PRODUCTION OF A CEREAL BASED PRODUCT (*OGI*): INFLUENCE OF CO-FERMENTATION WITH POWDERED GARLIC AND GINGER ON THE MICROBIOME

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ABSTRACT

Many African foods undergo lactic acid fermentation; alterations of these germane microorganisms during fermentation of *ogi* can affect its acceptability as a weaning food. Effects of garlic and ginger on microorganisms' load during the production of fermented cereal (*ogi*) from quality protein maize were explored. Powdered garlic and ginger individually and mixed at ratio (2 and 4%) resulted in 7 treatments. The microbial loads were enumerated, isolates identified, pH and total titratable acidity were also determined. O*gi* with 4% garlic-2% ginger had the lowest microbial count value (19.5×10^{7}cfu/ g). *Ogi* containing 4% garlic-2% ginger had the best effect on microbial loads. 4 Lactic acid bacteria were prominent while 6 yeast isolates were identified. Garlic inhibited the growth of *Candida utilis*, *Candida mycoderma, Candida tropicalis* and *Candida krusei.* Combination of ginger-garlic exerted better synergistic effect and addition of garlic and ginger did not affect important microorganisms involved in the fermentation of *ogi*.

KEYWORDS: *Ogi,* quality protein maize, sorghum, powdered garlic-ginger, co-fermentation, microbiome

INTRODUCTION

Cereal based foods are the main sources of cheap dietary energy in many developing countries (Greppi *et al.*, 2013, Olaniran *et al.,* 2019). High standards are set in the study of fermentation as a technique for the preparation of food by the World Health Organisation (WHO) food safety unit. The fermentation process for *ogi* production involves microorganisms and enzymes of distinct quality attributes that are quite different from the original raw materials (Solange *et al.*, 2014). Children in most developing countries are weaned with *ogi* because it is cheap and readily available. *Ogi* is a common fermented product consumed as breakfast cereal and weaning food. It is produced from sorghum, maize and millet (Adelekan & Oyewole, 2010, Omemu 2011, Olaniran & Abiose, 2018). Maize is among the oldest cultivated grains worldwide; a common diet of Nigerians for centuries and it constitutes about 90% of the cereals consumed globally (Kalui *et al.* 2010, Omemu *et al.*, 2018). Replacing with quality protein maize (QPM) will improve the nutritive value of consumers because it contains more lysine (30%) and tryptophan (55%) compared with ordinary maize (Cisse *et al.* 2013, Olaniran & Abiose, 2019). About 20% increase in the linear growth of poor children weaned in communities that maize served as staple food thus contributing to food and nutrition security (Eshetie, 2017). Garlic has nutritional and medicinal values besides the broad spectrum of anti-bacterial and anti-fungal activities (Ryu & Kang, 2017). Ginger commonly used as cooking spice due to it refreshing pleasant aroma and carminative property makes it an indispensable constituent in food processing (Olayiwola *et al.*, 2017). Ginger and garlic are reported safe for consumption (Olaniran *et al.* 2015). The preparation of complementary foods that are nutritious from maize has attracted a lot of attention with several innovative approaches being adopted to improve the quality of *ogi* amongst other foods. This study is therefore undertaken to assess the influence of garlic and/ ginger on key microflora associated with the spontaneous fermentation of *ogi* produced from quality protein maize grains.

MATERIALS AND METHODS

Preparation of *Ogi*

QPM were obtained from the Institute of Agricultural Research and Training (IAR&T), Ibadan. Ginger rhizomes and white variety of garlic bulbs were bought in Ile Ife, Nigeria. Ten (10) kilogram of quality protein maize was cleaned, steeped for 72h and wet-milled into slurry using an attrition mill. Distilled water was added to form slurry without sieving (Farinde, 2015).

