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Studies on Process Development and Quality Evaluation of Sour Dough Bread

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ABSTRACT

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The present investigation focuses on standardization the process for sour dough bread and its quality evaluation. Dough was fortified with LAB culture (*Lactobacillus plantarum* and *Lactobacillus brevis*) at 0.1% (T₁), 0.15% (T₂) and 0.2% (T₃) level of incorporation. The product prepared was evaluated for color, flavour, taste, texture, appearance and overall acceptability using semi-trained panel members on 9 point hedonic rating. It can be concluded that the LAB culture can be used successfully in preparation of sour dough bread at the level of 0.15% without any undesirable changes in physical, chemical and organoleptic attributes of bread.

Introduction

Bread products and their production techniques differ widely around the world. The foremost quality characteristics of leavened wheat breads are high volume, soft and elastic crumb structure, good shelf life, and microbiological safety (Cauvain, 2003; Chavan and Jana, 2008). Sourdough has a natural, additive-free image and lactic acid bacteria have been used in food for thousands of years and are "generally regarded as safe"

(Magnusson *et al.*, 2003). Sourdough is ancient way to improve flavor, texture and microbiological shelf life of bread, and is widely utilized in whole grain rye baking (Lorenz and Brummer, 2003). Sourdoughs are very complex biological ecosystems because of the microbial composition and all interactive effects among the bread-making processes and ingredients (Gobbetti *et al.*, 1999). The majority of species regularly isolated from sourdough or used as sourdough starter belong, with only few exceptions, to 1

of the 4 genera *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Weissella*. The highest number of different species (>23 species) is found in the genus *Lactobacillus*. Most of the yeast preparations for sourdoughs often contain LAB, especially *Lactobacilli* rather than *Pediococcus*, *Lactococcus* and *Leuconostoc* spp. (Jenson, 1998), which contributes a little to the aroma development through acidification of the dough during limited processing period (Rothe and Ruttloff, 1983). Yeasts are often associated with LAB in sourdough and the yeasts/LAB ratio is generally 1:100 (Gobbetti *et al.*, 1994; Ottogalli *et al.*, 1996). The utilization of sourdough has fundamental effects on dough rheology. Sourdough fermentation causes decreased elasticity and viscosity, less elastic and softer doughs, effects being more pronounced with longer fermentation times (Clarke *et al.*, 2004). In comparison to bread prepared with baker's yeast, the sourdough breads are characterized by moist, dense grains, and a rather chewy texture (Qarooni 1996). The application of sourdough to wheat breads has a positive impact on bread volume, which is a primary quality characteristic of bread (Collar *et al.*, 1994; Clarke *et al.*, 2004). The optimal use of sourdough can improve the taste and flavor of the bread (Rehman *et al.*, 2006). The flavor of sourdough wheat bread is richer and more aromatic than wheat bread, a factor that can be attributed to the long fermentation time of sourdough (Brummer and Lorenz, 1991). The flavoring compound diacetyl is produced in significant amounts by *Lactobacillus plantarum* and *Pediococcus pentosaceus*. The application of LAB in the form of sourdough has a positive effect on bread staling. One such effect is an improvement in loaf-specific volume, which is associated with the reduction in the rate of staling (Corsetti *et al.*, 2001). Sourdough-associated LAB produce many antimicrobial substances, such as organic acids, CO₂, ethanol, hydrogen peroxide, diacetyl, fatty

acids, phenyllactic acid, reuterin, and fungicins (Messens and De Vuyst, 2002). Also, *Lactobacillus plantarum* shows very broad antimicrobial activity, and the antifungal compounds 4-hydroxyphenyllactic and especially phenyl lactic acids have been identified as responsible for fungal inhibition (Ryan *et al.*, 2009). Sourdough-associated LAB are also effective against rope spoilage of bread induced by *Bacillus* spp., probably due to production of organic acids and other still unknown antibacterial substances (Valerio *et al.*, 2008). At present, the sourdough is employed in the manufacture of breads, cakes, and crackers (Chavan and Chavan, 2011). The use of sourdough is useful for making bread products with an increased level of flavor compounds, ultimately increasing the evenness in the batches and customer satisfaction. Sourdough technology can also be useful to reduce or eliminate the level of preservatives often used in baked products, as sourdough has shown antibacterial and antimold activity. Thus, sourdough could be useful in serving mankind with wholesome, tasty, and convenient foods.

