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Use of sourdough *lactobacillus plantarum* (ATCC 43332) to improve the quality and shelf life of toast soy bread

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ABSTRACT

Soy ingredients that have unique functional and nutritional properties have found wide application in bakery products .During storage of bread, several different physical and microbiological changes occur, lowering the quality of bread. The bread crumb becomes hard, the bread crust changes from crispy to leathery and the characteristic and favorable bread flavor disappears. Addition of sourdough in the bread recipe can be used to retard the staling process of the bread. Sourdough addition is the most promising procedure to preserve bread from spoilage, since it is in agreement with the consumer (demand for natural and additive free food) products. In this research, defatted soybean flour in 3, 5 and 10 % was mixed with wheat flour. For sourdough preparation, fresh microbial cells were collected by centrifugation from LAB primary cultures, and with wheat flour and tap water and 0.25% (w/w) active dry yeast extract, containing Saccharomyces cerevisiae were mixed, then sourdough Lactobacillus Plantarum (ATCC43332) was added to each of soy and wheat flour mixture in amount of 25% of flour weight. Specific volume, sensory characteristics, molds spoilage and firmness of bread, were measured in the period of 0,24,48 and72 hours after baking bread. In comparison with control bread, Specific volume in all of blends decreased. Results showed that by adding 25% sourdough, the rate of molds spoilage of bread decreased. During the storage of breads, increase in firmness was suppressed by adding soy flour and sourdough. The results showed that sourdough had significant effect ($p<0.05$) on shelf life of soy bread in comparison with control sample. Finally the results of sensory evaluation showed that toast bread which contains 5% soybean flour and sourdough, has the most acceptability regarding to the other samples.

Keywords: Lactic acid bacteria, Sourdough, Staling, Shelf life bread, Soy bread

INTRODUCTION

The unique properties of wheat flour to form a viscoelastic dough which is able to retain gas are due to protein characteristics of wheat gluten when it is mixed with water. However, wheat flour is considered nutritionally poor, as the cereal proteins are deficient in essential amino acids such as lysine and threonine [29,34]. Soy is the only legume that contains the nine essential amino acids in the correct proportion for human health. Therefore, soy protein is categorized as high quality complete protein. Also soy is a better source of tocopherols , an excellent natural antioxidant and B-vitamins compared to cereals, although it lacks B_{12} and vitamin C. Defatted soy flour, generally by a hexane extraction, is one of the richest isoflavone sources, with levels as high as 2.0 mg per gram of soy protein [20].Soy flour has three basic functions: it gives a white bread crumb, it contributes to gas retention through oxidation and it increases the level of water that needs to be added to the dough[18]. However, when defatted soybean flour was used, only about 10% soybean flour could be added successfully to the product, when incorporated simultaneously with higher amounts of water[25]. Soy isolate –whey blends have successfully replaced up to 100% of the nonfat dry milk in shortened cake products without affecting quality as long as the formula included additional water[17]. Lactic acid bacteria (LAB) are the most prominent non-pathogenic bacteria that play a vital role in our everyday life, from fermentation, preservation, and production of wholesome foods, and vitamins to prevention of certain diseases and cancer due to their antimicrobial action[33]. Sourdough has been used for leavening of bread dough for several hundreds of years, and sourdough bread was made in Egypt as early as 3000 B.C.[37].There is considerable consensus with regard to positive effects of sourdough addition for bread production, including improvement in bread volume and crumb structure[7,9] flavor[36], shelf-life[13].Sourdough fermentation with interaction of lactic acid bacteria and yeasts is considered to play a key role to get improved flavor and texture properties of bakery products[21]. The accumulation of amino acids during sourdough fermentation enhances the formation of flavor volatiles during baking[8].The use of selected LAB with specific activities like phytate degrading enzymes as starters for bread making could be a good alternative for obtaining whole wheat bread with low phytate content and in consequence with increased nutritional value regarding mineral bioavailability[11].Bakery products have a very short shelf-life and their quality is dependent on the period of time between baking and consumption. Firming is one of the most apparent changes occurring in bread during staling, which has been traditionally used to characterize staling rate. Factors such as retrogradation of amylopectin and changes in water states have been linked to the increased firming during prolonged storage[25].Changes during staling occur in both the crumb and the crust of the bread .The crust loses its brilliance and becomes dull, while its crispness also disappears. It may either harden or soften, depending on storage humidity, and it becomes less pleasant to the taste. The crumb loses it supple and mellow qualities and becomes more opaque. It also becomes granular or grainy to the touch, with a tendency to crumble. It is less easily moistened, and consequently is less pleasant to chew, while the taste changes and loses much of its appeal [2,6]. In some investigation, the addition of sourdough resulted in lower bread firming. However, sourdough wheat bread has higher bread volume and the measured resistance will thus be lower[21].Corsetti *et al*., (2007) showed that the addition of sourdough to wheat bread reduced crumb firmness and slowed down firming in comparison with breads made with no addition of sourdough[10].Bread and bakery products can be contaminated by a variety of molds, mainly *Aspergillus* and *Penicillium* [35],but sourdough is capable of controlling and inhibiting spoilage organisms during fermentation, due to different factors especially low pH value and antimicrobial compounds produced with LAB.

