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Evaluation of Additional SoyBean Flour and Sourdough *Lactobacillus Plantarum* (ATCC 43332) on Rheological and Quality Properties of Toast Breads

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ABSTRACT

Additional of defatted soybean flour was caused enriching bread by essential amino acids, vitamin and minerals. By using sourdough in bread production, properties of dough's texture, flavor and smell will be improved, in comparison with bread made by yeast. In this research, defatted soybean flour in 3, 5 and 10 percent were mixed with wheat flour then rheological properties of dough were evaluated by Farinograph and Extensograph. After this, with adding of 25 percent of Lactobacillus Plantarum sourdough on dough, some of rheological properties were evaluated again. Farinograph results showed that increasing rate of enrichment with soybean flour, was caused increasing rate of water absorption, development time, dough stability, resistance and valorimetry number. Furthermore, Extensograph results showed that the dough with different percent of soybean flour and sourdough had less energy than in comparison with dough that having soybean along. On the other hand, the rate of ash and protein in bread that enriched by soybean flour and also mixture of soybean flour and sourdough were more than in comparison with control bread. However, the rate of ash and protein in bread with sourdough were less than without it. 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: Soybean Flour, *Lactobacillus Plantarum***,** Toast Breads.

INTRODUCTION

Today in most countries of the world grains are regarded as the most important providers of calorie, protein, insoluble fibers, vitamins and minerals needed for human beings [5]. Of among grains wheat flour has unique viscoelastic properties and can preserve gases in the dough; this is related to its gluten protein. However wheat flour is poor at essential amino acid Lysine whereas soy flour contains significant amount of Lysine [17]. Soy is belonged to Leguminous family and contains nine different kinds which its agricultural variety is of Glycine max species. This product has been consumed for almost 5000 years and today it has different usages in dairy industry, meat preparation and bakery products [23]. It has been used for the first time in the U.S in 1940 in bread formulation due to food and medicinal properties [16]. At present time soy flour is regarded as the most inexpensive vegetable protein of high quality which contains 50% protein and all of essential amino acids needed for human beings. Tsen and Hoover (1973) found that enrichment of wheat flour with 12% high fat soy flour enhanced protein content and quality of bread [27]. Also Mc watters (1978) reported that inclusion of 10% soy flour in cookies formulation hadn't any negative effects on texture and sensory properties of the product [22]. Fulmer (1989) showed that soy isolate and whey can be replaced successfully with nonfat dry milk in cake production without any negative effects on product quality [11]. In order to determine rheological properties of soy, triticale and lupine flour replaced with wheat flour at 5 and10%. Doxastakis (2002) performed a research and found that inclusion of 5 and 10% lupine and soy flour increased dough resistance and decreased bread volume. It also led to intensification of crust colour and center colour of the bread [8]. Use of sourdough is one of the oldest biotechnological processes in bread production which results in an improvement of dough properties, dough texture, aroma and flavor as well as increase of shelf-life, retardation of mould growth and inhibition of rope decay in bread due to common activity of yeasts and lactic acid bacteria in the sourdough [24]. Gul (2005) studied optimal conditions of fermentation by the yeast, *Saccharomyces Cerevisiae* and lactic acid bacteria in sourdough and found that bread samples prepared with 1.5% *Lacto Bacillus Amylophilus* and 1.5% *Saccharomyces Cerivisae* had the best rheological properties[12]. Furthermore previous studies showed that lactic bacteria in sourdough have positive effects on bread texture due to production of metabolites such as organic acids, exopolysaccharides and enzymes during fermentation; for instance the obtained exopolysaccharids can be replaced with some hydrocolloids used as bread improver and even some of them contain peribiotic properties. Also organic acids influence protein and starch of the flour and result in pH reduction, increase of enzymatic activity (protease and amylase), staling retardation and improvement of the obtained bread. In addition, fermentation process in sourdough leads to increase of bioavailable organic materials, reduction of phytate remained at the bread and enhancement of aroma and flavor of the product [2]. In general, bread commercial preparations are digested easily and therefore have strong glucose and insulin response. So consumption of breads containing sourdough decrease insulin and glucose response compared to breads lacking sourdough. This is probably due to slowing stomach's defecation by acid lactic produced during fermentation of sourdough [16]. Bread is one of the most high consuming food products in the world and its enrichment has a significant role in improvement of people nutrition. Therefore in this research soy flour and sourdough were used for the said aims.

