LACTIC ACID BACTERIA AND YEAST DIVERSITIES IN SPONTANEOUSLY FERMENTED MILLET SOURDOUGHS

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ABSTRACT

The lactic acid bacteria (LAB) and yeast diversities in spontaneously fermented pearl millet (*Pennisetum glaucum*) and finger millet (*Eleusine coracona*) sourdoughs were investigated. Pearl and finger millet grains were processed into flour and used in spontaneous sourdough fermentation. LAB and yeast cultures were isolated, screened and identified based on morphological, biochemical and sugar utilisation pattern using API 50CHL and API 20 AUX kits. Titratable acidity, pH and Temperatures of sourdoughs developed were also monitored. Titratable acidity and Temperature increased with increasing days of fermentation and conversely the pH. and mesophilic bacterial count followed that of a normal bacterial growth curve. Dominant LAB and yeasts in pearl and finger millet spontaneously developed sourdoughs were *Lb. plantarum*, *Lb. plantarum* 1, *Pediococcus pentosaseus*, *Lb. pentosus*, and *Saccharomyces cereviasiae*. However, *Candida milleri* was found in pearl millet sourdoughs. The dominant cultures in millet sourdoughs could find applications as starter cultures if improved sourdough quality is desired.

Keywords: Pearl millet, Finger millet, Sourdough, Lactic acid bacteria, Yeast, Fermentation

INTRODUCTION

Millets are group of highly variable grasses with many small seeds. Pearl millet (*Pennisetum glaucum*) and Finger millet (*Eleusine coracona*) are among the most important ones grown in the tropics but underutilized. Sourdough is an acidic pastes laden with potentials to optimize desirable functional properties in flours. It is a technological process applied to non-gluten flours. It is the foremost cereal fermentation performed with almost any cereal (**De Vust et al., 2002; Valcheva et al., 2006; Edema, 2011).** Sourdough consists of mixture of flour and clean water. Sourness is achieved by the action of fermentative organisms such as the lactic acid bacteria genera. Lactic acid bacteria and yeasts metabolic activities accounts for the desirable changes observed in sourdoughs. Some metabolites of sourdough microflora includes among other; organic acids, carbondioxide, diacetyl and alcohols.

Spontaneous fermentation is the oldest form of fermentation, it is left to chance and it is the form of fermentation in most small scale fermentations in the developing countries (**Holpzapfel, 2002)**. Spontaneous fermentations results in inconsistent product quality and hazards. Several species of microgranisms ranging from bacteria and fungi have been reported to colonize most cereal fermentations. Lactic acid bacteria (LAB) belonging to the genus - Lactobacillus have been isolated from sourdoughs and identified (**Corsetti et al., 2004)**.

Sourdoughs are composed of diverse source of LAB and yeast species (**De Vuyst and Neysen, 2005)**. Microflora dominants in substrates are geographical region, processing and handling dependent. The LAB and yeasts developing in the dough may originate from selected natural contaminants in the flour, water, starters added or from previous fermentation vessel. LAB and yeasts are the predominant microorganisms present in sourdoughs (**Vogel et al., 1996; Steinkraus, 1996; 1997; Holzapfel et al., 1998; Lee, 1997; Oyewole, 1997)**. LAB's belong to several genera, this includes; *Lactobacillus spp., Lactococcus spp., Leuconostoc spp., Oenococcus spp., Pediococcus spp., Streptococcus spp., Tetragenococcus spp., Aerococcus spp., Carnobacterium spp., Enterococcus spp., Vagococcus spp. and Weissella spp* (**De Angelis et al., 2007)**. Great variety exist in yeast cultures found in sourdough fermentation depending on degree of dough hydration, leavening temperature, sourdough maintenance temperature and type of cereal used (**Hammes et al., 2004; Gobbetti et al., 1994)**. However, Lactobacillus and Saccharomyces genera have been reported to be the most dominant in sourdough fermentations.
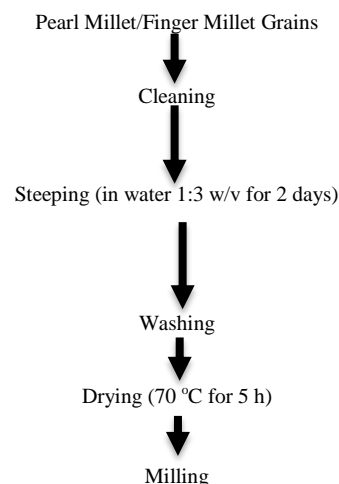
Studies on the ecology of sourdough microflora may help in the understanding of the microbial dynamics and differences between groups of closely related microbial population in cereal (sourdough) fermentations. In most natural fermentation, starters used are poorly known. Therefore, isolation, characterization and identification of the microorganisms involved in cereal fermentation with prospective selection as starter cultures is important hence our investigation.