Dal Bello *et al*.,(2007) reported that sourdoughs and breads produced with *Lactobacillus plantarum* showed consistent ability to retard the growth of mold spoilage microorganisms[13]. Moore *et al*.,(2008) showed that *L.plantarum* FST 1.7 can be used to produce gluten-free bread with increased quality and shelf-life[27].

The aim of this study was to evaluate the effects of sourdough *lactobacillus plantarum* (ATCC43332) to improve the quality and shelf life of toast soy bread.

MATERIALS AND METHODS

Initial ingredients

Wheat flour with extraction degree of 68% was prepared from Sahar Bread Co., defatted soy flour was purchased from Behpak Co., bakery dried yeast (*Saccharomyces Cerevisiae*) was prepared from Iran Molasses Co., vacuum dried of culture *Lactobasillus Plantarum* (ATCC 43332) was obtained from scientific and industrial research center of Iran. Treatments include: C: control (Wheat flour) , $S_{1:}$ 3% soy flour, $S_{2:}$ 5% soy flour, $S_{3\,:}$10% soy flour, $SD_{1\,:}$ 3% soy flour + 25% sourdough, $SD_{2\,:}$5% soy flour + 25% sourdough and $SD_{3\,:}$ 10% soy flour + 25% sourdough.

Chemical tests of wheat flour and soy flour

Chemical tests of the wheat flour were used: moisture, 13.33 % (according to international standard AACC 44-16), ash, 0.46% (according to international standard AACC 08-01), protein (N.5.7), 10.62 (according to international standard AACC 46-12), wet gluten, 29.75 (according to ICC 38-11), pH, 5.9 (according to AACC 52-02) and sedimentation value 35(according to AACC 116). Chemical tests were used of soy flour included: fat, 1.433% and fiber, 5.99 (according to AACC 32-10), moisture, 7.652% ash ,6.53% and protein (N .6.2) 53.13% (according to the said standard methods)[1,22].

Sourdough preparation method

At first, vacuum dried of *Lactobasillus Plantarum* (ATCC 43332) was transferred to culture environment of "Sourdough Media Broth" and then was incubated at 32°C for 48 hours according to direction issued by Iran industry and scientific research center. Biomass from actively growing lactic acid bacteria culture was harvested by centrifugation (5000g, 15 min and 4°C) washed twice and resuspended in 180 ml sterile tap water that was immediately mixed with 300g of wheat flour and o.25% active dry yeast extract, containing *S.cerevisiae* (w/w) of flour, until dough formation. Fresh cells were added to sourdough at a level 10^8 cfu/ml [13,24], and the LAB cell counts were determined by spectrophotometer. Wheat sourdough was prepared with the mentioned strain. The dough was covered and fermented at 32°C for 24h [31]. Finally 25% sourdough by soy and wheat flour weight was added to each blend with three percents of 3, 5 and 10%. The mixture was stirred for an additional a few minutes (60 rpm).

Baking test

At first, soy flour was added in amount of 3,5 and 10% to wheat flour, then sourdough mixture to each of soy and wheat flour in amount of 25% of flour weight with NaCl , sugar , oil and active dry yeast extract, containing *S. cerevisiae* mixed at 60 rpm for 20- 25 min. The amount of water was adjusted according to the water absorption (60%) determined by farinography [8].The dough was left for bulk fermentation for 30 min at 30°C and 80% relative humidity. Then the dough was re-mixed and was replaced for re-fermentation for 45min.Baking for each 400g dough piece was at 220 -230 °C for 45 min and then cooled in aseptic condition for 1h.