Preparation and Incorporation of Powdered Garlic and Ginger

Ginger rhizomes and garlic bulbs were equally sorted for cleaning, peeled and 500g of each dried for 12 h at 65 ^oC in a Gallenkamp oven (UK) then pulverized with Marlex grinder (PVT Mumbai). The resultant powders were passed through sieve with mesh size of 50 µm to get rid of shafts (Olaniran *et al.*, 2015). The weighed ginger and garlic powder were proportion into the *ogi* slurry (w/w), resulting in seven treatments and one control batch and labeled in this manner: A (control samples without garlic or ginger); B (2% garlic); C (4% garlic); D (2% ginger); E (4% ginger); F (2% garlic-2%ginger); G (2% garlic-4% ginger); H (4% garlic-2% ginger) before fermentation. The mixtures were each evenly homogenized using glass rods and allowed to ferment for 24h. Aliquot samples were taken at 12h interval for various analyses.

Microbial Analysis

The total viable, yeast/mould and lactic acid bacteria count were computed. Also, the microorganisms associated with *ogi* slurry were isolated and identified during fermentation. Dilution was carried out serially by mixing 5.0g of *ogi* slurry with 45 ml of maximum recovery diluents (MRD) to obtain 10^{-1} dilution. The mixture was subsequently diluted serially in MRD to the preferred level of dilution. From every dilution, 1.0 mL was pippeted into sterilized Petri dish prior to addition of 20 mL each of molten Nutrient agar (NA) for Total Viable Count (TVC). To other plates; molten Man de Rogosa and Sharpe (MRS) agar was added for Lactic acid bacteria (LAB) count also PDA (Potato dextrose agar) for yeast and mould count (0.5 ml of 3 mg/ml Streptomycin was incorporated to inhibit bacterial growth) respectively. All plates in triplicates were incubated at 37 ^oC for 24 and 72h for TVC and LAB count respectively. For 3 days plates for yeast count were incubated at 28 ± 2 ^oC. Counting of distinct colonies using colony counter was employed for enumeration of microbial load. Colonies counted (25-250) and multiplied with the reciprocal of the dilution factor was done to obtain the count (MClandsborough, 2005).

Classification and Identification of Microbes (Conventional Method)

Repeated streaking of distinct colonies on fresh agar plates to obtain pure culture was carried out; microscopic examination and biochemical test including catalase test, oxidase test, Gram stain, sugar fermentation and production of carbon dioxide. Applicable bacteria and yeast identification scheme were employed for identification (APHA, 2015).

Determination of pH and Titratable Acidity

The determination of total titratable acidity of *ogi* samples was measured during fermentation. Conversion factor of 0.09 was calculated as lactic acid. pH values of the samples were determined using Corning Scholar 425 pH meter (Shenzhen, China). Buffer 4.0 and 7.0 was used to calibrate or standardize the pH meter and the electrode of the pH probes was sanitized with 90% ethanol prior to placing in the sample. 10% slurry of the samples was made before the determination of pH (AOAC, 2010).

Statistical Analysis

Calculation of means were done, separated and recorded in MS Excel sheet (2014) and Duncan Multiple Range Test (DMRT) at 5% level of probability.

