

LACTIC ACID BACTERIA COMPOSITION OF NIGERIAN TYPE II SOURDOUGH

ABSTRACT

Aim: The study was aimed at determining the lactic acid bacteria composition of Nigerian type II sourdough produced from different brands of wheat flour.

Study Design: Sourdoughs were produced by spontaneous fermentation at 31°C and 40°C 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 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°C and 40°C for five days. Lactic acid bacteria counts, 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 show that the mean lactic acid bacteria counts are 6.462±0.74, 6.471±0.62 and 6.826±0.68 log CFU/g after five days of fermentation at 31°C while the counts are 6.878±0.99, 6.728±0.95 and 7.051±1.04 log CFU/g after five days of fermentation at 40°C. *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 published information on lactic acid bacteria composition of Nigerian type II sourdough; findings of this work have therefore bridged 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 continuous, 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°C [3, 4,5]. 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. delbrueckii*, *L. farciminius*, *L. plantarum*, *L. mindensis*, *L. crospectus*, *L. johnsonii*, *Pediococcus pentosaceus* etc [9,10,11]. 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. sanfriscensis*, *L. spicheri*, *L. zymae*, *L. plantarum* among others [9,11].

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 1000g of the flour with 1000ml of sterile tap water manually under aseptic conditions [12,4]. 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 room temperature (mean 31°C) and at 40°C 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 0hr, 24hours, 48hours, 96hours and 120hours of incubation. MRS plates were incubated anaerobically at 30°C for 72hrs, PCA plates were incubated at 35°C for 24hrs and PDA plates were incubated at room temperature for 72hrs.

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 (Biomérieux, Marcy l'Etoile France) [13,14,15].

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 show 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 is 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 is 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 are 6.551 ± 0.567 log CFU/g, 6.451 ± 0.585 log CFU/g and 6.305 ± 0.494 log CFU/g for Supreme flour, Dangote flour and Golden penny sourdoughs respectively while the total mean aerobic plate counts are 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°C, Supreme flour sourdough also have 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 is 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

is 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 are 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 are 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°C , there is 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°C sourdoughs ($P \leq 0.05$); the microbial counts of the 40°C 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; [16] 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. [17] 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. [18,19,20,21,22,23] have all reported higher lactic acid bacteria counts than fungal counts. [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 [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 reported lactic acid bacteria counts that tallies with the findings of this work [24,25,26,22,27]. The progressive increase in lactic acid bacteria counts and decrease in fungal and aerobic plate counts have also been reported by [19]. [28] reported aerobic plate counts that are similar to the findings of this work. The LAB and APC reported by [29] and [30] 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 [31]. 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 [32,33]. 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 [28,34]. 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 [35,36,37].

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°C while thirty-seven (37) isolates were obtained from dough fermented at room temperature. *Lactobacillus plantarum* is 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 pentosus*, *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 1).

Several workers in this field reported *Lactobacillus* species as the most dominant genera of LAB in sourdough [38,39,19,40,41,31,30,42,43,16,8,44,45,23] 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 [46,47,48,49,50,51,52]. 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 [21]. *Lactobacillus plantarum* is a ubiquitous species, found in several food ecosystems, including sourdoughs [53]. According to [50] *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

174 from that of other workers [38,54,55,42,56,57,58,45,53] who reported *Lactobacillus sanfranciscensis*
 175 as the dominant isolate in wheat flour sourdough.
 176 The other isolates obtained in this work have also been reported by a number of researchers in this
 177 field; *Lactobacillus brevis* [38,59,29,9,60,10,42,61,62,11,58,21,52], *Lactobacillus pentosus* [59,63],
 178 *Lactobacillus buchneri* [59,9,48,31,44] *Lactobacillus fermentum* [38,54,9,42,61,62,57,64,65],
 179 *Pediococcus pentosaceus* [59,66,10,9,60,42,67,21,68,58], *Pediococcus acidilactici* [66,60,42,69].
 180 There is no published information on the isolation of *Lactobacillus collinoides* from sourdough.
 181 *Lactobacillus collinoides* is reported to be commonly found in fermenting apple juice [70]. This is the
 182 first report of the isolation of this organism in sourdough.
 183 Although the lactic acid bacteria composition of sourdough produced in different parts of the world
 184 have been documented, there is no published information on the lactic acid bacteria composition of
 185 Nigerian sourdough.