Materials and Methods

Composite flour (70% of whole wheat flour and 30% refined wheat flour), Granulated cane sugar, Iodized salt, baker's compressed yeast, Shortenings were purchased from local market.

Starter cultures

Pure cultures of *Lactobacillus plantarum* and *Lactobacillus brevis* were purchased from National Chemical Laboratory, (NCL) Pune.

Purity of the cultures

The staining of the obtained pure cultures of *Lactobacillus plantarum* and *Lactobacillus brevis* was carried out by using Gram positive

staining technique for their identification (Harley and Prescott, 2002).

Preparation of starter culture

The starter culture was prepared with the help of the method described by Ghadge *et al.*, (2008) with suitable modifications.

Preparation of MRS Medium

All the ingredients were suspended in distilled water and heated to dissolve the medium completely. The medium was sterilized in autoclave at 15 lbs pressure for 15 minutes (De Mann *et al.*, 1960).

Ingredients	g/ lit
Proteose peptone	10.0
Yeast extract	5.0
Beef extract	10.0
Dextrose	20.0
Tween -80	1.0
Ammonium citrate	2.0
Sodium acetate	5.0
Magnesium sulphate	0.1
Manganese sulphate	0.05
Di-potassium phosphate	2.0

Sub-culturing of pure culture

The pure cultures i.e. *Lactobacillus plantarum* and *Lactobacillus brevis* were sub-cultured on slants prepared from MRS media in laminar flow. This was incubated at 37°C for 24 hours in incubator. After 24 hours there was growth on slant of cultures.

Preparation of sourdough bread

The production of sourdough bread was carried out in the Pilot Bakery Plant by using *Lactobacillus brevis*, *Lactobacillus plantarum* and *Saccharomyces cerevisiae* with some

modifications in the method described in A.A.C.C. (2000). The recipe used for preparation of bread mentioned below in Table 1 and pure LAB cultures used in recipe in different concentration is mentioned in Table 2.

Results and Discussion

Proximate analysis of flour

It is observed from the Table 3 that obtained flour found to contain 12.50 per cent of moisture. The lower moisture content of composite flour justifies the suitability for long term storage without deterioration (Peter and James, 2000). The protein content was recorded to be 11.85 per cent. The higher protein content is important for strong elastic dough which having high water absorptive capacity, excellent gas holding properties and will yield bread with good volume, grain and texture (Kent and Evers, 1997). The observed values for crude fat and ash content were 1.80 and 1.53 per cent respectively. The results obtained in the present study for the analysis of composite flour are also in close agreement with Yun *et al.*, (1996)

Bread making quality parameters of flour

It is evident from the above Table 4 that gluten content of flour was 11.14 per cent (On dry weight basis) and 31.54 per cent (On wet weight basis). The values clearly indicated that flour used was strong in nature. The alcoholic acidity of flour was 0.09% and the obtained results for sedimentation value of composite flour were 21.67 ml which represent good quality of flour. The Falling number of flour represents the viscous behavior of flour due to present of α -amylase. The obtained values of 278 sec for falling number represent the freshness of flour. The results are in comparable with William *et al.*, (1988).

Rheological characteristics of sourdough

It is observed from present investigation that the water absorption of sample T₁ containing 0.1 per cent of LAB starter culture was 61.21 per cent which increased with increased level of starter culture and reached up to 62.03 per cent in sample T₃ having 0.2 per cent of LAB starter culture. The water absorption of flour is an important factor influencing the handling properties and machinability of dough and is related to the quality of the finished baked product (Catterall, 1998). The arrival time is point where the top of curve reaches 500BU points and indicates the rate of absorption (min/BU). Arrival time for flour was recorded to vary in the range 1.94 (sample T₁) to 1.91(sample T₂) which were lower than control (2.0 min) (Table 5). The departure time is the point at which the top of the curve goes below 500BU line, which indicates the point at which the gluten is breaking down and dough has become over-mixed (Pomeranz, 1988). Sample C showed maximum time to break down (9.11) which decreased gradually with increasing level of LAB starter culture addition and the lowest departure time (8.49 min). The stability is time between arrival time and departure time and generally indicates the strength of flour which is function of gluten development (Collar *et al.*, 1994). The results revealed that stability of sourdough decreased with the increase in the level of LAB starter. Highest dough stability was recorded for the control (5.01 min), which gradually decrease from T₁ (4.58 min), T₂ (4.12 min) and T₃ (3.60 min). The findings of present investigation are similar with Hosoney (1994). Peak time reads the highest point on the curve and indicates the point they reached its maximum viscosity before gluten strands begin to breakdown (Pomeranz, 1988). Peak time values decreased with increase the level of LAB starter addition *viz.* 3.20, 3.17, 3.09 and 2.80 min for control, T₁, T₂ and T₃ respectively.

