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Lactic Acid Bacteria Composition of Type II Sourdough Produced in Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of the study was to determine the lactic acid bacteria composition of type II sourdough produced in Nigerian from different brands of wheat flour.
Study Design: Sourdoughs were produced by spontaneous fermentation at 31℃ and 40℃ for five days from three popular brands of Nigerian wheat flour. Lactic acid bacteria were isolated and identified from the sourdoughs using API 50 CH.
Place and Duration of Study: The work was carried out at the Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria between April 2014 to July, 2014.
Methodology: Doughs were prepared from three popular brands of wheat flour and allowed to ferment spontaneously at 31℃ and 40℃ for five days. Lactic acid bacteria cou nts, fungal counts and aerobic plate counts were carried out. The lactic acid bacteria were identified using the API 50 CH. Data obtained were subjected to statistical analysis using ANOVA. Significant differences among samples were evaluated by Duncan multiple – range test.
Results: The results obtained showed that the mean lactic acid bacteria counts were 6.462±0.74, 6.471±0.62 and 6.826±0.68 log CFU/g after five days of fermentation at 31℃ while the counts were 6.878±0.99, 6.728±0.95 and 7.051±1.04 log CFU/g after five days of fermentation at 40℃.

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Lactobacillus plantarum (34%), *Lactobacillus brevis* (29%), *Lactobacillus pentosus* (18%), *Pediococcus pentocaseus* (9%), *Lactobacillus buchneri* (3%), *Lactobacillus collinoides* (3%), *Lactobacillus fermentum* (3%) and *Pediococcus acidilactici* (3%) were isolated and identified.
Conclusion: There is no known published data on lactic acid bacteria composition of type II sourdough produced in Nigeria; findings of this work will assist to bridge this information gap. Knowledge of the lactic acid bacteria of the sourdough will help in the development of starter cultures for improvement of the nutritional and organoleptic qualities and shelflife of a wide variety of Nigerian baked products.

Keywords: Lactic acid bacteria; Nigerian type II sourdough; Isolation; Identification; fermentation.

1. INTRODUCTION

The spontaneous fermentation of dough leads to the development of sourdough. Lactic acid bacteria (LAB) and yeasts play a key role in sourdough fermentation processes [1,2]. Sourdoughs are grouped into three types, on the basis of technology; type I, type II and type III sourdough. Type I sourdough are produced traditionally and need continous, daily refreshments to keep the microflora active. Type II sourdough are semi-fluid and produced by long fermentation periods (2 to 5 days) at fermentation temperature of greater than 30℃ [3-5]. Sourdough is an intermediate product in bread production and contains a microbiota comprising of lactic acid bacteria and yeasts. Microbiological studies have revealed that 43 species of lactic acid bacteria, mostly species of the genus *Lactobacillus*, and more than 23 species of yeast occur in this ecological niche.

The metabolic activity of these microorganisms leads to an acidification of the dough and the development of aroma precursors and is therefore of major importance for the quality of the final product [6].

The use of sourdough in wheat breads has gained popularity as a mean to improve the quality and flavour of wheat breads. A vast array of traditional products relies on the use of sourdough fermentation to yield baked goods with peculiar quality characteristics. Some examples include the well-known Italian products associated with *Christmas*, *Panettone*, which originated from Milan. San Francisco sourdough, French breads, and soda crackers are other examples of wheat products that rely on the process of souring [5]. Spontaneous sourdough fermentation begins with aerobic fermentation immediately upon mixing flour and water. Once oxygen is depleted, anaerobic fermentation begins with the growth of LAB and yeasts. The production of acid by LAB enables their rapid growth when the pH of the sourdough has dropped to a level that is too low for other microorganisms to grow. The LAB become the dominant microflora in the sourdough and are therefore responsible for the final stages of sourdough development [7]

Lactic acid bacteria are food-grade organisms that possess GRAS status and are known to contribute positively to the taste, aroma and preservation of the final products [8].

Typical homofermentative lactic acid bacteria in spontaneous sourdough are *Lactobacillus casei*, *L. delbruecii*, *L. farciminis*, *L. plantarum*, *L. mindensis*, *L. crospactus*, *L. johnsonii*, *Pediococcus pentosaceus* etc [9-11]. While typical heterofermentative lactic acid bacteria in spontaneous sourdoughs include *L. brevis*, *L. buchneri*, *L. fermentum*, *L. frumenti*, *L. hilgardii*, *L. panis*, *L.pontis*, *L. sanfriciscencis*, *L. spicheri*, *L. zymae*, *L. plantarumI, Lactobacillus paralimentarius* among others [9,11,12]. Typical sourdough yeast species are *Candida humilis, Kazachstania exigua* and *Saccharomyces cerevisiae* [12].