Table 1: Microbial counts during spontaneous fermentation of the doughs

Length of fermentation (hrs)	Room Temperature (31°C)								
	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)
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	6.305±0.49 ⁱ	6.186±0.64 ^j	6.471±0.62 ^h	6.451±0.59 ^a	6.460±0.65 ⁱ	6.826±0.68 ^e	6.551±0.57 ^g	6.414±0.59 ^d
40°C									
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	4.900±0.64 ^c	4.968±0.88 ^f	6.728±0.95 ^g	4.922±0.79 ^g	4.937±0.85 ^e	7.051±1.04 ^b	4.925±0.64 ^h	4.794±0.58 ^k

All values are means of triplicate experiments. Values with the same superscript in the same column are not significantly different ($P \geq 0.05$). Values with the same subscript in the same row are not significantly different ($P \geq 0.05$).

Table 4.3: Frequency of occurrence of lactic acid bacteria isolates

Flour type	No. of samples analysed	Room temperature (%)								Total
		<i>Lactobacillus plantarum</i>	<i>Lactobacillus brevis</i>	<i>Lactobacillus pentosus</i>	<i>Lactobacillus buchneri</i>	<i>Lactobacillus collinoides</i>	<i>Lactobacillus fermentum</i>	<i>Pediococcus pentocaseus</i>	<i>Pediococcus acidilactici</i>	
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)
40°C										
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

4. Conclusion

This study have provided information on the lactic acid bacteria composition of Nigerian type II sourdough produced from 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 the application of the sourdough technology in the production of a wide variety of Nigerian baked products.

References

1. Sadeghi A, Shahidi F, Mortazavi A, Mahallati MN. Evaluation of *Lactobacillus sanfransicencis* (ATCC 14917) and *Lactobacillus plantarum* (ATCC 43332) effects on Iranian barbari bread shelf life. African J. Biotechnol. 2008;7:18:3346–3351.
2. Bolourin S, Khodaparast MH. Effect of lactic acid fermentation (*Lactobacillus plantarum*) on physicochemical, flavour, stalling and crust properties of semi volume bread (Baguette). World App.Sci. J. 2010;2: 5:490–498.
3. Ghulam M. Effect of wheat flour extraction rates on physico-chemical characteristics of sourdough flat bread. Unpublished Ph.D thesis, Department of Food Technology, University of Agriculture, Pakistan. 2009.
4. Saeed M. Isolation, characterization and utilization of starter culture for the production of sourdough bread. Unpublished Ph.D thesis, Department of Food Technology, University of Agriculture, Faisalabad. 2009.
5. Chavan RS, Chavan SR. Sourdough technology – A traditional way for wholesome foods: A review. Com.Rev. Fd Sci. & Fd Safety. 2011; 10:170–183.
6. Hertel C, Bello FD, Walter J, Roos S, Jonsson H. Inducible gene expression in *Lactobacillus reuteri* LTH5531 during type II sourdough fermentation. App. Env. Microbiol. 2005;71:10:5873 – 5878.
7. Zugic-Petrovic TD, Natasa MJ, Dragisa SS. The evolution of lactic acid bacteria community during the development of mature sourdough. APTEFF. 2009;40:115–121.
8. Saeed M, Anjum FM, Zahoor T, Nawaz H, Rehman S. Isolation and characterization of starter culture from spontaneous from spontaneous fermentation of sourdough. Int. J. of Agr. & Biol. 2009;11:329-332.
9. Korakli M, Ehrmann MA, Dousset X, Ganzle MG, Vogel RF. *Lactobacillus hammesii* sp. nov., isolated from French sourdough. Int. J.Sys.Evol.Microbiol. 2004;55:2:763-767.
10. Gerez CL, Rollan GC, de Valdez GF. Gluten breakdown by lactobacilli and pediococci strains isolated from sourdough. Lett. App.Microbiol. 2006; 42:456–464.
11. Corsetti A, Settanni L. Lactobacilli in sourdough fermentation. Fd Research Int. 2007;40: 539–558.
12. Teiking M. Production of prebiotic exopolysaccharides by lactobacilli. Unpublished Ph.D thesis, University of Munchen, Germany. 2005:22-26.
13. El Gaali El, Hamza AA, Mahdi AA. Use of the RAPD-PCR finger printing and API system for clustering lactic acid bacteria isolated from traditional Sudanese sour milk (Roab). African J. Biotechnol. 2009;8:15:3399–3404.
14. Vural HC, Ozgun D. Identification of *Lcatobacillus* strains isolated from faecal specimens of babies and human milk colostrums by API 50 system. J. Med.Gen. Genomics. 2011;3:3:46 – 49.