RESULTS AND DISCUSSION

The total viable counts (TVC) for all *ogi* samples increased throughout the fermentation period as presented in Table 1. The optimum TVC counts were recorded in the control sample (95.0×10^{10}cfu/ g) while *ogi* containing 4% garlic-2% ginger (19.5×10^{7}cfu/g) had the lowest TVC at the end of fermentation. Although there was no observable lactic acid bacteria growth in all *ogi* samples at 0 h, groups of bacteria appeared by the 12th hour and increased from 12 to 24 h. *Ogi* containing 4% garlic-2% ginger had the lowest LAB count (20.0×10^{7}cfu/ g) at the end of the 24 h fermentation. The steady increase in total viable counts and lactic acid bacteria count of *ogi* samples noted during the study were in agreement with previous studies by Izah *et al* (2016). There was no observable growth of yeast at 0 h of fermentation. However, the increase in yeast count was observed in all *ogi* treatment samples with or without garlic and ginger from 12 to 24 h of fermentation (Table 1). Low yeast counts were observed in all samples containing only 4% garlic; garlic-ginger at different concentrations compared with control (39×10^{4} cfu/g) at 12 h. Similar trends were observed in all *ogi* samples at the end of 24 h fermentation. An increase in yeast count of *ogi* has been reported by Akinleye *et al* (2014). Lower microbial growth in samples containing garlic and/or ginger during fermentation can be attributed to the presence of diffusible gingerols, allicin and shogaol with antimicrobial activities as reported by Olaniran *et al* (2015). Its incorporation also slows down the rate of increase yeast population and there was a synergy when combined by further reducing the load with the highest impacts recorded in the combination of garlic 4%-2%ginger during fermentation as presented. This may have implication on the flavour and sourness at the end of fermentation while prolonging the shelf life of the product by reducing ropiness and development of off flavour during storage. Thus, samples containing 4% garlic-2% ginger had the best effect on microbial counts during fermentation. *Leuconostoc mensenteroides, Lactobacillus plantarum, Lactobacillus amylovorus* and *Lactobacillus fermentum* while *Saccharomyces cerevisiae*, *Saccharomyces rouxii, Candida utilis*, *Candida mycoderma, Candida tropicalis*, *Candida krusei* were isolated. *Leuconostoc mensenteroides, Lactobacillus plantarum* and *Lactobacillus fermentum* mostly lactic acid bacteria were isolated (Tables 2). Lactic acid bacteria especially *Lactobacilli* were prominent in *ogi* during fermentation. *Saccharomyces cerevisiae, Candida utilis*, *Candida mycoderma, Candida tropicalis* were identified during fermentation from *ogi* (Table 3). It was also noted that the succession of these crucial isolates was not negatively affected by the integration of garlic and ginger during fermentation. Thus, garlic and ginger did not affect probiotically important microorganisms involved in the fermentation of *ogi. S. cerevisiae, C. mycoderma, C. utilis* and *C. tropicalis* have been isolated from *ogi* during fermentation (Greppi *et al.*, 2013). From the finding of this study the growth of *C. utilis*, *C. mycoderma* and *C. tropicalis* were inhibited in all *ogi* samples containing garlic during the study. Garlic has been reported to have high anticandidal activity (Olaniran *et al.*, 2015). Microbial successions during spontaneous fermentation are important. Since the overall quality of the final fermented products is connected to the populations for the transformation process attributed to the increase in lactic acid concentration caused by the activity of the LAB (Houngbédji *et al.*, 2018) . The total titratable acidity (TTA) values of all *ogi* samples increased during fermentation (Figures 1 and 2) while the pH of all *ogi* samples decreased throughout fermentation period (Figures 3 and 4). Fermentation of cereals has been reported to be accompanied with an increase in acidity due to the utilization of free sugars by *Lactobacillus* and yeast (Abioye, 2015). Adding either garlic or ginger singly or in combination to *ogi* slurry, improved the lactic acid bacteria flora and reduced the microbial loads during fermentation. This study has shown that garlic and ginger could be added effectively during fermentation of *ogi* produced either from sorghum or quality protein maize without affecting important microorganisms involved in the fermentation of *ogi*. Adding either garlic or ginger singly or in combination with *ogi* slurry, improved the lactic acid bacteria flora and reduced the microbial loads during the fermentation. Its addition of garlic and ginger during fermentation should be encouraged to eliminate unwanted microorganisms in *ogi* during production.

Table 1: Microbial Load (CFU/ g) of co-fermented *Ogi* with Garlic and Ginger

Time (h)	A	B	C	D	E	F	G	H
					Total viable Count			
0	20.7×10^{5}	17.7×10^{5}	17.3×10^{5}	18.9×10^{5}	20.8×10^{5}	32×10^{5}	10.5×10^{5}	11.9×10^{5}
12	83.0×10^{7}	91.0×10^{5}	51.0×10^{5}	36×10^{5}	73×10^{5}	77×10^{5}	31×10^{5}	28×10^{5}
24	95×10^{10}	28×10^{7}	22.5×10^{7}	45×10^{7}	42×10^{7}	79×10^{7}	82×10^{7}	19.5×10^{7}
					Lactic Acid Bacteria Count			
0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
12	35×10^{7}	75×10^{5}	65×10^{5}	21×10^{7}	16.1×10^{7}	51×10^{5}	38×10^{5}	31×10^{5}
24	92×10^{9}	57×10^{7}	42×10^{7}	13.7×10^{8}	12.9×10^{8}	37×10^{7}	29×10^{7}	20×10^{7}
					Yeast count			
0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
12	39×10^{4}	11.3×10^{3}	79×10^{2}	10.5×10^{4}	11.7×10^{3}	28×10^{2}	25×10^{2}	22×10^{2}
24	43×10^{5}	23×10^{4}	18.5×10^{4}	65×10^{4}	30×10^{4}	55×10^{3}	72×10^{3}	49×10^{3}