At present bakers in Nigeria are yet to adopt the sourdough technology in baking due to lack of awareness. The three flour types used in this work; Golden penny flour, Dangote flour and Supreme flour differ with respect to their proximate values and are all used in the baking of a wide range of bakery products in Nigeria such as bread, doughnuts, cakes, ‘chin-chin’, egg rolls and ‘puff-puff’.

2. MATERIALS AND METHODS

2.1 Sample Collection

Three (3) popular brands of wheat flour in Nigeria; Golden penny flour, Dangote flour and Supreme flour were purchased from Samaru market in Kaduna state, Nigeria and used in this study.

2.2 Preparation of Dough

Dough samples were prepared from the three (3) popular brands of flour by mixing 1000 g of each flour with 1000 ml of sterile tap water manually under aseptic conditions [13,4]. Six dough samples were prepared from each flour type; giving a total of 18 dough samples.

2.3 Fermentation of Dough to Yield Sourdough

The dough samples above were fermented spontaneously in triplicates at 31℃ and at 40℃ for 120 hours (five days).

2.4 Microbial Counts during Dough Fermentation

Total lactic acid bacteria count (TLABC), aerobic plate count (APC) and fungal counts were carried-out on de Man Rogosa Sharpes agar (MRS), Plate count agar (PCA) and Potato dextrose agar (PDA) respectively at 0 hr, 24 hours, 48 hours, 96 hours and 120 hours of incubation. MRS plates incorporated with cycloheximide were incubated anaerobically at 30℃ for 72 hrs, PCA plates were incubated at 35℃ for 24 hrs and PDA plates were incubated at room temperature for 72 hrs.

2.5 Identification of the Lactic Acid Bacteria Isolates

The lactic acid bacteria isolates were identified based on their Gram reaction, catalase test and carbohydrate fermentation profiles using API 50 CH system (Biomerieux, Marcy l'Etoile France) [14-16].

2.6 Statistical Analysis

The statistical analyses of the measured parameters were made using analysis of variance (ANOVA). Significant differences among samples were evaluated by Duncan multiple – range test ($P \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1 Microbial Counts during Dough Fermentation

The results of the microbial counts showed that the lactic acid bacteria are the most dominant population in all the sourdoughs. There was a progressive increase in the lactic acid bacteria counts while there was a progressive decrease in the aerobic plate counts and fungal counts with increase in the length of fermentation of all the sourdoughs. At room temperature fermentation; Supreme flour sourdough had the highest total mean lactic acid bacteria count (LABC) of 6.826±0.682 log CFU/g followed by Dangote flour sourdough with a total mean LABC of 6.471±0.620 log CFU/g and Golden penny flour sourdough with a total mean LABC of 6.462±0.620 log CFU/g. There was a significant difference in the total mean lactic acid bacteria count of Supreme flour sourdough and that of both Dangote flour and Golden penny flour ($P \leq 0.05$) but there was no significant difference in the mean lactic acid bacteria counts between Dangote flour and Golden penny flour sourdoughs ($P \geq 0.05$). The total mean aerobic fungal counts were 6.551±0.567 log CFU/g, 6.451±0.585 log CFU/g and 6.305±0494 log CFU/g for Supreme flour, Dangote flour and Golden penny sourdoughs respectively while the total mean aerobic plate counts were 6.460±0.640, 6.414±0.590 and 6.186±0.643 log CFU/g for Dangote flour, Supreme flour and Golden penny sourdoughs respectively.

For sourdoughs produced at 40℃, Supreme flour sourdough also had the highest total mean lactic acid bacteria count (LABC) of 7.051±1.035 log CFU/g followed by Golden penny flour sourdough with a total mean LABC of 6.878±0.989 log CFU/g and Dangote flour sourdough with a total mean LABC of 6.728±0.948. There was a significant difference in the total mean lactic acid bacteria count of Supreme flour sourdough and that of both Dangote flour and Golden penny flour ($P \leq 0.05$) but there was no significant difference in the mean lactic acid bacteria counts between Dangote flour and Golden penny flour sourdoughs ($P \geq 0.05$). The total mean fungal counts were 4.925±0.644 log CFU/g, 4.922±0.687 log CFU/g and 4.900±0.641 log CFU/g for Supreme flour, Dangote flour and Golden penny sourdoughs respectively. The total mean aerobic plate counts were 4.968±0.881 log CFU/g, 4.937±0.835 log CFU/g and 4.794±0.584 log CFU/g for Golden penny, Dangote flour and Supreme flour sourdoughs respectively.