15. Baratto CM, Gelinski JLN, Debastiani J, Dalbó MA. Molecular and phenotypic characterization of *Lactobacillus curvatus* isolated from handmade Brazilian salami. *Afr. J. Biotechnol.* 2012; 11:54:11724-11731.
16. Luangsakul N, Keeratipibul S, Jindamorakot S, Tanasupawat S. Lactic acid bacteria and yeasts isolated from the starter doughs for Chinese steamed buns in Thailand. *Fd Sci. Technol.* 2009; 42:8:1404-1412.
17. Hamtel M, Sana M, Mejri M. Microflora distribution and species of Tunisian fermented doughs for bakery industry. *Afri. J. Biotechnol.* 2007; 6:18:2122-2129.
18. Gobbetti M, Corsetti A, Lavermicocca P, Morea M, Tosti N. Phenotypic molecular identification and clustering of lactic acid bacteria and yeasts from wheat (species *Triticum durum* and *Triticum aestivum*) sourdoughs of Southern Italy. *Int. J. Microbiol.* 2001;64:1-2:95-104.
19. Hertel CB, Meroth J, Walter MJ, Brandt WP. Hammes, Monitoring the bacterial population dynamics in sourdough fermentation process by using PCR-denaturing gradient gel electrophoresis. *App. Env. Microbiol.* 2003; 69:1:475-482.
20. Scheirlinck RV, Meulen AV, Schoor M, Vancanneyt L, De Vuyst P, Vandame G, Huyst D. Influence of geographical origin and flour type on diversity of lactic acid bacteria in traditional Belgian sourdough. *App. Env. Microbiol.* 2007;73:19:6262-6269.
21. Iacumin, L, Manzano M, Cecchini F, Comi F. Lactic acid bacteria and yeasts involved in four different sourdoughs fermentation. *Annals Microbiol.* 2009; 59:64:1-2.
22. Zhang J, Liu W, Zhihong S, Bao Q, Wang F, Yu J, Chen W, Zhang H. Diversity of lactic acid bacteria and yeasts in traditional sourdoughs collected from western region in Inner Mongolia of China. *Fd Control.* 2011;22:5:767-774.
23. Annabelle V. Mai HL, Ve'ronique R, Yann D. Description of a French natural wheat sourdough over 10 consecutive days focussing on the lactobacilli present in the microbiota. *Antonie van Leeuwenhoek.* 2011;101:2:369-377.
24. Picozzi C, Gallini S, Della T, Fera D, Foschino R. Comparison of cultural media for the enumeration of sourdough lactic acid bacteria. *Annals Microbiol.* 2005;55:4:317-320.
25. Kim WJ, Jung SW, Lee KG, Kim CW, Noh WS. Fermentation characteristics of exopolysaccharides – producing lactic acid bacteria from sourdough and assessment of the isolates for industrial potentials. *J. Microbiol. Biotechnol.* 2008;18:7:1266-1273.
26. Banu I, Vasilean I, Aprodu I. Quality evaluation of the sourdough rye breads. *Fd Technol.* 2011;35:2:94-105.
27. Aplevicz KS, Oghari PJ, Sant'Anna ES. Influence of fermentation time on characteristics of sourdough bread. *Brazilian J. Pharm.Sci.* 2013;49:2:233-238.
28. Savic D, Savic T, Skrinjar M, Jokovic N. Profile of lactic acid bacteria in rye flour and sourdough. *J. Culture Collections.* 2006; 5:1:38-45.
29. Gatto V, Torriani S. Microbial population changes during sourdough fermentation monitored by DGGE analysis of 16S and 26S rRNA gene fragments. *Ann. Microbiol.* 2004;54:1:31-42.
30. Korakli M, Valcheva R, Ferchichi MF, Ganzle MG, Vogel RF, Ivanova I, Prevost H, Onno B, Dousset X. *Lactobacillus nantensis* sp. nov., isolated from French wheat sourdough. *Inter.J. Syst. Evol. Microbiol.* 2006;56:587-591.
31. De Vuyst L, Neysens P. The sourdough microflora: biodiversity and metabolic interactions. *Trends Fd Sci. Technol.* 2005;16:43-56.

