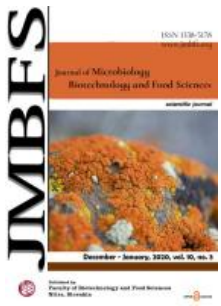

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GARLIC-GINGER AS POTENTIAL BIO-PRESERVATIVE IN FERMENTED MAIZE AND SORGHUM PASTES

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ABSTRACT

Garlic and ginger are natural spices with potentials as biopreservatives and allied health benefits. Fermented pastes either from maize or sorghum have a shelf life of fewer than 10 days except when refrigerated. In this study, garlic and ginger were added separately and in combinations to the fermented pastes prepared from maize and sorghum grains with a view to extending its shelf life resulted in 7 treatments. During storage for 4 weeks at ambient and refrigeration temperatures, the microbial load was enumerated and isolates identified using conventional methods. Physicochemical properties and shelf-life of the paste were also evaluated. Prominent in the fermented pastes during storage were 8 species of lactic acid bacteria and yeast (*Saccharomyces cerevisiae* and *Saccharomyces rouxii*). Garlic inhibited the growth of *Candida utilis*, *Candida mycoderma*, *Candida tropicalis*, *Candida krusei*, and *Rhodotorula glutinis* in paste during storage. Bio-preserved paste using 4% garlic with 2% ginger had the best preservative effect on total viable counts (8.29-11.30 CFU/g), lactic acid bacteria (8.3-9.70 CFU/g) and yeast (4.69-9.45 CFU/g) counts. The study established that fermented pastes produced from either maize or sorghum can be effectively bio preserved using garlic, ginger, and garlic-ginger at 2 or 4 % for 4 weeks without spoilage at ambient temperature (27± 2°C); thus, extending its shelf life.

Keywords: Fermented paste, Biopreservatives, Garlic, Ginger, Maize, Sorghum, Shelf life

INTRODUCTION

Quality protein maize (QPM) varieties are being encouraged in developing countries with maize as major constituents of their diets compared to the conventional maize (**Gunaratna**, ***et al.*, 2019**). Different varieties are used for the production of several traditional foods especially in Africa such as *ogi*, The main diets of poor people are cereals with scanty protein sources like eggs and meat **(Oluwakemi and Omodele, 2015)**. Quality Protein Maize (QPM) has a biological protein value of 80% compared with conventional maize (45%). Besides, It has more lysine (30%), tryptophan (55%) and less leucine (38%) with the potential in alleviating protein malnutrition (**Maseta *et al.*, 2017**). The superiority of nutritional assessment on consumption of quality protein maize by human being above other varieties has been demonstrated (**Tandzi *et al.*, 2017**). Sorghum is the world's fifth cereals food crop. A good source of starch and protein, its gluten-free serves as an alternative for gluten intolerance people **(Kulamarva *et al.*, 2009).** Fermentation is a traditional, age-old technique of transforming grains generally, into diverse forms of food that constitute the daily diets of most African populations**.** This processing technique has been well documented to improve shelf life, nutrient bioavailability and health benefits **(Olaniran and Abiose, 2019).** Some of the locally fermented products are *fura*, *ogi* and *tings*. Most of these fermented products especially *ogi* has relatively short shelf life of 10 days at room temperature depending on the moisture content and microorganisms present (**Olaniran *et al.*, 2019**). Refrigeration is a viable option when the power supply is regular for preservation if the family can afford the bill. Therefore there is a need to extend their shelf life to enhance its availability on the shelf and reduce the rigor of frequent production in small batches. Biopreservation is important in the food industry. Ginger powder compared well with potassium sorbate, citric acid, and sodium metabisulphite (**Singh, 2018**). Also, **Olaniran *et al.* (2015)** reported that garlic possesses antimicrobial activities in food. Garlic and ginger satisfy the questions of safety and generally regarded as safe (GRAS) **(Olaniran *et al.*, 2020).** They are indispensable in the preparation of daily food and reported to possess compounds with various benefits (**Tattari *et al.*, 2013**). Garlic and ginger are gaining increasing interest because of relatively safe status, acceptance by consumers, and a potential alternative to chemical preservatives **(Nath *et al.*, 2014)**. Hence, the study was designed in view of extending the shelf life of fermented pastes produced from maize and sorghum with garlic and ginger as biopreservative at ambient and refrigeration temperatures.

MATERIAL AND METHODS

Preparation of powder garlic and ginger

Garlic and ginger powder was prepared according to **Olaniran *et al.* (2015)** from fresh garlic bulbs and ginger rhizomes. Four hundred (400) gram of fresh garlic bulbs and ginger rhizomes were cleaned, peeled and dried in hot air oven (Gallenkamp, UK) at 65°C for 12h. Dried samples were pulverized with a grinder (Marlex Appliances PVT, India). The garlic and ginger powdered samples were then sieved in a mesh (50µm).