MATERIALS AND METHODS

Initial ingredients

Wheat flour with extraction degree of 68% was obtained from Sahar Bread Co., defatted soy flour was purchased from Behpak Co., bakery dried yeast (*Saccharomyces Cerevisiae*) was prepared from Iran Molasses Co., vaccum dried of culture *Lactobasillus Plantarum* (ATCC 43332) was obtained from scientific and industrial research center of Iran. Treatments include: C: control, $S_{1:}$ 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 flour and bread samples

Chemical tests carried on the wheat flour included the following parameters: moisture (according to international standard AACC 44-16), ash (according to international standard AACC 08-01), protein (according to international standard AACC 46-12), wet gluten (according to ICC 38-11), pH (according to AACC- 52-02) and sedimentation value (according to AACC 116). Chemical tests carried on the soy flour included fat and fiber (according to AACC 32-10), moisture, ash and protein (according to the said standard methods). Also chemical tests carried on the bread samples included moisture, ash and protein according to the said standard methods [1,18].

Dough rheological tests

In order to determine certain rheological properties of control dough samples as well as doughs prepared with 3, 5 and 10% soy flour, Farinograph test (according to AACC 54-21) was used. Also Extensograph test (according to AACC 54-10) was carried on three other dough samples (control, different amounts of soy flour and soy flour along with 25% sourdough).

Bread sensory test

In order to evaluate aroma and flavor of bread samples qualitatively, sensory was used. In this regard some experts, trained and untrained assessors were participated in the test. At first, samples were coded following cooling and cutting. Samples then were analyzed by four experts from Bread and Grain research center of Tehran. Sensory assessors (panelists) determined certain scores for aroma and flavor. The highest score for aroma was 10 and the highest score for flavor was 15.

Preparation method of microbial suspension

At first vaccum 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. Since a certain time is required for bacterial growth and colony incubation in order to inoculate microbial suspension to bread dough, therefore the said culture medium should be centrifuged (5000g, 15 min and 4°C). The obtained supernatant was then washed with physiologic serum followed by re-centrifugation. Absorbance was determined through spectrophotometer followed by bacterial count [10,19,26].

Preparation method of sourdough and its addition to bread dough

In order to obtain sourdough, 180 ml of microbial suspension (fresh cells were added to sourdough at level of 10^8 cfu/ ml) and 0.25% of *Saccharomyces Cerevisiae* were used per 300g of wheat flour. The obtained blend was then mixed in a mixer (60 rpm) followed by transferring to sterile bathes and incubation at 32°C for 24h. 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) [6,26].

Preparation method of toast bread and baking procedure

In order to obtain toast bread, raw materials (wheat flour, water, salt, the yeast *Saccharomyces Cerevisiae* sugar, liquid oil and improver) were first prepared and weighted. Three percents of soy flour (3, 5 and 10) were then added to wheat flour followed by mixing in making-dough tank for 10 min. Other dry and powdery materials were then added to the obtained blend. Mean while water was added to the mixture. After through mixing of flour and water and appearance of a shapeable mass (dough), initial resting of the samples was performed for 10 min. Some slices of dough with approximate weight of 450g were molded and medial fermentation was performed after a 10 min resting. Finally dough pieces were transferred to fermentation chamber so that final fermentation was performed at 30°C and relative humidity of 80% for 40 min. Bread loafs were entered into rotating oven at 220-230°C. Baking time of toast breads was about 45 min which of course decreased due to dough enrichment with soy flour.

Statistical analysis

Statistical analysis was performed by using completely randomized design and the mean comparison was done by Duncan's multiple range tests [8].

Table1. Mean value of chemical assessments of wheat flour

flour	Wet Gluten%	Ash %	Protein %	Moisture%	pH	Zeleny Number
Wheat flour	29.75	0.46	10.62	13.33	5.9	35

Table2. Mean value of chemical assessments of soy flour

flour	Moisture %	Ash %	Protein %	Fat%	Fiber
Soy flour	7.652	6.53	53.13	1.433	5.99

Table3. Mean comparison of chemical experimental bread (%)

Treatment	C	S_1	S_2	S_3	SD_1	SD_2	SD_3
Moisture	30.70^{c}	35.60^{b}	36.47^{b}	41.49^{a}	34.19^{b}	34.48^{b}	42.10^{a}
Protein	13.07^{e}	14.11^{cd}	15.26^{b}	17.44^{a}	13.42^{de}	14.52^{bc}	14.68^{bc}
Ash	0.6059^{d}	0.6630^{cd}	0.8062^{ab}	0.8145^{a}	0.5957^{d}	0.6774^{bcd}	0.7579^{abc}

Means within row followed by the same letter are not significantly different (P<0/01).

