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Lactic Acid Bacteria and Yeasts in Spontaneously Fermented Sorghum Sourdough

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Abstract **Introduction:** Generally, natural fermentations are carried out by yeast and lactic acid bacteria forming a complex microbiota that acts in cooperation. Yeast have a prominent role in the production of beverages, due to the ability to accumulate high levels of ethanol and to produce highly desirable aroma compounds, but lactic acid bacteria are particularly important in fermentation because they produce desirable acids, flavor compounds, and peptides that inhibit the growth of undesirable organisms [1]. 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 [2]. **Methodology:** Lactic acid bacteria and yeasts were isolated from Sourdoughsusing pour plating methods as described by Harrigan and McCance [3]. Mann Rogosa Sharpe (MRS) and potato dextrose medium were used for culturing lactic acid bacteria and yeasts respectively. Cultured MRS plates were incubated anaerobically at 30°C for 48h while that of PDA plates were incubated in an incubator at 25°C for 72h. The microbial populations of the sourdoughs were enumerated on each day of fermentation. Isolation and sub-culturing was done until pure cultures were obtained. **Result:** The spontaneously fermentative lactic acid bacteria and yeast populating the fermenting sorghum sourdough observed in this study increased rapidly as the fermentation time increased. The mutual or synergistic relationship between the duo confirmed and justified Wood (2004) report in fermenting food matrix. Wood and Hodge (1985), describe the co-existence of lactic acid bacteria and yeast in food processing as very crucial. **Conclusion:** The microbiological and physicochemical analysis of sorghum sourdough fermented spontaneously showed a synergistic relationship of lactic acid bacterial and yeast growing in it. The titratable acidity, pH, and temperature of the fermenting sorghum sourdough increased with increase days of fermentation with higher values observed in sorghum vulgare.

Keywords: *sorghum, sourdough, fermentation, yeast*

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1. Introduction

Fermented food products are not only rich in nutrients such as proteins, vitamins, essential aminoacids, sugars, fatty acids but are also good for digestion [4,5]. Most of the bacterial species associated with the fermented food do not possess health risk, they are designated as GRAS (generally recognized as safe) organism [6,7]. Sourdough is a mixture of flour and water fermented by lactic acid bacteria and yeast, with a large use in bakery products [8], for examples the production of sourdough bread, classical bread, snacks, pizza and sweet baked products [9]. The incorporation of sourdough in baking technological traits, enhances sensorial characteristics, increases shelf life and improves nutritional properties [10,11]. Sourdough is a technological process applied to non-gluten flours. It is the foremost cereal fermentation performed with almost any cereal [12,13,14]. 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 [2].

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 [15]. 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.

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 [16,17,18,19,20,21]. 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 Weissellaspp* [22]. Lactic acid bacteria (LAB) belonging to the genus - *Lactobacillus* have been isolated from sourdoughs and identified [11]. 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 [22]. However, *Lactobacillus* and *Saccharomyces* genera have been reported to be the most dominant in sourdough fermentations.

Generally, natural fermentations are carried out by yeast and lactic acid bacteria forming a complex microbiota that acts in cooperation. Yeast have a prominent role in the production of beverages, due to the ability to accumulate high levels of ethanol and to produce highly desirable aroma compounds, but lactic acid bacteria are particularly important in fermentation because they produce desirable acids, flavor compounds, and peptides that inhibit the growth of undesirable organisms (Faria-Oliveira *et al*., 2013).

1.1. Justification

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 [2]. 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 [22]. Morever, *Lactobacillus* and *Saccharomyces* genera have been reported to be the most dominant in sourdough fermentations. Also, the LAB from the sourdoughs naturally fermented may be used in the production of novel fermented foods such as sourdough bread, which is likely to have superior quality and long shelf life [23].

1.2. Study Objective

The objective of this study is to isolate, characterize and identify lactic acid bacteria and yeasts isolated from sourdough with prospective selection as starter cultures.

1.3. Research Questions

Research Question 1: What are the microorganisms involved in the natural fermentation of sorghum?

Research Question 2: Can these microorganisms serve as a starter cultures in sourdough production for use in bakery products?