These findings are in good conformity with the results of (Wehrle *et al.*, 1997). Softening of dough (SD) varied from 70.39, 90.01, and 120.33 BU for the samples T₁, T₂ and T₃ respectively. These findings revealed that dough became softer with the increase in the level of LAB starter. These values are in close agreement with the findings of Schober *et al.*, (2003).

It is evident from the Table 6 that highest value for mean distance at maximum force extensibility was noted for sample T₂ (i.e. 42.7mm) followed by T₁ and control (i.e. 38.1 and 37.2mm respectively) whereas lowest value was noted for sample T₃ *viz.* 34.4mm. Sample T₂ is best in extensibility over all other samples. The declined extensibility in sample T₃ can be attributed to presence of high concentration of organic acids that substantially decrease mixing time and weaken the dough (Wehrle *et al.*, 1997). The extensibility graph showing the effect of addition level of LAB starter culture is illustrated in Figure 1.

Physical properties of Sour Dough Bread with different levels LAB starter culture

It is evident from the Table 7 that loaf volume showed significant variation as a result of treatments. Loaf volume of control sample was recorded to be 586ml. samples T₁ and T₂ showed maximum volume *viz.* 587 and 590 ml respectively, followed by T₃ which produced minimum volume i.e. 583 ml. Thus it may be concluded that sample T₂ (0.15 per cent LAB starter) was able to produce superior loaf volume. These results in the present study are in line with the findings of Corsetti *et al.*, (2000). Similarly, the specific volume of the bread samples was calculated and the best treatment in this regard was found to be in sample T₂ (3.88cm³/g) whereas, T₃ sample was having the lowest specific volume *viz.*, 3.80 cm³/g. The results

pertaining to the specific volume of bread indicated that treatment resulted in breads with less density and high volume and hence, preferred by the consumers. The results obtained in present investigation are in close agreement with the findings of Salim-ur-Rahman *et al.*, (2007). Crust to crumb ratio was found to be in the range of 0.255 to 0.263 with control sample having 0.255 value for crust to crumb ratio, the highest value was obtained by sample T₃ while sample T₁ showed lowest value. The values obtained for crust to crumb ratio indicated that there was no much difference found in these values as no effect of different incorporation level of LAB starter culture on the crust to crumb ratio. These findings are comparable with those of Taranto (1983).

Effect of different incorporation level of LAB starter culture on external sensorial characteristics of sour dough bread

It is evident from the Table 8 that amongst various external characteristics, volume of bread, crust color, symmetry of form, character of crust, crumb color and aroma were significantly affected by the different levels of LAB culture (Fig. 2). The volume of bread was found to increase with increase in concentration of LAB starter culture (from control to T₃) and maximum volume was observed in sample T₂ containing 0.15 per cent of LAB starter culture. The volume then decreased with further addition of starter culture as seen in sample T₃. Crust color is an important sensory parameter concerning the consumer's acceptability of bread. Maximum score for the colour of crust in treated sample was obtained by sample T₂ (i.e. 8.07). This might be due to the production of lactic acid which improved bread color (Tarar, 1999). The 'Symmetry of form' is an important bread parameter in deciding the characteristics like uneven top, low ends and shrunken sides of the bread. It is observed