MATERIALS AND METHODS

Sample Collection

Pearl millet (*Pennisetum glaucum*) and finger millet (*Eleusine coracona*) were purchased from local market in Plateau state, Jos, Nigeria. Pearl and finger millet grains were cleaned, steeped, processed into flours and packaged in polyethylene bags for further analysis as shown in Fig. 1. The millet grains were steeped in water for two days (1:3 w/v ratios of seeds to the volume of steeping water), dried at 70 ºC for 4 h in hot air oven (Laboratory oven, DHG 9101.1SA) and milled into flour using attrition mill (Atlas exclusive, Alzico Ltd mill). Flours were sieved using 60 mesh Standard US sieve and packaged.
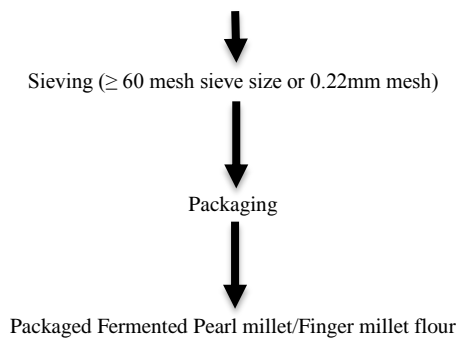
Pearl Millet/Finger Millet Grains

↓

Cleaning

↓

Steeping (in water 1:3 w/v for 2 days)

↓

Washing

↓

Drying (70 ºC for 5 h)

↓

Milling

Sieving (≥ 60 mesh sieve size or 0.22mm mesh)

Packaging

Packaged Fermented Pearl millet/Finger millet flour

Figure 1 Production of fermented pearl/finger millet flours

Spontaneous Sourdough Development

Sourdough were developed spontaneously from pearl and finger millet flours. Equal amounts of pearl and finger millet flours (100 g) and tap water (100 ml) were mixed together using a glass stirrer in glass jars until a soupy consistency was achieved. Fermentation was allowed to proceed at ambient temperature (29±2 °C) for seven days under anaerobic condition. Equal amount of fresh aliquots (millet flour and tap water) were added to the developing batter in the jars continously using a wooden spoon. This was done each day of the sourdough fermentation in order to refresh the fermentation medium (Edema and Sanni, 2006).

Determination of pH and Temperature

Changes in pH of spontaneously developed pearl and finger millet sourdoughs were monitored with increase in days of fermentation. Twenty milliliter of spontaneously developed pearl and finger millet sourdoughs were used for the determination. The pH was determined using a pH meter (ECO Testr pH 1) after standardising using pH buffer 4.0 and 7.0 respectively. The changes in temperature of the sourdough batters was determined from the same batch used for pH determination.

Determination of titratable acidity

Titratable acid produced in the sourdoughs was determined according to method described by **Lonner et al. (1986)**. Briefly, from the same batch used for the pH and temperature determination, one (1) g of the fermenting millet sourdoughs were measured using an electronic digital balance into a conical flask and made up with distill water to 9 ml mark. Ten (10) ml each of the homogenized samples were titrated against 1 N NaOH using 2-3 drops of phenolphthalein indicator until endpoint was reached. The amount of NaOH used during the titration was expressed as total titratable acid produced. Each ml of 1N NaOH used is equivalent to 90.08 mg of lactic acid.

Microbial analysis

Enumeration and isolation of lactic acid bacteria and yeast from Sourdoughs

The microbial analysis was carried out using pour plating methods as described by **Harrigan and McCane (1976)**. From the spontaneously developed pearl and finger millet sourdoughs, one gram of each was homogenised in 9 ml sterilized saline solution (0.85% NaCl) and further diluted till a factor of nine was obtained. One (1) ml each of serially diluted homogenized samples was plated on sterilized de Mann Rogosa Sharpe (MRS) (Oxoid, PM221, USA) and Potato-dextrose (EUR Pharm, Spain) agar. Cultured MRS plates were incubated anaerobically at 30 °C for 48 h in an anaerobic jar loaded with gas pak (BBL Gas Pak, H2 & CO2; Becton-dickinson, Cockeysville, MD, USA) while that of PDA plates were incubated in a fungi incubator at 25 °C for72 h. The microbial populations of the sourdoughs were enumerated each day of fermentation. Isolation and sub-culturing was done until pure cultures were obtained. Dominant presumptive lactic acid bacteria and yeast cultures were further screened on the basis of cell morphological characteristics and biochemical (Gram reaction, catalase) characteristics using standard microbiological techniques as described by **Harrigan and McCane, (1976); Fawole and Oso (2007)**.