2. Literature Review

2.1. Fermentation

Fermentation is a metabolic process that converts sugar to acids, gases, or alcohol. It occurs in yeast and bacteria, and also in oxygen-starved muscle cells, as in the case of lactic acid fermentation. Fermentation is also used more broadly to refer to the bulk growth of microorganisms on a growth medium, often with the goal of producing a specific chemical product. French microbiologist Louis Pasteur is often remembered for his insights into fermentation and its microbial causes. The science of fermentation is known as zymology (https://en.m.wikipedia.org/wiki/Fermentation).

To many people, fermentation simply means the production of alcohol: grains and fruits are fermented to produce beer and wine. If a food soured, one might say it was 'off' or fermented. Here are some definitions of fermentation. They range from informal, general usage to more scientific definitions [24].

1. Preservation methods for food via microorganisms (general use).
2. Any process that produces alcoholic beverages or acidic dairy products (general use).
3. Any large-scale microbial process occurring with or without air (common definition used in industry).
4. Any energy-releasing metabolic process that takes place only under anaerobic conditions (becoming more scientific).
5. Any metabolic process that releases energy from a sugar or other organic molecules, does not require oxygen or an electron transport system, and uses an organic molecule as the final electron acceptor (most scientific).

2.1.1. Fermentation in Food Processing

Fermentation in food processing is the process of converting carbohydrates to alcohol or organic acids using microorganisms (yeasts or bacteria) under anaerobic conditions. Fermentation usually implies that the action of microorganisms is desired. The science of fermentation is known as zymology or zymurgy. The term fermentation sometimes refers specifically to the chemical conversion of sugars into ethanol, producing alcoholic drinks such as wine, beer, and cider. However, similar processes take place in the leavening of bread (CO_2 produced by yeast activity), and in the preservation of sour foods with the production of lactic acid, such as in sauerkraut and yogurt. Other widely consumed fermented foods include vinegar, olives, and cheese. More localised foods prepared by fermentation may also be based on beans, grain, vegetables, fruit, honey, dairy products, fish, meat, or tea (https://en.wikipedia.org/wiki/Fermentation_in_food_processing).

Food fermentation is the conversion of sugars and other carbohydrates into alcohol or preservative organic acids and carbon dioxide. All three products have found human uses. The production of alcohol is made use of when fruit juices are converted to wine, when grains are made into beer, and when foods rich in starch, such as potatoes, are fermented and then distilled to make spirits such as gin

and vodka. The production of carbon dioxide is used to leaven bread. The production of organic acids is exploited to preserve and flavor vegetables and dairy products [25].

Food fermentation serves five main purposes: to enrich the diet through development of a diversity of flavors, aromas, and textures in food substrates; to preserve substantial amounts of food through lactic acid, alcohol, acetic acid, and alkaline fermentations; to enrich food substrates with protein, essential amino acids, and vitamins; to eliminate antinutrients; and to reduce cooking time and the associated use of fuel [26].

2.2. Fermented Foods

Culturalable and non-culturable microorganisms naturally ferment majority of global fermented foods and beverages. Fermented foods are the hub of consortia of microorganisms, since they are either present as natural indigenous microbiota in uncooked plant or animal substrates, utensils, containers, earthen pots, and the environment [27], or add starter culture(s) containing functional microorganisms (Holzapfel, 1995) which modify the substrates biochemically, and organoleptically into edible products that are culturally and socially acceptable to the consumers [18,28]. Microorganisms convert the chemical composition of raw materials during fermentation, which enrich the nutritional value in some fermented foods, and impart health-benefits to the consumers [29].

Several researchers have reviewed the microbiology, biochemistry, and nutrition of fermented foods and beverages from different countries of Asia [17,30] [17,28,31]; Africa [21,27]; Europe [32]; South America and North America. Many genera/species of microorganisms have been reported in relation to various fermented foods and beverages across the world.

Global fermented foods are classified into nine major groups on the basis of substrates (raw materials) used from plant/animal sources: (1) fermented cereals, (2) fermented vegetables and bamboo shoots, (3) fermented legumes, (4) fermented roots/tubers, (5) fermented milk products, (6) fermented and preserved meat products, (7) fermented, dried and smoked fish products, (8) miscellaneous fermented products, and (9) alcoholic beverages [18,28,31].

2.2.1. Fermented Cereal Foods

In Africa, fermented cereal foods are traditionally used as staples as well as complementary and weaning foods for infants and young children [33]. Yeasts and LAB conduct dough fermentation, mostly San Francisco sourdough, and the resultant products are generally called sourdough breads because they have higher contents of lactic acid and acetic acid due to the bacterial growth [34,35].

