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Effect of Using Sourdough and Frozen Dough for Preparation of Breads on Quality, Shelf Life and Staling

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ABSTRACT
The effect of sourdough and frozen dough on the shelf life and staling rate of bread was studied. In the present study malted barley and fermented milk was used for the preparation of sourdough and 25% of normal dough was substituted with sourdough for preparation of sourdough bread. Frozen dough was prepared by subsequent freezing and thawing of the dough. The prepared breads were tested for their overall characteristics. The present study found that the bread volume, crust and crumb structure, softness, texture, palatability and overall acceptability of the sourdough bread were better than the frozen dough incorporated and the normal bread. The bread prepared from the frozen dough also had a higher acceptability than the control one. The staling was least in the sourdough bread followed by the frozen dough bread and the greatest staling was observed in the control bread. Also the sourdough incorporated bread registered the highest shelf life of 8-9 days.

Key words: Sourdough, frozen dough, shelf life, staling

INTRODUCTION
The baking of breads represents one of the oldest biotechnological processes. Bread is a staple food around the world that is prepared by baking dough of wheat flour and water. It may be leavened or unleavened. Dough is usually leavened by bread yeast, which ferments 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 (Vollmar and Meuser, 1992; Bratovanova, 1996). The development of leavened bread can probably also be traced to prehistoric times. Sliced bread began to be developed from 1912 onwards. Fresh bread is prized for its taste, aroma and texture. Retaining its freshness is important to keep it appetizing. Bread that has stiffened or dried past its prime is said to be stale. The loss of perceived freshness is due to a number of factors, which may generally be categorized into one of the two groups, those that are due to a series of complex processes collectively known as staling and those that are attributed to microbial spoilage. A number of methods are applied to prevent or minimize staling and microbial spoilage of bread, e.g., addition of propionic acid and its salts, modified atmospheric packaging, irradiation, pasteurization of packaged bread (Dal Bello *et al.*, 2007) or biopreservation. Recently there has been an increasing interest in the application of biopreservation in the food industry. In this regard, LAB and in particular lactobacilli, are of special interest, since they have a long history of use in food and are

'generally regarded as safe'. Beside the weak organic acids, i.e., lactic and acetic acids (Gerez *et al.*, 2007), LAB produce a wide range of low molecular weight compounds and proteins with antifungal activity.

The application of Lactic Acid Bacteria (LAB) in the form of sourdough has been reported to have positive effects on wheat bread quality and staling (Dal Bello *et al.*, 2007). Sourdough is a mixture of flour and water that is dominated by a complex microflora composed of yeasts and lactic acid bacteria that are crucial in the preparation of bread dough. Lactic acid bacteria cause acidification by producing lactic acid that increases the shelf life of bread by preventing the growth of undesirable microorganisms and affects the nutritional value of bread by increasing the availability of minerals. The synergistic metabolic activities of microorganisms produce acidification or souring influencing the final characters of bread, notably the texture (Martinez-Anaya *et al.*, 1990; Rocken and Voysey, 1995; Corsetti *et al.*, 2000) and generate typical flavour compounds yielding typical sourdough sensory attributes (Gobbetti, 1998; Katina *et al.*, 2006). Associations of yeasts and LAB appear to be self-protecting and self-regulating. Compared to heterofermentative LAB, the homofermentative ones show greater inhibitory effects to coliforms (Ganzle *et al.*, 1998; Hansen and Schieberle, 2005; Gul *et al.*, 2005; Katina *et al.*, 2005; Simsek *et al.*, 2006). LAB such as *Lactobacillus delbrueckii* ssp., *bulgaricus* and *Lactobacillus helveticus* and the yeast *Kluyveromyces marxianus* have not been much tested as for sourdough bread making, although, the natural sourdough microflora comprises them (Plessas *et al.*, 2008). *L. delbrueckii* and *L. bulgaricus* is an important probiotic species and is currently used in the production of fermented milk products. Today, sourdough baking is an alternative to the use of additives (Katina *et al.*, 2005; Thiele *et al.*, 2003). Katina *et al.* (2005) have stated that sourdough fermentation can modify the healthiness of cereals in a number of ways, including improvement of texture and palatability of whole grain products, enrichment in fibre or reduction of gluten, stabilisation or increase of various bioactive compounds, improvement of mineral bioavailability, etc.

