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Effect of Sourdough Bacteria on the Quality and Shelf Life of Bread

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Abstract: Bread dough is usually fermented with yeast but in the present study sourdough lactic acid bacteria (*Lactobacillus bulgaricus*) alone and in combination with yeast (*Saccharomyces cerevisiae*) were used to determine their effect on the shelf life and sensory characteristics of bread at different intervals of storage. Lactic acid bacteria improved the sensory characteristics of bread such as volume, evenness of bake, character of crust, grain of bread, colour of bread crumb, aroma, taste and texture of bread and extended shelf life of bread by inhibiting the growth of microbes.

Key words: Lactic acid, microbes, bread, shelf life, sensory characteristics

Introduction

Dough is usually leavened by bread yeast, which ferment dough sugar and produces mainly carbon dioxide and alcohol. However, other gas producing microorganisms e.g., wild yeasts, coliform bacteria, saccharolytic Clostridium species, heterofermentative lactic acid bacteria and various naturally occurring mixtures of these organisms have been used for leavening of dough instead of bread yeast alone (Vollmer and Meuser, 1992; Bratovanova, 1996). Sourdough is an important modern fermentation of cereal flours and water based upon an earlier spontaneous process (Vogel *et al*., 1999). A slack flour dough is inoculated with microbial starter, "mother culture", which is constantly renewed in a cyclical way, using specified recipes and ripening conditions (Hammes and Ganzle, 1995; Ottogalli *et al*., 1996).

In addition to yeast, certain strains of the Lactic Acid Bacteria (LAB) play a key role in fermentation of bread dough. Although, yeast shows a high demand for amino acids but total amino acid concentrations are not affected by lactic acid bacteria (Thiele *et al*., 2001). The sourdough fermentation is central to acceptability in flavour, as chemically acidified breads prepared with pure commercial starter cultures are not well scored in sensory preference assessments (Lund *et al*., 1989; Rehman *et al*., 2006). The synergistic metabolic activities of microorganisms produce acidification or souring influencing the final characters of bread, notably the texture (Martinez *et al*., 1990; Röcken and Voysey, 1995; Corsetti *et al*., 2000) and generate typical flavour compounds yielding typical sourdough sensory attributes (Gobbetti, 1998; Katina *et al*., 2006). Sourdough bread may vary in flavouring compounds as a result of ingredients selection and lactic acid bacterial fermentation (Schieberle and Grosch, 1985; Schieberle and Grosch, 1992; Schieberle and Grosch, 1994). Moreover, lactic acid bacteria contribute to the production of safer foods by inhibiting the growth of pathogenic microbes or by removing chemicals or toxic contaminants. Certain lactobacillus bacteria, in the process of souring of dough, produce an enzyme that breaks down a protein to be toxic to people with celiac disease (Cagno *et al*., 2002; Gobbetti *et al*., 1995). Therefore, the present study was conducted to determine the effect of *Lactobacillus bulgaricus* alone and in combination with yeast (*Saccharomyces cerevisiae*) on the sensory characteristics and shelf life of bread.

Materials and Methods

Collection of raw materials: Pure culture of lactic acid bacteria (*Lactobacillus bulgaricus*) was isolated from yoghurt starter cultures purchased from the local market of Faisalabad. Wheat flour, salt, sugar and yeast were also procured from the local market.

Purification of cultures: Streak plate method was used to get pure culture of lactic acid bacteria. Then a single colony was transferred to the fresh medium and a pure culture (stock culture) was obtained (Cappuccino and Sherman, 1996).

Proximate analysis of flour: The commercial flour samples were analyzed for moisture, total ash, crude protein, crude fiber, crude fat and nitrogen free extract (AACC, 2000).

Rheological characteristics of dough: The rheological properties of flour samples were determined by Brabender Farinograph and Amylograph (AACC, 2000).

