Properties of Dough and Flat Bread Containing Wheat Germ

M. Majzoobi¹, S. Farhoodi¹, A. Farahnaky¹, and M. J. Taghipour²

ABSTRACT

Increasing the nutritional value of bread is important since the enriched product can be used for special target groups such as developing countries or special diets. Wheat germ, a highly nutritive part of wheat kernel, is a by-product of milling factories and has the potential to be used for food supplementation. The main aim of this research was to supplement flat bread (Barbari) with wheat germ and to study the quality and staling of the resultant bread. Therefore, processed (heated at 150°C for 45 minutes) and raw wheat germ were added at the rates of 0, 5, 10 and 15% (w/w) in bread recipe, as separate treatments. Using a Farinograph, it was found that the dough made with raw germ had less water absorption, lower consistency, and shorter stability time. Modeling of the data showed that increasing the germ level had negative correlation with bread volume and softness. Such effects were enhanced when raw germ was used. It was found that addition of germ could not delay bread staling; however, it had positive effects on its taste and general acceptability, particularly when 15% processed germ was used.

Keywords: Barbari bread; Dough empirical rheology; Flat bread; Wheat germ

INTRODUCTION

Wheat germ, the by-product of flour milling factories, is well-known for its high nutritional value. It supplies three times more protein, seven times more fat, fifteen times more sugars and six times more minerals and dietary fibers than does white flour, which is generally used for bread making (Matteuzzi et al., 2004). Moreover, wheat germ is the richest plant source of vitamin E. It is also high in thiamin, riboflavin, and niacin. Cereal proteins are known to be poor qualitatively and quantitatively, while wheat germ proteins are placed on equivalence with high quality animal proteins (Qarooni, 1996; Sidhu et al., 1999; Al-Hooti et al., 2002). The proteins in wheat germ are rich in lysine, methionine, and threonine, which are limited in cereals. Wheat germ also contains high levels of phytochemicals (e.g. ferulic acid, glutathione and phytosterols) and provides good palatability in many food products (Qarooni, 1996; Zhu et al., 2006). Therefore, it has the potential to be used as supplement for bakery products. However, the presence of wheat germ in flour can reduce the shelf-life and baking quality of the flour. This is mainly due to the presence of high levels of unsaturated fatty acids and enzymes, particularly lipase, in wheat germ. Therefore, wheat germ should be separated during milling (Pomeranz et al., 1970). Accordingly, the shelf-life of the separated wheat germ is very short at ambient temperature, a problem which has limited its applications in many food products. However, some methods have been introduced to prolong wheat germ shelf-life such as autoclaving, dry heating or freezing methods in order to inactivate enzymes or prevent the contact between enzymes and substrates (Shurpalekar and Haridas Rao, 1978; Pomeranz, 1988). Furthermore, the presence of glutathione in wheat germ has some adverse

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effects on dough and bread quality. Glutathione is a tripeptide consisting of glycine, glutamic acid and cysteine. This component can reduce disulphide bonds in the gluten and hence decrease the stability and strength of the dough resulting in a reduction in bread quality (Shurpalekar and Haridas Rao, 1978; Al-Hooti et al., 2002). To inhibit glutathione activity, a number of methods such as roasting, autoclaving, soaking, fermentation and addition of some oxidants have been used with different degrees of success (Shurpalekar and Haridas Rao, 1978; Pomeranz, 1988; Sidhu et al., 1999).

Production of high nutritive foods is important since the enriched products can be used for special target groups such as low-income groups, particularly for developing countries, disaster relief operations, and child-feeding programs (Clauthon and Pearce, 1989). Bread is the most consumed food around the world. Therefore, it offers an appropriate medium to convey wheat germ to human diet. Accordingly, flat breads are of great interest since they are the most common type of bread in many developing countries. Earlier studies signified that the wheat germ loaves had superior nutrition value, however, their quality and consumer acceptability were inferior. Reduction of the quality of wheat germ loaves was mainly due to the increase in bread hardness and decrease in bread volume (Pomeranz et al., 1970). Since the quality attributes of loaves and flat breads are different to some extent, addition of wheat germ to either of these bread types may have different effects on their quality. Such effects on loaves have been investigated before (see e.g. Sidhu et al., 1999; Al-Hooti et al., 2002 and Sidhu et al., 2007), while there is a lack of information on the effects of wheat germ on flat bread quality and shelf-life.