Means (n=3)

A (control samples without garlic or ginger); B (2% garlic); C (4% garlic); D (2% ginger); E (4% Ginger); F (2% garlic-2%ginger); G (2%garlic-4%ginger); H (4%garlic-2%ginger)

Table 2: Biochemical and morphological Characteristics of bacteria isolated from co-fermented cereal with ginger and garlic

Test	Isolates					
	1	2	3	4	5	6
Morphology	Rods	Rods	Cocci	Rods	Rods	Rods
Colour of growth	Cream	Cream	Cream	Cream	Cream	Cream
Gram reaction	+	+	+	+	+	+
Catalase test	-	-	-	-	-	-
Growth at:						
15ºC	+	+		-	-	-
45ºC	-	-		+	+	+
Production of CO_2	-	+	+	-	+	
Dextran production	-	-	+	-	-	-
fermentation of:						
Glucose	+	+	+	-	+	-
Lactose	+	-	-	-	+	+
Salicin	+	-	-	-	-	+
Galactose	+	+	-	-	+	+
Sucrose	+	+	-	+	+	+
Raffinose	+	+	-	-	-	+
Probable identity of organism	*Lactobacillus plantarum*	*Lactobacillus brevis*	*Leuconostoc mensenteroides*	*Lactobacillus delbrueckii*	*Lactobacillus fermentum*	*Lactobacillus acidophilus*

Key +: positive; -: negative

Table 3. Biochemical and morphological Characteristics of yeast isolates from co-fermented cereal with ginger and garlic

Test	Isolates			
	1	2	3	4
Morphology:				
Colour	Cream	Cream	Grey	Cream
Shape	Ovoid	Cylindrical	Cylindrical	Ovoid
Reproduction (Budding)	Polar	Multilateral	Multilateral	Multilateral
Fermentation:				
Glucose	+	+	-	+
Sucrose	+	+	-	+
Maltose	+	-	-	+
Galactose	+	-	-	+
Raffinose	+	+	-	-
Lactose	-	-	-	-
Sugar assimilation				
Glucose	+	+	+	+
Sucrose	+	+	-	+
Maltose	+	+	-	+
Galactose	+	-	-	+
Raffinose	+	+	-	-
Lactose	-	-	-	-
Trehalose	-	-	-	-
Pellicle	-	-	+	+
Nitrate assimilation	-	+	-	-
Vit. free s Medium	+	+	+	+(w)
Probable identity of organism	*Saccharomyces Cerevisae*	*Candida utilis*	*Candida mycoderma*	*Candida tropicalis*

Key: -: negative; +: positive; w: weak; vit: vitamin

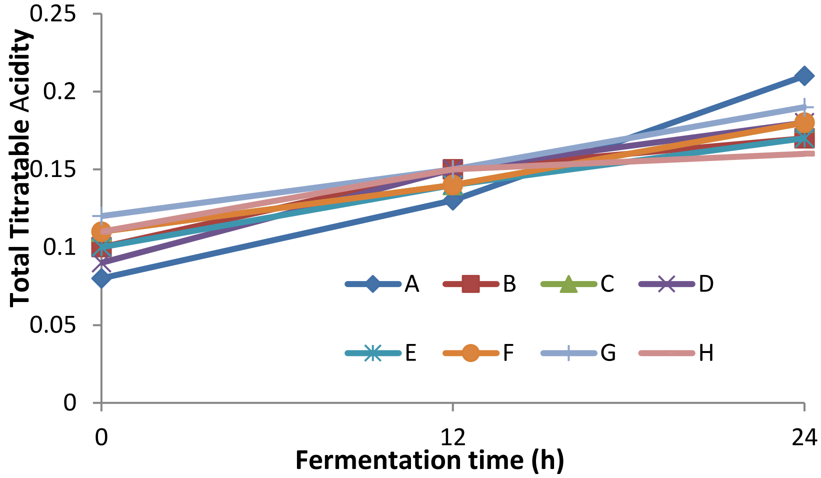

Figure 1: Total Titratable Acidity of co-fermented *Ogi* (maize) with Garlic and Ginger