Cereal fermentation is mainly represented by species of LAB and yeasts [36]. Enterococcus, Lactococcus, Lactobacillus, Leuconostoc, Pediococcus, Streptococcus, and Weissella are common bacteria associated with cereal fermentations [35,37,38]. Native strains of Saccharomyces cerevisiae are the principal yeast of most bread fermentations [39], but other non-Saccharomyces yeasts are also significant in many cereal fermentations including Candida, Debaryomyces, Hansenula, Kazachstania, Pichia, Trichosporon, and Yarrowia [40,41].

2.3. Microorganisms in Fermented Foods

Lactic acid bacteria (LAB) are widely present in many fermented foods and beverages [28,42]. Major genera of the LAB such as Alkalibacterium, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, ecdPediococcus, Streptococcus, Tetragenococcus, Vagococcus, and Weissella [43,44,45] have been isolated from various globally fermented foods and beverages.

Bacillus is present in alkaline-fermented foods of Asia and Africa [46,47]. Species of Bacillus that are present, mostly in legume-based fermented foods, are Bacillus amyloliquefaciens, Bacillus circulans, Bacillus coagulans, Bacillus firmus, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus subtilis, Bacillus subtilis variety natto, and Bacillus thuringiensis [48,49], while strains of Bacillus cereus have been isolated from the fermentation of Prosopisafricana seeds for the production of okpehe in Nigeria [27]. Some strains of B. subtilis produce λ-polyglutamic acid (PGA) which is an amino acid polymer commonly present in Asian fermented soybean foods, giving the characteristic of a sticky texture to the product [50,51].

The association of several species of Kocuria, Micrococcus (members of the Actinobacteria), and Staphylococcus (belonging to the Firmicutes) has been reported for fermented milk products, fermented sausages, meat, and fish products [52,53]. Species of Bifidobacterium, Brachybacterium, Brevibacterium, and Propionibacterium are isolated from cheese, and species of Arthrobacter and Hafnia from fermented meat products [54]. Enterobacter cloacae, Klebsiellapneumoniae, K. pneumoniae subsp. ozaenae, Haloanaerobium, Halobacterium, Halococcus, Propionibacterium, Pseudomonas, etc. are also present in many global fermented foods [28].

Genera of yeasts reported for fermented foods, alcoholic beverages and non-food mixed amylolytic starters are mostly Brettanomyces, Candida, Cryptococcus, Debaryomyces, Dekkera, Galactomyces, Geotrichum, Hansenula, Hanseniaspora, Hyphopichia, Issatchenkia, Kazachstania, Kluyveromyces, Metschnikowia, Pichia, Rhodotorula, Rhodosporidium, Saccharomyces, Saccharomycodes, Saccharomycopsis, Schizosaccharomyces, Sporobolomyces, Torulaspora, Torulopsis, Trichosporon, Yarrowia, and Zygosaccharomyces [55,56,57].

Major role of filamentous molds in fermented foods and alcoholic beverages is the production of enzymes and the degradation of anti-nutritive factors [30] Species of Actinomucor, Amylomyces, Aspergillus, Monascus, Mucor, Neurospora, Parcilomyces, Penicillium, Rhizopus, and Ustilago are reported for many fermented foods, Asian non-food amylolytic starters and alcoholic beverages [30]

2.3.1. Yeast Diversity and Metabolism

Yeasts are unicellular fungi, being the simplest eukaryotes. Present in a great number of environments, yeast can be found not only in decomposing fruit, trees, and soils but also in commensal relationships with higher

eukaryotes, humans included, and even saltwater. The high diversity of species, almost 1500 species have been described (Kurtzman *et al*., 2011), is closely related to this wide distribution. Some of these yeast are adapted to extreme environments, such as high salt concentrations [58], low pH [59], or extremely cold temperatures [60,61]. The genus *Saccharomyces*, particularly *Saccharomyces cerevisiae*, is strongly associated with the production of fermented products for human consumption, namely, bread, wine, and beer [62]. After several millennia of close coexistence, through phenotypic selection, these species evolved to produce goods with organoleptic properties pleasant to humans. However, given the high degree of diversity found in nature, it is expected to find yeast with new and more interesting characteristics for the industry in new and unexplored niches [63,64].