Production of biopreserved *ogi* paste

The paste produced from clean sorghum (red variety) and quality protein maize grains from the Institute of Agricultural Research and Training (I.A.R.T.), Ibadan, Nigeria. The grains soaked in clean water for 72h individually, drained, wetly milled to smooth paste in attrition mill using Thomas Wiley Model ED-5 **(Akanbi *et al.*, 2003, Olaniran and Abiose, 2018)**. The smooth paste divided to 8 portions (800g each); Fermented paste without biopreservative served as control and labeled PWB. The garlic and ginger powder added to the slurry in different concentrations (2 and 4%). Other portions labeled as follows: BPG_1 (2% garlic), BPG_2 (4% garlic); BPG_3 (2% ginger), BPG_4 (4% ginger), BPG_5 (2% garlic with 2% ginger), BPG_6 (2% garlic with 4% ginger), and BPG_7 (4% garlic with 2% ginger). The mixtures were steadily homogenized in containers with a glass rod and fermented at ambient temperature (27± 2°C) for 24h. It was decanted and packaged in plastic containers stored at ambient temperature (27 ± 2 °C) and refrigeration (4 ± 1°C) temperatures. Samples were selected at random in 4 weeks in replicates for futher studies

Microbial Analyses

Enumeration of Microbes

Enumeration of microbes by serial dilution of fermented paste was carried out during storage. Mixing of the paste (5g) with 45ml of maximum recovery diluents (MRD) resulting in 10^{-1} dilution. The stock was further diluted till a preferred dilution was obtained. From each dilution, 1ml was pipetted into sterile petri dish prior to adding 20mL each of molten Nutrient agar (NA) for enumeration microorganisms. Molten agar of de Man Rogosa-Sharpe (MRS) was added for Lactic acid bacteria (LAB) count and Potato dextrose agar (PDA) for yeast and mold count (to inhibit bacterial growth, 0.5ml of 3 mg/ml Streptomycin was incorporated). Petri dish in triplicates were incubated at 37^{o}C for 24h for the total viable count (TVC) and LAB count while for yeast and mold count media were incubated at 28 ± 2^{o}C for 72h. The microbial load in paste as storage progressed was determined to compute distinct colonies using a colony counter. Incubated Petri dish with 25-250 colonies was counted and numbers of colonies multiplied with reciprocal dilution factor **(Harrigan, 1998; APHA, 2015)**.

Characterization and Identification of Isolates

Distinct colonies were streaked on solidified agar repeatedly to obtain pure isolate and subjected to microscopic examination for identification. The isolates were identified based on cultural, morphological characteristics and biochemical assays according to **Taubeneck (2007)**, **Wood, and Holzapfel (1995)**. Shape and elevation of the colonies were examined, Gram reaction, cell morphology and cell arrangement of the isolates were observed under a microscope. Identification of yeast and bacteria was done using conventional method carrying out catalase test, oxidase test, Gram staining, sugar fermentation and production of carbon dioxide. Relevant scheme were consulted **(Harrigan, 1998; Stöckheim, 2007; Taubeneck, 2007; Wood and Holzapfel, 1995)** for the identification of isolates strain.

Determination of Titratable acidity and pH

The total titratable acidity (TTA) of fermented paste was done using 25 ml of the slurry and 3 drops of phenolphthalein as indicator and titrated against 0.1N Sodium hydroxide (NaOH) to achieve pink color. The acidity of paste was calculated as lactic acid with conversion factor 0.09. pH meter (Corning Scholar 425, UL Laboratories, Shenzhen, China) was used for determination of pH values. Buffer 4.0 and 7.0 used to calibrate the pH meter and the electrode probes sanitized by swabbing with 90% ethanol before dipping in paste **(AOAC, 2010)**.

Preparation of extracts for total free amino acid, total reducing sugars, total soluble sugars and individual sugars assay

Extraction of the paste was carried out according to **Omafuvbe (2006)**. Paste (5g) was weighed into 250ml conical flask and 50ml of 80% ethanol (v/v) was added. The suspension was mixed and 10ml of petroleum ether was added to extract the fat in paste. The ethanol- petroleum ether was stirred using magnetic stirrer at room temperature for 30 min and centrifuged at 5000 rpm for 30 minutes. The petroleum ether phase was discarded and clear ethanolic phase was collected and used for further analysis.

Determination of total free amino acid

Ninhydrin colorimetric method according to **Omafuvbe (2006)** to 1ml of the extract in labeled test tube, 0.5 ml of cyanide acetate buffer (pH 5.4) and 0.5ml of 3.0% Ninhydrin solution in 2-methoxyethanol were added. The tubes were heated in a water bath for 15 min, 10ml of isopropyl alcohol: water mixture (1:1) was rapidly added then cooled to room temperature. The Optical Density (O.D) of the solution was read at 570 nm in a spectrophotometer (Model SP9, PyeUnican UK). The concentration of free amino acids in the extract was extrapolated from a standard curve of known concentrations of glycine.

Determination of total reducing sugars

The total reducing sugar content was determined using the dinitrosalicyclic acid (DNSA) reagent method described by **Adepoju et al. (2016)**. One (1) milliliter of ethanolic extract was dispensed into test tubes and 2 ml of DNSA was added. The mixture boiled for 5 min, rapidly cooled under running water and 7ml of distilled water was added. The absorbance was read at 540 nm in a UV Spectrophotometer (Model SP9, PyeUnican UK) against reagent blank. Results were extrapolated from concentrations of a standard glucose calibration curve.