Gram positive and catalase negative presumptive LAB isolates were screened for nitrate, urease, indole utilisation, gas production from glucose, spore formation, growth at different temperatures (10 °C, 15 °C, 30 °C and 45 °C), NaCl salt concentrations (2%, 4% and 6.5%) and growth at different pH (4.5 and 6.0). The presumptive yeast cells were screened for growth at different NaCl salt concentration (2%, 4% and 6.5%), growth at 37 °C and utilisation of urease. Cultures were further characterised using carbohydrate fermentation pattern through the Analytical Profile Index (API Kit). The dominant presumptive lactic acid bacteria were characterised using API 50 CH and API 50 CHL medium (Biomerieux, France) while API 20 E was used for the presumptive yeast isolates. The tray was moistened with sterile distilled water to prevent drying out

and inoculum (actively growing culture) was dispensed into wells after equal McFarland concentration was achieved. McFarland concentration was achieved using UV-visible spectrophotometer at 290 nm wavelenght. Anaerobiosis was achieved in the API kits by overlaying inoculated tubes on trays with sterilised paraffin oil and incubated at 30 °C for 48 h while API 20E at 36 °C for 72 h. Isolates were identified with reference to Bergey's Manual of Systematic Bacteriology **(Wood and Holzapfel, 1995)** and apiweb™ identification software (Biomerieux).

RESULTS AND DISCUSSION

Sourdough paste developed spontaneously from pearl and finger millet flour had a soupy consistency as the days of fermentation increased however, more frotting was observed in sourdough developed from finger millet which might be due to the metabolic activity of yeast in the sourdough. The finger millet flour absorbed more water compared to the pearl millet flour and this might be due to higher carbohydrate and fibre content in finger millet compared to pearl millet. **Obilana (2003)** reported the carbohydrate content of whole pearl and finger millet flour as 70 g/100 g and 74 g/100 g respectively and the crude fibre content as 2.3 g/100 g and 3.6 g/100 g respectively. Higher water absorption capacity is attributed to loose structure of starch polymers **(Adebowale et al., 2008; Akinola et al., 2016)**.

Table 1 presents the morphological and biochemical characteristics of presumed lactic acid bacteria and yeast isolates from spontaneously developed pearl and finger millet sourdough. About 62.5% and 58.8% of presumptive bacteria isolates from pearl and finger millet sourdoughs were circular while 37.5 % and 41.2% were irregular in shape respectively. This findings supports the previous work of **Valík and Görner (1995) and Hutkins (2006).** The colony surfaces of presumptive bacteria isolates were generally smooth except in P3, P4, F2 and F6 that were wrinkled. However, F8 had rough surface. The colony colour of presumptive bacteria isolates ranged from white, creamy, dull white and orange in finger millet isolates. All presumptive yeast isolates were non-motile, butyrous, creamy, smooth in surface. Most isolates from pearl and finger millet sourdoughs were catalase negative. Catalase negative is indicative of the isolates ability to hydrolyse hydrogen peroxide in producing catalase enzyme. All isolates were gram positive. The morphological characteristics observed in many of the isolates corroborate the findings of previous authors on lactic acid bacteria. **Wood and Holzapfel, (1995)** described lactic acid bacteria as Gram-positive, non-spore forming, catalase-negative cocci or rods that are anaerobic, micro-aerophilic or aero-tolerant. Lactobacilli are Gram-positive, catalase-negative, non-sporing rods (whose cell length varies between 1.5 μm and 5 μm. The cell may be slender, curved or bend when viewed under the microscope and may be in chains or network **(Edema and Sanni, 2006; 2008)**. The shape of isolates under the light microscope were short rods in clusters, short rods, long rods in network, long rods and cocci. The isolates were non-spore formers and indole negative. This imply they are unable to hydrolyze tryptophan in producing indole. The isolates were unable to utilize urea and nitrate while some produced nitrogen gas. Homofermentative organisms produces only lactic acid from glucose, examples include *L. lactis* subsp *lactis, L. lactis subsp diacetylactis and L. cremoris* while the Heterofermentative produces lactic acid, carbondioxide and aroma compounds as product of fermentation, examples include *Leuconostoc mesenteroides, Lactobacillus spp* **(Sanni et al., 1998)**. Many of the isolates had ability to produced gas from glucose. The isolates thrived at 15 °C and above. This current findings agrees with that of **Hutkins, (2006)** describing most bacteria isolates in sourdough as mesophilic, with some genuses having some species that are psychrotrophic, thermoduric or thermophilic. Some species are salt tolerant, acid tolerance, growing below pH 4.0. Lactic acid bacteria grows at an optimum pH of 5.5 - 6.5. Some strains are reported to be ethanol tolerant, bile-tolerant, aero-tolerant and some requiring strict anaerobic conditions **(Petra et al., 2011)**.