Yeast, as other heterotrophic organisms, have the anabolism coupled with catabolism. In one hand, the oxidation of organic molecules, as sugars, yields adenosine 5-triphosphate (ATP) that, in turn, is used as an energy resource for the cell. On the other hand, such organic molecules can also be used as building blocks or to generate intermediary compounds for the synthesis of other compounds, some of which with high commercial value. The high diversity of environments where yeast can be found is closely related to the variety of carbon sources that can be used. Hexoses such as glucose, fructose, galactose, or mannose are the most common substrates, but some species can use pentoses like xylose or arabinose. Several industrial relevant species can metabolize disaccharides as maltose, lactose, or sucrose, and some, as *Saccharomyces diastaticus*, can even metabolize dextrins (glucose polymers) [65] Nevertheless, glucose and fructose, to a lesser extent, are the preferred substrates.

2.3.2. Lactic acid Bacteria

Lactic Acid Bacteria (LAB) are gram positive, typically non- sporulating rod or coccus shaped. They lack catalase and strictly fermentative, producing either a mixture of lactic acid, carbon dioxide, acetic acid and/or ethanol (heterofermentation) or almost entirely lactic acid (homofermentation) as the major metabolic end-product [66,67,68,69,70,71].

Lactic acid bacteria (LAB) constitute an ubiquitous and heterogeneous group capable of fermenting carbohydrate with the production of lactic acid as a major end product [72]. LAB are found in diverse nutrient-rich habitats associated with plant and animal's matter, as well as in respiratory, gastrointestinal, and genital tracts of humans [73,74]. A typical LAB is Gram positive, present a GC content below 55%, generally nonsporulating, usually nonmotile, fastidious, catalase negative (pseudocatalase may occur in some LAB), aerotolerant, and acid tolerant [72].

Taxonomic parameters have distributed LAB members into two phyla, *Firmicutes* and *Actinobacteria*. Within the *Firmicutes* phylum, LAB members belong to the order *Lactobacillales* and comprise the following genera: *Aerococcus*, *Alloiococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Symbiobacterium*, *Tetragenococcus*, *Vagococcus*, and *Weissella*. Regarding LAB members belonging to the *Actinobacteria* phylum, the only species belongs to the *Bifidobacterium* genus [42,73,75]. Nevertheless, it is worth mentioning that *Bifidobacterium* is poorly phylogenetically related to typical LAB. These bacteria have been considered as LAB given its physiological similarity and the shared biochemical properties [76].

Usually, LAB members are nonpathogenic organisms with a reputed generally recognized as safe (GRAS) status. The *Lactobacillus* genus includes some of the most important GRAS species involved in food microbiology and human nutrition [77,78]. The remarkable ability of these bacteria to adapt to different environments resulted in a large number of industrially relevant strains. Among these are *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, and *Bifidobacterium* [73,79,80]. Furthermore, given that LAB greatly contribute to the effective acidification of the matrix and consume rapidly fermentable sugars, these bacteria are frequently predominant in the natural fermentation microbiota [80].

Lactic acid bacteria are among the most important groups of microorganisms used in food fermentations. They contribute to the taste and texture of fermented products and inhibit food spoilage bacteria by producing growth-inhibiting substances and large amounts of lactic acid. As agents of fermentation LAB are involved in making yogurt, cheese, cultured butter, sour cream, sausage, cucumber pickles, olives and sauerkraut, but some species may spoil beer, wine and processed meats.

2.3.2.1. LAB as Starter Culture

Lactic Acid Bacteria (LAB) are the most important bacteria used in food fermentations. Apart from general demands for starter cultures from the view of safety, technological effectiveness and economics, numerous specific aspects have to be considered when selecting strains for the different food fermentations. Therefore, selection criteria for LAB depend on the type and the desired characteristics of the final product, the desired metabolic activities, the characteristics of the raw materials and the applied technology [81]. A starter culture can be defined as a microbial preparation of large numbers of cells of at least one microorganism to be added to a raw material to produce a fermented food by accelerating and steering its fermentation process. The group of Lactic Acid Bacteria (LAB) occupies a central role in these processes and has a long and safe history of application and consumption in the production of fermented foods and beverages [4,32,82,83]. They cause rapid acidification of the raw material through the production of **organic acid**s, mainly **lactic acid**. Also, their production of acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides and several enzymes is of importance. In this way they enhance shelf life and microbial safety, improve texture and contribute to the pleasant sensory profile of the end product.