The objectives of this study were to determine the optimum level of processed and raw wheat germ in production of a flat bread, namely, Barbari bread, and to study the effects of wheat germ on the properties of dough, bread, and its staling.

MATERIALS AND METHODS

Materials

Raw wheat germ (RG) and wheat flour with extraction of 87% (according to the manufacturer) were obtained from Sepidan Milling Factory, Zarghan, Iran. Active dried bakery yeast and salt (NaCl) were purchased from local market. Other chemicals used in this study were obtained from Merck Company (Germany) and were of analytical grade.

Preparation of RG

The particle size of RG was reduced to 350-400 μm using a laboratory cutter (model Alexanderwerck, Germany) and then sieving. The chemical composition (including moisture, fat, protein, fiber, and ash content) of the RG was determined according to the AACC methods (AACC, 2000). To inhibit enzyme activity, RG was packed in sealed polyethylene bags and stored in freezer at -18ºC before further experiments.

Preparation of Processed Wheat Germ (PG)

In order to inactivate enzyme activity and destroy glutathione, a portion of RG (with particle size of 350-400 μm) was further processed by dry heating at 150°C for 45 minutes in an electrical oven according to Shurpalekar and Haridas Rao (1978). The PG was then packed in polyethylene bags and stored at room temperature (20±2°C).

Determination of the Total Reducing Substances of RG and PG

The AACC method for determination of total reducing substances was used as an indication of the glutathione content of wheat germ (AACC method 10-01, 2000) with slight modification. The reducing
substances in PG and RG (5g) were extracted with 50 ml of trichloroacetic acid (10 g l\(^{-1}\)). After centrifugation at 5000g for 15 minutes, 20 ml of clear supernatant and 3 ml of standard iodine was titrated against 0.005 ml l\(^{-1}\) sodium thiosulfate.

**Sensory Analysis**

To determine the appropriate levels of the wheat germ to be added in the bread recipe, breads were made suing 0, 5, 10, 15 and 20% (w/w) wheat germ (see dough and bread preparation section) and were tested for the sensory analysis. To do the experiments, 30 in-house panelists were selected according to the pre-tests explained by Watts et al. (1989). Information about the best quality of the bread and how to evaluate bread samples in terms of hardness, taste, color and general acceptability were given to the panelists. Characteristics of a standard Barbari bread were explained to the panelists as follows: crust color should be light golden brown, the texture should be soft and the bread should be palatable without any off-flavor and torn easily by hand and, when chewing, it should not be sticky or grainy in the mouth. Later, the samples were coded with three random digits and were given to the panelists simultaneously in a standard booth under day light illumination. The panelists were asked to rank the samples and give the highest score i.e. 5, to the most acceptable sample, while the lowest score i.e. 1, to the least acceptable sample. Further experiments including dough empirical rheological properties and bread physical tests were performed on the samples made with the appropriate germ levels as determined by the sensory evaluation results.

**Empirical Rheological Properties of Wheat Germ Bread Dough**

To study the effects of addition of wheat germ on empirical rheological properties of bread dough, PG and RG were added to the flour separately at 0, 5, 10 and 15% (w/w, flour basis) in the bowl of a Brabender Farinograph (model FE022-NK, Germany) with capacity of 50 g. The control was 50 g wheat flour of 14% moisture content. Addition of wheat germ to the flour increased the dry weight of the samples, which could affect the water absorption and empirical rheological properties of the dough. Therefore, samples made with wheat flour having weights equal to the samples containing germ were also prepared and tested with the Farinograph. Water was then added to the mixture until the dough was formed with a consistency of 500BU. The empirical rheological properties of the dough, including dough arrival and stability times, mixing tolerance, and softening after 12 min were determined from the obtained Farinogram according to the AACC (2000).

**Dough Preparation and Bread Making**

Wheat flour, activated bakery yeast (2%, w/w, flour basis) and salt (1.5% w/w, flour basis) were mixed with appropriate amount of water (determined by Farinograph) in a single z-blade laboratory dough mixer (Iypto, Model EB12, Germany) at 140 revolution/min (rpm) for 15 min. The dough was then proofed in a proofing cabinet with relative humidity of 80% at 38\(^\circ\)C for 45 minutes, divided into portions of 400 g, rounded by hand and placed in the proofing cabinet once again for 45 minutes. The dough pieces were shaped in a circular pan with a thickness of 1 cm and diameter of 40 cm and baked in an electrical baking oven (Model Karl Welkerkg, Germany) set at 210\(^\circ\)C for 30 minutes. Afterwards, the breads were cooled down to room temperature and packed in polyethylene bags for further experiments.