The carbohydrate fermentation pattern of presumed lactic acid bacteria isolated from sourdough is presented in Table 2. The result showed that *Lb. pentosus* and *Lb. plantarum* was most dominant cultures representing about twenty-five percent (25%), *P. pentosaseus*, *Micrococcus varians*, *Lb. buchneri* and *Lb amylovorus* (12.5%) each while *Lb. plantarum* and *Lb. pentosus* was the most dominant LAB in finger millet sourdough. The genera *Lactobacillus*, *Lactococcus*, *Pediococcus* and *Leuconostoc* have been reported important in food technology **(Harrigan and McCane, 1976)**. The carbohydrate fermentation pattern of presumed yeasts isolates from pearl and finger millet sourdoughs is presented in Table 3. Results showed the dominance of *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* had weak reaction to mannitol, sorbose, sorbitol and esculin while *Candida quercitrusa* and *C. milleri* had weak reaction to trehalose and 2-keto-gluconate. Several yeast species has been reported in sourdoughs by various authors **(Hammes et al., 2004; Rossi, 1996; De Vuyst and Neysens, 2005)**. The observed lactic acid bacteria and yeast isolates agrees with those observed in previous similar works. **Jakobsen and Lei (2004); Achi (2005) and Kalui et al. (2009)** reported the occurrence of *L. plantarum, L. casei, L.sakei, L. acidophilus* and *L. salivarius* in cereal based fermented food. Also, **Kalui et al. (2009)** reported the isolation of *L. fermentum, P. pentosaceus, L. plantarum, W. confusus* and *L. rhamnosus* from *ikii*- a traditional fermented

Table 1 Morphological and biochemical characteristics of dominant presumed lactic acid bacteria and yeasts isolates from spontaneously developed pearl and finger millet sourdoughs