2.4. SORGHUM

2.4.1. Cultivation and Uses

Sorghum bicolor [84], native to Africa with many cultivated forms, is an important crop worldwide, used for

food (as grain and in sorghum syrup or "sorghum molasses"), animal fodder, the production of alcoholic beverages, and biofuels. Most varieties are drought- and heat-tolerant, and are especially important in arid regions, where the grain is one of the staples for poor and rural people. These varieties form important components of pastures in many tropical regions. *S. bicolor* is an important food crop in Africa, Central America, and South Asia, and is the fifth-most important cereal crop grown in the world. Some species of sorghum can contain levels of hydrogen cyanide, hordenine, and nitrates lethal to grazing animals in the early stages of the plants' growth. When stressed by drought or heat, plants can also contain toxic levels of cyanide and/or nitrates at later stages in growth [85]. Sorghum is efficient in converting solar energy to chemical energy, and also uses less water compared to other grain crops [86]. Biofuel, using sweet sorghum as a high sugar content from its stalk for ethanol production, is being developed with biomass which can be turned into charcoal, syngas, and bio-oil [87].

2.4.2. Sorghum Bicolor

Sorghum bicolor, commonly called sorghumand also known as great millet, *durra*, jowari, or milo, is a grass species cultivated for its grain, which is used for food, both for animals and humans, and for ethanol production. Sorghum originated in northern Africa, and is now cultivated widely in tropical and subtropical regions [88]. Sorghum is the world's fifth most important cereal crop after rice, wheat, maize and barley. *S. bicolor* is typically an annual, but some cultivars are perennial. It grows in clumps that may reach over 4 m high. The grain is small, ranging from 2 to 4 mm in diameter. Sweet sorghums are sorghum cultivars that are primarily grown for foliage, syrup production, and ethanol; they are taller than those grown for grain [89].

Sorghum bicolor is the cultivated species of sorghum; its wild relatives make up the botanical genus *Sorghum*. The leading producers of sorghum bicolor in 2011 were Nigeria (12.6%), India (11.2%), Mexico (11.2%) and the United States (10.0%). Sorghum grows in a wide range of temperature, high altitudes, toxic soils and can recover growth after some drought. It has four features that make it one of the most drought-resistant crops:

- It has a very large root-to-leaf surface area ratio.
- In times of drought, it will roll its leaves to lessen water loss by transpiration.
- If drought continues, it will go into dormancy rather than dying.
- Its leaves are protected by a waxy cuticle.

In many parts of Asia and Africa, its grain is used to make flat breads that form the staple food of many cultures [90,91]. The grains can also be popped in a similar fashion to popcorn. The species can be used as a source for making ethanol fuel, and in some environments may be better than maize or sugarcane, as it can grow under harsher conditions. It typically has protein levels of around 9%, enabling dependent human populations to subsist on it in times of famine, in contrast to regions where maize has become the staple crop. It is also used for making a traditional corn broom.

3. Materials and Methods

3.1. Sample Collection

Sorghum (white variety) and maize (yellow variety) were purchased from Kuto market in Abeokuta, Ogun State, All the grains were cleaned, steeped, processed into flours and packaged in polyethylene bags for further analysis as shown in Figure 1. The grains were steeped in water for two days (1:3 w/v ratios of seeds to the volume of steeping water), dried at 70°C for 4 h in hot air oven and dry milled into flour. Flours was sieved and packaged.

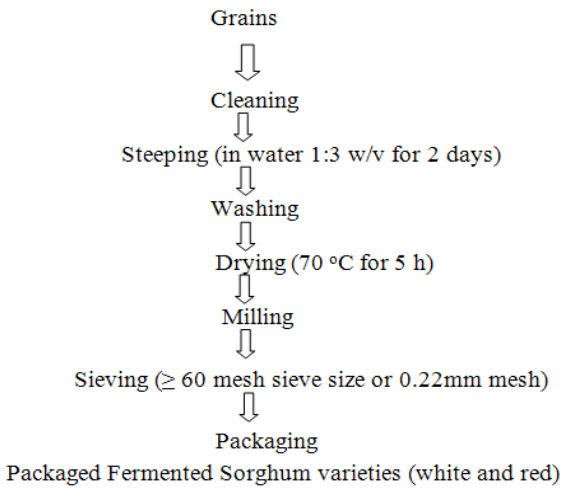

Figure 1. (Flow chart): Production of fermented Sorghum varieties (white and red) flours