**Determination of the Moisture Content of the Samples**

The moisture content of the bread samples was determined after baking and cooling of
the samples in an oven at 130°C until constant weight.

**Determination of the Volume of Fresh and Stored Breads**

The volume of the bread samples was determined using the rapeseed displacement method (AACC, 2000). Since mold growth was observed on the samples stored for longer than 48 hours, the volume of these samples was not determined.

**Determination of Bread Hardness**

A penetration test using a metallic cylindrical probe with diameter of 10 mm was performed using a Texture Analyzer (Model Stevens-LFRA, UK). A piece of bread was placed in the instrument where the probe moved towards the sample with speed of 1 mm s\(^{-1}\) and penetrated in the crust to the depth of 5 mm. To determine crumb hardness, first, the crust was removed carefully using a sharp knife and, then, the penetration test was conducted as explained before, except that the penetration depth was 10 mm. For the samples stored for longer than 48 h, mold growth could be observed on some part of the sample. Therefore, mold free pieces of bread were selected for the study. The experiment was performed at five different points of the bread crust and crumb of three individual bread samples and the average value was recorded.

**Microscopic Structure of Fresh and Stored Breads**

To study the microscopic structure of the bread crumb, scanning electron microscope (SEM) (Model 5526, Cambridge, UK) was used. To prepare each sample, a thin layer of bread was first freeze dried and then a tiny part of the sample was sputter coated with gold. Finally the sample was transferred to the microscope where it was observed at 20 kV.

**Statistical Analysis**

The experiments were performed in a completely randomized design. All experiments were conducted in triplicates and the mean values and standard deviations were recorded. Analysis of variance (ANOVA) was performed and the results were separated using the Multiple Ranges Duncan’s test (α< 0.05) using statistical software of Statistical Package for the Social Sciences (SPSS) (SPSS, Inc. New Jersey, USA). Software of Design-Expert 6.0.2 (State Ease, USA) and D-optimal Response mode was used in order to model and estimate any non-linearity in the relationships between the parameters under study and to obtain the best model i.e. quadratic model. In each model, all variables and their interactions with significant effect (α < 0.05) were kept and any variable or interactions without significant effect were removed as indicated by the software (Farahnaky and Hill, 2007).

**RESULTS AND DISCUSSION**

**Chemical Composition of the Flour and Wheat Germ**

Determination of the chemical compositions of the flour and RG showed that the flour had 13.30 % moisture, 11.03 % protein, 1.31% fat, 0.37% fiber, 0.66% ash, and 35.75% wet gluten. The RG had 11.68% moisture, 30.64% protein, 11.70% fat, 5.34% fiber, and 4.55 % ash. Determination of the total reducing agents of the wheat germ indicated that it reduced from 4±0.1 in RG to 1.8±0.1 (ml 0.05 mol l\(^{-1}\) S\(_2\)O\(_3\)^2/5 g germ) in PG as a result of processing. Therefore, the PG had less than half of the reducing substances compared to the RG.