Comparing the microbial counts of the sourdough produce at room temperature and at 40℃, there was a significant difference in the total mean lactic acid bacteria count, fungal count and aerobic plate counts between the room temperature sourdoughs and the 40℃

sourdoughs ($P \leq 0.05$); the microbial counts of the 40℃ sourdoughs being significantly higher in all cases (Table 1).

The higher counts of lactic acid bacteria compared to fungal and aerobic plate counts agrees with the findings of other workers in this area; Luangsakul et al. [17] reported lactic acid bacteria counts of log 4.255 to 8.000 colonies/g sample, and low fungal counts of log 1.000 to 2.361 colonies/g sample. Hamtel et al. [18] reported higher lactic acid bacteria count of log 5.477 to 8.477 compared to fungal and aerobic plate counts ranging from log 4.699 to 7.602 and log 2.000 to 8.707 respectively. [Gobbetti et al. [19]; Hertel et al. [20]; Scheirlinck et al. [21]; Iacumin et al. [22]; Zhang [23]; Annabelle et al. [24] have all reported higher lactic acid bacteria counts than fungal counts. Saeed [4] reported lactic acid bacteria as the dominant bacteria in sourdough with counts in the range of log 4.795 CFU/g to 7.840 CFU/g this also agrees with the findings of this work with respect to lactic acid bacteria count for Dangote flour at room temperature. The fungal count reported by Saeed [4] also agrees with the findings of this work while the aerobic plate count was much higher than that obtained in this work. A number of other researchers in this field have reported lactic acid bacteria counts that tallies with the findings of this work [25-28]. The progressive increase in lactic acid bacteria counts and decrease in fungal and aerobic plate counts have also been reported by [19]. Savic et al. [29] reported aerobic plate counts that are similar to the findings of this work. The LABC and APC reported by Gatto and Torriani, [30] and Korakli et al. [31] differ from the findings of this work.

Several factors account for the dominance of lactic acid bacteria during dough fermentation. First, their carbohydrate metabolism is highly adapted to the main energy sources (maltose and fructose) in dough [32]. Second, sourdough lactic acid bacteria possess several stress response mechanisms to overcome acid, high/low temperatures, high osmolarity/dehydration, oxidation, and starvation. Important mechanisms to resist acid conditions include intracellular proton removal through H^+-ATPase activity, alterations in cell membrane composition, and amino acid conversions [33,34]. Third, production of lactic and acetic acids by the LAB which enhances their rapid growth when the pH value has dropped too low for other microorganisms to grow [29,35]. Fourth, the production of antimicrobial compounds, both organic acids (lactate, acetate, and others) and proteinaceous compounds (for instance, bacteriocins), improves their competitiveness and may contribute to their stable persistence in sourdough fermentations [36-38].

3.2 Lactic acid Bacteria Isolated from the Type II sourdough

A total of seventy – nine (79) isolates of lactic acid bacteria comprising of six (6) species of the genera *Lactobacillus* sp. and two species of the genera *Pediococcus* sp. were obtained. Forty-two (42) isolates were obtained from dough fermented at 40^0C while thirty-seven (37) isolates were obtained from dough fermented at 31℃. *Lactobacillus plantarum* was the most predominant isolate in both fermented dough (sourdough) with an overall frequency of occurrence of 27 representing 34.17%, followed by *Lactobacillus brevis* with an overall frequency of occurrence of 23 representing 29.11%. *Lactobacillus pentoses, Lactobacillus buchneri, Lactobacillus collinoides* and *Lactobacillus fermentum* all have overall frequency of occurrence of 2 (2.53%). *Pediococcus pentosaceus* and *Pediococcus acidilactici* have overall frequency of occurrence of 7 (8.86%) and 2 (2.53%) respectively. *Lactobacillus buchneri* was isolated from only Golden penny flour sourdough while both *Lactobacillus collinoides* and *Lactobacillus fermentum* were isolated from only supreme flour sourdough (Table 2).

