococcus pentosaceus*, 1 strains of *Lactobacillus plantarum* and 1 strain of *Leuconostoc pseudomesenteroides*. *Kunu* showed inhibitory activities against various diarrhoeagenic strains of *E. coli,* when co-cultured in comparison to control. Enteroinvasive *E.coli* strain reduced by 4 log (from initial counts of 3.5 x 10^7 cfu/mL at 0 h to 3.8 x 10^3 cfu/mL after 24 h co-incubation with *kunu),* while Enteroaggregative *E.coli* strain reduced by 2 log (from 2.05 x 10^6 cfu/mL at 0 h to 1 x 10^4 cfu/mL after 24 h co-incubation with *kunu)* Shigella-toxin producing *E. coli* strain reduced by 5 log (from 3.7 x 10^7 cfu/mL at 0 h to 1.05 x 10^2 cfu/mL after 24 h co-incubation with *kunu),* while Enteropathogenic *E. coli* strain reduced by 3 log (from 5.9 x 10^7 cfu/mL at 0 h to 1.5 x 10^4 cfu/mL after 24 h co-incubation with *kunu)* and Enterotoxigenic *E. coli* reduced by 5 log (from 1.25 x 10^6 cfu/mL at 0 h to 2.5 x 10^1 cfu/mL after 24 h co-incubation with *kunu).* All the control strains increased in viable counts after 24 h incubation in saline (Fig. 2).

Table 1. Informal consumers acceptability level of stored *kunu* at different days and storage conditions

	ROOM TEMPERATURE		COLD	
DAY	TASTE	SMELL	TASTE	SMELL
0 hour	Excellent	Good	-	-
Day 1	Excellent	Good	-	-
Day 2	Very good	Good	Excellent	Good
Day 3	Fair	Fair	Very good	Good
Day 4	Very sour	Bad	Good	Fair
Day 5	-	-	Fair	Bad

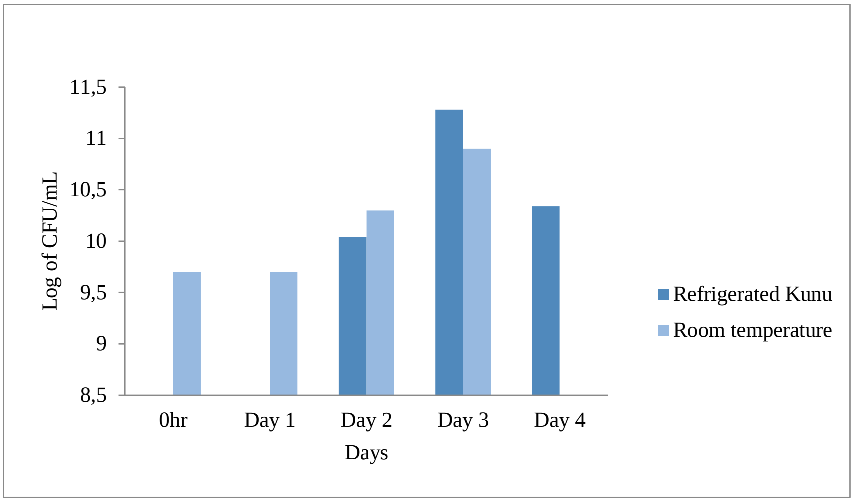

Fig. 1. Viability counts of lactic acid bacteria in *kunu* at different storage conditions