form Table 8, symmetry of breads prepared from different starter cultures increased non significantly with the increase in addition of LAB starter culture. The 'evenness' of bake reflects that all sides including top and the bottom are uniformly baked and it also reflects the intensity of baking whether the sides having lighter or darker shade. The mean values for the evenness of bake of breads given in Table 8 indicated that evenness of bake among breads varied from 7.34 to 7.38 prepared from different level of LAB starter cultures. The 'character of crust' represents the hardness of bread crust. Higher crust hardness represents superior quality as it can sustain minor impact or attrition during transportation and handling. Addition of LAB starter culture does not have significant impact on the character crust of bread. The results in the Table 8 indicate that the scores assigned to aroma of breads ranged from 7.12 to 7.89 among the breads prepared from different level of starter cultures. The breads prepared from T₃ got significantly higher score (i.e. 7.89). However, the lowest scores for the aroma were given to breads produced from T₁ and T₂ followed by control. The results of present study are in conformity with the findings of Martinez (1996). It is observed that the 'overall acceptability' in terms of external characteristics of bread improved linearly with increase in concentration of LAB starter culture and maximum score was secured for sample T₂ containing 0.15 per cent of LAB starter. The Sour Dough provides aromatic and pleasing flavor, and improves overall acceptability and shelf life of whole grain breads (Katina *et al.*, 2005).

Effect of different levels of LAB starter culture on internal characteristics of sour dough bread

It was found that LAB starter culture results in enhancement of grain formation. Maximum grain score was obtained by the sample T₂

while control sample got the minimum score. These findings are similar with those obtained by Katina *et al.*, (2006). LAB starter culture addition found to improve the color of crumb with sample T₂ obtaining the highest score. Maximum score for 'taste' was noted in the sample T₂ followed by T₃ and T₁ while the control sample scored the lowest value. The results for taste scores of Sour Dough bread were much higher than that of control breads, which suggested that the addition of Sour Dough improved the bread's taste. The scores for taste in the present study are in agreement with the findings of Siddique (1989). Textural properties of all the samples were found to increase with increase in concentration of LAB starter culture. The Sour Dough addition resulted in softer breads than control. Maximum textural scores were secured by sample T₂ while the minimum values were observed in control sample C. The desirable texture obtained by sample T₂ was soft and pliable. The results of the present study are in concordance with the findings of Crowley *et al.*, (2002) who found that the breads containing Sour Dough starter culture maintained superior textural properties. The 'overall acceptability' for internal characteristics of bread found to increase with increase in LAB starter culture concentration, however maximum score for overall acceptability was also observed in sample T₂ having 0.51 per cent of LAB starter culture. The results obtained in the present study are in agreement with the findings of Shah *et al.*, (1999) (Fig. 3 and Table 9).

Effect of different level of addition of LAB starter culture on pH and titrable acidity of sour dough bread

The mean values for the pH and titrable acidity of Sour Dough breads are given in Table 10, showing that yeast leavened control bread showed the highest pH (5.4) and the lowest acidity (2.75) values. The pH of Sour

Dough bread decreased with an increase in level of LAB starter culture with T₁, T₂ and T₃ pH value to be 4.5, 4.1 and 3.9 respectively. It may be due to the acidification of dough by LAB starter culture. The results for pH and acidity are in accordance with findings of Park *et al.*, (2006).

Chemical composition of bread prepared with different addition level of LAB starter culture

The results for moisture content of breads prepared from different level of LAB starter cultures as given in Table 11 indicated that moisture content increased significantly with the increase in addition of LAB starter culture. The results indicated that breads prepared from control sample showed the lowest moisture content (31.02 per cent). Sample T₃ possessed the highest moisture content (36.53 per cent) followed by T₂ (35.80 per cent) and T₁ (35.13 per cent). The results of the present study are also in conformity with the findings of Corsetti *et al.*, (2001). The breads were a good source of protein (8.02 ± 0.2), low in fat (0.81 ± 0.1), ash content (2.01 ± 0.04) and higher in the crude fiber (9.91 ± 0.01). These findings conforms the earlier study of Dubravka *et al.*, (2011). It is observed from the Table that resistant starch of breads increased significantly with increase in the addition level of LAB starter culture. Control sample showed the lowest content of RS (3.21 per cent). Sample T₃ showed the highest content of RS (6.04 per cent) followed by T₂ (5.54 per cent) and T₁ (4.76 per cent). This is in accordance with Brighenti *et al.*, (1998).

Effect of different addition level of LAB starter culture on organic acid content of bread

The Sour Doughs bread of sample T₃ yielded significantly the highest lactic acid content

(0.493 g/100g) followed by the Sour Doughs of sample T₂ and T₁ having lactic acid to be 0.350 and 0.224 g/100g respectively. The production of lactic acid increased significantly by increasing the addition level of LAB starter cultures. The lactic acid content found in the Sour Doughs is similar to the study reported by Robert *et al.*, (2005). The acetic acid content of Sour Doughs of sample T₃ produced significantly the highest

acetic acid content (0.093 g/100g) followed by T₂ (0.072 g/100g) and T₁ (0.041 g/100g). The acetic acid was found to be least in the control sample i.e. 0.01 g/100g. The results indicated that the production of acetic acid increased significantly with the increased addition level of starter cultures. The results of the present study are in agreement to the findings of Katina (2004) (Table 12).