Several workers in this field have reported *Lactobacillus* species as the most dominant genera of LAB in sourdough [39,40,20,41,42, 32,31,43,44,17,8,45,46,24] this is in agreement with the findings of this work. The occurrence of *Lactobacillus plantarum* as the dominant isolate in this study agrees with the findings of other workers [47-53]. In a study of four different types of sourdough obtained from bakeries in Northern Italy, *Lactobacillus plantarum* was found to be most dominant isolate in two of the four sourdoughs [22]. *Lactobacillus plantarum* is a ubiquitous species, found in several food ecosystems, including sourdoughs [54]. According to De Vuyst et al. [51] *Lactobacillus plantarum* is more acid tolerant and often dominate fermentation processes of vegetables and cereals in particular because of its ability to transport and metabolize different carbohydrates. However, this finding differs from that of other workers [39,55,56,43,57,58,59,46,54] who reported *Lactobacillus sanfranciscensis* as the dominant isolate in wheat flour sourdough.

Table 1. Microbial counts during spontaneous fermentation of the doughs

Length of fermentation (hrs)	Golden penny flour			Dangote flour			Supreme flour		
	LABC (Log CFU/g)	FC (Log CFU/g)	APC (Log CFU/g)	LABC (Log CFU/g)	FC (Log CFU/g)	APC (Log CFU/g)	LABC (Log CFU/g)	FC (Log CFU/g)	APC (Log CFU/g)
31℃									
0	5.524±0.08^{a}	6.701± 0.05^{a}	7.061±0.02^{a}	4.930±0.56^{a}	6.557±0.39^{a}	7.020±0.08^{a}	5.860±0.07^{a}	6.876±0.06^{a}	6.977±0.06^{a}
24	5.729±0.02^{a}	7.095±0.06^{a}	7.071±0.04^{a}	5.760±0.05^{b}	6.851±0.17^{b}	6.881±0.11^{a}	5.877±0.13^{a}	6.908±0.09^{a}	6.890±0.02^{a}
48	5.950±0.16^{a}	6.032±0.04^{b}	6.001±0.04^{b}	6.391±0.24^{c}	6.951±0.03^{b}	6.957±0.07^{a}	7.188±0.03^{b}	6.977±0.02^{a}	6.802±0.36^{a}
72	7.095±0.07^{b}	6.438±0.43^{c}	5.895±0.76^{b}	7.143±0.05^{d}	7.346±0.41^{c}	6.805±0.09^{a}	7.291±0.03^{b}	7.037±0.08^{a}	6.945±0.05^{a}
96	7.168±0.06^{b}	5.924±0.10^{b}	5.590±0.12^{c}	7.250±0.03^{d}	5.957±0.24^{d}	5.611±0.11^{b}	7.287±0.08^{b}	5.827±0.12^{b}	5.787±0.04^{b}
120	7.303 ±0.05^{b}	5.637±0.15^{d}	5.508±0.21^{c}	7.354±0.07^{d}	5.645±0.14^{e}	5.485±0.03^{c}	7.453±0.02^{c}	5.683±0.06^{c}	5.483±0.05^{c}
Total Mean	6.462±0.74$_{h}^{c}$	6.305±0.49$_{i}^{c}$	6.186±0.64$_{j}^{b}$	6.471±0.62$_{h}^{c}$	6.451±0.59$_{h}^{a}$	6.460±0.65$_{h}^{f}$	6.826±0.68$_{l}^{e}$	6.551±0.57$_{k}^{g}$	6.414±0.59$_{h}^{d}$
40℃									
0	5.770±0.02^{a}	5.489±0.04^{d}	5.778±0.07^{c}	5.775±0.08^{b}	5.615±0.19^{e}	5.755±0.05^{b}	5.954±0.02^{a}	5.725±0.10^{b}	5.774±0.03^{e}
24	6.002±0.06^{a}	5.601±0.12^{d}	5.811±0.04^{c}	5.841±0.07^{b}	5.654±0.11^{e}	5.804±0.12^{b}	5.956±0.05^{a}	5.514±0.06^{d}	5.512±0.16^{c}
48	6.071±0.02^{a}	5.528±0.05^{d}	5.687±0.05^{c}	5.886±0.03^{b}	5.548±0.08^{e}	5.625±0.11^{b}	6.339±0.05^{e}	5.432±0.07^{d}	5.451±0.03^{c}
72	7.177±0.11^{b}	4.300±0.16^{e}	4.551±0.18^{d}	7.059±0.09^{d}	4.143±0.46^{f}	4.548±0.06^{c}	7.331±0.06^{b}	4.461±0.03^{e}	4.525±0.13^{f}
96	8.024±0.07^{d}	4.279±0.12^{e}	4.577±0.12^{d}	7.665±0.04^{e}	4.324±0.07^{f}	4.367±0.08^{c}	8.290±0.07^{d}	4.254±0.23^{f}	4.217±0.08^{g}
120	8.225±0.01^{d}	4.201±0.14^{e}	3.405±0.16^{e}	8.144±0.05^{f}	4.246±0.09^{f}	3.525±0.04^{d}	8.433±0.02^{d}	4.165±0.09^{f}	3.286±0.08^{h}
Total Mean	6.878±0.99$_{a}^{c}$	4.900±0.64$_{c}^{f}$	4.968±0.88$_{c}^{f}$	6.728±0.95$_{a}^{g}$	4.922±0.79$_{c}^{g}$	4.937±0.85$_{c}^{e}$	7.051±1.04$_{i}^{b}$	4.925±0.64$_{c}^{h}$	4.794±0.58$_{k}^{i}$