A (control samples without garlic or ginger); B (2% garlic); C (4% garlic); D (2% ginger); E (4% Ginger); F (2% garlic-2%ginger); G (2%garlic-4%ginger); H (4%garlic-2%ginger)

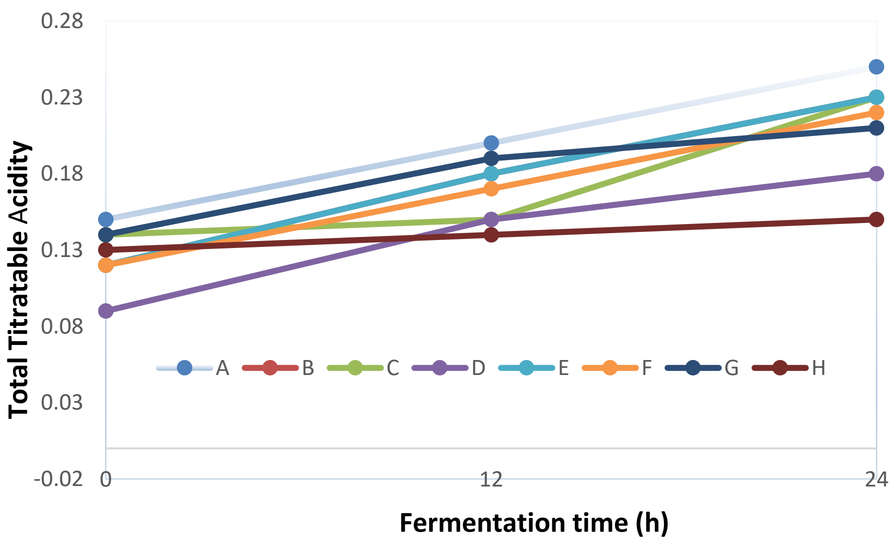

Figure 2: Total Titratable Acidity of co-fermented *Ogi* (sorghum) with Garlic and Ginger

A (control samples without garlic or ginger); B (2% garlic); C (4% garlic); D (2% ginger); E (4% Ginger); F (2% garlic-2%ginger); G (2% garlic-4% ginger); H (4% garlic-2% ginger)