Effect of Biopreservative on individual sugars in fermented paste

Thin layer chromatography described by Adeniran and Abiose **(2012)** in the identification of individual sugars was used. Newly prepared standard sugars containing 0.5 % w/v maltose, glucose, and galactose were stored in bottles. Micropipette was used to spot the standard sugar solution on chromatogram sheet (pre-coated silica gel adsorbent for thin-layer chromatography) and the extracts. Prepared solvent system mixing 60 % ethyl acetate, 15 % glacier acetic acid, 15 % ethanol and 10 % distilled water together. The dried spotted plate was immersed in the saturated chromatography tank containing the solvent system, uprightly positioned and allowed to develop for 1 h. The developed sheet was gently removed and dried at room temperature. The detection reagent prepared by mixing naphthol resorcinol (20 mg), 90% ethanol (10 ml), and concentrated sulphuric acid (0.2 ml). Detection reagent was sprayed on the dried plate, dried in the oven at 100^{o}C for 10 min resulting in defined and distinct spots on dried chromatogram sheet.

RESULTS AND DISCUSSION

Effects of addition of biopreservative on fermented paste

The total viable counts (TVC) of PWB (paste without preservative) increased ranging from 11.98-16.97 CFU/g (maize) and 11.99-15.99 CFU/g (sorghum) during storage (Table 1). The viable counts of BPG_1 (bipreserved paste with 2% garlic) and BPG_2 (4% garlic) gradually increased from 8.45-9.81 CFU/g and 8.35 to 11.59 CFU/g respectively in 4 weeks at ambient shelf life. The viable counts recorded in all biopreserved pastes in week 4 were lower than counts in week 2 compared to that of PWP (control) at week 2. The preservative effect in BPG_1 in week 4 (11.68 CFU/g) was comparable to that of BPG_3 in week 2 (11.71CFU/g) in pastes produced from maize at ambient temperature as documented in table 1. Both BPG_2 (11.59 CFU/g) and BPG_5 (11.40 CFU/g) showed the nearly equivalent preservative effect in week 4. The use of garlic- ginger in combination with refrigeration further reduced total viable counts of preserved paste during the 4 weeks of storage by extending the shelf life of the paste by 200%. Also, TVC of BPG_7 was stable at refrigeration temperature during storage. The lowest TVC count (1.27 CFU/ g) was recorded during storage in BPG_7 (preserved paste with 4% garlic-2% ginger) which was prepared from maize (supplementary 1).

Table 1 Total Viable Count (CFU/ g) of biopreserved fermented pastes at ambient temperature

Time (weeks)	PWB	BPG_1	BPG_2	BPG_3	BPG_4	BPG_5	BPG_6	BPG_7
Maize								
0	11.98±0.11^{a}	8.45±0.10^{c}	8.35±0.13^{c}	8.65±0.14^{c}	8.62±0.10^{c}	8.90±0.10^{b}	8.91±0.10^{b}	8.29±0.10^{d}
2	15.56±0.10^{a}	9.81±0.11^{c}	9.77±0.15^{c}	11.92±0.12^{b}	11.85±0.13^{b}	9.69±0.03^{d}	9.40±0.15^{e}	9.32±0.11^{f}
4	16.97±0.10^{a}	11.68±0.15^{b}	11.59±0.13^{d}	11.89±0.10^{c}	11.71±0.15^{b}	11.40±0.15^{b}	11.34±0.12^{b}	11.30±0.15^{b}
Sorghum								
0	11.99±0.15^{a}	11.63±0.10^{c}	11.57±0.10^{c}	11.76±0.12^{b}	11.65±0.10^{c}	11.43±0.10^{d}	11.34±0.15^{c}	11.45±0.11^{d}
2	13.94±0.12^{a}	11.91±0.15^{b}	11.87±0.11^{c}	11.96±0.15^{b}	11.94±0.12^{b}	11.64±0.12^{d}	11.49±0.12^{c}	11.54±0.15^{d}
4	15.99±0.11^{a}	13.40±0.15^{c}	11.94±0.15^{d}	13.81±0.12^{b}	13.67±0.10^{c}	11.93±0.10^{d}	11.78±0.11^{c}	11.86±0.15^{de}

Means followed by different superscripts are significantly different across rows ($p < 0.05$). Keys: PWB (paste without biopreservative), BPG_1 (2% garlic), BPG_2 (4% garlic); BPG_3 (2% ginger), BPG_4 (4% ginger), BPG_5 (2% garlic-2% ginger), BPG_6 (2% garlic-4% ginger), and BPG_7 (4% garlic-2% ginger).

Lactic acid bacteria counts for all samples during storage for 4 weeks ranged from 8.30 to 16.96 CFU/g (maize) and 8.40-13.93 CFU/g (sorghum) at ambient temperature. LAB counts of biopreserved paste increased all through the 4 weeks of storage (Table 2). The LAB counts of PWP (13.82 CFU/g), BPG_3 (13.77 CFU/g) and BPG_4 (13.80 CFU/g) observed in fermented paste produced using sorghum; at ambient temperature showed no significant difference at the end of week 4. Also, the use of only garlic and garlic-ginger showed stabilizing effect on lactic acid bacteria growth in 4 weeks. The significant distinction in

LAB counts of garlic-ginger as biopreservatives as the storage of paste progressed was n*oticeable.* This demonstrated more antimicrobial activity of garlic against gram-positive bacteria and gram-negative bacteria.