All values are means of triplicate experiments. Values with the same superscript in the same column are not significantly different (P ≥ 0.05).
Values with the same subscript in the same row are not significantly different (P ≥ 0.05). Key: LABC= Lactic acid bacteria count; FC= Fungal counts; APC= Aerobic plate count

Table 2. Frequency of occurrence of lactic acid bacteria isolates

Flour type	No. of samples analysed	*Lactobacillus plantarum*	*Lactobacillus brevis*	*Lactobacillus pentosus*	*Lactobacillus buchneri*	*Lactobacillus collinoides*	*Lactobacillus fermentum*	*Pediococcus pentocaseus*	*Pediococcus acidilactici*	Total
31ºC (%)										
Golden penny flour	36	3 (8.33)	3 (8.33)	2 (5.56)	2 (5.56)	-	-	-	-	10 (12.66)
Dangote flour	36	5 (13.88)	4 (11.11)	3 (8.33)	-	-	-	1 (2.78)	-	13 (16.46)
Supreme flour	36	4 (11.11)	4 (11.11)	2 (5.56)	-	2 (5.56)	-	2 (5.56)	-	14 (17.72)
Total	108	12 (32.43)	11(29.73)	7 (18.91)	2 (5.40)	2 (5.40)	0 (0.00)	3 (8.10)	0 (0.00)	37 (46.84)
40ºC										
Golden penny flour	36	5 (13.88)	4 (11.11)	1 (2.78)	-	-	-	1 (2.78)	1 (2.78)	12 (15.19)
Dangote flour	36	4 (11.11)	3 (8.33)	3 (8.33)	-	-	-	2 (5.56)	-	12 (15.19)
Supreme flour	36	6 (16.67)	5 (13.88)	3 (8.33)	-	-	2 (5.56)	1 (2.78)	1 (2.78)	18 (22.78)
Total	108	12 (32.43)	11(29.73)	7 (18.91)	2 (5.40)	2 (5.40)	0 (0.00)	3 (8.10)	0 (0.00)	42 (53.16)
Overall Total	216	27 (34.17)	23 (29.11)	14 (17.72)	2 (2.53)	2 (2.53)	2 (2.53)	7 (8.86)	2 (2.53)	79

The other isolates obtained in this work have also been reported by a number of researchers in this field; *Lactobacillus brevis* [39,60,30,9,61, 10,43,62,63,11,59,22,53], *Lactobacillus pentosus* [60,64], *Lactobacillus buchneri* [60,9,49,32,45] *Lactobacillus fermentum* [39,55,9,43,62,63,58, 65,66], *Pediococcus pentosaceus* [60,67,10,9, 61,43,68,22,69,59], *Pediococcus acidilactici* [67,61,43,70]. There appears to be no known published data on the isolation of *Lactobacillus collinoides* from sourdough. *Lactobacillus collinoides* is reported to be commonly found in fermenting apple juice [71]. This could be the first report of the isolation of this organism in sourdough.

Although the lactic acid bacteria composition of sourdough produced in different parts of the world have been documented, there is no known published information on the lactic acid bacteria composition of type II sourdough produced in Nigerian.

4. CONCLUSION

This study have provided information on the lactic acid bacteria composition of type II sourdough produced in Nigeria from popular wheat flours an information that was lacking. Knowledge of the lactic acid bacteria composition of Nigerian sourdough is relevant for the development of starter cultures and also for the application of sourdough technology in the production of a wide variety of Nigerian baked products.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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