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Bacterial ecology and rheological parameters of multigrain gluten-free sourdoughs

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ABSTRACT

The microbial diversity and pasting properties of three sourdoughs produced from composite gluten-free flours were investigated using 16S rRNA gene clone libraries and the Rapid Viscoanalyser. Finger millet-pearl millet (FP), Pearl millet-sorghum (PS) and Finger millet-sorghum (FS) sourdoughs were produced. Eleven aerobic bacteria and twelve lactic acid bacteria (LAB) were randomly selected from the sourdoughs. Presumptive *Bacillus subtilis* and *Pediococcus* spp. were identified in all the sourdoughs after 48 h of fermentation, while yeast was not detected in any of the products. The LAB population and pH ranged from log 7.70 CFU g^{-1} to log 10.52 CFU g^{-1} and 3.8 to 4.2 respectively. The findings showed that well-developed sourdough could be produced from these composite flours by spontaneous fermentation. Significant differences were observed in the pasting properties of all the sourdoughs. Decline in the tendency to retrograde occurred in all sourdoughs, thereby justifying the lower staling rate of final products. This study enhanced the corpus of existing knowledge on the microbial diversity of gluten-free sourdough and provided a basis for the possible application of *Pediococcus* spp. and *Weisella* spp. as a starter culture(s) in fermented products.

1. Introduction

Fermented food possesses an ecosystem that comprises lactic acid bacteria (LAB), acetic acid bacteria and other Gram positive/negative and/or fungi that contribute to its several beneficial characteristics, such as prolonged shelf-life, improved texture and organoleptic properties (Wood, 1998). Cheese, yoghurt and sourdough are some of the examples of myriads of fermented food commonly consumed. Sourdough is a mixture of milled cereal and water that is spontaneously fermented (Gobbetti, 1998; Vrancken, Rimaux, Weckx, Leroy, & De Vuyst, 2011) by the action of LAB and yeasts leading to improved dough structure, aroma, palatability, nutritional value and prolonged shelf-life (Moroni, Dal Bello, & Arendt, 2009). Previous studies have affirmed that the positive effects of LAB on dough include the release of small peptides and free amino acids, which are essential for pH reduction, rapid growth of microorganisms, precursors for flavour development (Rollan, De Angelis, Gobbetti, & De Valdez, 2005), larger and evenly distributed gas cells, higher loaf volume (Edema, Emmambux, & Taylor, 2013), accumulation of bioactive peptides (Hu et al., 2011) and metabolite production (Galle, 2013).

Specifically, it was reported that the presence of *Leuconostoc* species had improved the visco-elastic properties of sour maize dough (Edema, 2010). According to Salovaara (2004), some common LAB species found in sourdoughs include *Lactobacillus acidophilus, Lactobacillus farciminis, Lactobacillus delbrueckii* (obligate homofermentative), *Lactobacillus casei, Lactobacillus plantarum, Lactobacillus rhamnosus* (facultative heterofermentative), *Lactobacillus brevis, Lactobacillus sanfransicencis and Lactobacillus fermentum* (obligate heterofermentative). Studies have shown that the dominant microbial species in sourdough is influenced by temperature and type of flour (Vrancken et al., 2011; Ercolini et al., 2013; Harth, Van Kerrebroeck, & De Vuyst, 2016; Ogunsakin et al., 2017). Hence, it becomes imperative in product development to scrutinize the flour to be used, so as to ascertain the microbial community present in it. This will not only ensure the safety of targeted consumers, but also help food scientists to develop healthy starter cultures that can be of immense benefit to the food production sector.

Pearl millet (*Pennietum glaucum*), finger millet (*Eleusine coracana*) and sorghum (*Sorghum bicolor*) are gluten-free cereals known to be rich sources of energy and used in the production of fermented foods (Akinola, Badejo, Osundahunsi, & Edema, 2017; Nazni & Shalini, 2010). In addition to these benefits, flour-blends have the characteristic advantage of synergistically combining the strengths of individual

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grains, thereby making up for possible weaknesses of the individual flour. Three multigrain flours, each containing two of the listed cereals in equal proportion, were screened for microbial diversity and pasting properties. This work is part of a large-scale and ongoing research aimed at producing bread from underutilized gluten-free African cereals using sourdough technology. As with the development of new food products, several components that could affect the resultant product's nutritional value, acceptability and organoleptic quality must be ascertained. The key objective of this research was to identify predominant fermenting organisms in the dough, which could be utilized for the development of a starter culture, and to understand the rheological parameters of the fermented multigrain sourdough.

2. Materials and methods

2.1. Sample collection

Flours made up of Finger millet (*Eleusine coracana*) of the KNE 1149 variety (F), Pearl millet (*Pennisetum glaucum*) of the ICMV 221-White variety (P) and Sorghum (*Sorghum bicolor*) of the KARI MTAMA 1 variety (S) were used. These grains were sourced from the International Crops Research Institute on Semi-Arid Tropics (ICRISAT) in Nairobi, Kenya. The grains were cleaned, milled through a knife mill and sieved to a particle size of ≤0.2 mm. The flours were stored in labelled airtight containers for further analyses. The proximate composition (dry matter basis) of the grains was obtained using the AOAC (2005) method. The respective values for F, P and S are as follows; protein (N × 6.25),18.26 ± 0.88%, 18.58 ± 0.02% and 9.84 ± 0.91%, fat; 8.90 ± 0.01%, 9.65 ± 0.14% and 6.94 ± 0.42%, crude fibre; 2.22 ± 0.17%, 1.31 ± 0.12% and 1.62 ± 0.09%, ash; 2.12 ± 0.08%, 1.58 ± 0.11% and 1.34 ± 0.24%, total carbohydrate; 68.49 ± 1.13%, 68.88 ± 0.07% and 80.26 ± 1.48%.