Yeast and mould counts in PWP ranged from 8.81-13.76 CFU/g (maize) and 8.39-15.68 CFU/g (sorghum). Addition of garlic and garlic-ginger as biopreservatives significantly reduced yeast and mould counts in 4 weeks at ambient temperature from 13.76 and 15.68 CFU/g to range of 9.45-11.59 CFU/g and 11.39-13.92 CFU/g in pastes produced form maize and sorghum respectively. Yeast and mould counts in BPG_6 were relatively constant (4.86-4.95 and 5.91-5.99 CFU/g) in paste from maize and sorghum at refrigeration temperature (Table 3). Garlic was found to be more effective than ginger against yeast and mould count during storage. Ajoene, a garlic-derived sulfur-containing compound reported by **Olaniran *et al.* (2020)** to be responsible for this observation. Low LAB and yeast count in preserved pastes at both temperatures may be due to the antimicrobial properties of garlic and ginger **(Asimi *et al.*, 2017)**. Similar reports by **Oladipo and Jadesimi**, **(2013)** and **Olaniran *et al.* (2015)** during the storage of food products preserved singly with either ginger or garlic was documented. BPG_6 (4% garlic-2% ginger) had the best effect on the total viable, Lactic acid bacteria and yeast count during storage of paste produced from maize and sorghum.

Table 2 Lactic Acid Bacteria Count (CFU/ g) of biopreserved fermented pastes at ambient temperature

Time (weeks)	PWB	BPG_1	BPG_2	BPG_3	BPG_4	BPG_5	BPG_6	BPG_7
Maize								
0	9.34 ± 0.15^{bc}	8.76 ± 0.10^{b}	9.43 ± 0.01^{b}	9.13 ± 0.11^{c}	9.63 ± 0.15^{a}	8.90 ± 0.10^{d}	8.46 ± 0.03^{e}	8.30 ± 0.02^{f}
2	13.92 ± 0.10^{a}	9.70 ± 0.15^{d}	9.17 ± 0.12^{f}	11.60 ± 0.05^{b}	9.99 ± 0.01^{c}	9.34 ± 0.02^{e}	9.03 ± 0.05^{g}	9.01 ± 0.03^{g}
4	16.96 ± 0.02^{a}	9.94 ± 0.04^{d}	9.80 ± 0.03^{c}	11.95 ± 0.01^{b}	11.45 ± 0.03^{c}	9.74 ± 0.01^{e}	9.62 ± 0.06^{f}	9.70 ± 0.01^{e}
Sorghum								
0	11.60 ± 0.11^{a}	9.43 ± 0.12^{ab}	8.51 ± 0.15^{d}	9.91 ± 0.10^{b}	9.82 ± 0.10^{b}	8.64 ± 0.11^{c}	8.40 ± 0.15^{e}	8.48 ± 0.15^{f}
2	13.93 ± 0.10^{a}	11.68 ± 0.13^{c}	11.63 ± 0.01^{c}	11.80 ± 0.03^{b}	11.89 ± 0.05^{b}	10.73 ± 0.05^{e}	10.45 ± 0.01^{e}	10.69 ± 0.05^{d}
4	13.82 ± 0.05^{a}	11.86 ± 0.03^{b}	11.76 ± 0.05^{c}	13.77 ± 0.05^{a}	13.80 ± 0.01^{a}	11.71 ± 0.08^{c}	11.58 ± 0.02^{d}	11.67 ± 0.00^{d}

Means followed by different superscripts are significantly different across rows ($p < 0.05$). Keys: PWB (paste without biopreservative), BPG_1 (2% garlic), BPG_2 (4% garlic); BPG_3 (2% ginger), BPG_4 (4% ginger), BPG_5 (2% garlic-2% ginger), BPG_6 (2% garlic-4% ginger), and BPG_7 (4% garlic-2% ginger).

Table 3 Yeast count (CFU/ g) of biopreserved fermented pastes at ambient temperature