Isolates/Description	Colony surface	Pigment	Shape	Cell morphology	Arrangement	Motility	Catalase	Gram reaction	Spore	Urease	Nitrate	Indole	Homo/Hetero	Growth at 10 °C	Growth at 15 °C	Growth at 30 °C	Growth at 37 °C	Growth at 45 °C	Growth at 2% NaCl	Growth at 4% NaCl	Growth at 6.5% NaCl	pH 4.5	pH 6.0	Probable organism
P1	Sm	Wh	Cir	Rods	SS	-	-	+	-	-	-NG	-	Ho	-	-	+	nd	-	+	-	-	+	+	*Lactobacillus pentosus*
P2	Sm	Wh	Cir	Cocci	CL	-	-	+	-	-	-NG	-	Het	-	+	+	nd	-	+	+	-	+	+	*Pediococcus pentosaseus*
P3	W	Wh	Irreg	Rods	SL	-	-	+	-	-	±G	-	Het	-	-	+	nd	+	-	+	+	+	±	*Lactobacillus plantarum*
P4	W	C	Irreg	Rods	SL	-	-	+	-	-	±G	-	Het	-	+	+	nd	+	+	+	-	+	±	*Lactobacillus buchneri*
P5	Sm	Wh	Cir	Cocci	CL	-	-	+	-	-	-NG	-	Het	-	+	+	nd	-	+	+	-	+	-	*Micrococcus varians*
P6	Sm	Wh	Cir	Rods	SC	-	-	+	-	-	-NG	-	Ho	-	-	+	nd	+	+	+	-	+	±	*Lactobacillus amylovorus*
F1	Sm	Wh	Cir	Rods	SS	-	-	+	-	-	-NG	-	Ho	-	-	+	nd	-	+	+	-	+	+	*Lactobacillus pentosus*
F2	W	C	Irreg	Rods	SL	-	-	+	-	-	+G	-	Het	-	+	+	nd	+	+	+	-	+	±	*Lactobacillus plantarum***1**
F3	Sm	C	Cir	Rods	SS	-	-	+	-	±	+G	-	Ho	-	+	+	nd	-	+	+	+	+	+	*Lactobacillus farciminis*
F4	Sm	C	Cir	Rods	SS	-	-	+	-	±	+G	-	Ho	-	+	+	nd	-	+	+	+	+	+	*Lactobacillus farciminis*
F5	Sm	Wh	Cir	Rods	SC	-	-	+	-	-	-NG	-	Ho	-	-	+	nd	+	+	+	-	+	±	*Lactobacillus amylovorus*
F6	W	Wh	Irreg	Rods	SL	-	-	+	-	-	±G	-	Het	-	-	+	nd	+	-	+	+	+	±	*Lactobacillus plantarum*
F7	Sm	Wh	Irreg	Rods	SC	-	-	+	-	-	-NG	-	Het	-	+	+	nd	-	+	+	+	-	-	*Lactobacillus alimentarius*
F8	R	Wh	Cir	Cocci	CL	-	-	+	-	-	-NG	-	Het	-	+	+	nd	+	-	-	-	+	+	*Pediococcus pentoaseus* **1**
F9	Sm	Wh	Cir	Rods	SS	-	-	+	-	-	-NG	-	Ho	-	-	+	nd	+	-	-	-	+	+	*Lactobacillus crispatus*
PY1	Sm	C	Cir	Oval	EL	-	+	+	-	+	nd	nd	nd	nd	nd	nd	+	nd	+	+	+	nd	nd	*Saccharomyces cerevisiae*
PY2	Sm	C	Cir	Oval	EL	-	+	+	-	+	nd	nd	nd	nd	nd	nd	+	nd	+	+	+	nd	nd	*Candida quercitrusa*
PY3	Sm	C	Cir	Oval	EL	-	+	+	-	+	nd	nd	nd	nd	nd	nd	+	nd	+	+	+	nd	nd	*Candida milleri*
PY4	Sm	C	Cir	Oval	EL	-	+	+	-	+	nd	nd	nd	nd	nd	nd	+	nd	+	+	+	nd	nd	*Saccharomyces cerevisiae*
FY1	Sm	C	Cir	Oval	EL	-	+	+	-	+	nd	nd	nd	nd	nd	nd	+	nd	+	+	+	nd	nd	*Saccharomyces cerevisiae*
FY2	Sm	C	Cir	Oval	EL	-	+	+	-	+	nd	nd	nd	nd	nd	nd	+	nd	+	+	+	nd	nd	*Saccharomyces cerevisiae*
FY3	Sm	C	Cir	Oval	EL	-	+	+	-	+	nd	nd	nd	nd	nd	nd	+	nd	+	+	+	nd	nd	*Saccharomyces cerevisiae*

Key: + = positive reaction, - = negative reaction, ± = Weak reaction, +G = Positive and gas production, -NG = Negative and no gas production, ±G = Weak reaction and gas production, **Sm= Smooth, W= wrinkled, R= Rough, Cir = Circular, Wh = White, C= Creamy, Irreg = Irregular, SS = Short and Singly, CL= Clusters, SL=Short and long, SC= Short and in Clusters, EL = Ellipsoidal, nd = not determined.**

Table 2 The carbohydrate fermentation pattern of dominant presumed lactic acid bacteria isolates obtained from spontaneously developed pearl and finger millet sourdoughs