**Sensory Evaluation of the Samples**

Sensory evaluation of the samples (Table 1) showed that addition of processed or raw wheat germ to the bread recipe could improve the taste and flavor of the samples. This can be due to the wheatty and sweet taste of the germ.
Table 1. Sensory evaluation of the bread samples made by addition of different levels of raw (RG) and processed (PG) germ*.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Taste and flavor</th>
<th>Crust color</th>
<th>Texture</th>
<th>General acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.0±0.2</td>
<td>3.0±0.1</td>
<td>4.0±0.1</td>
<td>2.8±0.1</td>
</tr>
<tr>
<td>5% RG</td>
<td>3.4±0.2</td>
<td>3.0±0.1</td>
<td>4.0±0.1</td>
<td>3.0±0.1</td>
</tr>
<tr>
<td>10% RG</td>
<td>4.2±0.1</td>
<td>4.2±0.2</td>
<td>3.2±0.1</td>
<td>3.5±0.2</td>
</tr>
<tr>
<td>15% RG</td>
<td>4.5±0.2</td>
<td>4.1±0.1</td>
<td>2.5±0.2</td>
<td>4.0±0.1</td>
</tr>
<tr>
<td>20% RG</td>
<td>4.5±0.1</td>
<td>2.4±0.3</td>
<td>2.2±0.2</td>
<td>3.2±0.2</td>
</tr>
<tr>
<td>5% PG</td>
<td>4.0±0.2</td>
<td>3.0±0.2</td>
<td>4.2±0.1</td>
<td>3.0±0.2</td>
</tr>
<tr>
<td>10% PG</td>
<td>4.0±0.1</td>
<td>3.5±0.1</td>
<td>4.0±0.2</td>
<td>4.0±0.2</td>
</tr>
<tr>
<td>15% PG</td>
<td>4.5±0.2</td>
<td>4.2±0.2</td>
<td>3.5±0.3</td>
<td>4.5±0.2</td>
</tr>
<tr>
<td>20% PG</td>
<td>4.5±0.1</td>
<td>2.8±0.3</td>
<td>3.0±0.2</td>
<td>3.5±0.3</td>
</tr>
</tbody>
</table>

*Values in the table are the average of Triplicates±Standard deviation. Different letters in each column show significant differences between the data (α<0.05).

which can affect the taste and flavor of the bread positively. Moreover, the germ contains sugars and proteins that can interact with each other through Maillard and caramelization reactions. The products of these reactions are coloring and flavoring agents that can enhance the color of the crust (Purlis and Salvadori, 2007). Therefore, the crust color of the samples received higher scores when the germ was added. The texture of the samples was affected negatively when wheat germ was added particularly when RG was used. This can be due to the increase in bread hardness as a result of glutathione activity and hence reduction of the amount of gluten. Furthermore, an increase in the general acceptability of the samples was observed, which can be due to their better taste, flavor, and color. The results also indicated that the general acceptability of the PG samples was observed, with increasing the dry material. For instance, with increasing germ levels (processed or raw) and wheat flour, the water absorption increased significantly. Samples prepared with addition of wheat flour to the control had the highest water absorption followed by the PG and RG. At the same level of either of the germs and flour (e.g. 10%), the water absorption of wheat flour sample was higher than the PG followed by the RG samples. Differences in the water absorption of the samples can be due to the presence of gluten in the flour which can absorb a large amount of water. In the PG and RG the proteins are mainly albumins and globulins (not gluten), which have little effects on increasing the water absorption of the dough. Gluten can absorb water almost 60% of its weight (Pomeranz, 1988), while the water absorbing components in the wheat germ (such as hydrocolloids and sugars) do not have such ability and absorb less water.

It has been reported that the glutathione present in the germ can degrade gluten network and, hence, reduce its water absorption ability (Shurpalekar and Haridas Rao, 1978; Srivastava et al., 2007). Accordingly, the sample containing PG had higher water absorption than the sample made with RG since the latter had higher amount of glutathione. Moreover, the presence of high levels of lipids in the germ can reduce water absorption ability of gluten by interacting with gluten and preventing it

Water Absorption of the Dough

The results (Table 2) showed that the water absorption of the samples increased with increasing the dry material. For instance, with increasing germ levels (processed or raw) and wheat flour, the water absorption increased significantly. Samples prepared with addition of wheat flour to the control had the highest water absorption followed by the PG and RG. At the same level of either of the germs and flour (e.g. 10%), the water absorption of wheat flour sample was higher than the PG followed by the RG samples. Differences in the water absorption of the samples can be due to the presence of gluten in the flour which can absorb a large amount of water. In the PG and RG the proteins are mainly albumins and globulins (not gluten), which have little effects on increasing the water absorption of the dough. Gluten can absorb water almost 60% of its weight (Pomeranz, 1988), while the water absorbing components in the wheat germ (such as hydrocolloids and sugars) do not have such ability and absorb less water.