Time (weeks)	PWB	BPG_1	BPG_2	BPG_3	BPG_4	BPG_5	BPG_6	BPG_7
Maize								
0	8.81 ± 0.03^{a}	5.36 ± 0.01^{d}	5.27 ± 0.00^{c}	6.63 ± 0.02^{b}	5.78 ± 0.01^{b}	4.74 ± 0.04^{d}	4.86 ± 0.03^{d}	4.69 ± 0.00^{d}
2	11.60 ± 0.03^{a}	8.74 ± 0.03^{c}	8.56 ± 0.03^{c}	9.86 ± 0.03^{b}	9.60 ± 0.03^{b}	8.45 ± 0.03^{d}	8.40 ± 0.03^{d}	8.30 ± 0.03^{e}
4	13.76 ± 0.03^{a}	9.86 ± 0.03^{c}	9.81 ± 0.03^{c}	11.53 ± 0.03^{b}	11.39 ± 0.03^{b}	9.72 ± 0.03^{e}	9.57 ± 0.03^{d}	9.45 ± 0.03^{d}
Sorghum								
0	8.39 ± 0.01^{a}	6.67 ± 0.02^{b}	6.48 ± 0.01^{b}	6.61 ± 0.05^{b}	6.73 ± 0.03^{b}	6.32 ± 0.00^{c}	5.91 ± 0.08^{c}	5.98 ± 0.05^{c}
2	11.87 ± 0.03^{a}	8.85 ± 0.03^{c}	8.81 ± 0.03^{c}	9.94 ± 0.03^{b}	9.91 ± 0.03^{b}	8.83 ± 0.03^{c}	8.36 ± 0.03^{d}	8.74 ± 0.03^{c}
4	15.68 ± 0.03^{a}	11.93 ± 0.03^{c}	11.73 ± 0.03^{c}	13.92 ± 0.03^{b}	13.85 ± 0.03^{b}	11.67 ± 0.03^{c}	11.39 ± 0.03^{d}	11.50 ± 0.03^{d}

Means followed by different superscripts are significantly different across rows ($p < 0.05$). Keys: PWB (paste without bio-preservative), BPG_1 (2% garlic), BPG_2 (4% garlic); BPG_3 (2% ginger), BPG_4 (4% ginger), BPG_5 (2% garlic-2% ginger), BPG_6 (2% garlic-4% ginger), and BPG_7 (4% garlic-2% ginger).

Identification and occurrence pattern of microorganisms in fermented paste

Eight lactic acid bacteria isolates were identified as *Lactobacillus plantarum, Lactobacillus amylovorus, Leuconostoc mensenteroides, Lactobacillus delbrueckii, Lactobacillus brevis, Lactobacillus fermentum, Pediococcus pentosaceus,* and *Pediococcus acidilactici*. *Lactobacillus plantarum* was dominant throughout the period of storage at ambient and refrigeration temperature in paste produced from sorghum. *Lactobacillus amylovorus* was present for first 2 weeks at ambient temperature and throughout at refrigeration temperature. *Leuconostoc mensenteroides* was also present throughout storage at refrigeration temperature but only in the first week at ambient temperature. *Lactobacillus brevis, Pediococcus pentosaceus,* and *Pediococcus acidilactic* isolated in week 2 at both temperatures as presented in Table 4. They have been reported as major lactic acid bacteria isolated from fermented cereal dough **(Adesulu-Dahunsi *et al.*, 2017**; **Bello *et al.*, 2018**; **Okoronkwo, 2014; Olayiwola *et al.*, 2017; Wakil and Ajayi, 2013)**. Yeast isolates were identified as *Saccharomyces cerevisiae, Saccharomyces rouxii, Candida utilis, Candida mycoderma, Candida tropicalis, Candida krusei, Hansenula anomala,* and *Rhodotorula glutinis* (Table 5). *Saccharomyces cerevisiae* and *Saccharomyces rouxii* were dominant for 4 weeks as shown in the occurrence pattern of yeast isolates. The growth of *Candida utilis, Candida mycoderma, Candida tropicalis, Candida krusei,* and *Rhodotorula glutinis* were inhibited during storage in pastes having garlic. Garlic has been reported to have high anti-candida activity and fungicidal effects against *Rhodotorula* **(Ali and Mustafa, 2009; Ogunsakin *et al.*, 2015)**.

Total Titratable Acidity and pH of paste

The total titratable acidity (TTA) value of the pastes increased at ambient and refrigeration temperatures during the 4 weeks of storage respectively. Control sample from paste produced from sorghum had the highest titratable acidity (0.25) while lowest was observed in BPG_7 (0.15) in week 4 at ambient temperature. The TTA and pH of BPG_6 and BPG_7 were stable during storage (figures 1 and 2). BPG_7 total titratable acidity and pH were stable at refrigeration temperature. The variations in acid tolerance of microorganisms could be attributed to their relative ATPase activities as the pH decreases **(Wakil and Ajayi, 2013)**. Effective inhibition of microorganisms depends on numbers of lactic acid bacteria sufficient to decrease the pH rapidly to levels that inhibit growth of pathogen. Decreased in pH and increase in TTA in the study was in agreement with **Oyedeji *et al.* (2013)**.

Table 4 Morphological and Biochemical Characteristics of Bacteria Isolates from of biopreserved fermented pastes

Test	**Isolates**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Morphology	Rod	Cocci	Rod	Rod	Cocci	Rod	Rod	Cocci	Cocci	Rod
Colour of growth	Cream	Yellow	Cream	Cream	Cream	Cream	Cream	Cream	Cream	Cream
Gram reaction	+	+	+	+	+	+	+	+	+	+
Catalase test	+	+	-	-	-	-	-	-	-	-
Growth at:										
15 ^{o}C	-	+	+	-	+	-	+	+	+	-
45 ^{o}C	+	+	-	+	-	+	-	-	+	+
Production of CO_2	+	Nd	-	-	+	-	+	-	-	-
Starch	+	-	-	+	-	-	-	-	-	-
Nitrate	-	-	-	-	-	-	+	Nd	Nd	+
Dextran production	+	+	-	-	+	-	-	-	-	-