2.2. Sourdough preparation

Preparation of sourdough was done using the Type I sourdough technique, which does not require the use of starter culture(s). Three composite flours, namely finger millet-pearl millet (FP), pearl millet-sorghum (PS) and finger millet-sorghum (FS), were mixed in equal proportion (50:50) to allow for equal expression of its unique properties, whereby none of the sourdough predominates the other quantitatively. The sourdough from of each of the developed blends were than produced, as previously described by Edema et al. (2013), with slight modifications. The flour-to-water ratio was 1:2 due to the dough's consistency. The flour and tap water were thoroughly mixed and allowed to ferment naturally at room temperature (27 °C) for 48 h. Preparations were carried out in triplicate.

2.3. pH determination, enumeration and isolation of cultivable bacteria and yeast

Prepared samples were analysed every 12 h for a total duration of 48 h so that each sample was analysed four times within the stated period. The pH was determined using a Model pHs-25 pH meter, and all analyses were carried out in triplicates. Ten grams of each sample was homogenized with 90 ml of sterile 0.85% (wt./vol.) NaCl solution. Viable bacteria and lactic acid bacteria were enumerated at 37 °C for 24 h under aerobic conditions and 37 °C for 48 h under anaerobic conditions, respectively. The media used for the former was Nutrient agar (NA) with cycloheximide (0.1 g L^{-1}), while the latter used the de Mann Rogosa and Sharpe (MRS) agar. Rose Bengal Chloramphenicol (RBC) agar was used for enumerating yeasts at 30 °C for 72 h under aerobic conditions. Culture dependent approaches were used for investigating the sourdough microbiota. At least 11 colonies of presumptive bacteria were randomly selected from plates containing the three highest sample dilutions with distinct colonies. The isolates were re-streaked on Nutrient agar with cycloheximide (0.1 g L^{-1}) and cultivated in Nutrient broth at 37 °C for 24 h. About three randomly selected colonies of Gram positive, catalase negative rod and coccus from plates containing the three highest sample dilutions with distinct colonies were re-streaked and sub-cultured on MRS broth at 37 °C for 72 h. Stock cultures were stored at - 20 °C in 10% (vol/vol) glycerol.

2.4. Genotypic identification of bacteria

The Genomic DNA of bacteria was extracted using the Wizard Genomic DNA purification Kit (Promega Corporation) according to manufacturers' instruction. For identification of presumptive bacteria and lactic acid bacteria, two primer pairs, namely 27F (5′-AGAGTTTG ATCMTGGCTCAG-3′) and 1492R (5‘-GGTTACCTTGTTACGACTT-3’), were used for amplifying the 16S rRNA genes. Electrophoresis was carried out on 1.5% agarose gel, while the amplicon was purified using Wizard SV Gel and PCR Clean Up System (Promega Corporation; USA). The amplicon was then sequenced using the Dye Terminator. Sequence alignments were carried out using the multiple-sequence alignment method called ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2) and the sequence was identified by a BLAST search in the GenBank (http://www.ncbi.nlm.nih.gov/GenBank/).

2.5. Pasting properties

Pasting properties were determined using the Rapid Viscoanalyzer (RVA Super 4, Australia), as previously described by Akinola et al. (2017). The procedure was carried out according to the operational manual. Prior to loading the sample in to the RVA, 2 g of flour each were dried in an oven at 105 °C to obtain a constant weight. A programmed heating and cooling cycle was used at a constant shear rate, where the sample was held at 50 °C for 1 min, heated from 50 °C to 95 °C at 6 °C/min, held at 95 °C for 5 min, cooled to 50 °C at 6 °C/min and held at 50 °C for 5 min.

2.6. Statistical analyses

Data were subjected to one-way Analysis of Variance (ANOVA), while Duncan's Multiple range test was used to separate the mean at a significance level of $P < 0.05$. All data were determined in triplicate and analyses were performed using the Statistical Package for Social Sciences (SPSS) 16.0 version software.

3. Results and discussion

3.1. Microbial population and acidification dynamics during sourdough fermentation

Colony counts on nutrient agar at the beginning of the fermentation revealed the presence of bacteria in all the sourdoughs produced (Fig. 1). A drastic increase in bacterial growth ($P < 0.05$) was observed in all sourdoughs within the 12 h fermentation period. The significant difference in bacterial population between the 0–12 h of fermentation is indicative of a favourable environmental condition. The peak cell density of FS was attained at 12 h fermentation, while that of FP and PS were attained at 24 h fermentation. The sourdoughs generally recorded cell densities within the range of log 9.60 (PS) and log 11.48 (FS) CFU g^{-1}. A decline in the bacterial count was observed in the sourdoughs following the attainment of maximum cell densities. This waning in bacterial count is attributed to the corresponding increase in the dough's acidity (Fig. 3). Corsetti and Settanni (2007) and Weckx et al. (2010) had independently stated that some microbes die off with an increase in fermentation time, owing to its inability to survive in acidic medium.

Final bacterial loads in the sourdoughs at 48 h fermentation were slightly different, having values of log 7.95, log 8.30 and log

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