The need for increasing the shelf life of bread has made the bakery industry to exploit all possible technologies like modified atmospheric packaging, freezing, etc. (Degirmencioglu *et al.*, 2011) of which, freezing process plays a major role (Selomulyo and Zhou, 2007). Application of freezing temperatures to the bread making process is a major alternative for the bread industry (Barcenas *et al.*, 2003). Frozen dough has become an increasingly popular alternative to that made directly from unfrozen dough. The products made from frozen dough do not require specialized workers and hence make them to be available fresh at any time of the day (Matuda *et al.*, 2005). Bread quality made from frozen dough depends on a number of factors like freezing temperature, storage time, thawing rate etc., (Neyreneuf and Delpuech, 1993). Though there are some problems associated with frozen dough like long proof time, low volume, poor texture and variable performance, (Kenny *et al.*, 1999) but these problems have been mostly overcome by the advances in technology and formulation.

Therefore, the objectives of the present investigation were to study the effect of sourdough and frozen dough on various quality parameters of bread and on the shelf life and staling of bread.

MATERIALS AND METHODS

Preparation of the experimental breads: All raw materials and chemicals for preparation of breads were supplied by Modern Foods India Limited. Bread was prepared following AACC (1983) method. An amount of 0.4% NaCl, 5% yeast was added to 250 g of maida (refined wheat flour) and were mixed for 15 min. The amount of water added was according to the water absorption

(Sadeghi *et al*., 2008). The dough was left for bulk fermentation at 30°C for 15 min. After fermentation, the dough was rounded before molding by hand. The molded dough was then proofed again for another 30 min. The proofed dough was then baked at 250°C for 30 min. The control for the sourdough and frozen dough breads was the wheat bread without the sourdough or the frozen dough.

Sourdough preparation from barley (MBIB1) (bread prepared by inoculating with 7.5% mash) Preparation of malt involved steeping of the barley grains (at 14°C) followed by germinating (at 16-21°C for 5-7 days) and then finally kiln drying. The sprouts were then removed and finally malt was obtained. The malt was then mixed in water (to form the mash) and then left for overnight fermentation (which decreased the initial pH of the solution from 7.0-3.5). The mash (7.5%) was then incorporated to the dough which was let to ferment further for a period of 15-17 h to obtain the sourdough. After the preparation of sourdough, 25% of control dough was substituted by sourdough for preparation of bread.

Sourdough preparation from barley (MBIB2) (along with a further chemical acidification): The process of preparation of the mash was the same with the only difference that here the acidity was further lowered (to pH 4.5) by chemical acidification, i.e., by the addition of lactic acid to the mash. The mash was then allowed to ferment overnight which decreased the pH to 2.2. This low acidic mash was then incorporated to the dough to make sourdough.

Sourdough preparation from barley (MBIB3) (by inoculating in milk): The process of preparation of malt was same. But for the preparation of the mash instead of inoculating the malt in water it was done in milk. The preparation of the sourdough was same as in the other two cases.

Preparation of the frozen dough (FDIB): The dough was prepared in the same way as that for the normal bread. After preparation of the dough it was kept in the refrigerator at -5°C overnight. After that the dough was let to thaw at 25°C. This dough was used for the preparation of the bread.

Determination of physical quality characteristics, shelf life and staling of bread: Bread volume was measured by the rapeseed displacement method (Hallen *et al*., 2004). Dough raising capacity was measured by volumetric method. The shelf life of the bread was determined as per the method described by Wassermanns (1969). The prepared breads were packed and stored at room temperature (25±2°C) and at a relative humidity of 75±1%. Staling was determined by the loss of moisture content of the breads over a period of time.

Chemical analysis: The breads thus prepared were then analyzed for moisture content, ash content and alcoholic acidity (AOAC, 1995).

Microbiological analysis: Microbiological shelf lives of samples kept at room temperature of 25±3°C were determined by plating serial dilutions on to PDA (potato dextrose agar) media (Katina *et al*., 2002). Microbes in the sourdough was identified and isolated by using the MRS and PDA media.