NH_3 from arginine fermentation of:	-	+	-	-	Nd	-	+	+	-	+
Glucose	+	+	-	-	+	+	+	+	+	+
Lactose	+	-	+	-	+	+	+	+	+	+
Maltose	+			+	+	+	+	+	+	+
Arabinose	-	-	+	+	+	-	+	-	+	+
Trehalose	+	-	+	+	+	+	-	+	+	+
Salicin	-	-	+	+	+	-	-	+	+	-
Sucrose	+	+	+	-	+	+	+	-	-	+
Raffinose	-	-	+	-	+	-	+	+	+	+
Mannitol	+	+	Nd	-	+	-	-	+	+	Nd
Xylose	-	+	+	-	+	-	+	-	+	+
Fructose	+	+	Nd	-	+	Nd		+	+	
Galactose	+	+	+	+	+	-	+	+	+	+
Citrate	-	+			+					
Probable identity of the organism	*Corynebacterium spp.*	*M. luteus*	*L. plantarum*	*L. amylovorus*	*L. mensenteroides*	*L. delbrueckii*	*L. brevis*	*P. pentosaceus*	*P. acidilactici*	*L. fermentum*

Table 5 Morphological and Biochemical Characteristics of Yeast Isolates from of biopreserved fermented pastes

Test	**Isolates**							
	1	2	3	4	5	6	7	8
Morphology:								
Colour	Cream	Cream	Red	Cream	Cream	Yellow	Cream	Grey
Shape	Ovoid	Ovoid	Ovoid	Spherical	Ovoid	Cylindrical	Cylindrical	Cylindrical
Reproduction (Budding)	Polar	Polar Budding	Multilateral	Polar Budding	Multilateral	Multilateral Budding	Multilateral	Multilateral
Fermentation:								
Glucose	+	+	-	+	+	+	+	-
Sucrose	+	-	-	+	+	-	+	-
Maltose	+	+(w)	-	+(w)	+	-	-	-
Galactose	+	-	-	+	+	-	-	-
Raffinose	+	-	-	+(w)	-	-	+	-
Lactose	-	-	-	-	-	-	-	-
Sugar assimilation								
Glucose	+	+	+	+	+	+	+	+
Sucrose	+	-	+	+	+	-	+	-
Maltose	+	+	+	+	+	-	+	-
Galactose	+	+	+	+	+	-	-	-
Raffinose	+	-	+	+	-	-	+	-
Lactose	-	-	-	-	-	-	-	-
Trehalose	-	-	+	+	-	-	-	-
Pellicle	-	-	-	+	+	+	-	+
Nitrate assimilation	-	-	-	+	-	-	+	-
Probable identity of organism	*Saccharomyces Cerevisiae*	*Saccharomyces Rouxii*	*Rhodotorula glutinis*	*Hansenula anomala*	*Candida tropicalis*	*Candida krusei*	*Candida utilis*	*Candida mycoderma*

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

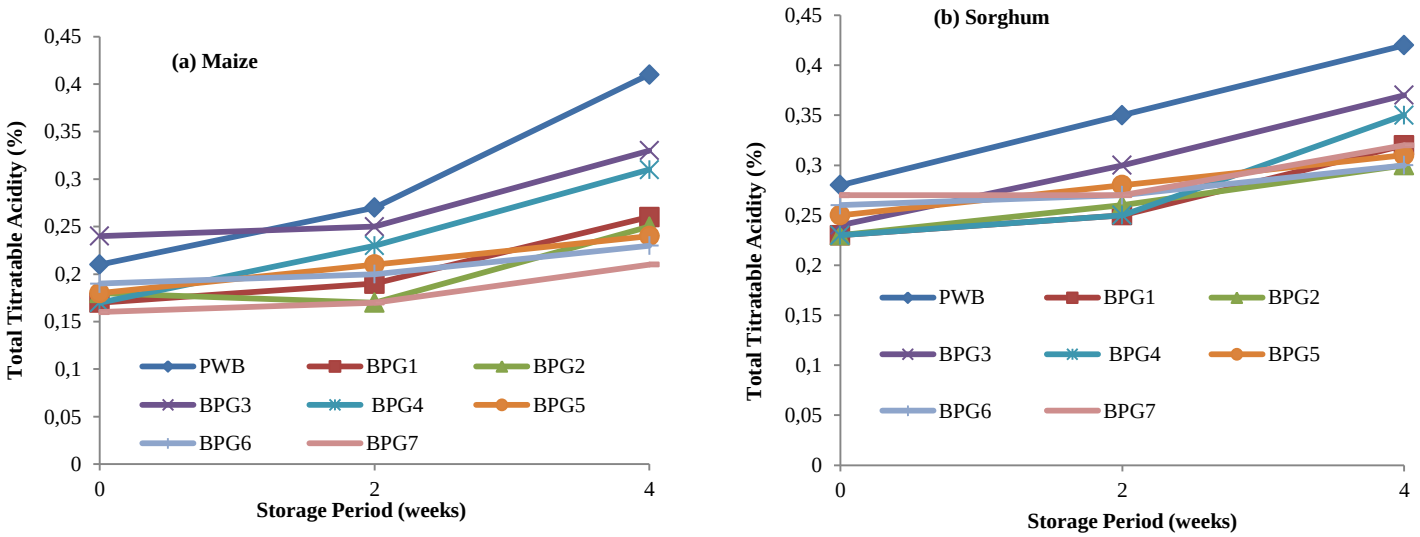


Figure 1 Total titratable acidity of Biopreserved fermented pastes at Ambient Temperature (a) maize, (b) Sorghum