Table4. Comparison of results farinography test on bread dough containing soy

Treatment	Water Adsorption (%)	Dough Development time (min)	Dough Stability time (min)	Dough softening after 10 min (B.U)	Dough Softening after 12 min (B.U)	Quality Number
C	60.59^{c}	4.85^{b}	6.25^{b}	61.5^{a}	92^{a}	73.5^{b}
S_1	61.17^{c}	4.93^{b}	6.8^{a}	58.5^{ab}	88^{a}	75.5^{b}
S_2	62^{b}	5.3^{b}	7^{a}	53.5^{bc}	94^{a}	84.5^{a}
S_3	64.35^{a}	6.45^{a}	7.1^{a}	47.5^{c}	94.33^{a}	85^{a}

Means within column followed by the same letter are not significantly different (P<0/01).

Table5. Mean comparison of results extensograph test on breads dough containing soy and sourdough + soy

Treatment	Energy (cm²)			Resistance to Extension (B.U)			Dough Extensibility (mm)			Resistance to Extension / Extensibility		
	45	**90**	**135**	**45**	**90**	**135**	**45**	**90**	**135**	**45**	**90**	**135**
C	83^{a}	108^{a}	108.7^{a}	22.8^{c}	448^{a}	532^{a}	154^{ab}	138^{c}	128^{c}	1.9^{b}	3.26^{a}	4^{b}
S_1	82^{a}	95^{b}	108.7^{a}	264^{b}	337^{c}	385^{cd}	168^{a}	159^{a}	155^{a}	1.58^{c}	2.13^{c}	2.46^{e}
S_2	87^{a}	88^{b}	94.3^{b}	301^{a}	333^{c}	391^{c}	160^{a}	150^{ab}	142^{b}	1.87^{b}	2.23^{c}	2.8^{d}
S_3	74^{b}	92^{b}	97.6^{b}	261^{b}	317^{cd}	394^{c}	161^{a}	163^{a}	146^{ab}	1.66^{c}	2^{c}	2.66^{de}
SD_1	33.3^{e}	47.5^{d}	47^{c}	182^{d}	300^{cd}	336^{e}	120^{c}	110^{d}	98^{de}	1.53^{c}	2.76^{b}	3.43^{c}
SD_2	40^{d}	58^{c}	54.6^{c}	228^{c}	370^{b}	430^{b}	93^{d}	108^{de}	88^{f}	3.1^{a}	3.36^{a}	4.8^{a}
SD_3	50.6^{c}	55.6^{c}	56.3^{c}	260^{b}	293^{d}	282^{cd}	124^{c}	119^{d}	108^{d}	2^{b}	2.73^{b}	3.3^{c}

Means within column followed by the same letter are not significantly different (P<0/01).

RESULTS AND DISCUSSION

Tables 1, 2 and 3 show properties of flour samples subjected to chemical tests. Adding 25% sourdough reduced protein and content of produced breads and had significant effect on the moisture content. Results obtained from Farinograph test (table 4) showed that water absorption, development time and stability of dough as well as quality Number (valorimetric value) increased due to increasing enrichment with soy flour.

Chemical tests of bread samples

Table 3 shows results related to bread samples which were subjected to chemical test. It was shown that increase in enrichment with soy flour caused an increase in moisture content of the obtained breads. S_3 and SD_3 treatments showed the highest moisture content and had significant difference ($P<0.01$) with the other treatments. The samples had more ash and protein content leading to higher water absorption; this is probably due to addition of defatted soy flour in their formulation. The least moisture content was related to control sample. These results were in accordance with findings of Brewer et al., 1992 [3]. Also no significant difference was observed between SD_2, SD_1, S_2 and S_1 treatments regarding moisture factor. However there was a significant difference between control treatment and treatments containing soy flour and sourdough probably due to addition of soy flour to bread formulation since it had more ash and protein. However sourdough hadn't any significant effect on bread's moisture. The effect of sourdough on the moisture content of the product is depended on fermentation condition. As you can see from table 3 as enrichment of soy flour increased, protein content of the obtained breads increased; S_3 treatment had the highest protein content and a significant difference with the other treatments ($P<0.01$) where as control treatment showed the lowest protein content. In fact adding defatted soy flour which contained 50% protein led to an increase in protein content of the obtained breads. These results agree with studies of Dhingra and Jood (2001) [7]. Also protein content of bread samples containing soy flour and sourdough is more than that of control samples due to presence of soy protein. However bread samples containing just soy flour had a little more protein than bread samples containing soy flour and sourdough. This is probably due to activity of protease enzymes in sourdough which resulted in decomposition of proteins[2]. It should be mentioned that bread sample containing 3% soy flour and sourdough was the only sample which hadn't a significant difference with control bread due to application of less soy flour (consequently less protein). Based on table 3 it was shown that as enrichment increased, the as content of the obtained breads increased significantly so that the highest amount was related to S_3 and S_2 treatment and the lowest a moist was related to control treatment. It is due to presence of lots of minerals in soy flour which causes an increase in the ash content of the enriched product. These results are in accordance with findings of Dhingra and Jood (2001). However addition of sourdough to bread formulation resulted in a decrease in the ash content probably due to presence of organic acid produced during fermentation.