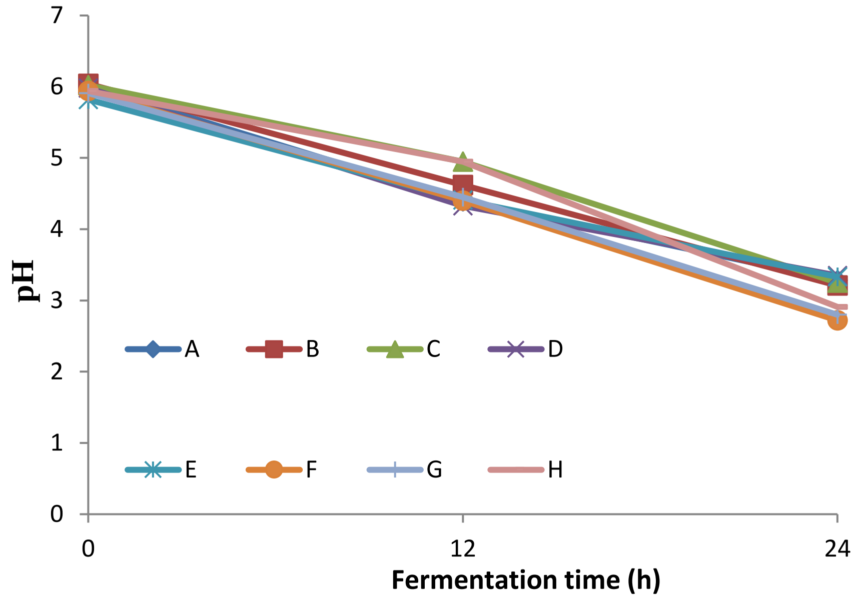

Figure 3: pH of co-fermented *Ogi* (maize)with Garlic and Ginger
A (control samples without garlic or ginger); B (2% garlic); C (4% garlic); D (2% ginger); E (4% Ginger); F (2% garlic-2%ginger); G (2% garlic-4% ginger); H (4% garlic-2% ginger)

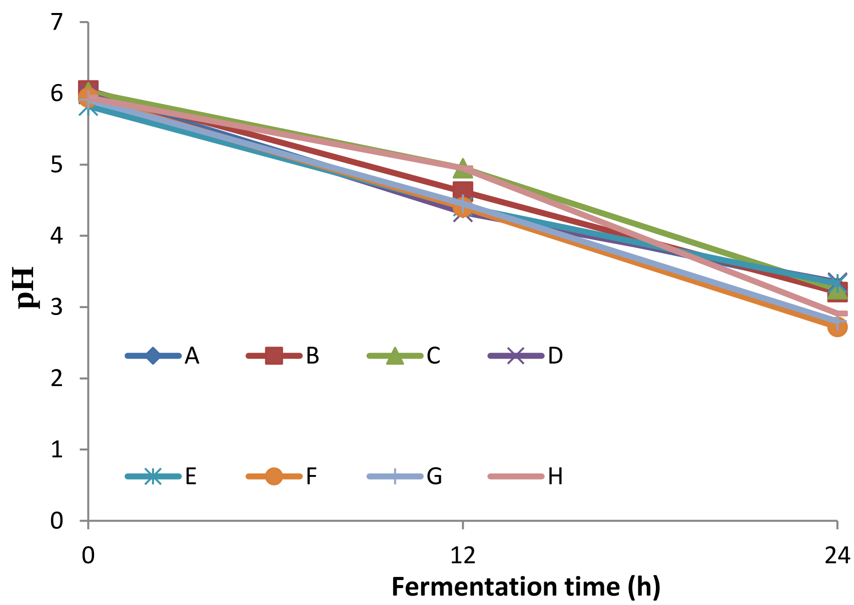

Figure 4: pH of co-fermented *Ogi* (sorghum) with Garlic and Ginger

A (control samples without garlic or ginger); B (2% garlic); C (4% garlic); D (2% ginger); E (4% Ginger); F (2% garlic-2%ginger); G (2% garlic-4% ginger); H (4% garlic-2% ginger).

CONCLUSION

The microflora present during spontaneous fermentation of cereals plays a key role in the acceptability of the final product. This study shows that addition of garlic and ginger at 2 or 4% (w/w) during fermentation of *ogi* produced from quality protein maize did not have a negative significant effect on the natural microflora involved in the production of *ogi*. Co-fermentation of the cereals with garlic, ginger, or ginger and garlic improved the lactic acid bacteria flora and reduced the microbial loads during the fermentation. Thus, its addition during fermentation is recommended to inhibit unwanted microorganisms in *ogi* during production.

REFERENCES

Abioye F.V. (2015). Proximate composition and sensory properties of Moringa fortified Maize-Ogi. ***Journal of Nutrition and Food Sciences,*** S12, 1-4.

Adelekan, A. O. and Oyewole, O. B. (2010). Production of Ogi from germinated sorghum supplemented with soybean. ***African Journal of Biotechnology***, 9(42), 7114-7121

Akinleye, O. M., Fajolu, I. O., Fasure, A. K., Osanyinpeju, O. S., Aboderin, A. O. and Salami, O. O. (2014): Evaluation of micro-organisms at different stages of production of Ogi in Alimosho Community, Area Southwest, Lagos, Nigeria. ***American Journal of Research Communication*****,** 2(10), 215-230.