Sensory analysis: Sensory evaluation of bread was conducted using a 9-point hedonic scale rating as per Vulicevic *et al*. (2004). The texture "grain of crumb" was judged through the sense of touch and by observing crumb structure. The flavour/aroma "aroma of crumb" was judged by the sense of smell and taste. The mouthfeel "texture of crumb" was judged by the tactile character and eating quality (compressibility, resilience, elasticity, mouthfeel and moistness) of the bread. The coded samples per bread type were presented individually to 20 panelists. Water was used for mouth rinsing before and after each sample testing.

Statistical analysis: Five replications were conducted for all the experiments. Single factor Analysis of Variance (ANOVA) was conducted using Microsoft Excel. Fisher's Least Significant Difference (LSD) was applied to observe the statistical difference between the treatment conditions for the responses.

RESULTS AND DISCUSSION

Effect of sourdough and frozen dough on physical and chemical quality of bread: The physical and chemical characteristics of breads prepared by various sourdoughs and frozen dough are presented in Table 1. The study found that the quality of the wheat bread improved by the addition of the sourdough and frozen dough. The metabolic products of the yeasts and the LAB improved the properties of the flour as well as the aroma, taste, nutritive value and the shelf life of the bread. The present study confirmed that addition of the sour dough caused a distinct change in the properties of the dough. This can be attributed to the fermentation of the dough by the *Lactobacillus* species. Activity of *Lactobacillus* cultures against bacteria and fungi is widely observed and frequently reported. Isolation of antimicrobial compounds from cell-free supernatant identified lactic acid, phenyllactic acid and the two cyclic dipeptides cyclo (l-Leu-l-Pro) and cyclo (l-Phe-l-Pro) are the major components responsible for this activity (Strom *et al*., 2002; Dal Bello *et al*., 2007).

Improved volume of sourdough breads has been suggested to be dependent on the nature and intensity of the acidification process (Clarke *et al*., 2002). Studies on the analysis of loaf volume, confirmed that the sour dough addition in the bread caused a more pronounced volume than the normal bread. Similar results were also observed by Corsetti *et al*. (2000) in sourdough bread. Also, the frozen dough bread had a volume more than that of the normal bread but lesser than the sourdough incorporated bread. The positive effect of sourdough in bread volume has been linked to better gas holding capacity of gluten in acidic dough containing sourdough and frozen dough (Gobbetti *et al*., 1995).

Table 1: Different physical and chemical quality characteristics of the experimental breads

	Formulations				
Quality characteristics	Normal bread*	MBIB1	MBIB2	MBIB3	FDIB
Dough raising capacity (%)	1.2±0.03$^{b\#}$	1.4±0.01^{c}	1.1±0.04^{a}	1.1±0.04^{a}	1.1±0.03^{a}
Loaf volume (mL)	310.0±5.25^{a}	340.0±4.47^{d}	335.0±3.16^{cd}	333.0±3.39^{c}	316.0±2.92^{b}
Ash (%)	1.4±0.03^{a}	1.6±0.02^{c}	1.4±0.03^{a}	1.5±0.02^{b}	1.4±0.03^{a}
Alcoholic acidity (%) (Initial)	6.3±0.06^{b}	6.1±0.08^{a}	7.1±0.11^{d}	6.9±0.09^{c}	6.3±0.10^{b}
Alcoholic acidity (%) (Final)	10.0±0.10^{d}	9.0±0.13^{b}	8.6±0.09^{a}	9.4±0.12^{c}	9.5±0.10^{c}
Shelf life (days)	5.0±0.44^{a}	9.0±0.70^{d}	8.0±0.70^{c}	8.0±0.44^{c}	6.0±0.44^{b}

MBIB1: Malted barley incorporated bread (mash inoculated with water), MBIB2: Malted barley incorporated bread (acidic mash), MBIB3: Malted barley incorporated bread (mash inoculated with milk), FDB: Bread prepared from frozen dough, values of responses are expressed as Mean±SD (n = 5), *Bread prepared without incorporation of sourdough or frozen dough, #Values of responses followed by same letter in a row are not significantly different (p>0.05)

A continuous decrease in the moisture content of crumb was noticed. The study found that bread prepared from the frozen dough had the highest initial (40.95%) and the final (38.65%) moisture content. On the other hand the loss of moisture content in case of MBIB2 bread was found to be minimum (4.87%).