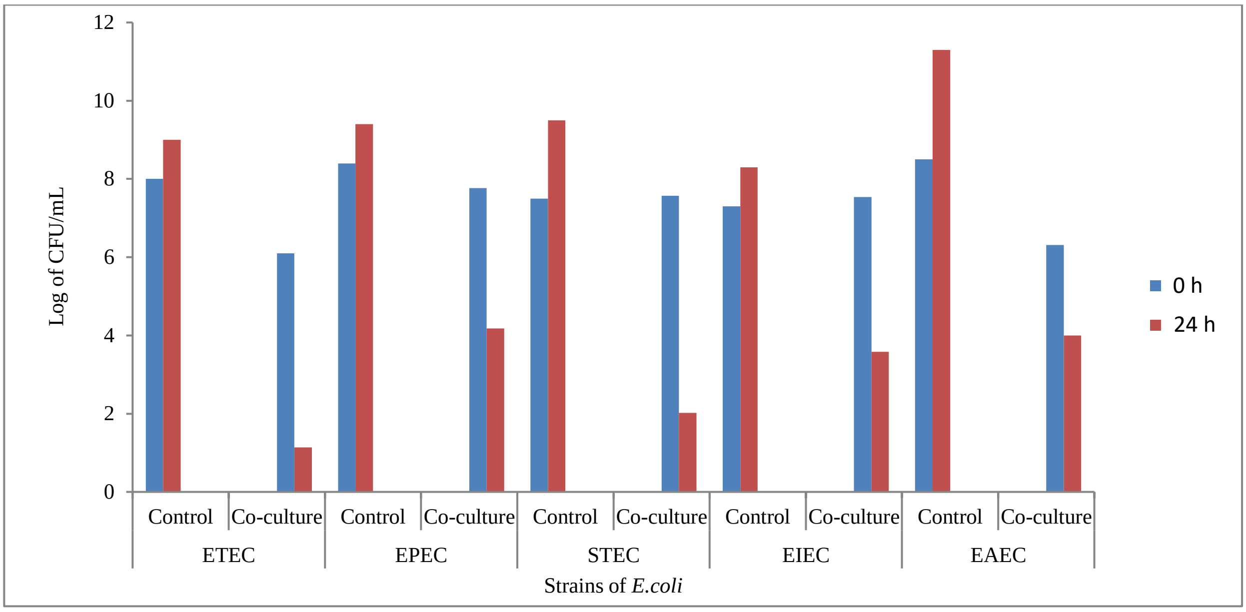

Fig. 2. Reduction in viable counts of *E. coli* after co-culture with *kunu* for 24 h

Discussion

The occurrence of LAB in fermented foods is known to be of high benefits to human health, acting as prophylaxis or therapeutics against various intestinal pathogens as well as maintaining the balance occurring in the gut microbiota (Afolayan and Ayeni, 2017). *Kunu* has got high viable LAB occurring at its different storage conditions. This is especially important in some developing countries that do not have regular power for refrigeration. The consumer acceptability and high LAB viability of 5.0 x 10^9 up to the 3rd day of storage at room temperature is encouraging for homes without refrigeration. The shelf life of refrigerated k*unu* is just a day more than the one stored at room temperature. However, refrigerated conditions made the product to have a longer shelf life of 4 days, and this could, due to the low temperature, reduce the rate of fermentation, thereby preventing high rate of acid production and spoilage.

Viable LAB in *kunu* varies according to preparation, fermentation time and source. Okoronkwo (2014) has previously reported LAB count of 10^7cfu/mL during 48 h fermentation of *kunu*, while Olonisakin et al. (2017) reported a 10^4 cfu/mL viability count of LAB from *Kunu*. However, the LAB count in this study was higher (ranging between log 9.7-11.28 cfu/mL) than in previous studies, which could be the result of traditional preparation of *Kunu* used in this study as opposed to laboratory preparation used in other studies. Also, the methods adopted (such as the amount of time allowed for sorghum to ferment pre (before milling) and amount of time left to ferment post (after preparation) could all play a major role in the lower count reported in the previous studies. There is also a steady increase in the viable counts of LAB in *kunu* over the period of three days before a reduction in counts on day 4. This is comparable to results obtained by Okoronkwo (2014) who reported a steady LAB count in *kunu* over a period of 48 hours.

Studies conducted on microbial population in African non-alcoholic fermented foods such as *Ogi*, fermented dairy foods amongst others have shown the dominance of LAB when compared to other genera with various health effects (Adeniyi et al., 2006, Afolayan et al., 2017, Kwasi et al., 2019, Audu et al., 2019, Sunmola et al., 2019, Omeiza et al., 2020). The presence of *Lactobacillus plantarum* (BOC8), *Leuconostoc pseudomesenteroides* and *Pediococcus pentosaceus* (W22409) in *kunu* ascertains that it is not only a refreshing drink, but could also have health benefits to the consumers. Although lactobacilli are usually isolated from fermented foods, *Pediococcus* and *Leuconostoc* are also associated with *kunu* as reported by Oguntoyinbo and Narbad (2012), Olonisakin et al., (2017) as well as in this study. Ezekiel et al., (2019) also reported dominant community of *Leuconostoc, Pediococcus, Lactobacillus, Lactococcus,* and *Weissella* in the studied *kunu* product, which is related to their adaptation to the acidic pH of *kunu*.

Interestingly, k*unu* is not just a refreshing drink, but it could be an antimicrobial beverage for diarrhoea caused by *E. coli* as it inhibited the growth of different strains of diarrhoeagenic *E. coli* with great log reduction (between 2-5 log). Enterotoxigenic *E. coli* and Shiga toxin producing *E. coli* both had the highest sensitivity to *kunu* followed by Enteroinvasive *E. coli*, Enteropathogenic *E. coli* and Enteroaggregative *E. coli* strains. The drastic reduction in *E. coli* counts, when compared to the growth in normal saline control (where there was increase in the growth of each strain of diarrhoeagenic *E. coli* strain), indicates that viable LAB and their metabolites in *kunu* produced some antimicrobial effects which have potent activity against diarrhoeagenic strains of *E. coli*. Other studies have reported the inhibitory properties of LAB isolated from fermented foods against various pathogens (Adeniyi et al., 2006, Ayeni et al., 2009, Ayeni et al., 2011, Ayeni and Afolayan, 2017, Afolayan et al., 2017, Ayeni et al., 2019). Ojo et al. (2017) has also reported the antimicrobial activity of a local brand of yogurt (Quincy) against diarrhoeagenic *E. coli* strains and Kwasi et al. (2019) also reported antimicrobial activity of *Ogi*, another fermented cereal, against diarrhoeagenic *E. coli* strains. However, to the best of our knowledge, this is the first study reporting the antimicrobial activities of *kunu* against diarrhoeagenic *E. coli* strains. This is especially useful in resource limited areas, where antibiotics may be too expensive. In these areas, locally fermented foods like *kunu* have the potential of being a good alternative.