32. De Angelis M, Bini L, Pallini V, Cocconcetti PS, Gobbetti M. The acid-stress response in *Lactobacillus sanfranciscensis* CB1. *Microbiology*. 2001; 147:1863–1873.
33. Weckx S, Vrancken G, De Vuyst L, Rimaux T, Allemeersch J. Adaptation of *Lactobacillus plantarum* IMDO 130201, a wheat sourdough isolate, to growth in wheat sourdough simulation medium at different pH values through differential gene expression. *App. Env. Microbiol.* 2011; 77:10:3406–3412.
34. Gerokova P, Petrulakova Z, Sturdik E. Importance of lactobacilli for bread-baking industry. *Acta Chimica Slovaca*. 2011;4:2:118 - 135
35. Gobbetti M. The sourdough microflora: interactions of lactic acid bacteria and yeasts. *Trends Fd Sci. Technol.* 1998;9:267–274.
36. Gañzle MG, Vogel RF. Contribution of reutericyclin production to the stable persistence of *Lactobacillus reuteri* in an industrial sourdough fermentation. *Inter. J. Fd Microbiol.* 2002;80:31–45.
37. Messens W, De Vuyst L. Inhibitory substances produced by lactobacilli isolated from sourdough—a review. *Inter. J. Fd Microbiol.* 2002;72:31–43.
38. Corsetti A, Lavermicocca P, Morea M, Baruzzi M, Totsi N, Gobbetti M. Phenotypic and molecular identification and clustering of lactic acid bacteria and yeasts from wheat (species *Triticum durum* and *Triticum aestivum*) sourdoughs of Southern Italy. *Inter. J. Fd Microbiol.* 2001;64:95–104.
39. Corsetti A, De Angelis MM, Dellaglio M, Paparella A, Fox P, Settanni FL, Gobbetti M. Characterization of sourdough lactic acid bacteria based on genotypic and cellwall protein analyses. *J. App. Microbiol.* 2003;94:641–654.
40. Gobbetti M, Corsetti A, Rossi J. The sourdough microflora: interactions between lactic acid bacteria and yeasts. *App. Microbiol. Biotechnol.* 1994;41:456–460.
41. Korakli M, Ehrmann MA, Dousset X, Ganzle MG, Vogel RF. *Lactobacillus hammesii* sp. nov., isolated from French sourdough. *Inter. J. Syst. Evol. Microbiol.* 2004;55:2:763–767.
42. Rehman S, Peterson A, Piggott JR. Flavour in sourdough bread: A review. *Trends Fd Sci. Technol.* 2006;17:557–566.
43. Herve R, Valérie G, Fontagné-Faucher C. Biodiversity of lactic acid bacteria in French wheat sourdough as determined by molecular characterization using species – specific PCR. *Inter. J. Fd Microbiol.* 2009;135:1:1–2.
44. Rollan G, Gerez CI, Dallagnol AM, Torino G, Font G. Update in bread fermentation by lactic acid bacteria. *Curr. Research, Technol. Educ. Topics App. Microbiol., Microbial Biotechnol.* 2010;1168–1171.
45. De Vuyst L, Vrancken G, Rimaux T, Weckx S, Leroy F. Influence of Temperature and Backslopping Time on the Microbiota of a Type I Propagated Laboratory Wheat Sourdough Fermentation. *App. Environ. Microbiol.* 2011;77:8:2716–2726.
46. Ricciardi A, Parente E, Piraino P, Paraggio M, Romano P. Phenotypic characterization of lactic acid bacteria from sourdoughs for Altamura bread produced in Apulia (Southern Italy). *Inter. J. Microbiol.* 2005;98:1:63–72.
47. Van der Meulen R, Scheirlinck I, Van Schoor A, Huys G, Vancanneyt M, Vandamme P, De Vuyst L. Population dynamics and metabolite target analysis of lactic acid bacteria during