Table 1: Treatments

Treatments	
T_0	Control bread (1% yeast)
T_1	3 ml lactic acid starter culture
T_2	0.25% yeast + 2 ml lactic acid starter culture
T_3	0.50% yeast + 1.5 ml lactic acid starter culture
T_4	0.75% yeast + 1 ml lactic acid starter culture.

1 ml = 1.5×10^6 (*L. bulgaricus*) lactic acid producing bacteria

Table 2: Proximate composition of commercial flour

Constituent	Quantity (%)
Moisture	12.20
Ash (db)	0.65
Crude protein (db)	12.87
Crude fibre (db)	0.54
Crude fat (db)	0.94
NFE (db)	85.0

db: dry basis

Table 3: Farinographic and amylographic characteristics of flour

Characteristics	Quantity
Water absorption (%)	64.0
Arrival time (min)	2.0
Departure time (min)	9.0
Peak time (min)	3.0
Dough stability (min)	7.0
Softening of the dough (BU)	50.0
Tolerance index (BU)	30.0
Amylographic (BU)	1500.0

Table 4: Effect of different treatments on volume, weight and weight to volume ratio of bread

Treatments	Volume (CC)	Weight (g)	Weight to volume ratio
T_0	585^{b}	153	3.83^{ab}
T_1	587^{ab}	152	3.86^{a}
T_2	588^{a}	153	3.84^{b}
T_3	590^{a}	154	3.80^{c}
T_4	583^{bc}	153	3.81^{cd}

Table 5: Effect of lactic acid starter culture on sensory characteristics of bread

Characteristics (score)	T_0	T_1	T_2	T_3	T_4
Volume (10)	8.2^{b}	8.5^{a}	8.0^{c}	8.0^{c}	8.0^{c}
Crust Colour (8)	7.0^{b}	7.5^{a}	6.8^{c}	6.9^{cd}	7.0^{b}
Symmetry of form (5)	4.0^{c}	4.5^{a}	4.2^{b}	4.3^{b}	4.2^{b}
Evenness of bake (3)	2.2	2.2	2.3	2.2	2.3
Character of crust (4)	3.5^{b}	3.8^{a}	3.7^{a}	3.3^{c}	3.3^{c}
Grain of bread (15)	13.2^{c}	13.7^{b}	13.4^{c}	13.4^{c}	14.0^{a}
Colour of crumb (10)	8.5^{c}	8.9^{a}	8.8^{b}	8.8^{b}	8.5^{c}
Aroma (10)	8.0^{d}	8.7^{a}	8.4^{b}	8.2^{c}	8.2^{c}
Taste (20)	16.0^{c}	18.0^{a}	17.0^{b}	16.5^{c}	16.2^{d}
Texture (15)	11.8^{d}	12.5^{b}	12.0^{c}	11.3^{e}	12.8^{a}

Bread making process: Bread was prepared with some modification in the method as described in AACC (2000), by using lactic acid bacteria (*Lactobacillus bulgaricus*). Flour (100 g), sugar (4 g), oil (4 g), salt (1 g), water (64 ml; farinographic water absorption) and lactic acid starter culture/yeast were mixed together with Hobart mixer. Lactic acid starter culture was used in different proportions (Table 1). The dough was kneaded till clean up stage and allowed to ferment for two and a half hours. Then the dough was divided into 100 g dough ball, moulded, placed in a pan and kept in a proofer at 30°C and 85% relative humidity for 60 minutes. The bread was baked in a gas heated oven at 220°C for 15 minutes. The bread was depanned and allowed to cool for 2 hours. The bread was sliced and packed in a polythene bag.

Objective evaluation of bread: Bread was evaluated for its weight, volume and weight to volume ratio. The volume of a loaf was measured by rape seed displacement method using loaf volume meter (AACC, 2000) and weight of fresh loaf was measured with a digital balance.

Sensory evaluation of bread: The bread was evaluated for sensory characteristics at 0, 24, 48, 72, 96 h storage intervals by panel of judges (Land and Shepher, 1988).

