



Sourdough bread preparation using selected lactic acid bacterial starter cultures

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Received: May 18, 2014; Revised received: August 07, 2014 Accepted: October 03, 2014

Abstract: The lactic fermentation of cereals is known to improve the food quality through the development of flavor, enhancement of the nutritional value and shelf life, and by removing toxic or antinutritional factors of food products. Lactic acid bacteria (LAB) strains are able to improve the shelf life of several food products. The efficiency of the LAB cultures determined in *in vitro* assays was confirmed in bread manufacture. The sourbread prepared using 50 per cent yeast and 50 per cent LAB starter (based on cell density) was found to be superior to the conventional bread in textural characteristics, flavor, appearance and even taste. It contained enough protein (10.15%) and the least fat value (7.68%). It scored the highest acceptability index of 81.70. These results point out the advantages of using selected LAB strains as starter cultures for sourdough fermentation.

Keywords: Bread, Lactic acid bacteria, Nutritional profile, Sensory characters, Sourdough, Starter culture

INTRODUCTION

Lactic acid bacteria (LAB) have a long history in preserving foods from spoilage microorganisms, they are commonly used in food fermentation, produce several metabolites with beneficial health effects and thus, are generally recognized as safe (GRAS) (Diop *et al.* 2007). For several thousands of years, bread has been one of the major constituents of the human diet, making the baking of yeast-based breads one of the oldest biotechnological processes. Sourdough is an intermediate product between dough and traditional bread preparation, containing flour, water and metabolically active microorganisms, mainly lactic acid bacteria (LAB) and yeast. During sourdough fermentation, lactic acid bacteria (LAB) produce a number of metabolites which have been shown to have a positive effect on the texture and staling of bread (Tieking and Gänzle, 2005). Exopolysaccharides (EPS) produced by LAB have the potential to replace more expensive hydrocolloids used as bread improvers. Organic acids affect the protein and starch fractions of flour. Additionally, the drop in pH associated with acid production causes an increase in the protease and amylase activities of the flour, thus leading to a reduction in staling. While improving the textural qualities of bread, sourdough fermentation also results in increased mineral bioavailability and reduced phytate content (Lopez *et al.*, 2000). Recent results have demonstrated the effectiveness of sourdough fermentation (Petra Gereková, 2011). The sourdoughs fermented with the hetero fermentative *Lactobacillus* spp. had higher acid

equivalents, lower pH-values and also gave bread with stronger taste of acidity and acetic acid than those fermented with the homo fermentative organisms. The sourdough bread was shown to have a stronger taste of acidity and also a more aromatic and crispy crust. The bread baked with sourdoughs fermented with the heterofermentative organisms, showed the best ability even to prevent growth of the mould, *Eurotium rubrum* (Paola Lavermicocca, 2000). Keeping these in view, the present investigation was conducted to prepare sourdough bread using selected lactic acid bacterial starter cultures.

MATERIALS AND METHODS

The Department of Agricultural Microbiology, UAS, Dharwad has a large collection of lactic acid bacteria, isolated from diverse sources. These isolates were screened for production of CO₂, lactic acid and exopolysaccharides (EPS), and the potent strains selected. Refined wheat flour, sugar, salt, vanaspati and baker yeast (*Saccharomyces cerevisiae*) were procured from the local market of Dharwad.

Testing the LAB isolates for functional traits: All the 22 isolates were tested for production of CO₂ (Zuniga *et al.*, 1993), lactic acid (Zuniga *et al.*, 1993) and EPS (Garcia and Marshall, 1991). Based on these values, promising isolates were selected for sourbread preparation.

LAB strains used for bread preparation: Three LAB strains were used for bread preparation. They were LAB 49, LAB 72 and LAB 74. They were

individually grown in de Man, Rogosa and Sharpe (MRS) broth medium for three days, centrifuged and a suspension prepared and mixed at 1:1:1: ratio (based on the cell population).

Preparation of bread: Maida (1 kg) was mixed with yeast (20 g), vanaspati (100 g) and sugar solution (200g in 600 ml). A table spoon of salt was added to maida flour and kneading done for 30 min to get a dough having a consistency suitable for producing a good textured bread. The kneaded dough was kept for raising (proofing) for 45 min. The raised dough was filled in to a mould, pressed and incubated for 60 min. The bread was baked by keeping the dough tins in an oven at 400⁰ F (205⁰ C) temperature for 30 min., depanned, allowed to cool for 4 h, sliced and packed in polythene bags.

The bread was prepared by using the following treatments. Yeast and LAB cultures were taken at different ratios. This was based on the cell density of the yeast used (10⁸ cfu /g).

T₁ - 100% Yeast (present practice), T₂ - 50% Yeast + 50% LAB, T₃ - 50% Yeast and T₄ -100% Yeast + 50% LAB.

Sensory evaluation of bread: The bread was prepared as above and subjected for sensory evaluation for appearance, color, texture, flavor and taste by hedonic score system as described by Meilgaard *et al.* (2007). For sensory evaluation of the bread, as many as 30 panelists were selected covering faculties from teaching, research and extension wings.

Nutritional analysis: The bread was analyzed for moisture, protein, fat, ash and crude fibre content (AACC, 2000).