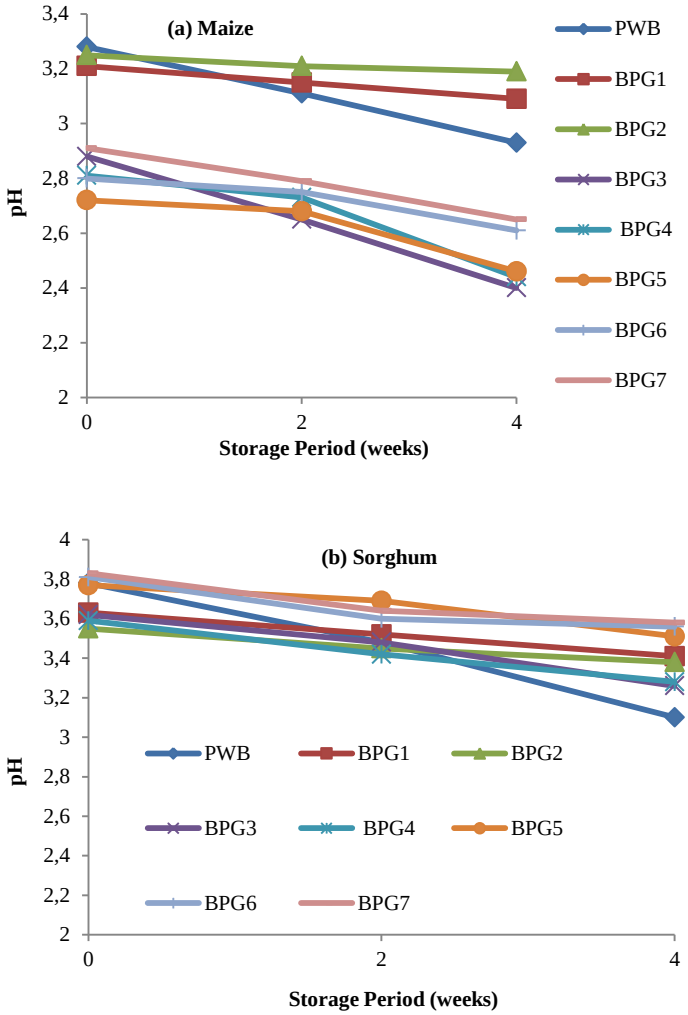
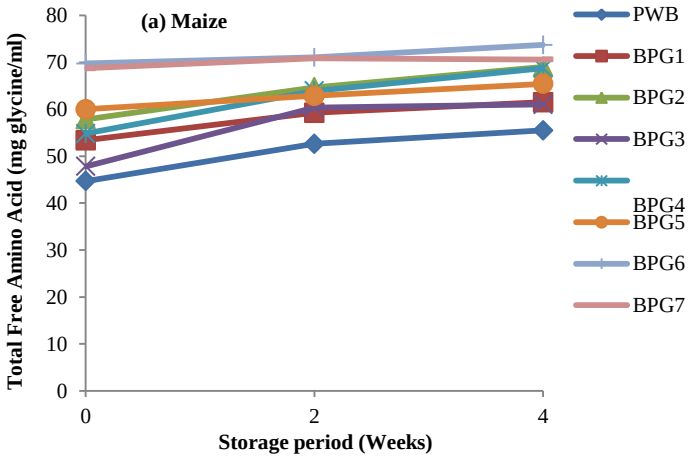


Figure 2 pH of Biopreserved fermented pastes at Ambient Temperature (a) maize, (b) Sorghum

The total free amino acid of fermented paste

The total free amino acid (TFAA) content of pastes ranged from 44.96 to 73.70 mg/ml and 37.18-102.56 mg/ml for pastes form maize and sorghum at ambient temperature respectively. Stable TFAA observed during storage of BPG_1, BPG_2, BPG_4, BPG_5 and BPG_7 at ambient temperature (Figure 3). The TFAA content of PWB increased throughout the 4 weeks of storage at a refrigeration temperature from 44.69 to 50.62 mg/ml and 37.18 to 51.00 mg/ml in pastes form maize and sorghum respectively. **Rojas-Molina *et al.* (2008)** reported a similar observation of increased in free amino acid.