Microbial identification: Total bacterial and mould colony counts and identification of moulds were carried out at 0, 24, 48, 72, 96 h storage intervals (Cappuccino and Sherman, 1996).

Statistical analysis: Data obtained on different parameters were subjected to statistical analysis using analysis of variance technique. When F-test was statistically significant a least significant difference ($LSD_{0.05}$) was used to test the treatments effect (Steel *et al.*, 1997).

Results and Discussion

Wheat flour contained moisture 12.20%, crude protein 12.87%, crude fibre 0.54%, crude fat 0.94%, ash 0.65% and NFE 85% (Table 2). The results were in close agreement with the findings of Ali (1980) who used amylolytic enzymes in wheat flour. The data on rheological studies are presented in Table 3. The perusal of the farinogram showed that flour had 64% water absorption, 2.0 min arrival time, 9.0 min departure time, 0.7 min dough stability, 3.0 min peak time, 30 B.U tolerance index and 50 B.U. softening of the dough.

The results pertaining to objective assessment of bread are given in Table 4. Loaf volume showed significant variation as result of treatments. T_1 and T_3 showed maximum volume followed by T_2 and T_4 which produced minimum volume. Weight to volume ratio showed significant differences among treatments. Analysis showed that individual LAB used as starter culture was able to produce a characteristic sourdough. As the application of sourdough had a positive impact on bread volume (Clarke *et al.*, 2002), it could be used in bread making process in combination with yeast in order to provide bread with desirable qualities (Robert *et al.*, 2006).

Table 6: Effect of storage on sensory characteristics of bread

Characteristics	0 h	24 h	48 h	72 h	96 h
Volume	8.20^{a}	8.03^{a}	7.72^{b}	7.48^{bc}	7.36^{c}
Crust Colour	7.04^{a}	6.84^{ab}	6.84^{ab}	6.66^{b}	6.36^{c}
Symmetry of form	4.14^{a}	3.98^{b}	3.76^{c}	3.58^{d}	3.22^{c}
Character of crust	3.64^{a}	3.44^{b}	3.30^{c}	3.12^{d}	2.94^{c}
Grain of bread	13.32^{a}	13.14^{ab}	13.00^{ab}	12.88^{b}	12.18^{c}
Colour of crumb	8.54^{a}	8.46^{a}	8.42^{a}	7.78^{b}	7.400^{c}
Aroma	7.46^{a}	8.40^{a}	7.58^{b}	7.08^{c}	6.30^{d}
Taste	15.64^{a}	16.46^{ab}	16.10^{b}	14.82^{c}	12.88^{d}
Texture	10.56^{a}	11.40^{a}	11.06^{b}	10.66^{c}	10.02^{d}

Table 7: Total bacterial count of bread at different storage intervals

Treatments	0 h	24 h	48 h	72 h	96 h
T_0	5×10^{1}	7×10^{1}	1.9×10^{2}	2.5×10^{2}	3×10^{2}
T_1	-	-	5.3×10^{1}	9.1×10^{1}	2×10^{2}
T_2	-	-	5.5×10^{1}	9.5×10^{1}	2.3×10^{2}
T_3	-	-	8.9×10^{1}	2.1×10^{2}	2.4×10^{2}
T_4	-	4×10^{1}	1.2×10^{2}	2×10^{2}	2.7×10^{2}

Table 8: Total mould count of bread at different storage intervals

Treatment	0 h	24 h	48 h	72 h	96 h
T_0	1.5×10^{2}	1.8×10^{2}	2.5×10^{2}	2.8×10^{2}	3.9×10^{2}
T_1	5×10^{1}	9×10^{1}	1×10^{2}	2.4×10^{2}	3.3×10^{2}
T_2	9×10^{1}	1×10^{2}	1.6×10^{1}	2.1×10^{2}	2.3×10^{2}
T_3	7×10^{1}	1.2×10^{2}	2.5×10^{2}	2.5×10^{2}	2.5×10^{2}
T_4	1×10^{2}	1.4×10^{2}	2.2×10^{2}	2.6×10^{2}	3.3×10^{2}