3.2. Spontaneous Sourdough Development

Sourdough was developed spontaneously from Sorghum (white) and (red) flours. Equal amounts ofSorghum (white) and (red) flour that is 100g Sorghum sample and tap water (100ml) were mixed together using a glass stirrer in glass jars until a soupy consistency is achieved. Fermentation was allowed to proceed at ambient temperature (29±2°C) for seven days under anaerobic condition. Equal amount of fresh aliquots (Sorghum (white) and (red) flour and tap water) was added to the developing batter in the jars continuously using a wooden spoon. This was done each day of the sourdough fermentation in order to refresh the fermentation medium [92].

3.3. Determination of pH and Temperature

Changes in pH of spontaneously developed Sorghum (white) and (red) sourdoughs were monitored with increase in days of fermentation. Twenty milliliter of spontaneously developed Sorghum (white) and (red) sourdoughs were used for the determination. The pH was determined using a pH meter after standardizing using pH buffer 4.0 and 7.0 respectively. The changes in temperature of the sourdough batters were determined from the same batch used for pH determination.

3.4. Determination of Titratable Acidity

Titratable acid produced in the sourdoughs was determined according to method described by Lonner *et al*. [93]. One (1)g of the fermenting Sorghum (white) and (red) sourdoughs was measured using an electronic digital balance into a conical flask and made up with distilled water to 9ml mark. Ten (10)ml each of the homogenized samples was titrated against 1 N NaOH using 2-3 drops of phenolphthalein indicator until endpoint is reached. The amount of NaOH used during the titration was expressed as total titratable acid produced.

3.5. Microbial Analysis

Enumeration and isolation of lactic acid bacteria and yeasts from Sourdoughs

Lactic acid bacteria and yeasts were isolated from Sourdoughsusing pour plating methods as described by Harrigan and McCance [3]. Mann Rogosa Sharpe (MRS) and potato dextrose medium were used for culturing lactic acid bacteria and yeasts respectively. Cultured MRS plates were incubated anaerobically at 30°C for 48h while that of PDA plates were incubated in an incubator at 25°C for 72h. The microbial populations of the sourdoughs were enumerated on each day of fermentation. Isolation and sub-culturing was done until pure cultures were obtained. Presumptive lactic acid bacteria and yeast cultures were further screened on the basis of cell morphological and biochemical characteristics using standard microbiological techniques as described by Harrigan and McCance [3]; Fawole and Oso [94]. Gram positive and catalase negative presumptive LAB isolates were screened for nitrate, urease, indole utilization, gas production from glucose, spore formation, growth at different temperatures (10°C, 15°C, 30°C and 45°C), growth at NaCl concentrations (2%, 4% and 6.5%) and growth at different pH. The presumptive yeast cells were screened for growth at different NaCl concentration (2%, 4% and 6.5%), growth at 37°C and utilization of urease. Cultures were further characterized using carbohydrate fermentation pattern through the Analytical Profile Index (API Kit). The dominant presumptive lactic acid bacteria were characterized using API 50 CH and API 50 CHL medium while API 20C was used for the presumptive yeast isolates. Inoculum (actively growing culture) were dispensed into wells after equal Mc Farland concentration is achieved. McFarland concentration was achieved using UV-visible spectrophotometer at 290 nm wavelength. Isolates were identified with reference to Bergey's Manual of Systematic Bacteriology [4] and apiwebTM identification software (Biomerieux).

3.6. Production of Sour Bread Using the isolated microorganisms

The isolated and identified yeasts were used to ferment dough by baking bread in order to test for their fermentative ability. Samples of the dough was prepared according to the basic method described by the samples were left in a warm place about 30°C and baked in an oven at 180°C for 8 minutes.

4. Results

Table 1 shows the temperature, pH and TTA had values ranged from 27.2°C - 28.5°C, 3. 51 - 5.12 and 0.08% - 0.38% for White Sorghum *(Sorghum vulgare)* and for Red Sorghum *(Sorghum bicolor)* 27.0°C - 28.4°C, 3.40 - 4.31, and 0.12% - 0.59% respectively.