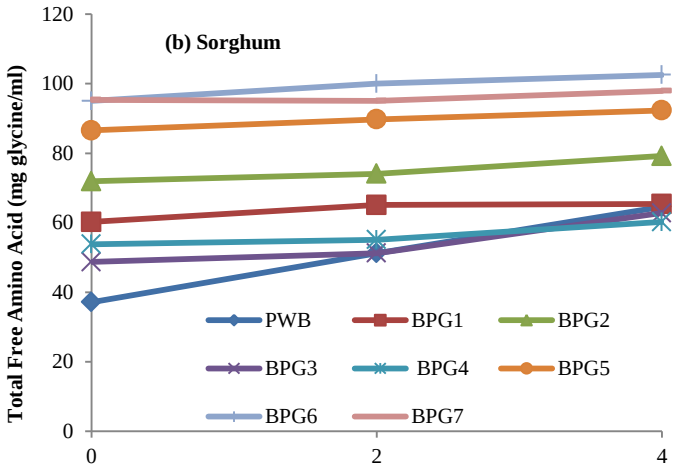


Figure 3 Total free amino acid of biopreserved fermented pastes at Ambient Temperature

Total Reducing Sugar Content of fermented paste

The total reducing sugar (TRS) of paste during storage ranged from 6.21 - 41.87 mg/ml (maize) and 19.97-51.32 mg/ml (sorghum) at ambient temperature. Relatively stable total reducing sugar content was documented in BPG_4 and BPG_5 (Figures 4). PWB (Control paste from maize) had the lowest total reducing sugar (6.21 mg/ml) in 4 weeks while BPG_5 from sorghum had the highest (51.32 mg/ml) at ambient temperature. Increased total soluble sugar may be due to maximum breaking down of starch substrates to simpler sugars by amylolytic enzymes produced during fermentation **(Adesulu *et al.* 2014; Okafor *et al.* 2018; Oyarekua and Adeyeye, 2009)**. Low reducing sugar content in fermented pastes has been reported as a good attribute because methods of preparing weaning foods involve heat processing by Oyerekau and Adeyeye **(2009)**.

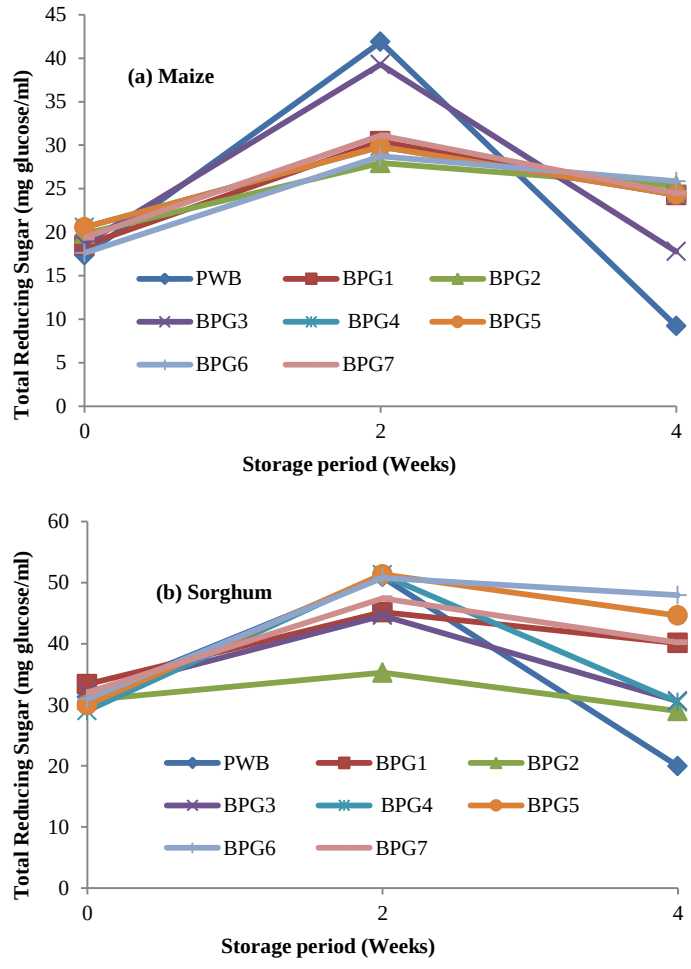


Figure 4 Total reducing sugar of biopreserved fermented pastes at Ambient Temperature

Identification of individual sugars in fermented paste extract

Individual sugars were not present in all paste extracts at week 0. However in paste produced from maize; glucose was the main sugar present in PWB, BPG_1, BPG_2, and BPG_4, BPG_5, BPG_6 and BPG_7 at week 4 at ambient temperature. Also in pastes made from sorghum, sucrose and glucose with calculated refractive index (Rf value) 0.31 and 0.41 were present in BPG_6 in 4 weeks at ambient temperature. Fructose with calculated refractive index (R_f value) of 0.46 was identified in BPG_3, BPG_5, BPG_6, and BPG_7 in 4 weeks at ambient temperature. Individual sugars were not present in all paste extracts stored at refrigeration temperature. Glucose reported as one of the major monosaccharide present in significant quantity in cereal grains such as maize and sorghum by **Omemu (2018)** and **Akinrinola *et al.* (2014)**.

CONCLUSION

This study established that fermented paste produced from maize and sorghum can be effectively biopreserved with garlic-ginger for 4 weeks at ambient temperature without deteriorating. The most effective combination was 4% garlic and 2% ginger in maize and sorghum as biopreservatives. Thus, garlic, ginger addition are highly recommended as biopreservatives in fermented paste for extending shelf life in our bid to sustain its availability in remote communities with inconsistent power supply and probable application for industrial production.

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