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Table 2. Rheological parameters of different dough samples obtained by Brabender Farinograph.*

<table>
<thead>
<tr>
<th>Addition of Flour/Wheat germ (w/w)</th>
<th>Water absorption (%)</th>
<th>Dough arrival time (min)</th>
<th>Dough development time (min)</th>
<th>Dough stability time (min)</th>
<th>Dough softening after 5 min (BU)</th>
<th>Dough softening after 12 min (BU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>60.1±0.1*</td>
<td>1.20±0.10</td>
<td>1.55±0.30</td>
<td>3.33±0.12</td>
<td>85.03±0.03</td>
<td>120.05±0.05</td>
</tr>
<tr>
<td>5% flour</td>
<td>64.3±0.1†</td>
<td>1.54±0.08</td>
<td>1.75±0.15</td>
<td>3.41±0.20</td>
<td>85.03±0.05</td>
<td>120.05±0.05</td>
</tr>
<tr>
<td>10% flour</td>
<td>68.2±0.1†</td>
<td>1.50±0.10</td>
<td>1.91±0.20</td>
<td>3.25±0.31</td>
<td>87.50±3.50</td>
<td>120.03±0.03</td>
</tr>
<tr>
<td>15% flour</td>
<td>72.7±0.1†</td>
<td>1.62±0.12</td>
<td>2.02±0.22</td>
<td>3.25±0.20</td>
<td>85.02±0.05</td>
<td>120.05±0.05</td>
</tr>
<tr>
<td>5% RG</td>
<td>63.9±0.1†</td>
<td>1.20±0.10</td>
<td>1.50±0.20</td>
<td>2.33±0.30</td>
<td>137.50±3.50</td>
<td>140.00±0.05</td>
</tr>
<tr>
<td>10% RG</td>
<td>67.1±0.1†</td>
<td>1.40±0.15†</td>
<td>1.87±0.16†</td>
<td>1.83±0.21†</td>
<td>175.00±5.00</td>
<td>195.00±7.00</td>
</tr>
<tr>
<td>15% RG</td>
<td>70.5±0.1†</td>
<td>1.60±0.10</td>
<td>2.20±0.54†</td>
<td>1.40±0.20†</td>
<td>222.50±3.50</td>
<td>252.50±3.50</td>
</tr>
<tr>
<td>5% PG</td>
<td>64.1±0.3†</td>
<td>1.25±0.22†</td>
<td>1.58±0.23†</td>
<td>2.35±0.20†</td>
<td>127.50±3.50</td>
<td>135.00±0.05</td>
</tr>
<tr>
<td>10% PG</td>
<td>67.6±0.1†</td>
<td>1.42±0.12†</td>
<td>1.84±0.40†</td>
<td>2.04±0.15†</td>
<td>167.50±3.50</td>
<td>187.50±3.50</td>
</tr>
<tr>
<td>15% PG</td>
<td>70.9±0.0†</td>
<td>1.55±0.20†</td>
<td>2.10±0.10†</td>
<td>1.85±0.10†</td>
<td>212.50±3.50</td>
<td>240.00±1.03</td>
</tr>
</tbody>
</table>

*Values are average of Triplicates±Standard deviation. Different small letters show significant differences in each column (α< 0.05).

†Brabender unit.

from absorbing more water. Contradictive reports can be found in the literature; for instance, Qarooni (1996) reported that substituting raw wheat germ (1-3%) with flour could slightly increase the water absorption of the dough. However, Srivastava et al. (2007) found that replacement of raw wheat germ (5, 10, 15 and 20%) had no significant effect on the water absorption of the dough. They also reported that inactivation of glutathione by different methods (e.g. roasting) increased water absorption of the dough.

Empirical Rheological Properties of the Dough

The results (Table 2) show that by addition of either wheat flour or germ, dough arrival and development time increased and the highest values were observed for the samples containing different levels of wheat flour. Dough stability time is an indication of dough strength. The results show that with increasing the level of flour replacement in the dough, the stability time remained unchanged and the values were similar to that obtained for the control. However, with increasing the level of PG or RG in the samples, this parameter decreased significantly, probably due to the dilution effect of germs on gluten content of the samples. Moreover, the samples made with RG had shorter stability time since they contained glutathione, which could degrade gluten and cause further reduction in dough stability time. The presence of high amount of lipids in wheat germ can also have some effects in reducing dough stability time since fat can have lubricating effect on the dough and can soften the dough (Bloksma and Bushuk, 1988). Lipids are also involved in oxidation during dough mixing. Lipoxygenase present naturally in the wheat flour, particularly in the germ, can oxidize linoleic acids in the form of fatty acids and monoglycerided during dough mixing. The primary oxidation products of lipoxygenase are hydroperoxides and free radicals that can oxidize disulfide bonds of the gluten and strengthen the dough (Talit and Galliard, 1988). However, such effects should not be overestimated since the oxidized lipids and sulfhydryl groups react only minimally. Apparently, the presence of hydrocolloids and sugars in the germ, which can absorb water, had no significant effect on dough.
empirical rheology at the beginning of the dough mixing in the Farinograph.