Flowsheet-1: Preparation of bread by addition of sourdough

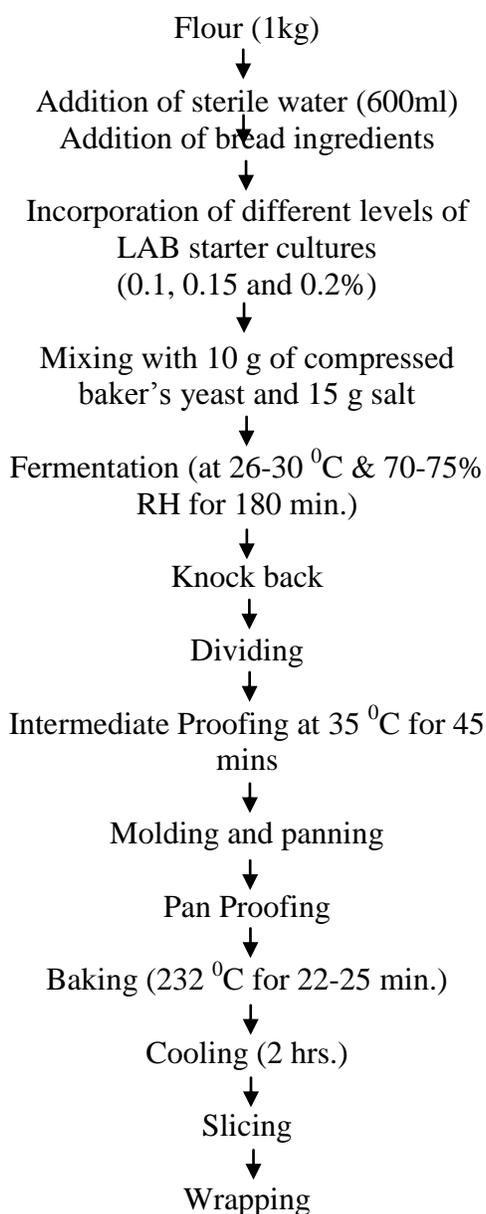


Table.1 Bread recipe used in bread production

Sr. No.	Ingredients	Weight (g)
1	Flour (70% whole wheat flour + 30% Maida)	100.0
2	Water	60.0
3	Sugar	2.0
4	Shortening	2.0
5	Salt	1.5
6	Yeast	1.0
7	GMS	0.3

Table.2 Different levels of addition of starter culture

Sample	Lactic acid starter culture*
T ₁	0.1% LAB starter culture
T ₂	0.15% LAB starter culture
T ₃	0.2% LAB starter culture

Table.3 Proximate analysis of flour

Chemical Constituent (%)	Mean value
Moisture	12.50
Crude fat	1.80
Crude protein	11.85
Ash	1.53
Crude fiber	2.04
NFE	70.28

Table.4 Bread making quality parameters of flour

Parameters	Result
Alcohol acidity (%)	0.09
Gluten content: % (Wet basis)	31.54
Gluten content: % (Dry basis)	11.14
Peleshnke value (min)	108
Sedimentation value (ml)	21.67
Falling number (Sec)	278

Table.5 Rheological characteristics of sourdough prepared with different addition level of LAB starter cultures

Sample	Water Absorption (%)	Arrival time (min)	Departure time (min)	Peak Time (min)	Dough stability (min)	Softening of dough (BU)
Control	60.71	2.0	9.11	3.20	5.01	50.03
T ₁	61.21	1.94	8.90	3.17	4.58	70.39
T ₂	61.50	1.92	8.74	3.09	4.12	90.01
T ₃	62.03	1.91	8.49	2.80	3.60	120.92
Mean	61.3625	1.9425	8.81	3.065	4.3275	82.8375
SE	0.092	0.006	0.043	0.030	0.101	5.064
CD at 5%	0.275	0.020	0.130	0.091	0.303	15.091