The breads prepared from different treatments were sensory evaluated for internal and external characteristics (Table 5, 6). The results revealed that the sensory characteristics of bread including volume of bread, crust colour, symmetry of form, character of crust, grain of bread, crumb colour, aroma, taste and texture were significantly affected by the different treatments (Corsetti *et al.*, 2000). Maximum score for volume was noted in treatments T_1 and minimum in treatments T_2, T_3 and T_4. Maximum score for colour of crust was noted in T_1 and minimum inT_2. The effect of T_4 was found non-significant with respect to T_0. In ranking order, the mean value for T_1 was at the top followed by T_3 and T_4, T_2 and T_0 for the symmetry of form of bread loaf. There was no effect of treatments and storage intervals on evenness of bake. For the character of crust, the T_1 was placed at the top followed by T_2 and T_4. Although, T_4 was most effective for grain formation but grain was adversely affected during storage. This might be due to staling effect. T_0 was found to be least effective for grain formation. It has been generally acknowledged that holes of relatively small size were preferred in bakery products, whereas, large voids crumb distributions were undesirable (Cauvain, 1998).T_1 was found best for the colour of bread crumb as well as for pleasant bread aroma. It was found that the taste of T_1 was observed best followed by T_2. Minimum score was obtained by T_0. T_4 was found best for texture of bread followed by T_1 and T_2. The score for various characteristics of bread decreased as storage period increased except evenness of bake which remained unchanged. The results are in close agreement with the findings of Maleki *et al.* (1980) who reported that larger loaf size produced softer bread. Moreover, the sourdough breads showed to have lower crumb firmness values (Corsetti *et al.*, 2000; Clarke *et al.*, 2002).

The data on the bacterial colony counts in breads at different storage intervals are given in Table 7. Maximum numbers of bacterial colonies were observed in T_0. T_1 proved to be the most effective in inhibiting bacterial spoilage in bread. The colonies appeared after 48 h of storage in T_1, T_2 and T_3. In T_4, bacterial colonies appeared after 24 h (4×10^{1} cfu/g) and increased (2.7×10^{2} cfu/g) in bread at 96 h of storage. It indicated that lactic acid starter culture (*L.bulgaricus*) was more effective against the growth of other bacteria in bread (Menteş *et al.*, 2007).

The mould colony counts in bread were observed at different storage intervals on saboraud's agar media (Table 8). Maximum number of mould colonies were recorded in bread (T_0) fermented only with yeast culture. T_1 proved to be the most effective in inhibiting mould spoilage in bread followed by T_2 (2 ml lactic acid starter culture+0.25% yeast) and T_3 (1.5 ml lactic acid starter culture+0.5% yeast). Total colony count at 96 h of storage in treatment T_2 was 2.3×10^{2} cfu/g of bread. The results showed that sourdoughs of different combinations of lactic acid starter culture and yeast were effective in enhancing the shelf life of bread by inhibiting the mould growth. The antifungal phenomenon might be due to the production of organic acids by lactic acid starter culture during sourdough fermentation (Röcken and Voysey, 1995). The results suggest that use of sourdough in bread production is beneficial in improving sensory properties, delaying firmness and preventing mould and bacterial spoilage (Martinez *et al.*, 1990; Hammes and Gänzle, 1995; Gobbetti, 1998). Microscopic examination and staining of the samples with lactophenol cotton blue or lectophenol picric acid revealed that majority of the moulds; isolated during study belonged to *Aspergillus flavous*, *Peniciellium* and *Rhizopus* in all the spoiled breads.

Conclusion: It was concluded that T_1 (*Lactobacillus bulgaricus*) was most effective to inhibit microbial spoilage and extended the shelf life of bread. Sourdough affected the physical property of bread such as volume which was assessed objectively. However, the key differences between control and sourdough breads were observed in sensory characteristics i.e. Aroma, taste and texture.

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