The resistance of the dough to mixing is shown by determination of the dough softening after 5 and 12 minutes. In general, stronger dough with high gluten content has lower dough softening (Bloksma and Bushuk, 1988). The results show that with addition of the wheat flour, dough softening after 5 and 12 minutes remained unchanged and the values were similar to those of the control. However, addition of germs could increase the softening values of the dough. On the other hand, the dough became softer 5 and 12 minutes after the peak consistency. This can be due to the presence of high amount of lipids in the germ that soften the dough (Pomeranz, 1988). Moreover, degradation of gluten network as a result of glutathione in RG caused further increase in the dough resistance against mixing.

**Bread Moisture Content**

The water added during dough formation is absorbed mainly by gluten and polysaccharides and damaged starch granules. During baking, at high temperature, gluten denatures and discharges the absorbed water and gelatinize. Therefore, the moisture content of the bread is mainly stored by the gelatinized starch, non-starch polysaccharides, and sugars. The components of the wheat germ may have some effects on the gelatinization behavior of starch. Previous studies have shown that sugars and lipids can influence the thermal and pasting properties of starch (Watanabe et al., 2002; Chang et al., 2004; Torley and van der Molen, 2005). However, depending on the type and concentration of the sugars and lipids, their impact on the properties of starch is different. Therefore, these components (present in the germ) may also have some effects on the water absorption of the final bread by changing the degree of swelling, water uptake, and gelatinization process of the starch granules during baking.

The results showed that with addition of RG or PG to bread recipe, no significant changes in bread moisture content were observed. The results (data not given) showed that in all samples made with different levels of PG or RG, the moisture content was nearly 32.5±1.1 %. Determination of the water absorption of the samples by Farinograph showed that samples made with addition of wheat germ had higher water absorption than the control. However, after baking, all samples had similar moisture contents. Therefore, the wheat germ bread had higher baking weight loss than the control.

**Bread Volume**

The average height of the bread made in this study was 2 cm. Since Barbari bread is a kind of flat bread, its volume is not as important as it is for loaves. However, determination of the bread volume can be an indication of the number of gas cells in the bread which affect its softness.

Determination of the volume of bread samples during storage time (Figure 1) showed that the volume of the samples decreased slightly (but not significant) with increasing the storage time. Maleki et al. (1980) and Gray and Bemiller (2003) indicated that decrease in volume could be due to the shrinkage of the gas cells that occurs during bread staling. Addition of PG or RG caused further reduction in bread volume. The results are in agreement with Siddiq et al. (2009) who reported a reduction in bread volume with replacement of defatted corn germ. Arshad et al. (2007) also observed a reduction in the volume of cookies made with wheat germ. Despite the presence of proteins, fibers, and sugars in wheat germ, theses components cannot have significant contribution to bread volume as compared to the gluten. Moreover, lipids are known to have influence on bread volume, and there is high amount of lipids in the
Figure 1. The volume of the bread samples stored at 25°C: (A) Bread containing raw germ, (B) Bread containing processed germ.

wheat germ. The positive influence of polar lipids (as present in the germ) on bread volume is attributed to their ability to form lipid mono-layer at gas-liquid interphase of the gas cells, thus, increasing the gas retention of the dough. In this study, it seemed that the lipids present in the wheat germ could not compensate the reduction of the volume of germ breads caused mainly by the glutathione.

The results also indicated that the samples containing PG had higher volume than those made with RG. This can be related to the reduction of glutathione in PG and the presence of more intact gluten in the resulting dough. The empirical model obtained from Design Expert software (Table 3) indicates the negative effect of wheat germ level on the bread volume with a high coefficient of determination ($R^2 > 0.95$). According to the equation, the effect of storage time was not significant ($\alpha < 0.05$) on bread volume and, hence, it was eliminated from the equation.