Table.6 Extensibility of sour dough prepared with different level of LAB starter culture

Sample	Mean Max. force 'resistance to extension' (g)	Mean Distance at Max. Force 'Extensibility'(mm)
Control (C)	84.6	37.2
T ₁	69.9	38.1
T ₂	67.6	42.7
T ₃	76.2	35.4
Mean	74.575	38.35
SE	1.276	0.521
CD at 5%	3.804	1.554

Table.7 Effects of different treatments on volume, specific volume, crust to crumb ratio of sour dough bread

Treatments	Loaf volume (ml)	Specific volume (cm ³ /gm)	Crust to crumb ratio
Control	586	3.83	0.255
T ₁	587	3.85	0.259
T ₂	590	3.88	0.260
T ₃	583	3.80	0.263
Mean	586.5	3.84	0.25925
SE	0.484	0.005	0.0005
CD at 5%	1.443	0.016	0.0016

Table.8 Effect of different incorporation level of LAB starter culture on external sensorial characteristics of sour dough bread

Sample	Volume	Crust colour	Symmetry of form	Evenness of bake	Character of crust	Aroma	Overall acceptability
Control	7.52	7.81	7.34	7.34	7.75	7.12	7.53
T ₁	7.83	7.86	7.35	7.38	7.76	7.70	7.58
T ₂	7.95	8.07	7.37	7.39	7.78	7.81	7.67
T ₃	7.80	7.80	7.36	7.37	7.65	7.89	7.59
Mean	7.775	7.885	7.355	7.370	7.735	7.630	7.592
SE	0.030	0.021	0.002	0.003	0.009	0.058	0.0097
CD at 5%	0.090	0.063	0.006	0.010	0.029	0.174	0.028

Table.9 Effect of different levels of LAB starter culture on internal characteristics

Sample	Grain of bread	Colour of crumb	Taste	Texture	Overall acceptability
Control	7.35	7.81	7.15	7.57	7.43
T ₁	7.48	7.85	7.53	7.63	7.65
T ₂	7.53	7.87	7.90	7.69	8.00
T ₃	7.49	7.86	7.72	7.64	7.66
Mean	7.4625	7.8475	7.575	7.6325	7.685
SE	0.013	0.004	0.053	0.008	0.039
CD at 5%	0.039	0.013	0.160	0.024	0.117

Table.10 Effect of different level of addition of LAB starter culture on pH and titrable acidity of sour dough bread

Treatments	pH	Titrable acidity (ml NaOH)
Control	5.4	2.75
T ₁	4.5	4.71
T ₂	4.1	5.20
T ₃	3.9	6.31
Mean	4.475	4.7425
SE	0.111	0.249
CD at 5%	0.332	0.743

Table.11 Chemical composition of bread prepared with different addition level of LAB starter culture

Sample	Moisture (%)	Protein (%)	Fat (%)	Total Carbohydrates (%)	Resistant Starch (% RS)	Ash (%)	Crude Fiber (%)
Control	31.02	8.02	0.81	45.02	3.21	2.01	9.91
T ₁	35.13	7.91	0.73	39.51	4.76	2.11	9.84
T ₂	35.80	8.01	0.7	37.90	5.54	2.14	9.90
T ₃	36.53	7.9	0.76	36.7	6.04	2.15	9.91
Mean	34.62	7.96	0.75	39.7825	4.8875	2.1025	9.89
SE	0.415	0.010	0.007	0.618	0.208	0.010	0.005
CD at 5%	1.233	0.031	0.023	1.838	0.618	0.031	0.016

Table.12 Effect of different addition level of LAB starter culture on organic acid content of bread

Treatments	Lactic acid (g/100g)	Acetic acid (g/100g)
Control	0.007	0.010
T ₁	0.224	0.041
T ₂	0.350	0.072
T ₃	0.493	0.093
Mean	0.2685	0.054
SE	0.034	0.006
CD at 5%	0.103	0.018

Table.13 Total plate count at different storage intervals in bread samples

Treatments	Different storage intervals (hr.)				
	0	24	48	72	96
Control	4.2× 10 ¹	8.5×10 ¹	1.9×10 ²	2.7× 10 ²	3.5× 10 ²
T ₁	-	4 ×10 ¹	1.0×10 ²	1.4×10 ²	1.9× 10 ²
T ₂	-	-	5.5 × 10 ¹	9.5 × 10 ¹	1.3× 10 ²
T ₃	-	-	4.3× 10 ¹	8.2× 10 ¹	1.0× 10 ²