AOAC International., William W. H and George W. L. (2010). Official methods of analysis chemistry. Gaithersburg.

American Public Health Association (APHA) (2015). Compendium of Methods for the Microbiological Examination of Foods. Fifth edition, American Public Health Association Washington, DC., USA.

Cisse, M., Zoue, L. T., Soro, Y. R., Megnanou, R. and Niamke, S. (2013). Physicochemical and functional properties of starches of two quality protein maize (QPM) grown in Côte d’Ivoire. ***Journal of Applied Biosciences***, 66, 5130– 5139

Eshetie, T. (2017). Review of quality protein maize as food and feed: In alleviating protein deficiency in developing countries. ***American Journal of Food and Nutrition***, 5(3), 99–105.

Farinde, E. O. (2015). Chemical and sensory properties of sieved and unsieved fortified *Ogi*. ***Nature and Science***, 13 (1), 49-53.

Greppi A., Rantsiou K., Padonou W., Hounhouigan, J., Jespersen L., Jakobsen M. and Cocolin, L. (2013). Determination of yeast diversity in *ogi*, *mawè*, *gowé* and *tchoukoutou* by using culture-dependent and independent methods. ***International Journal of Food Microbiology***, 165(2), 84–88.

Izah S.C., Kigigha L.T. and Okowa I.P. (2016). Microbial quality assessment of fermented maize Ogi (a cereal product) and options for overcoming constraints in production, ***Biotechnological Research***, 2, 81-93.

Houngbédji, M., Johansen, P., Padonou, S. W., Akissoé, N., Arneborg, N., Nielsen, D. S., Hounhouigan, D. J., and Jespersen, L. (2018). Occurrence of lactic acid bacteria and yeasts at species and strain level during spontaneous fermentation of mawè, a cereal dough produced in West Africa. ***Food Microbiology***, 76, 267–278.

Kalui, C. M., Mathara, J. M. and Kutima, P. M. (2010). Probiotic potential of spontaneously fermented cereal based foods – A review. ***African Journal of Biotechnology***, 9(17), 2490-2498

MClandsborough, L.A. (2005). Food Microbiology Laboratory, CRC Press, Boca Raton, FL.

Olaniran, A.F., Abiose, S.H., Adeniran, A.H. (2015). Biopreservative effect of Ginger (*Zingiber Officinale*) and Garlic powder (*Allium Sativum*) on tomato paste. ***Journal of Food Safety***, 35(4), 440–452.

Olaniran, A.F. and Abiose, S.H. (2018). Proximate and Antioxidant activities of bio-preserved flour with garlic and ginger ogi. ***F1000Research***,7(1936),1-16.

Olaniran, A.F. and Abiose, S.H. (2019). Nutritional evaluation of enhanced unsieved *Ogi* Paste with Garlic and Ginger. ***Preventive Nutrition and Food Science***, 24(3), 348-356.

Olaniran, A.F., Abiose, S.H. and Gbadamosi, S.O. (2019). Quality attributes and acceptability of *ogi* flour biofortified with garlic and ginger. ***Journal of Health Science***, 7, 101-109.

Olayiwola, J. O., Victoria, I., Mobolaji, A. B. (2017). Bacteriological and proximate evaluation of ginger-fortified fermented Maize (Ogi). ***American Journal of Food Technology***, 12(6), 374–378.

Omemu, A. M. (2011). Fermentation dynamics during production of ogi, a Nigerian fermented cereal porridge. ***Report and Opinion***, 3(4), 8–17.

Omemu, A. M., Uchechukwu, I. O., Adewale, O. O., Mobolaji, O. B., Samuel, A. O. A. (2018). Microbiological assessment of maize ogi cofermented with Pigeon Pea. ***Food Science and Nutrition***, 6(5),1238–53.

Ryu, J. H., Kang, D. (2017). Physicochemical properties, biological activity, health benefits, and general limitations of aged black Garlic: A Review. ***Molecules***, 22(6), 919.

Solange, A., Konan, G., Fokou, G., Dje, K. M., Bonfoh, B. (2014). Review on African traditional cereal beverages. ***American Journal of Research Communication***, 2(5), 103–153.