Organoleptic evaluation

Most acceptable blended breads were used for sensory evaluation. Samples were coded following cooling and cutting. Their organoleptic characteristics were evaluated by panel group (4 judges) for crust color, shape, texture, chewiness, flavor, taste, loaf volume and finally staling of breads were determined in the period of 24,48 and72 hours after baking bread. The highest point for texture and taste was 15, for chewiness, flavor and loaf volume was 10 , crust color was 8 , shape was 3 and finally the highest point for staling was 6.

Crumb firmness measurements

Crumb firmness was measured at 0, 24, 48 and 72h after baking. Bread crumb firmness during storage was determined as maximum compression force (40% compression, AACC 1998 modified method 74-09) Height of each bread slice was 2.5cm and edges of the slice were cut off before measurement.

Specific loaf volume

Specific loaf volume was measured by rape seed displacement method after cooling.

Molds spoilage measurements

Bread mold spoilage were measured according to standard and industry of Iran(10899-2).The breads were stored for 5 days in room condition prior to the application. At first step, liquid Ringer (90ml) were made for each 10g of bread samples and added to the samples. Then after 10 min, products were cultured in Dichloran 18% glycerol Agar according to pour plate method and incubated at 25°C for 3 days .Finally mold counts were determined.

Statistical analysis

The statistical analysis was performed by SPSS V.16 software and using completely randomized design. Duncan's multiple range test was used to determine the differences among means.

RESULTS AND DISCUSSION

Result of staling assay

Staling is an important property which affects consumption and acceptability of the product. Force needed to aggregate produced breads 0, 24, 48 and 72 hours after baking was evaluated by Instron. Based on data presented at table 1, addition of soy flour and sourdough led to a significant decrease in bread firmness during storage. Immediately after baking, control sample showed a significant firmness compared to other treatments also, there was no significant effect between S_2, S_3, SD_1, SD_2 and SD_3.

24 hours after baking control treatment had the highest firmness in comparison with other treatments. In general adding soy flour decreased firmness. Also, 24 hours after baking there was no significant difference between S_2, S_3, SD_1, SD_2 and SD_3 treatments. 48 and 72 hours after baking, bread sample with 10% soy flour was significantly tender than other treatments. This decrease in firmness in attributed to the role of soy flour in staling retardation due to absorption of moisture by protein and ash present at soy flour [5]. By increasing storage time (72h) samples containing soy flour and sourdough had tough texture compared to samples just with soy flour. Based on results obtained from studies of Baraber *et al*.,(1992) after storage firmness of bread samples containing sourdough increased compared to control sample. Acidity of sourdough is responsible for tenderness of bread's texture so that strong acidity leads to firmness of the crumbed [4].

Table1.Staling of breads measured as crumb hardness during 72(h) after baking.

Treatment	0	24(h)	48(h)	72(h)
C	1.15[c]	1.365[d]	1.450[d]	1.465[d]
S_1	0.885[b]	0.9150[c]	0.9500[bc]	1.065[b]
S_2	0.635[ab]	0.6500[ab]	0.8850[b]	1.050[b]
S_3	0.5500[a]	0.5850[a]	0.7350[a]	0.7500[a]
SD_1	0.6220[ab]	0.6850[ab]	1.185[c]	1.25[c]
SD_2	0.560[a]	0.585[a]	0.965[bc]	1.365[cd]
SD_3	0.571[a]	0.665[ab]	0.95[bc]	1.065[b]

Means within column followed by the same letter are not significantly different (P<0.01).

Results of specific volume

Results of specific volumes are presented in table 2.This results showed that enrichment with soy flour decreased volume of bread samples. Control treatment had the highest specific volume and as except with S_1 and SD_2 had a significant difference with the other treatments ($p<0.01$). Among soy flour breads, S_3 treatment had the lowest specific volume, probably due to addition of soy flour. Soy flour has negative rheological effects such as volume reduction (due to weakening gluten network and replacement of soy proteins in the network), enhancement of density of SH groups and creating porosity in dough which leads to decrease in gas holding capacity in gluten network. These results are in agreement with Doxastakis *et al.*, (2002), Roccia *et al.*, (2009) and Maforimbo *et al.*, (2007) [15,26,29]. In addition SD_1 and SD_3 treatments had less specific volume than control treatment ($p<0.01$). Specific volume of treatments with sourdough was higher than treatments without sourdough (S_2, S_3) but this different was not significant. This finding agreed of the report Robert *et al.*, (2006) who studied on wheat bread [28]. However opposite reports on volume reduction [3,12,30,32] and volume enhancement[19] are available. Katina *et al.,* (2006) reported that volume of bread sample varied with acidification, microbial strains and technology used for dough preparation. The amount of organic acids produced during fermentation of sourdough has a significant effect on specific volume since acidic compounds can enhance CO_2 gas holding capacity in gluten. Also, bacteria present in sourdough enhance metabolic activity of yeast thus producing more CO_2 for dough expansion [23].