- laboratory fermentation of wheat and spelt sourdough. *App. Environ. Microbiol.* 2007;73:15:4741–4751.
48. Zotta T, Piranto P, Parente E, Salzano G, Ricciardi A. Characterization of lactic acid bacteria isolated from sourdoughs for cornetto, a traditional bread produced in Basilicata (Southern Italy). *World J. Microbiol. Biotechnol.* 2008;24:9:1785–1795.
49. Minervini F, Lattanzi A, Di Cagno R, Diviccaro A, Antonielli L, Cardinali G, Capelle S, De Angelis M, Gobbetti M. The lactic acid bacteria and yeast microbiota of eighteen sourdoughs used for the manufacture of traditional Italian sweet leavened baked goods. *Inter. Fd Microbiol.* 2010;5:12-16.
50. De Vuyst L, Van der Meulen R, Allermeeersch J, Huys G, Vandamme P, Van Hummelen P, Weckx S. Community dynamics of bacteria in sourdough fermentations as revealed by their metatranscriptome. *App. Environ. Microbiol.* 2010;76:16:5402–5408.
51. Moroni AV, Arendt EK, Dal Bello F. Biodiversity of lactic acid bacteria and yeasts in spontaneously – fermented buck wheat and teff sourdoughs. *Fd Microbiol.* 2011;28:3:497-502.
52. Wan Aida WM, Kam WY, Sahilah AM, Maskat MY. Volatile compounds in spontaneous fermented dough. *Sains Malaysians.* 2011; 40:2:135–138.
53. Minervini F, Di Cagno R, Lattanzi A, De Angelis M, Cardinali G, Capelle G, Capelle S, Gobbetti M. LAB and yeast microbiotas of nineteen sourdoughs used for traditional/typical Italian breads: Interactions between ingredients & microbial species diversity. *App. Environ. Microbiol.* 2012;78:1251-1264.
54. Thiele C, Ganzle MG, Vogel RF. Contribution of sourdough lactobacilli, yeast and cereal enzymes to the generation of amino acids in dough relevant for bread flavour. *Cereal Chem.* 2002;79:1:45–51.
55. Coppola R, Reale A, Trementa P, Succi M, Sorrentiro P. Exploration of lactic acid bacteria ecosystems of sourdoughs from Molise region. *Annals Microbiol.* 2005;55:1:17–22.
56. Ferchichi M, Rosica V, Herve P, Bernand O, Xavier D. Molecular identification of the microbiota of French sourdough using temporal temperature gradient gel electrophoresis. *Fd Microbiol.* 2007;24:7:43–47.
57. Arendt EK, Moroni AV, Dal Bello F. Sourdough in gluten – free breadmaking: An ancient technology to solve a novel issue? *Fd Microbiol.* 2009; 26:7:676–684.
58. De Angelis M, Siragusa S, Di Cagno R, Ercolini D, Minervini F, Gobbetti M. Taxonomic structure and monitoring of dominant population of lactic acid bacteria during wheat flour type I propagation using *Lactobacillus sanfranciscensis*. *App. Environ. Microbiol.* 2009;77:10991013.
59. Thiele C. Hydrolysis of gluten and the formation of flavour precursors during sourdough fermentation. Unpublished Ph.D thesis, Department of Microbiology, University of Munchen, Germany. 2003:55–75.
60. Sagdic O, Certel M, Ozcelik S, Gul H. Sourdough bread production with lactobacilli and *Saccharomyces cerevisiae* isolated from sourdoughs. *Pro. Biochem.* 2005; 40:2:691–697.
61. Spier MR, Rapacci M, Dutcosky SD, Tedrus GS. Sweet bread produced by lactic acid bacteria *L. brevis* and the yeast *S. cerevisiae*. *Int. J. Fd Eng.* 2007; 3:5:1–15.
62. De Vuyst L, Van der Meulen R, Scheirlinck I, Schoor AV, Huys G, Vandamme P, Vancanneyt M. Population dynamics and metabolite target analysis of lactic acid bacteria during