Bread Texture

Figure 2 revealed that addition of RG increased the hardness of both crust and crumb of the fresh bread (time zero). Moreover, with increasing the storage time,

Table 3. Equations obtained in the D-Optimal mode of response surface methodology (given by Design Expert software) in terms of actual factors: effect of wheat germ and storage time on each measured parameter in Barbari bread. Coefficient of determination ($R^2$) between the actual and predicted values are given.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Equations in terms of actual factors: Effect of %wheat germ and storage time</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (RG) (cm$^3$)</td>
<td>$1011.43 - 38.58 \text{RG} + 1.3 (\text{RG})^2$</td>
<td>0.96</td>
</tr>
<tr>
<td>Volume (PG) (cm$^3$)</td>
<td>$1010.74 - 29.20 \text{PG} + 0.97 (\text{PG})^2$</td>
<td>0.95</td>
</tr>
<tr>
<td>Crust hardness (RG) (g-force)</td>
<td>$143.23 + 37.25 \text{RG} + 11.64 t - 1.19 (\text{RG})^2 - 0.06 t - 0.03 \text{RG}.t$</td>
<td>0.95</td>
</tr>
<tr>
<td>Crumb hardness (RG) (g-force)</td>
<td>$68.40 + 19.75 \text{RG} + 6.60 t - 0.76 (\text{RG})^2 - 0.03 t + 0.05 \text{RG}.t$</td>
<td>0.97</td>
</tr>
<tr>
<td>Crust hardness (PG) (g-force)</td>
<td>$159.20 + 7.70 \text{PG} + 9.90 t + 0.40 (\text{PG})^2 - 0.05 t$</td>
<td>0.96</td>
</tr>
<tr>
<td>Crumb hardness (PG) (g-force)</td>
<td>$84.70 + 6.00 \text{PG} + 5.70 t - 0.02 t + 0.03 \text{PG}.t$</td>
<td>0.98</td>
</tr>
</tbody>
</table>

$^a$ Time.
crust and crumb hardness increased as a result of bread staling. Starch retrogradation, water migration from bread crumb to the crust, and inside the starch granules, from the amorphous regions to the crystalline areas, and some interactions between starch and gluten are the main reasons for bread staling (Gray and Bemiller, 2003).

As shown in Figure 3, the texture of these samples became harder when PG was added in bread recipe. Moreover, the hardness of bread increased during the storage time as a result of staling. Storage time had greater negative impact on bread hardness than addition of PG.

Comparison of the bread texture of the samples made with PG and RG showed that the PG samples had softer texture and the rate of hardness increased slower. Increase in the hardness of germ bread samples could be related to the reduction of the amount of gluten as a result of increase in wheat germ level. Gluten is the main protein that has a significant role in bread texture and volume. Therefore, when the amount of gluten in the dough is reduced, it may result in a harder

**Figure 2.** The hardness of the bread containing raw germ stored at 25°C: (A) Crust hardness, (B) Crumb hardness.

**Figure 3.** The hardness of the bread containing processed germ stored at 25°C. (A) Crust hardness, (B) Crumb hardness.
bread texture. The results also indicated that the texture of bread made with PG was softer than that made with RG. This can be attributed to the decrease of glutathione in PG and, consequently, less destruction of gluten network of this sample. It seems that the adverse effect of glutathione on gluten was more significant on bread hardness than the positive effects of lipids on bread softness. The empirical model obtained from Design Expert software (Table 3) indicates the simultaneous positive effects of storage time and wheat germ level on the crust and crumb hardness with a high coefficient of determination ($R^2 > 0.95$). The positive coefficients of the model (Table 3) are much greater than the negative ones.

**Bread Microstructure**

The microstructure of the cell walls of the air bubbles of the bread samples are presented in Figure 4. Some deformed starch granules can be observed that were embedded in the denatured gluten matrix for the fresh control (1 hour after baking) (Figure 4-A). Furthermore, some holes and spaces can also be observed in this sample. However, during the storage time (72 hours), the structure became dense and shrunk as a result of bread staling (Figure 4-B). When fresh RG bread was observed under the electron microscope (Figure 4-C), a compact structure with no holes was observed. Moreover, it seemed that the starch granules became more apparent than those of the other samples.