Table.14 Mold colony count at different storage intervals (hr.) in bread samples

Treatments	Different storage intervals (hr.)				
	0	24	48	72	96
Control	1.4× 10 ²	1.9× 10 ²	2.3× 10 ²	2.9× 10 ²	3.8× 10 ²
T ₁	9 × 10 ¹	1.2 × 10 ²	1.6× 10 ²	2.1× 10 ²	2.5× 10 ²
T ₂	7× 10 ¹	1.0× 10 ²	1.3× 10 ²	1.7× 10 ²	2.3 × 10 ²
T ₃	5× 10 ¹	8.1× 10 ¹	1.0× 10 ²	1.4× 10 ²	2.0× 10 ²

Table.15 Theoretical energy value of sour dough bread

Sample	Energy (Kcal)			Total energy (Kcal)
	Protein (Kcal)	Carbohydrate (Kcal)	Fat (Kcal)	
Control	32.08	180.08	7.29	219.45
T ₁	31.64	158.04	6.57	196.25
T ₂	32.04	151.6	6.3	189.94
T ₃	31.6	146.8	6.84	185.24

Fig.1 Extensibility curve of sour dough

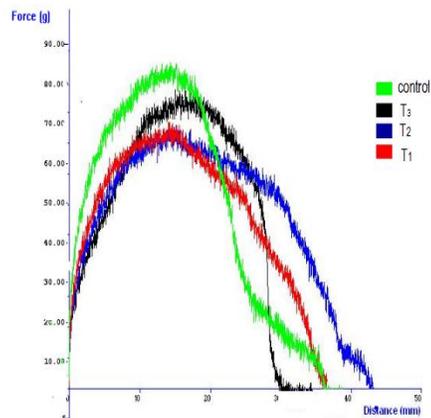


Fig.2 External sensorial characteristics of sour dough bread

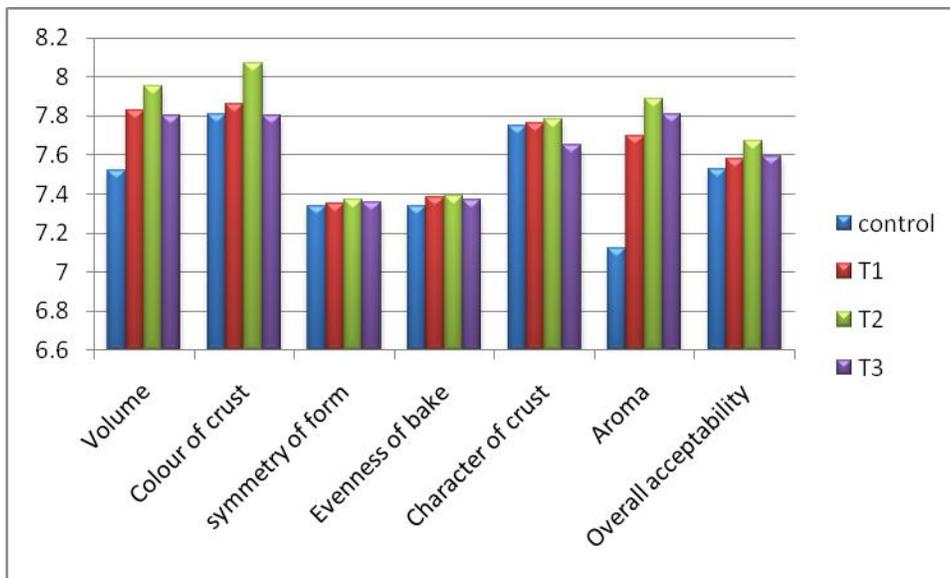
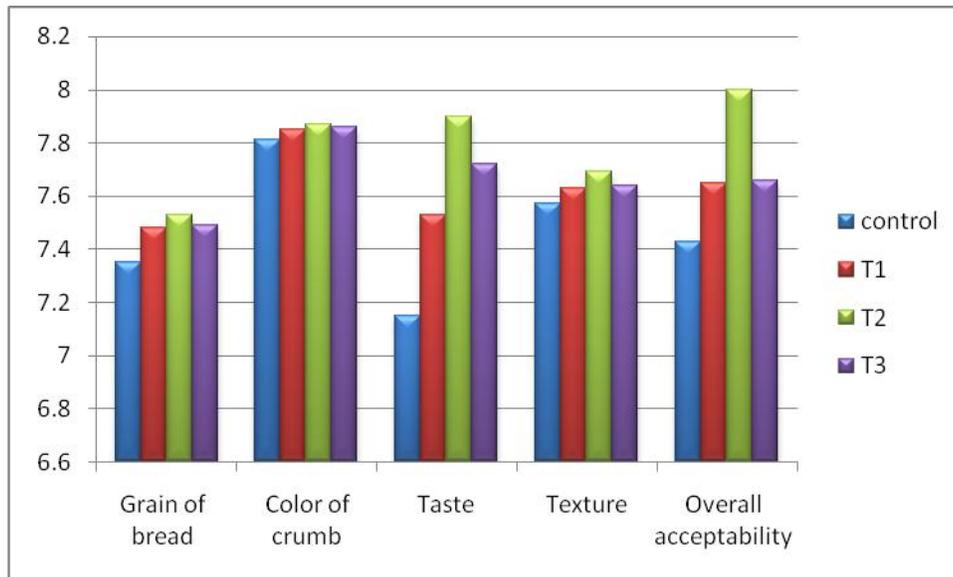


