Effect of Flour Age on Sensory Evaluation of Whole Wheat Bread
Katherine Kopfler, Laura Prevo, Tessa Thralls, Kali Tupper

Abstract
Aging or chemically treating refined flour to increase the number of disulfide bonds in the glutenin and gliadin protein components and thus optimize gluten development is standard practice in bread making. However, in contrast to refined wheat flour, whole wheat flour has a shorter shelf life and is prone to rancidity which may reduce its flavor profile. The objective of this study was to determine the effect of the age of flour on the sensory evaluation of whole wheat bread, particularly changes in color, flavor, texture and overall preference of the finished baked product. The study was divided into the following steps: preparation of three bread samples using flour aged for 0, 3, or 10 weeks; objective and sensory analysis of the bread; and statistical analysis of the data gathered. Bread made from flour aged for 0, 3, or 10 weeks showed no significant difference in texture, flavor, or preference. There was a significant difference in color between the 3 and 10 week variations, possibly the result of sampling error. Given that there is no significant difference in the sensory evaluation of flavor, texture, or overall preference associated with bread made from whole wheat flour aged 0, 3, or 10 weeks, when practical, bread may be made with whole wheat flour as fresh as possible to minimize nutrient loss. However, given the small sample size, further experiments are warranted.

Introduction
The practice of aging wheat flour is a method that has been employed for centuries to improve gluten structure and optimize physical properties of the finished baked product.\footnote{1} During maturation, oxidation occurs by way of atmospheric exposure of oxygen to flour, which changes the molecular structure of gluten fractions and results in the strengthening of gluten bonds.\footnote{1} The outcome of the increase in cross-linking of flour proteins is a stronger, more elastic dough with increased volume potential and a lighter texture in the final product.\footnote{1,2}

Of the gluten constituents found in freshly milled flour, oxidation primarily affects sulfur containing amino acids, such as cysteine, found in glutenin and gliadin.\footnote{3} During the maturation of flour, a gradual decrease of free sulfhydryl groups occurs due to the oxidation of two adjacent thiol groups resulting in the formation of disulfide bridges between the polypeptide chains of gluten proteins.\footnote{4} As flour ages, the degree of polymerization of gluten proteins increases, and leads to a tightly cross-linked protein structure with improved physical properties such as elasticity, gas retaining ability, and crumb structure.\footnote{5}

While aging is known to improve regular refined wheat flour, Doblado-Maldonado et al. stated that the shelf-life of milled whole wheat flour is shorter than refined white flour.\footnote{6} White flour solely contains the endosperm, whereas whole wheat flour is prepared from the intact grain and contains the nutrient-rich bran, germ, and endosperm. These nutrients
include thiamin, riboflavin, niacin, vitamin B6, folate, pantothenic acid, choline, betaine, vitamin E, lysine, phosphorus, potassium, magnesium, iron, zinc, manganese, selenium, fiber, proteins, and unsaturated fats.7–8

Stone milled (SM) wheat flours are more nutrient dense and flavorful than roller milled (RM) flours since they contain the original proportions of the endosperm, bran, and germ. 7–9,1 Also, stone grinding is a slower process, which doesn’t create as much heat.7 Excessive heat temperatures from milling can destroy vitamins in the flour due to oxidation and subsequent rancidity of the fat in the germ.7 A 2008 study compared the vitamin E content of SM wheat flour to RM wheat flour after 297 days of storage and found the SM wheat flour retained greater vitamin E content.9 Total vitamin E losses in the SM wheat flour were 25%, compared to 50% for the RM wheat flour after the storage period.9 The drawback of SM wheat flour is a shorter shelf life, due to the germ and bran content, making it more susceptible to rancidity.1

Although whole wheat flour contains substantially more nutrients, many of these compounds are subject to biochemical changes that reduce flour functionality during storage.6 The primary point of destabilization during storage is the degradation of lipids through lipase-mediated hydrolysis and, to a lesser extent, oxidation via lipoxygenase. Products of hydrolytic and/or oxidative rancidity can diminish the baking and sensory qualities of whole wheat flour.6

The B vitamins in wheat flour are particularly susceptible to destruction due to light and air exposure.7 Additionally, light and air can cause lipoproteins to become denatured and polymerized, phospholipids to undergo hydrolysis and auto-oxidation of their unsaturated fatty acids, and the Maillard Reaction to occur, leading to carotene, vitamin E, and aroma losses.7,9,10

Rancidity in flour has been detected as soon as 2 to 14 days after milling, meaning lipid oxidation and nutrient losses occur quite soon after processing.7 Therefore, it would seem beneficial from a nutritional standpoint to consume whole wheat flour very soon after it has been milled, preferably by stone-milling, to ensure the most desirable nutrient profile of the bread.

The purpose of the present study was to determine whether the length of aging whole wheat flour affects flavor and/or texture of its finished baked product. In the experiment, whole wheat flour was utilized in a basic flatbread recipe at three varying ages—10 weeks, 3 weeks, and 0 weeks. The age of the flour (independent variable), thus, had three conditions under which the dependent variables (flavor, texture, color, and overall desirability) were measured in order to determine if there were significant sensory and/or objective differences between varying ages of whole wheat flour. The hypothesis was that while aging flour may improve the texture of flatbread, the flavor would be less desirable. It was predicted that as the age of flour increased, the volume and crumb structure of the flatbread would improve and the texture would be perceived as chewier. Conversely, it was expected that as the age of the flour increased, the flavor of the flatbread would diminish and overall, be qualified as less desirable.
Methods

This study was divided into the following steps: preparation of three different bread samples, objective evaluation, sensory analysis of the bread, and statistical analysis of the data gathered.