Farinograph test of flour samples

Based on table 4 a significant difference was observed between S_1 and control treatments with the other treatments. Furthermore S_3 treatment showed the highest water absorption followed by S_2 and S_1 treatments. The lowest water adsorption was observed of C treatment. In general increasing soy flour content and consequently more protein and ash increased water absorption. The studies show that higher amount of ash which is an indication of presence of more bran particles in flour leads to increase in water absorption [25]. Also presence of hydroxyl groups in structure of each kind of fiber causes more hydrogenise bonds and consequently more exchange with water leading to enhanced water absorption. Regarding the factor of dough development time, there was no significant difference between C, S_1 and S_2 treatments; however S_3 treatment which had the highest dough extension time showed a significant difference with the other treatments ($P<0.01$). No significant difference was observed between S_1, S_2 and S_3 treatments with respect to dough stability time; however all of them showed a significant difference with C treatment so that the least dough stability was related to control treatment and the highest of this index was belonged to S_3 treatment followed by S_1 and S_2 treatments. Regarding relaxation degree of dough samples after 10 min, there was no significant difference between S_1 and S_2 treatments but other treatments showed significant difference so that S_3 treatment had the lowest relaxation degree and C treatment had the highest relaxation degree followed by S_1 and S_2. In fact enrichment with soy flour decreased dough relaxation degree. It should be mentioned that for measuring dough based on farinograph properties, dough stability times were signified as follows: between 0-2 min very poor, 2-4 min poor, 4-7 min intermediate to strong, 7-10 min strong and 10-15 min very strong [28]. Consider to dough relaxation degree after 12 min there was no significant difference between treatments.

Furthermore dough relaxation degree increased after 12 min over 10 min determining bakery value or flour quality number is an important factor in farinograph test. In this research it was shown that as enrichment with soy flour increased, bakery value increased so that the highest quality number is related to S_3 treatment followed by S_2 and S_1 treatment. Control treatment had the lowest quality values; however there was no significant difference between S_1 and C. A significant difference was observed between control with S_2 and S_3 treatments ($P<0.01$). In general increase in enrichment with defatted soy flour containing 50% protein led to increase in water absorption, development time, stability and quality value, and decrease in relaxation degree.

Extensograph test of flour samples

Table 5 shows that at each three times of 45, 90 and 135 min, bread samples containing soy flour have more sub-curve area (energy) compared to bread samples containing soy flour and sourdough due to presence of soy proteins which leads to dough strengthening. Addition of sourdough decreased sub – curve area or samples energy leading to dough softening, reduced viscosity due to enzymatic activity (especially protease), formation of some acids and dilution of gluten network [2]. Also dough stretch resistance during 45 min increased was related to SD_1 treatment as sourdough added to samples and this treatment showed a significant difference with the other ones probably due to less soy flour percent and consequently reduced protein as well as presence of enzymes formed during fermentation. During the times of 90 and 135 min a reduced resistance to dough stretch was observed at all treatments compared to control treatment. Decrease in this index is an indication of dough stability reduction. Based on presented data extensibility of dough samples containing soy flour was more than that of control treatment at times of 45, 90 and 135 min and this trend increased significantly by addition of sourdough to samples ($P<0.01$). Extensibility indicates gas and water retention properties of dough which reduces staling and retains bread freshness. Based on table 5 you can see that SD_2 treatment had the highest relative value (proportion of resistance to extension) at three time intervals of 45, 90 and 135 min. The lowest relative value at 45 min was related to SD_1 treatment and at 90 and 135 min was related to S_3 treatment. In general by increasing fermentation time, proportion of resistance to extension increased in all treatments especially in treads containing sourdough. Results obtained in the present study agree with studies of other researchers [6, 8, 21].