Table 1. The physico-chemical properties of sourdough at different fermentation duration

	Temperature(°C)		pH		TTA(%)	
Fermentation Period (Day)	***White Sorghum (Sorghumvulgare)***	***Red Sorghum (Sorghumbicolor)***	***White Sorghum (Sorghumvulgare)***	***Red Sorghum (Sorghumbicolor)***	***White Sorghum (Sorghumvulgare)***	***Red Sorghum (Sorghumbicolor)***
1	27.2	27.0	5.12	4.31	0.08	0.12
2	27.6	27.3	3.88	3.66	0.14	0.21
3	28.5	28.4	3.71	3.52	0.18	0.26
4	28.2	28.1	3.63	3.48	0.23	0.33
5	28.5	28.4	3.58	3.43	0.35	0.56
6	27.9	28.0	3.51	3.4	0.38	0.59
7	27.7	27.7	3.64	3.57	0.33	0.44

Table 2. The occurrence of lactic acid bacteria in sourdough at different fermentation period.

	White Sorghum *(Sorghumvulgare)*			Red Sorghum *(Sorghumbicolor)*		
Fermentation Period (Day)	**Actual Noof Colonies**	**Dilutionfactor**	**Count (cfu/ml)**	**Actual Noof Colonies**	**Dilutionfactor**	**Count (cfu/ml)**
1	264	10^4	$2.64X10^6$	210	10^4	$2.10X10^6$
2	343	10^4	$3.34X10^6$	312	10^4	$3.12X10^6$
3	168	10^5	$1.68X10^7$	118	10^5	$1.18X10^7$
4	284	10^5	$2.84X10^7$	236	10^5	$2.36X10^7$
5	197	10^5	$1.97X10^7$	137	10^5	$1.37X10^7$
6	128	10^5	$1.28X10^7$	102	10^5	$1.02X10^7$
7	180	10^5	$1.80X10^7$	156	10^5	$1.56X10^7$

cfu/ml-colony forming unit permilliliter

Table 3.

	White Sorghum (Sorghumvulgare)			*Red Sorghum (Sorghumbicolor)*		
Fermentation Period (Day)	**Actual Noof Colonies**	**Dilutionfactor**	**Count (sfu/ml)**	**Actual Noof Colonies**	**Dilutionfactor**	**Count (sfu/ml)**
1	22	10^3	$0.22X10^5$	67	10^3	$0.67X10^5$
2	111	10^3	$1.11X10^5$	218	10^3	$2.18X10^5$
3	284	10^3	$2.84X10^5$	411	10^3	$4.11X10^5$
4	470	10^3	$4.7X10^5$	136	10^4	$1.36X10^6$
5	164	10^4	$1.64X10^6$	174	10^4	$1.74X10^6$
6	148	10^4	$1.48X10^6$	262	10^4	$2.62X10^6$
7	163	10^4	$1.63X10^6$	274	10^4	$2.74X10^6$

Sfu/ml-Spore forming unit permillimter.

Table 2 shows the lactic acid bacteria load of the white and Red Sorghum *(Sorghum bicolor)*ranged between 2.64 x 10^6cfu/ml - 2.48 x 10^7cfu/ml and 2.10 x 10^6cfu/ml - 2.36 x 10^7cfu/ml respectively.

Table 3 shows the occurrence of yeast in spontaneously fermented sorghum sourdough. The occurrence of yeast in white and Red Sorghum *(Sorghum bicolor)* ranged between 0.22 x 10^5sfu/ml - 1.63 x 10^6sfu/ml and 0.67 x 10^5sfu/ml - 2.74 x 10^5sfu/ml respectively.

The occurrence of lactic acid bacteria in spontaneously fermented White *(Sorghum vulgare)* and Red Sorghum *(Sorghum bicolor)* shows the properties of colonies on M R S agar. All colonies are positive to gram reaction, short, long and circular in shape while some are irregular and short clusters in shape. The colony surface varies from smooth, wrinkled and white. All colonies are negative to catalase, and indole, produced glucose and had ability to grow at 45°C. The probable organisms are *Lactobacillus pentosus, Lactobacillus plantarum, Lactobacillus ailmentaruis, and Micrococcus varians.*

Properties of colonies of colonies on yeast extract agar also revealed that all colonies are oval, ellipsoidal and circular in shape while the colonies are smooth on surface. All colonies are catalase negative, and grow at 2 - 6.5% NaCl and 37°C. Probable organisms identified are *Saccharomyces cerevisiae* and *Candida milleri.*