- laboratory fermentations of wheat and spelt sourdoughs. *App. Env. Microbiol.* 2007;73: 15:4741–4750.
63. Catzeddu P, Mura E, Parente E, Sanna M, Farris GA. Molecular characterization of lactic acid bacteria from sourdough bread produced in Sardina (Italy) and multivariate statistical analyses of results. *Syst. App. Microbiol.* 2005;29:2:138–144.
 64. Vogelmann SA, Seitter M, Singer U, Brandt MJ, Hertel C. Adaptability of lactic acid bacteria and yeasts to sourdoughs prepared from cereals, pseudocereals and cassava and use of competitive strains as starters. *International J. Fd Microbiol.* 2009; Vol. 130:3:205–212.
 65. Stefan W, Roel VM, Dominique M, Itse S, Geert H, Peter V, Luc D. Lactic acid bacteria community dynamics and metabolite production of rye sourdough fermentation share characteristics of wheat and spelt sourdough fermentations. *Fd Microbiol.* 2010; 27:8:100–108.

66. Stolz P. Biological fundamentals of yeast and lactobacilli fermentation in bread dough. In *Handbook of Dough Fermentations*, edited by Kulp, K. & Lorenz, K. USA, Marcel Dekker, Inc, 2003.
67. Corsetti A, Settanni L, Valmorri S, Mastrangelo M, Giovanna A. Identification of subdominant sourdough lactic acid bacteria and their evolution during laboratory – scale fermentations. *Fd Microbiol.* 2007; 24:6:1-3.
68. Sterr Y, Weiss A, Schmidt H. Evaluation of lactic acid bacteria of sourdough fermentation of amaranth. *Int. J. Fd Microbiol.* 2009;20:1-5.
69. Wakil SM, Onilude AA, Adetutu EM, Ball AS. PCR-DGGE finger prints of microbial succession changes during fermentation of cereal-legume weaning foods. *Afri. J. Biotechnol.* 2008; 7:24:4643-4652.
70. Sauvageot N, Gouffi K, Laplace JM, Auffray Y. Glycerol metabolism in *Lactobacillus collinoides*: Production of 3-hydroxypropionaldehyde, a precursor of acrolein. *Int. J. Fd Microbiol.* 2000;10:55:167–170.