Fig.3 Internal sensorial characteristics of sour dough bread



Microbial analysis of sour dough bread

Total plate count for Sour Dough breads at different storage intervals is given in the Table 13. It is evident that the total plate count was the highest (3.5×10^2) in breads prepared from control yeast culture. However, the total plate count was the lowest in Sour Dough bread sample T₃ (1.0×10^2) followed by T₂ (1.3×10^2) after 96 hours of storage. The microbiological analysis of breads at different storage intervals showed that the microbial loads decreased with the increase in the addition level of LAB starter cultures ranging from 1.9×10^2 CFU/g to 1.0×10^2 CFU/g from 0 to 96 hour. In T₁, microbial colonies appeared after 24 h (4×10^1 CFU/g) and increased (1.9×10^2 CFU/g) in bread at 96 h of storage. The colonies appeared after 48 h of storage in T₂ and T₃ ranging from 5.5×10^1 to 1.3×10^2 CFU/g, 4.3×10^1 to 1.0×10^2 for the sample T₂ and T₃ respectively. The total plate count for the control ranged from 4.2×10^1 to 3.5×10^2 CFU/g. The Sour Dough breads produced with the addition of LAB starter cultures showed resistance against the growth of the contaminating microorganisms. The results of present study are in line with the study of Latif *et al.*, (1996)

Yeast and mold count at different storage intervals in bread samples

Maximum number of yeast and mould colonies were recorded in control bread (3.8×10^2). However, the fungal count was the lowest in breads prepared with the sample T₃ (2.0×10^2) followed by breads prepared by sample T₂ (2.3×10^2) even after 96 hours of storage. The microbiological analysis of breads at different storage intervals showed that the microbial load decreased with increased addition level of LAB starter cultures. The mold count ranged from 1.4×10^2 to 3.8×10^2 for the control, 9×10^1 to 2.5×10^2 for T₁, 7×10^1 to 2.3×10^2 for T₂, 5×10^1 to 2.0×10^2 for the Sour Dough breads of sample T₃. The results are in line with the findings of Hansen and Hansen (1994) (Table 14).

Theoretical energy value of Sour Dough bread

It is evident from the Table 15 that total energy value of control sample is 219.45 Kcal while total energy value of Sour Dough bread T₁, T₂ and T₃ is 196.25, 189.94 and 185.24 Kcal respectively. Results showed that the

total energy value of control was found to be higher than Sour Dough bread samples. This is due to increase in the resistant starch content in Sour Dough bread.

In conclusion, dough was fortified with LAB culture (*Lactobacillus plantarum* and *Lactobacillus brevis*) at 0.1% (T₁), 0.15% (T₂) and 0.2% (T₃) level of incorporation. The product prepared was evaluated for color, flavour, taste, texture, appearance and overall acceptability using semi-trained panel members on 9 point hedonic rating. It can be concluded that the LAB culture can be used successfully in preparation of sour dough bread at the level of 0.15% (T₂) without any undesirable changes in physical, chemical and organoleptic attributes of bread.

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