Isolates/Description	Glucose	Sucrose	Maltose	D-xylose	Sorbitol	Glycerol	Starch	Arabinose	Mannitol	Galactose	Raffinose	Esculin	Lactose	Trehalose	Melebiose	Inositol	Melezitose	α-methyl-mannoside	Salisin	Ribose	Cellobiose	Inulin	Fructose	Dextrose	Mannose	Rhamnose	Xylose	Sorbose	Malonate	Citrate	Glucosamine	Dulcitol	Adonitol	α-methyl-glucoside	Xylitol	ONPG	Saccharose	Probable Microorganism
P1	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	+	-	+	-	-	-	-	-	V	-	+	*Lb. pentosus*
P2		+	-	V	-	-	-	+	-	+	-	+	V	+	V	-	-	-	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	*P. pentosaseus*
P3	+	+	+	+	+	-	-	-	-	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	*Lb. plantarum*
P4	+	+	+	+	-	-	-	+	-	V	-	V	V	-	+	+	-	-	-	+	-	+	+	+	-	-	V	-	-	-	-	-	-	-	-	-	-	*Lb. buchneri*
P5	+	+	+	+	-	-	-	V	-	-	-	V	V	V	-	-	-	+	+	-	-	V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*M. varians*
P6	+	+	+	+	-	+	+	-	-	+	-	+	-	+	-	-	V	-	+	-	+	+	+	+	-	-	-	+	-	-	-	-	-	-	V	-	+	*Lb. amylovorus*
F1	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	-	+	-	+	-	+	-	-	-	-	-	-	V	-	+	*Lb. pentosus*
F2	+	+	+	+	+	-	-	-	-	V	-	+	+	+	+	+	+	-	+	+	+	+	+	+	V	-	-	-	-	-	-	-	-	-	-	-	+	*Lb. plantarum 1*
F3	+	+	+	V	-	-	-	-	+	-	-	+	+	-	-	-	-	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	*Lb. farciminis*
F4	+	+	+	V	-	-	-	-	-	+	-	+	+	-	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	*Lb. farciminis*
F5	+	+	+	+	+	+	-	+	+	+	+	+	+	-	V	-	+	+	+	-	+	V	-	-	+	-	V	-	-	-	-	-	-	V	-	+	*Lb. amylovorus*
F6	+	+	+	+	+	-	-	-	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	*Lb. plantarum*
F7	+	+	+	+	-	-	-	V	-	+	-	+	-	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	*Lb. alimentarius*
F8	+	+	-	V	-	-	-	+	-	+	-	+	V	+	V	-	-	-	+	+	-	+	-	+	V	-	-	+	-	-	-	-	-	-	-	V	*P. pentoaseus 1*
F9	+	+	+	+	-	+	-	-	+	+	-	+	+	-	-	-	-	+	-	+	+	+	+	-	-	+	-	-	-	-	V	-	+	*Lb. crispatus*

Keys: + = Positive reaction, - = Negative reaction, **V**= weak reaction,

Table 3 The carbohydrate fermentation pattern of dominant presumed yeasts isolates obtained from spontaneously developed pearl and finger millet sourdoughs

Isolates/Description	Glucose	Maltose	Mannitol	D-xylose	Sorbitol	Glycerol	Actidione	L-arabinose	Mannitol	Galactose	Raffinose	Esculin	Lactose	Trehalose	Melebiose	Inositol	Melezitose	Ribose	Cellobiose	Rhamnose	D-Xylose	Sorbose	Erythritol	N- acetyl glucosamine	α-methyl-glucoside	Saccharose	DL-lactate	2-keto-gluconate	Palatinose	Glucoronate	Gluconate	Levulinate	Glucosamine	Probable microorganism
PY1	+	-	V	+	V	-	-	-	V	+	+	V	-	-	-	-	-	-	-	-	V	-	-	V	+	-	V	-	-	-	-	-	-	*S. cerevisiae*
PY2	+	+	+	+	+	+	-	-	+	+	-	-	-	+	-	-	+	-	-	V	+	-	+	-	+	-	+	+	-	+	+	+	+	*C. quercitrusa*
PY3	+	-	+	+	+	+	-	+	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-	*C. milleri*
PY4	+	-	V	+	V	-	-	+	+	+	V	-	V	-	-	-	-	-	-	V	-	V	+	-	V	-	-	-	-	-	-	-	-	*S. cerevisiae*
FY1	+	-	V	+	V	-	-	-	V	+	+	V	-	-	-	-	-	-	-	V	-	V	+	-	V	-	-	-	-	-	-	-	-	*S. cerevisiae*
FY2	+	-	V	+	V	-	-	-	+	+	+	V	-	V	-	-	-	-	-	V	-	V	+	-	V	-	-	-	-	-	-	-	-	*S. cerevisiae*
FY3	+	-	V	+	V	-	-	-	+	+	+	V	-	V	-	-	-	-	-	V	-	V	+	-	V	-	-	-	-	-	-	-	-	*S. cerevisiae*

Keys: + = Positive reaction, - = Negative reaction, **V**= weak reaction.

maize porridge in Kenya. The number and type of yeast species found in sourdoughs depends on several factors including the degree of dough hydration and type of cereal used. The dominance of *S. cerevisiae* might be due to its ability to utilise simple sugar in the cereals faster during fermentation.

Fig. 2 and 3 presents the mean pH, temperature and titratable acidity of spontaneously developed sourdoughs from pearl and finger millet. From Figure 2 and figure 3, the pH of the spontaneously developed sourdoughs decreased with increasing days of fermentation. The pH ranged from 4.9 to 3.5 in pearl millet sourdough while in finger millet sourdough it ranged from 5.7 to 3.3. This agrees with results obtained by **Sanni et al. (1998)** in a work on sour maize bread production using starter cultures. The significant decrease in pH might be due to increase acid production by lactic acid bacteria in the sourdough. No significant difference was observed in the pH of the pearl millet sourdoughs on the 6th and 7th day of fermentation.