Although microorganisms in sourdough produce CO_2 gas, but the effect of sourdough on bread volume depends mainly on enzymatic reactions during fermentation. Baraber *et al.,* (1992) reported that excess increase in sourdough acidity led to decrease in bread volume [4].

Table 2. Specific volumes of breads (cm^3/g)

Treatment	C	S_1	S_2	S_3	SD_1	SD_2	SD_3
Specific volumes	585.8[a]	530.3[ab]	495.2[b]	480.1[b]	513[b]	518.4[ab]	509.3[b]

Means within row followed by the same letter are not significantly different (P<0.01).

Results of mold count

Results obtained from mold count are presented at figure 1, the highest count of mold was observed at S_3 treatment followed by S_2 with a significant difference with the other treatments ($p<0.05$). Control treatment had the lowest mold count. By adding soy flour, water absorption and mold counts increased. In general sourdough addition led to significant decrease in mold count, probably due to bacteriocins produced by lactic acid bacteria during fermentation. Dal Bello *et al.,* (2007) and Moore *et al.*, (2008) found similar results[13,27]. Anti- fungal effects of *lactobacillus plantarum* in plates containing bread and sourdough compared to bread without sourdough are indicated at figure 2.

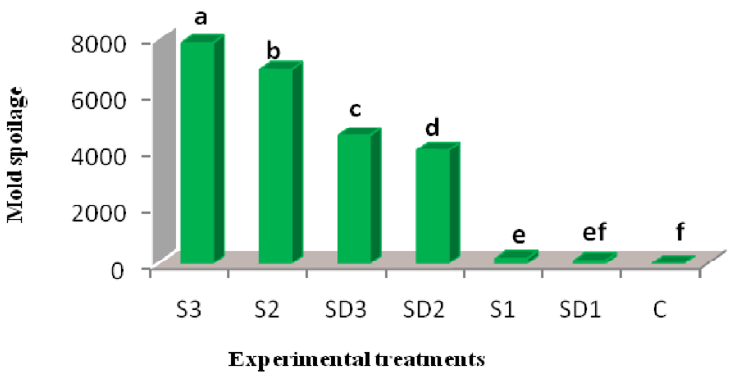

Fig.1. Results of mold count.
Means within column followed by the same letter are not significantly different (P<0.05).

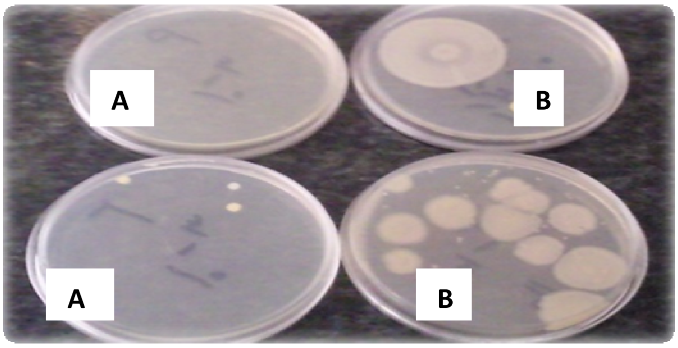

Fig.2. Antifungal effect of sourdough fermented by *L. plantarum* (ATCC 43332) in plates with soy bread and sourdough (A) compared to soy bread without sourdough (B).

Table 3. Sensory evaluation of breads

Treatment	shape	Crust color	chewiness	texture	flavor	taste	Loaf volume
C	2.5^{a}	6.25^{d}	8^{bc}	13.13^{bc}	8.875^{ab}	13^{a}	9^{a}
S_1	2^{a}	7^{bc}	8.5^{ab}	13^{bc}	8.375^{abc}	12.2^{ab}	8.375^{ab}
S_2	2^{a}	7.375^{ab}	8.5^{ab}	12.88^{bc}	8^{bc}	11.88^{ab}	8^{abc}
S_3	1.125^{b}	6.375^{cd}	7.25^{c}	10.63^{d}	7.375^{c}	10.75^{b}	6.625^{cd}
SD_1	2.188^{a}	7.313^{ab}	8.25^{ab}	13.38^{ab}	9^{ab}	10.75^{b}	7.625^{abcd}
SD_2	2.438^{a}	7.875^{a}	8.813^{a}	14.25^{a}	9.313^{a}	13.25^{a}	7.250^{bcd}
SD_3	1.25^{b}	6.5^{cd}	7.313^{c}	12.13^{c}	7.875^{bc}	11.88^{ab}	6.375^{d}

Means within column followed by the same letter are not significantly different (p<0.05).