Alcoholic acidity determines the rate of chemical changes taking place within the bread. The alcoholic acidity was found to be the highest in the MBIB2 bread (7.05%) and the lowest in the MBIB1 bread (6.13%).

Ash content was recorded the highest in the MBIB1 bread (1.58%) and the lowest in the normal bread (1.35%).

Effect of sour dough and frozen dough on staling of bread: The degree of staling was measured by the difference of the initial and final moisture content of the crust and crumb of the bread. It was found that the MBIB2 bread produced the least staling in the crumb moisture amongst all the experimental breads followed by the FDIB bread, MBIB3 bread, MBIB1 bread, whereas the normal bread noticed the highest staling (Table 2). Similarly crust staling was observed least in the MBIB2 bread followed by the FDIB bread, MBIB3 bread, MBIB2 bread and the highest staling in the normal bread. The staling of crust is defined as the gaining of moisture from the environment and crumb, resulting in the formation of leathery crust. Moisture will migrate from the centre of the bread to the surface by the mechanism, called diffusion. Sourdough fermentation was effective in delaying starch retrogradation. The effect depended on the level of acidification and on the lactic acid bacteria strain (Corsetti *et al*., 2000). Biological acidification may aid in maintaining bread freshness because it influences moisture redistribution throughout the loaf during storage (Corsetti *et al*., 2000).

Effect of sour dough and frozen dough on shelf life and microbial quality of bread: Shelf life analysis (Table 1) showed that the MBIB1 bread had the highest shelf life (9 days) followed by the MBIB2 and the MBIB3 bread (8 days each) and the FDIB bread (6 days). The control sample, non-fermented with sourdough, had the lowest microbiological shelf life (5 days). Similar results were observed in the case of sourdough bread by Sadeghi *et al*. (2008). Dal Bello *et al*. (2007) found that the microorganisms in the sourdough preparation produce a wide range of low molecular weight compounds and proteins with antifungal activity. Also the production of the organic acids by those microorganisms was capable of increasing the shelf life of the bread (Gerez *et al*., 2007).

Table 2: Loss of moisture (% wet basis) of experimental breads during storage at room temperature of 25±2°C

	Crust		Crumb		Overall	
Bread samples	5th day	8th day	5th day	8th day	5th day	8th day
Normal bread*	$-0.64\pm0.03^{\$a\#}$	--	8.2 ± 0.11^{e}	--	8.2 ± 0.11^{d}	--
MBIB1	-0.24 ± 0.03^{b}	-0.12 ± 0.04^{a}	5.8 ± 0.10^{d}	6.1 ± 0.09^{c}	5.7 ± 0.11^{c}	6.1 ± 0.10^{d}
MBIB2	-0.11 ± 0.05^{d}	-0.09 ± 0.03^{ab}	4.4 ± 0.08^{a}	4.9 ± 0.09^{a}	4.4 ± 0.10^{a}	4.9 ± 0.13^{a}
MBIB3	-0.19 ± 0.04^{bc}	-0.07 ± 0.03^{b}	5.6 ± 0.09^{c}	5.9 ± 0.15^{b}	5.6 ± 0.12^{c}	5.9 ± 0.09^{c}
FDIB	-0.16 ± 0.03^{cd}	-0.05 ± 0.03^{b}	5.3 ± 0.11^{b}	6.6 ± 0.15^{d}	5.3 ± 0.15^{b}	5.6 ± 0.10^{b}

MBIB1: Malted barley incorporated bread (mash inoculated with water), MBIB2: Malted barley incorporated bread (acidic mash), MBIB3: Malted barley incorporated bread (mash inoculated with milk), FDB: Bread prepared from frozen dough, values of responses are expressed as Mean±SD (n = 5), *Bread prepared without incorporation of sourdough or frozen dough, $Negative (-ve) sign indicates gaining of moisture, # Values of responses followed by same letter in a column are not significantly different (p>0.05)