Conclusion

Viable lactic acid bacterial strains are present in high quantities in *kunu*. *Kunu* tends to have longer shelf life when kept in refrigerated conditions than room temperature. However, the storage at room temperatures does not greatly reduce the viability of lactic acid bacteria present within *kunu*. *Kunu* possesses antimicrobial activities against diarrhoeagenic E. *coli* strains. *Kunu,* therefore, has gotthe potential of being a functional food.

Limitation of the study: A formal organoleptic test was not done in this study, but rather an informal acceptability level test of the stored *kunu* by the local consumers.

Author Contributions: Sowemimo AF did the laboratory work and drafted some parts of the manuscript, Obisesan AO did some of the laboratory works and wrote some aspects of the manuscript. Ayeni FA designed and supervised the laboratory work and edited the final manuscript.

Funding: This research received no external funding

Acknowledgement: We acknowledge Prof. Iruka N. Okeke of the molecular laboratory unit of Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeriafor the provision of already characterised diarrhoeagenic *E. coli* strains used in this study.

Conflicts of Interest: The authors declare no conflict of interest.

References

Adeniyi, B.A., Ayeni, F.A., Ogunbanwo, S.T. (2006): Antagonistic activity of lactic acid bacteria isolated from Nigeria fermented diary food against organisms implicated in urinary tract infections. *Biotechnol.* 5, 183-188. https://dx.doi.org/10.3923/biotech.2006.183.188

Afolayan, A.O., Ayeni, F.A. (2017): Antagonistic effects of three lactic acid bacterial strains isolated from Nigerian indigenous fermented *Ogi* on *E. coli* EKT004 in co-culture. *Acta Aliment.* 46, 1–8. https://doi.org/10.1556/066.2017.46.1.1

Afolayan, A.O., Ayeni, F.A., Ruppitsch, W. (2017): Antagonistic and quantitative assessment of indigenous lactic acid bacteria in different varieties of *Ogi* against gastrointestinal pathogens. *Pan. Afr. Med. J.* 27, 22. https://doi.org/10.11604/pamj.2017.27.22.9707

Audu H.J. Abiodun O.O., Ayeni F.A. (2019): Beneficial Effects of a Fermented Maize product with Its Supernatant, *Lactobacillus fermentum* and *Lactobacillus brevis* in Rat Model of Colitis. *Nor. Afr. J. Food Nutr. Res.* 3 (6), 195-200. https://doi.org/10.5281/zenodo.3540383

Ayeni, F.A., Adeniyi, B.A., Ogunbanwo, S.T., Tabasco, R., Paarup, T., Peláez, C. *et al.* (2009): Inhibition of uropathogens by lactic acid bacteria isolated from dairy foods and cow's intestine in western Nigeria. *Arch. Microbiol.* 191, 639-648. https://doi.org/10.1007/s00203-009-0492-9

Ayeni, F.A., Sánchez, B., Adeniyi, B.A., de los Reyes-Gavilán, C.G., Margolles, A., Ruas-Madiedo, P. (2011): Evaluation of the functional potential of *Weissella* and *Lactobacillus* isolates obtained from Nigerian traditional fermented foods and cow´s intestine. *Int. J. Food Microbiol.* 147, 97-104. https://doi.org/10.1016/j.ijfoodmicro.2011.03.014

Ayeni, A.O., Ruppitsch, W., Ayeni, F.A. (2019): Characterization of bacteria in Nigerian yogurt as promising alternative to antibiotics in gastrointestinal infections. *J. Diet. Suppl.* 16, 141-151. https://doi.org/10.1080/19390211.2018.1440684

Bamidele, T.A., Adeniyi, T.A., Ayeni, F.A., Fowora, M.A., Smith, S.I. (2013): The antagonistic activities of Lactic acid bacteria isolated from Nigerian salad vegetables against methicillin resistant *Staphylococcus aureus*. *Glob. Res. J. Microbiol* 3 (1), 18-23.