Table 4. Sensory evaluation staling of breads

Treatment	C	S_1	S_2	S_3	SD_1	SD_2	SD_3
24(h)	4^{b}	5^{a}	4.5^{ab}	4.75^{a}	4.5^{ab}	4.75^{a}	4.75^{a}
48(h)	3.5^{a}	4^{a}	3.75^{a}	3.75^{a}	3.5^{a}	3.5^{a}	3.75^{a}
72(h)	2^{ab}	2.25^{ab}	2.75^{ab}	3^{a}	1.75^{b}	1.75^{b}	2^{ab}

Means within row followed by the same letter are not significantly different (p<0.05).

Results of sensory analysis

As shown at table 3, control treatment had the best shape fitness although no significant difference was observed between control and S_1, S_2, SD_1 and SD_2 treatments. The lowest score was related to SD_3 treatment followed by S_3 which had a significant difference with the other treatments ($p<0.05$).It is probably due to more incorporation of defatted soy flour into bread formulation. The highest score of crust color was observed in SD_2 followed by S_2 and SD_1 treatments which had a significant difference in compared with control treatment ($p<0.05$). In other words addition of sourdough in to formulation of soy flour breads led to improvement of quality and color of crust. The reason is that some acids and metabolites produced by lactic bacteria in sourdough have important role in bread quality and can enhance appearance characteristics of bread samples. On the other hand addition of defatted soy flour containing 50% protein leads to caramelisation and Maillard reactions between sugars and amino acids produced during bread preparation. Also this reactions leads to improvement of crust color[14]. Results of texture and chewiness showed that the highest score was related to SD_2 and the lowest was related to S_3 treatment. Dhingra and Jood(2001) found that crispiness and texture quality correlated with appearance shape of bread surface and enrichment with soy flour decreased crispiness compared to control sample[14].Also, based on literature enrichment with soy flour led to bolding and coarsening bubbles of bread [16]. Results of flavor showed significantly different between seven treatments. SD_2 had the highest score among all treatments and SD_3 had the lowest score among sourdough treatments. The accumulation of amino acids during sourdough fermentation enhances the formation of flavor volatiles during baking and flavor improve. Also, proteolytic enzymes present at sourdough and proteolysis provides precursor compounds for the formation of aroma volatiles during baking. Results of taste showed that the highest score was related to SD_2 treatment and had no significant difference with control treatment, the lowest score was related to S_3 ($p<0.05$). Flavor quality decreased when bread enriched with 10% soy flour. This might be due to the beany flavor of soy flour. Results obtained in this study agreed report of Dhingra and Jood (2001)[14]. Control treatment had the highest volume and the lowest score was related to SD_3 treatment therefore it is clear that results of sensory evaluation by panelists correspond to mechanical results. Sensory evaluation of staling rate are presented in table 4. Results showed that control treatment had the lowest value and the highest staling rate (24h), but after 48 h no significant difference was observed between other treatments at this time. After 72 hours of storage, SD_1 and SD_2 treatments showed the highest staling rate and S_3 had the lowest staling rate between seven experimental treatments. Results showed that texture of bread in treatments with soy flour and sourdough was hardness than other treatments(72h) and bread sample with 10% soy flour was tender than other treatments . Therefore it is clear that results obtained from sensory evaluation by panelists correspond to results obtained from mechanical evaluation.

Their Sensory evaluation were determined by a panel of 4 judges and acceptability using a six-point. The best point was (6) and lowest point was (1).

CONCLUSION

Results of this study showed that soy flour bread contains a sufficient amount of minerals. According to reports high amount of ash present at soy flour and phytase enzyme present at sourdough increased bioavailability of minerals in produced bread. Results obtained in the present study showed that addition of soy flour and 25% sourdough led to significant improvement of texture properties and retardation of staling process. Addition of Soy flour increased moisture in produced bread therefore enhanced mold spoilage but by adding 25% sourdough mold spoilage decreased. Finally, Results of this study showed that sourdough addition at 25% combined with soy flour at 5% improved appearance characteristics, internal and sensory properties of produced breads.

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