Table 3: Sensory quality score of experimental breads (9-point hedonic scale, n = 20 panelists)

Parameters	Normal Bread*	MBIB1	MBIB2	MBIB3	FDIB
Colour	6.0±1.48$^{a\#}$	7.0±0.89^{b}	6.5±1.34^{a}	6.5±1.67^{a}	6.5±0.77^{a}
Flavour	5.5±0.95^{a}	7.0±1.22^{c}	6.0±0.95^{ab}	6.5±1.34^{bc}	6.5±1.61^{bc}
Appearance	6.0±0.71^{a}	6.5±0.55^{a}	6.5±0.84^{a}	6.5±1.10^{a}	6.5±1.14^{a}
Softness	5.5±1.26^{a}	6.5±1.34^{b}	6.0±0.95^{ab}	5.5±1.38^{a}	6.0±1.41^{ab}
Taste	5.5±1.70^{a}	7.0±1.34^{b}	7.0±1.45^{b}	6.6±0.55^{b}	6.5±0.95^{b}
Crumb colour	5.4±1.22^{a}	6.5±0.84^{b}	6.2±1.61^{ab}	6.0±1.58^{ab}	6.5±1.10^{b}
Crust colour	6.0±1.64^{a}	6.5±0.71^{a}	6.0±0.45^{a}	6.0±0.89^{a}	6.0±0.32^{a}
Crumb texture	6.0±0.71^{a}	6.9±0.95^{b}	7.0±0.55^{b}	7.0±0.63^{b}	6.0±1.00^{a}
Crust texture	6.0±1.34^{a}	7.0±0.95^{b}	6.3±0.71^{a}	6.5±1.10^{ab}	6.5±1.22^{ab}

MBIB1: Malted barley incorporated bread (mash inoculated with water), MBIB2: Malted barley incorporated bread (acidic mash), MBIB3: Malted barley incorporated bread (mash inoculated with milk), FDB: Bread prepared from frozen dough, values of responses are expressed as Mean±SD (n = 20), *Bread prepared without incorporation of sourdough or frozen dough, #Values of responses followed by same letter in a row are not significantly different (p>0.05)

The use of sourdough has been established as very important in prolonging the shelf life of bread (Ganzle *et al*., 1998). The enhanced shelf life in case of FDIB bread was due to the availability of lower free water content. The free water content is mainly responsible for the microbial growth and as it is low in bread the shelf life is comparatively more.

The micro-organisms in the mash produced during fermentation of the barley were identified as the Lactic Acid Bacteria (LAB). Further plating using the MRS media showed them to be *Lactobacillus plantarum*. Also yeast colonies were detected in the sourdough upon plating onto PDA media. The results for mould colonies during storage at ambient temperature have shown that there is a variation among the different incorporated breads. The highest yeast and mould count was found in the FDIB bread (20×10^{3} CFU mL^{-1}) followed by MBIB3 bread (15×10^{3} CFU mL^{-1}), normal bread (14×10^{3} CFU mL^{-1}) and the MBIB2 bread (10×10^{3} CFU mL^{-1}).

Effect of sour dough and frozen dough on sensory quality of bread: Sensory results (Table 3) revealed that the sourdough incorporated bread had the highest acceptance generally followed by frozen dough and then the normal bread. The overall sensory characteristics like colour, texture, appearance, softness etc., were the most acceptable in the sourdough incorporated bread. It was found by earlier workers that 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 (Salim-ur-Rehman *et al*., 2007). Controlled acidity level of wheat sourdough bread is premise for improved sensory properties.

CONCLUSION

The research on improvement in quality and shelf life of bread has always been an area of interest. The study evinced the influence of sourdough and frozen dough in the reduction of staling and increasing the shelf life of the bread. Based on the observations it was found that the sourdough bread was successful in improving the overall characteristics of the bread. The results suggest that use of sourdough and frozen dough in bread production is beneficial in improving sensory properties, delaying firmness and preventing mould and bacterial spoilage.

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