**Figure 4.** The electromicrographs of the air bubble cell walls of the bread samples: (A) Fresh control; (B) Control after 72 hours; (C) Fresh raw germ bread; (D) Raw germ bread after 72 hours; (E) Fresh processed germ bread; (AF) Processed germ after 72 hours.
control, probably due to the reducing effect of the glutathione of the RG, which could split the integrity of the gluten network. After 72 hours, the crumb structure became more shrunk and denser than the control (Figure 4-D). This can be attributed to the staling of bread, which was enhanced by the destruction of gluten as a result of glutathione activity. On the other hand, the gluten of RG sample had weaker structure than the control and, hence, the staling became more obvious for this sample. For the fresh PG sample, a more solid structure than the control and less visible starch granules than the RG sample was observed (Figure 4-E). This can be related to the effect of the remaining glutathione in this sample. During storage for 72 hours, the staling process occurred but the structure was less shrunk than the RG sample (Figure 4-F). The microstructures of these samples give evidence for the softer and smoother texture of the PG samples compared to the RG samples during storage time. These results are in agreement with the results obtained from the Texture Analyzer.

CONCLUSIONS

Previous studies have shown the effects of wheat germ on loaves, while no published work of such effects on flat bread are available. The results of this study showed that Barbari bread can be used as a means to include high nutritious wheat germ in human diet, particularly in developing countries where flat bread is very popular. Nevertheless, it may have some undesirable effects on the quality of the dough and bread. These effects are almost similar to those observed for loaves by other researchers. In general, the dough became softer when wheat germ was added to the dough. Such effect was more pronounced when raw germ was used, probably due to the reducing effect of glutathione on gluten. Therefore, to obtain proper dough, destruction of glutathione in the germ is necessary.

The volume of bread is affected negatively by increasing the germ level. This is a great concern for loaves; however, it is of less importance for Barbari bread. Therefore, if wheat germ is to be added to the bread, flat bread is a better option. Moreover, when PG was used, the undesirable effects diminished.

The undesirable effects of wheat germ on dough and bread are in general due to the dilution of gluten proteins by wheat germ components, among which glutathione seems to have great influence on the dough and bread properties. Therefore, glutathione should be destroyed prior to addition of the germ to the bread. The method used in this study could destroy more than half of the glutathione and, hence, the quality of the bread samples made with processed germ was in general better than its unprocessed counterpart.

The results also indicated that addition of wheat germ to the Barbari bread cannot retard its staling as the main cause of bread loss since the rates of bread hardness and reduction of bread volume, as indicators of bread staling, were even higher than the control. Nevertheless, wheat germ could improve the taste and flavor as well as crust color of the bread, as determined by sensory evaluation. This can partly compensate the unfavorable effect of wheat germ on bread texture. Based on the panelists’ judgment, the sample made with 15% PG was the most acceptable sample. Therefore, this formulation can be introduced as wheat germ Barbari bread. If raw germ is to be used for Barbari bread production, lower levels (< 15%) may give acceptable results.

REFERENCES

 dokhtar آراش غذایی نان از اهمیت بسیاری برخوردار است زیرا نان غذای شده می‌تواند برای تغذیه افراد خاصی مانند مدخن کشته‌ها در حال توسعه و همین‌طور مزیت غذایی خاص استفاده گردد. جوانه گندم، بخشی از ارزش غذایی بالایی دانه گندم، یک محصول جانی کار آشته‌ها از طریق می‌باشد که دارای قابلیت بالایی برای استفاده جهت غذای سازی محصولات غذایی می‌باشد. هدف اصلی از انجام این تحقیق غذای سازی نان مسطح (پیری) با جوانه گندم و بررسی کیفیت و پذیرایی نان حاصل بود. به این منظور جوانه گندم فرآیند شده (حرارت دیده در دمای 1500 به مدت 25 دقیقه) و جوانه گندم خام با درصد‌های 50، 10 و 15٪ (وزنی/وزن) در فرمول نان به کار رفته. استفاده از فاریون‌گراف نشان داد که خمیر نهایی شده از جوانه گندم خام جذب آب، قوام و پایداری گمتری نسبت به سایر نمونه‌ها بود. مدل سازی داده‌ها نشان داد که افرادی که درصد جوانه و زمان ماندگاری تأثیر معنی‌دار دارند، می‌توانند نان با کیفیت بیشتری ساختند. نتایج نشان داد که استفاده از جوانه گندم باعث تعویض بیماری نان نشد، اگرچه استفاده از جوانه خاصی در غلظت 15٪ اثرات مثبت بر طعم و مزه و پذیرش کلی نان داشت.