Ezekiel, C.N., Ayeni, K.I., Ezeokoli, O.T., Sulyok, M., van Wyk, D.A.B., Oyedele, O.A., Akinyemi, O.M., Chibuzor-Onyema, I.E., Adeleke, R.A., Nwangburuka, C.C., Hajšlová, J., Elliott, C.T., Krska, R. (2019): High-Throughput sequence analyses of bacterial communities and multi-mycotoxin profiling during processing of different formulations of *Kunu*, a traditional fermented beverage. *Front. Microbiol.* 9, 3282. https://doi.org/10.3389/fmicb.2018.03282

Frank, C., Werber, D., Cramer, J.P. (2011): Epidemic profile of Shiga-toxin– producing *Escherichia coli* O104: H4 outbreak in Germany. *N. Engl. J. Med.* 365, 1771-1780. https://doi.org/10.1056/NEJMoa1106483

Gaffa, T., Jideani, I.A., Nkama, I. (2002): Traditional production, consumption and storage of *kunu* a non-alcoholic cereal bevereage plant food for human consumption. *Afr. J. Food Sci.* 57, 73- 81. https://doi.org/10.1023/A:1013129307086

Gomes, T.A.T., Elias, W.P., Scaletsky, I.C.A., Guth, B.E.C., Rodrigues, J.F., Piazza, R.M.F. (2016): Diarrheagenic *Escherichia coli*. *Brazil. J. Microbiol.* 47, 3–30. https://doi.org/10.1016/j.bjm.2016.10.015

Kwasi, R.E., Aremu, I.G., Dosunmu, Q.O., Ayeni, F.A**.** (2019): Viability of Lactic Acid Bacteria in Different Components of *Ogi* with Anti diarrhoeagenic *E. coli* Activities. *Nor. Afr. J. Food Nutr. Res.* 3 (6), 206-213. https://doi.org/10.5281/zenodo.3560326

Mokomane M, Kasvosve I, de Melo E, Pernica J.M, Goldfarb D.M. (2018): The global problem of childhood diarrhoeal diseases: emerging strategies in prevention and management. *Ther. Adv. Infect. Dis.* 5 (1), 29-43. https://doi.org/10.1177%2F2049936117744429

Oguntoyinbo, F.A., Narbad, A. (2012): Molecular characterization of lactic acid bacteria and in situ amylase expression during traditional fermentation of cereal foods. *Food Microbiol.* 31, 254-262. https://doi.org/10.1016/j.fm.2012.03.004

Oguntoyinbo, F.A., Tourlomousis, P., Gasson, M.J., Narbad, A. (2011): Analysis of bacterial communities of traditional fermented West African cereal foods using culture independent methods. *Int. J. Food Microbiol.* 145, 205-210. https://doi.org/10.1016/j.ijfoodmicro.2010.12.025

Ojo, O.E, Sowemimo, A., Ayeni, F.A. (2017): Evaluation of viability of lactic acid bacteria in a Nigerian commercial yogurt and its antagonistic effects on selected strains of diarrheagenic *Eschericia coli*. *Niger. J. Pharm. Res.* 13, 175-180.

Olonisakin, O.O., Jeff-Agboola, Y.A., Ogidi, C.O., Akinyele, B.J (2017): Isolation of antifungal lactic acid bacteria from "*Kunu*" against Toxigenic *Aspergillus flavus Prev. Nutr. Food Sci.* 22, 138-143. https://doi.org/10.3746/pnf.2017.22.2.138

Okoronkwo, C.U. (2014): Isolation and characterization of lactic acid bacteria involved in the fermentation of millet and sorghum sold in Nkwo-Achara market, Abia State. *J. Environ. Sci. Toxicol. Food Technol.* 8, 42-45.

Omeiza, F.O., Ademowo, G.O., Ayeni F.A. (2020): Evaluation of in vivo anti-malarial potential of *omidun* obtained from fermented maize in Ibadan, Nigeria. *Malar. J.* 19, 414. https://doi.org/10.1186/s12936-020-03486-0

Pinloche, E., McEwan, N., Marden, J.P., Bayourthe, C., Auclair, E., Newbold, C.J. (2013): The effects of a probiotic yeast on the bacterial diversity and population structure in the rumen of cattle. *PLOS One* 8 (7), e67824. https://doi.org/10.1371/journal.pone.0067824

Sunmola A.A, Ogbole O.O, Faleye T.O.C, Adeniji J.A Ayeni F.A. (2019): Antiviral Activities of Supernatant of Fermented Maize *(Omidun)* against Selected Enteroviruses. *FUDMA J. Sci.* 3 (3), 540 –545.

Temba, M.C., Njobeh, P.B., Adebo, O.A., Olugbile, A.O., Kayitesi, E. (2016): The role of compositing cereals with legumes to alleviate protein energy malnutrition in Africa. *Int. J. Food Sci. Technol.* 51, 543–554. https://doi.org/10.1111/ijfs.13035