The temperature of the spontaneously developed pearl and finger millet sourdoughs increased with increasing days of fermentation. This might be due to increased rate of reaction in the sourdough as metabolites production increased. Temperature increases from 27 °C to 32 °C in sour maize bread produced by **Sanni et al. (1998)** however, after 48 h of fermentation the temperature was constant at 32 °C and the pH reduced further to 3.7. **Hounhouigan et al. (1993)** reported titratable acidities ranging from 1.2 - 1.4 in `mawe', a fermented maize dough. Titratable acidities increased significantly with increasing days of fermentation. Titratable acidity ranged from 1.5 ml to 3.4 ml and 1.1 ml to 3.6 ml in spontaneously developed pearl and finger millet sourdoughs. The dominant metabolite of lactic fermentation is lactic acid although acetic acid also contributes to acidification **(Petra et al., 2011)**. Acetic acid is important for strong aroma, fungicidal and antimicrobial effect in fermented products while lactic acid influences product texture **(Corsetti and Settanni, 2007).**

Fig 4 and 5 presents the microbial population of mesophilic bacteria and fungi of spontaneously developed pearl and finger millet sourdoughs. The mesophilic bacterial count increased with increasing fermentation days. The bacteria count of pearl and finger millet sourdough ranged from 53 x 10^8 - 280 x 10^8 Cfu/ml and 28 x10^9 cfu/ml - 50 x10^9 cfu/ml respectively. The bacterial count of developed finger millet sourdough was highest on day 4 of fermentation. The fungi growth increased with increasing days of fermentation. The growth pattern of associated microorganisms followed a normal bacterial growth curve. This could be that the microbes were still in an adaptation phase (Lag phase) between the first two days of fermentation. At day 2 and 3 the mesophilic bacterial count increased (exponential phase) which might be due to the activity of the microbes in adjusting to the new environment of fermentation. At day 4 and 5, the mesophilic bacterial count declined, this might be due to accumulation