Sensory evaluation

The effect of adding sourdough and different concentration of soy flour on aroma

Figure 1 shows that control treatment had better aroma than treatments containing soy flour. Comparing these treatments with treatments contain soy flour and sourdough show that SD_2 treatment gained the highest aroma score and the lowest score was related to SD_3. In fact by increasing enrichment of dough with defatted soy flour, aroma score decreased; it is probably due to grass or bean aroma of soy flour. However breads containing sourdough had better aroma compared to soy flour breads due to presence of proteolysis enzymes and decomposition of some of dough proteins. Proteolysis leads to formation of free amino acids which in turn enhance aroma.

Not only free amino acids had a role in formation of good aroma but also some aldehydes and ketones had a determining role in this area so that they are regarded as basic sources of producing aromatic compounds. In other words sourdough breads had more volatile compounds and consequently higher sensory score. Generally lactic bacteria can produce different aromatic materials so that basic properties of sourdough breads (sourdough's aroma and flavor, formation of suitable metabolites) are function of microbial species used, raw materials, presence of carbohydrates and baking process. In addition products resulted from fermentation in sourdough are lactic and acetic acids which produce. Flavor and it seems that acetic acid intensifies the effect of other aromatic compounds. However kind of aromatic compounds of the bread varies by used stub [4,9,14].

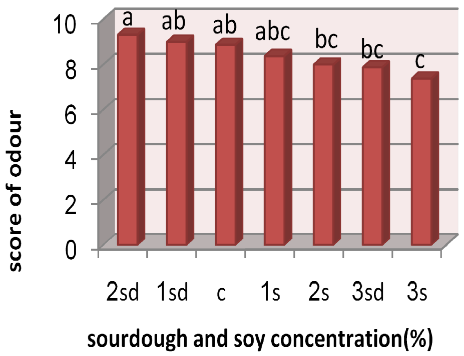

Fig1. Mean comparison of fragrance between treatments

Columns marked by same letter are not different at(P<0.05).

The effect of adding sourdough and different amounts of soy flour on taste and flavor

Based on figure 2, SD_2 treatment gained the highest taste score and the lowest score was related to SD_1 and S_3 treatments; there was no significant difference between them but they showed a significant difference with all other treatments(P<0.05). By increasing enrichment of dough with 10% soy flour, taste quality reduced probably due to bean taste of soy flour however sourdough addition gave a better taste to SD_3 breads compared to S_3 bread which is due to positive effect of lactic and acetic acid in sourdough. However if acetic acid in sourdough increases more than permit table extent, growth of yeasts presented at sourdough is stopped leading to unpalatable taste. The lowest taste quality score was related to SD_1 treatment which contained the least amount of soy flour and addition of 25% sourdough to this treatment led to formation of a sour taste. Results of previous studies showed that, taste of sourdough is depended on stutter culture, flour's ash content and fermentation time and dough efficiency which influence bread taste [13,15,20] .

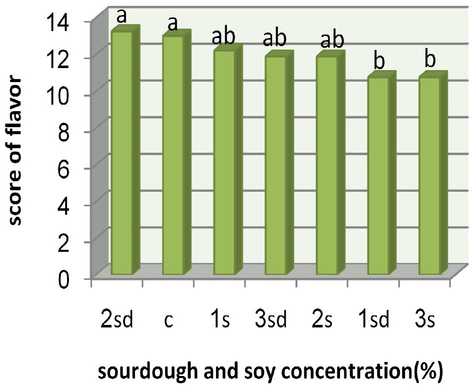

Fig2. Mean comparison of taste and flavor between treatments
Columns marked by same letter are not different at(P<0.05).

CONCLUSION

Results showed that protein and ash content of toast breads (soy flour as well as soy flour in addition to sourdough) was more than those of control bread (wheat flour), therefore using the said breads improves individual's diet and can enhance protein content of the bread by paying a little price. Furthermore required mineral materials in such breads are available due to presence of phytase enzyme in consumed sourdough. This leads to increase of bioavailability of mineral elements in soy flour. Regarding farinograph and extensograph properties, it was found that addition of defatted soy flour to wheat flour resulted in an increase in water absorption, development time and stability of dough. Moreover sub-curve area was decreased with addition of 25% sourdough to all of treatments containing soy and wheat flour. Other results obtained in this research showed that inclusion of 25% sourdough in addition to 5% soy flour improved aroma, taste and flavor of obtained breads due to proteolysis process and formation of different aromatic compounds.

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