5. Discussion

The spontaneously fermentative lactic acid bacteria and yeast populating the fermenting sorghum sourdough observed in this study increased rapidly as the fermentation time increased. The mutual or synergistic relationship between the duo confirmed and justified Wood (2014) report in fermenting food matrix. Wood and Hodge (1995), describe the co-existence of lactic acid bacteria and yeast in food processing as very crucial. Higher number of the LAB than yeast observed during the fermentation is an indication of LAB producing acids to dominate the fermentation medium. This align with the report of Ottogalli *et al* [95]. The symbiotic association between the spontaneous fermenting LAB and yeast is confirmed proto cooperation, which is common in many fermenting food; the yeast provides vitamins that enhances the LAB growth and in return the LABs producing acids for yeast growth [96]. The predominating LAB isolated in this study during spontaneous fermentation were homo-fermenters, with L. pentosus having larger population and few numbers of heterofermenters. This support the report made by Mbata *et al* [97], who observed both homofermentative and heterofermentative lactic acid bacteria at the end of fermention of maize flour fortified with Bambara nuts.

Among the highest yeast dominating the sorghum sourdough under spontaneous fermentation was *S. cerevisiae*. This is in agreement with Ottgalli *et al* (1996), who reported that *S. cerevisiae* is one of the most frequently yeast found in African fermented foods and beverages. Starter culture in food processing can be pure culture or mixed culture. Starter culture was used in this study in order to have a defined outcome by isolating yeast and LAB in pure form and combinations. *L. pentosus* with the highest frequency of occurrence among the LAB and S. cerevisiae of the yeast were selected based on their dominance and reported leavening ability [98].

A significant decrease in pH were observed in the spontaneously fermented sorghum sourdough from 5.12 - 3.64 (white sorghum) and 4.31 - 3.57 (red sorghum) this is as a result of the LABs producing acids in order to dominate the medium. A significant difference was observed in the pH of the white and red sorghum sourdoughs on the 7th day of fermentation indicating an increase as the temperature decreased.

The acidity level of sourdough is the main factor that determines the flavour and inhibitory activity of LAB against rope formation and other spoilage factors [36]. The temperature of the spontaneously developed white sorghum and red sorghum sourdoughs increased with increasing days of fermentation. This might be due to increased rate of reaction in the sourdough as metabolites production increased. However on the 6th and 7th day the temperature reduced.

Titratable acidities increased significantly with increasing days of fermentation. Titratable acidity ranged from 0.08 to 0.38 and 0.12 to 0.59 in spontaneously developed White sorghum and Red sorghum sourdoughs. The dominant metabolite of lactic fermentation is lactic acid although acetic acid also contributes to acidification Acetic acid is important for strong aroma, fungicidal and antimicrobial effect in fermented products while lactic acid influences product texture [36]. The microbial population of bacteria and fungi of spontaneously developed white and red sorghum sourdoughs are mesophilic (growing within 27 - 28°C). The mesophilic bacterial count increased with increasing fermentation days. The bacteria count of white and red sorghum sourdough ranged from 2.64X106 - 1.80X107 and 2.10X106 - 1.57X107; the yeast count ranged from 0.22 x 10 5 - 1.63 x 10 6 Cfu/ml and 0.67 x10 5 cfu/ml - 2.74

x10 6 cfu/ml respectively. The bacterial and the yeast count developed in the two sorghum sourdough increased with increasing days of fermentation. The bacteria involved in sourdough fermentations are mainly mesophilic [9].

6. Conclusion

The microbiological and physicochemical analysis of sorghum sourdough fermented spontaneously showed a synergistic relationship of lactic acid bacterial and yeast growing in it. The titratable acidity, pH, and temperature of the fermenting sorghum sourdough increased with increase days of fermentation with higher values observed in sorghum vulgare. Furthermore, homolatic fermenters (lactobacillus pentosus) and heterofermenter (Lactobacillus plantarum) predominated in the spontaneous fermentation of the sourdough while yeast (S. cerevisiae, candida milleri) are dominant. The use of such homo-fermentative lactic acid bacteria as starter culture was able to improve the shelf life of the sour bread as the acid produce by the LABs lowers the pH, hence bringing about inhibitory factors to spoilage and enhancement of sourdough flavour.

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