of metabolites, increased competition for limited nutrient resulting in death of the microorganisms. The bacteria involved in sourdough fermentations are mainly mesophilic **(De-vuyst and Neysens, 2005)**. The microflora population of cereals flours ranged from 2x10^4–6x10^6 CFU/g **(Stolz, 1999)**. The occurrence of yeasts up to 2x10^3 CFU/g have been reported in cereals flours **(De-vuyst and Neysens, 2005)**. The values obtained in this study are slightly higher than those reported by **Stolz, (1999)**. The higher population of microbial counts might be due to pretreatment of the grains (fermentation).
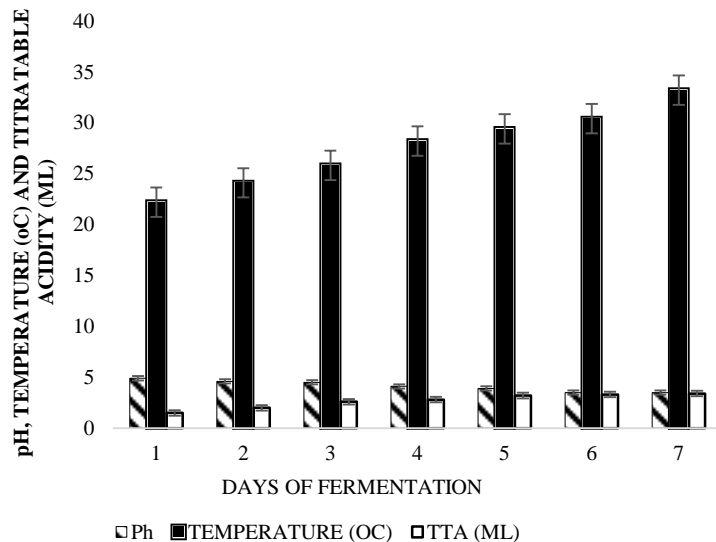
Figure 2 pH, Temperature (°C) and Titratable acidity (ml) of spontaneously developed sourdough from Pearl millet flour
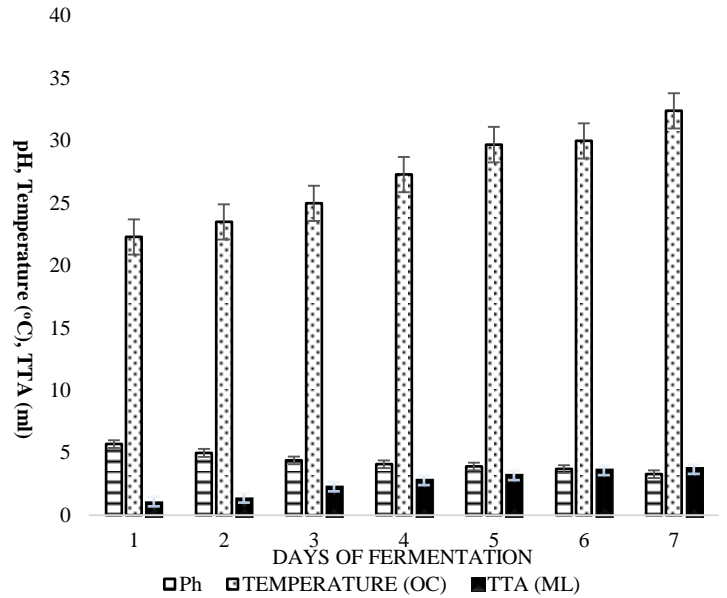
Figure 3 pH, Temperature (°C) and Titratable acidity (ml) of spontaneously developed sourdough from Finger millet flour
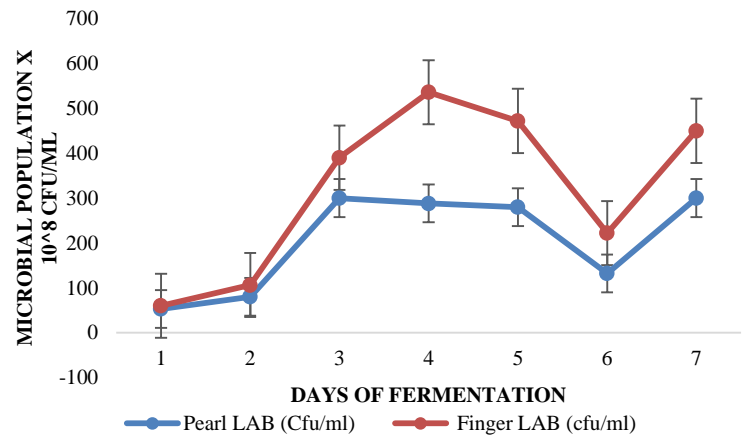
Figure 4 The microbial population of LAB isolated from spontaneously developed sourdough using pearl and finger millet flour
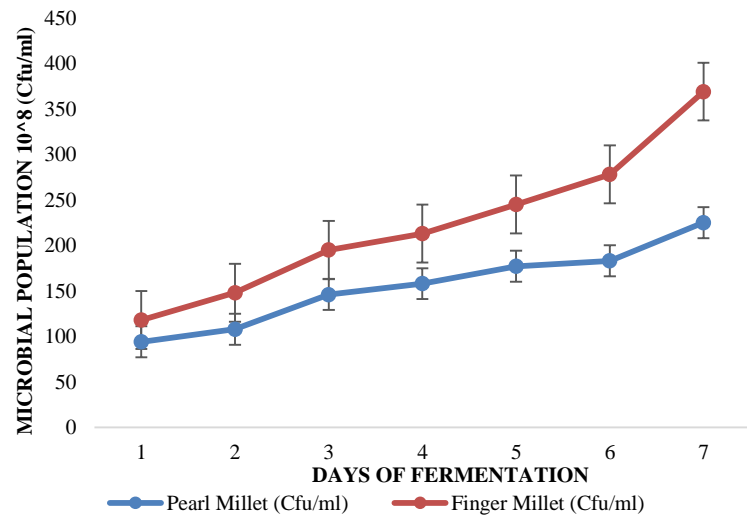
Figure 5 The microbial population of yeast isolated from spontaneously developed finger and pearl millet flour sourdough

CONCLUSION

Pearl and finger millet flour are rich sources of beneficial microbial consortia especially LAB and yeast. The titratable acidity, pH, temperatures of fermenting millet meals increased with increasing days of fermentation. Spontaneously developed pearl millet sourdoughs were dominated by *Lb. plantarum* (Heterofermenters) and *Lb. pentosus*, *P. pentosaseus* (Homofermenters) while

spontaneously fermented finger millet sourdoughs is dominated by *Lb. plantarum* 1, *Lb. pentosus* and *P. pentosaseus*. *S. cerevisiae* and *Candida milleri* are dominant yeast cultures in millet sourdoughs. Further studies should be done on the characterisation of microbial isolates using molecular techniques and screening of isolated cultures for desirable sourdough functional properties.

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