RESULTS AND DISCUSSION

Table 1. reveals that many LAB isolates produced CO₂. Although all the isolates produced lactic acid, LAB 72 produced the highest value (8.3%). Similarly, the highest EPS was produced by LAB 49 (81.7 µg/ml). LAB 74 produced higher amounts of both EPS (76.50 µg/ml) and lactic acid (7.5%). All these three strains were selected for bread preparation.

From the results, it is evident that T₂ treatment *i.e.* 50% Yeast + 50% LAB performed better than T₁ (conventional bread) in terms of the acceptability index (Table 2). The T₂ sourbread scored higher values in all the sensory parameters than the traditional bread. That means, by using only 50% population from the commercial yeast culture and the remaining 50 % from the LAB consortium, bread could be produced whose acceptability level is higher than the currently produced bread. Similar observations were also made by Deshpande *et al.* (2013). They prepared sourdough bread using selected lactic acid bacterial cultures. They obtained a nutritionally superior sourdough bread using *Lactobacillus brevis* and *L. palntarum* along with the yeast. Superior quality bread was produced by using a mixture of yeast and LAB by others also

(Martinez-Anaya and Devesa., 2000). Proteolysis in sourdoughs has been reported to be higher than in yeasted doughs. LAB is known to improve taste and flavour of fermented products via proteolysis and lipolysis activities which cause production of aromatic compounds (acetate, ethanol, di acetyl and acetaldehyde, 3-methyl-1-butanol, 2-phenylethanol) (Galle *et al.*, 2011). EPS is known to improve the volume, texture, and shelf life of bread (Zuzana *et al.*, 2009). Pérez-Chabela *et al.* (2012) observed that *Propioni bacterium acidilacti* UAM15c and *Leuconostoc plantarum* UAM10a secreted exopolysaccharides which was related to high moisture stability and better textural properties, as compared with non-inoculated samples.

Nutritionally, the bread prepared using 50 per cent LAB and 50 per cent yeast cells was superior in that it possessed the least fat and higher ash content (Table 3). It is known that the low pH values associated with microbiologically acidified wheat dough leads to solubilization of the phytate complex, thus increasing mineral bioavailability, accounting to higher ash content (Larsson and Sandberg, 1991). The improvement in the mineral availability of sourdough baked goods has also been reported by Lopez *et al.* (2003). Oral administration of *Lactobacillus gasserii* NT strain reduced fat synthesis in adipose tissue, suggesting its potential to prevent the development of obesity

Table 1. Testing of Lactic acid bacterial isolates for functional traits.

S. N.	Strain code no.	CO ₂ production	Lactic acid production (%)	EPS production (µg/ ml)
1	LAB 04	+	6.20	66.50
2	LAB 05	-	5.70	46.25
3	LAB 10	+	6.40	50.16
4	LAB 45	-	7.90	69.83
5	LAB 46	+	7.00	60.16
6	LAB 48	+	6.70	50.16
7	LAB 49	+	5.90	81.70
8	LAB 50	+	7.60	81.50
9	LAB 51	+	6.00	65.41
10	LAB 60	-	7.30	25.10
11	LAB 63	+	7.60	58.16
12	LAB 65	+	5.90	50.16
13	LAB 68	+	7.00	34.16
14	LAB 72	+	8.30	76.50
15	LAB 74	+	7.50	74.50
16	LAB 75	-	7.20	53.83
17	LAB 76	+	7.70	61.83
18	LAB 77	-	7.70	61.25
19	LAB 82	-	7.10	44.83
20	LAB 83	+	7.30	72.10
21	LAB 89	-	7.40	62.75
22	LAB 91	+	7.00	49.58

Table 2. Sensory evaluation of four different breads prepared.

S. N.	Particulars	Treatments			
		T ₁ - 100% Y	T ₂ - 50% Y: 50% L	T ₃ - 50% Y	T ₄ - 100% Y: 50% L
1	Color of crumb	7.22	7.42	7.07	7.25
2	Color of crust	7.04	7.54	6.98	7.24
3	Texture of crumb	7.02	7.31	6.90	7.25
4	Texture of crust	6.95	7.44	7.02	7.15
5	Appearance	7.12	7.38	7.00	7.32
6	Flavor	7.00	7.10	6.95	7.14
7	Taste	6.87	7.30	6.82	7.18
8	Overall acceptability	7.05	7.34	6.98	7.51
9	Total score	56.27	58.83	55.72	58.04
10	Acceptability index (%)	78.15	81.70	77.38	80.61

Y- Yeast; L- LAB strains

Table 3. Nutritional composition of bread prepared using LAB and yeast cultures.

S. N.	Treatments	Moisture (%)	Ash (%)	Protei (%)	Fat (%)	Crude Fiber (%)
1	100% Y	32.52	6.20	14.16	8.60	ND
2	50(y): 50% (L)	30.68	7.70	10.15	7.68	ND
3	50% Y	26.61	4.55	10.15	10.84	ND
4	100%(Y):50% (L)	28.01	1.50	10.32	9.86	ND
	CD at 1%	1.34	0.18	1.45	0.84	
	S Em	0.28	0.04	0.31	0.18	

Y- Yeast; L- LAB strains; ND- Not detected

(Yonejima et al., 2013).

Conclusion

Thus, the results of the present study have indicated that the sourbread prepared using yeast and LAB cultures (1:1) were superior in textural characteristics, flavor, appearance and even taste. It contained enough protein (10.15%) and the least fat value (7.68%). It scored the highest acceptability index of 81.70.

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