Materials

For the 0 week and 3 week variations, organic hard red spring wheat berries from Bluebird Grain in the Upper Methow Valley of Washington State were purchased at PCC and ground using a Magic Mill stone grinder. Flour ground three weeks prior to bread preparation was stored on an uncovered sheet pan at cool room temperature (62-66 degrees F) and stirred twice per day. Flour ground the day of bread preparation was used within five minutes of grinding.

For the 10 week variation, hard red spring wheat flour was purchased already milled from King Arthur Flour. The grind date, as stamped on the package, was exactly 10 weeks prior to the date of bread preparation. The flour contained no bleach, no bromate, or any other added chemicals and included the entire wheat berry.

All variations were made with clover honey (Naturally Preferred), California olive oil (Napa Valley Naturals), iodized salt (Morton), and instant yeast (SAF).

Bread Preparation

For each bread variation 345 grams warm water, 65 grams honey, 20 grams olive oil, 300 g whole wheat flour (aged either 0, 3 or 10 weeks), 12 grams salt, and 12 grams yeast were combined in a Zojirushi Home Bakery Supreme Bread Machine and kneaded for 30 minutes on the dough cycle. After kneading, the dough was removed from the bread machine and allowed to rise in a covered glass bowl for 70 minutes. Following the initial rise, the dough was punched down and stretched to fit a 9 x 13 inch glass pan. The dough was covered and allowed to rise for 30 minutes then baked for 20 minutes in a 375 degree F oven. The complete recipe was the following:

**Basic Flatbread Recipe**\textsuperscript{11,12}

**Ingredients**

345 grams warm water (37-45C)
65 grams honey
20 grams olive oil
300 g whole wheat flour (0 weeks, 3 weeks, or 10 weeks)
12 grams salt
12 grams dried yeast
Procedures
1. Measure water, honey, and oil into bread machine.
2. Add flour, salt, and yeast.
3. Knead for 30 minutes on dough cycle.
4. Remove dough from bread machine. Place dough in an oiled bowl and turn the dough to coat it with oil.
5. Cover the bowl with a towel and let rise in a warm, draft-free location for 70 minutes.
6. Punch dough down and place it in a lightly oiled 9 x 13 inch glass pan.
7. By hand, gently stretch and press the dough until it covers the bottom of the pan.
8. Cover with a towel and let rise for 30 minutes in a warm, draft-free location.
9. Brush 1 Tbsp of olive oil over the top of the bread.
10. Bake 10-15 minutes, until brown or until toothpick comes out clean. Allow to cool for 5 minutes.
   • Cut bread samples in half and measure the height in triplicate.
   • Evaluate all the bread variations for crumb structure.
   • Label bread samples to be cut immediately before sensory evaluation.

Objective Evaluation
Each of the three bread variations was measured in triplicate for height.

Sensory Evaluation
Each of the three bread variations was rated in triplicate for pore uniformity of crumb structure and pore size of crumb structure by the researchers using the scorecard shown in Figure 1.

Figure 1: Scorecard for Sensory Evaluation of Crumb Structure

<table>
<thead>
<tr>
<th>Rank the samples according to the following characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Flour aged 0 weeks</td>
</tr>
<tr>
<td>Flour aged 3 weeks</td>
</tr>
<tr>
<td>Flour aged 10 weeks</td>
</tr>
</tbody>
</table>

To characterize the flavor, color, and texture of the three bread samples, descriptive sensory tests were administered to a panel using a 1 to 9 scale for each characteristic as
shown in Figure 2. It is important to note that a rating of five was considered the most desired rating in each of the three categories.

**Figure 2.** Scorecard for Descriptive Sensory Tests

In addition, to discriminate perceived chewiness and taster preference of the bread samples, rank order difference and affective tests were also administered to the panel (Figure 3).

**Figure 3.** Scorecard for Rank Order Difference and Preference Tests

<table>
<thead>
<tr>
<th>Please rank each sample from 1 to 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Which sample is the chewiest?</strong></td>
</tr>
<tr>
<td><strong>Which sample do you like best?</strong></td>
</tr>
<tr>
<td>Sample A</td>
</tr>
<tr>
<td>Sample B</td>
</tr>
<tr>
<td>Sample C</td>
</tr>
</tbody>
</table>
With the exception of the evaluation of crumb structure, the sensory evaluation was done by 25 adult male and female untrained testers on the same day as the bread was prepared. Approximately 60% of the panel was made up from students at Bastyr University and the other 40% were non-Bastyr University associated adults in Seattle, WA.

All subjects received a coded sample for each of the three variations, where Sample A was the 3 week variation, Sample B was the 0 week variation, and Sample C was the 10 week variation.

**Statistical Analyses**
The descriptive scale scorecards for flavor, color, and texture were analyzed using one-way between subjects ANOVAs with Tukey’s HSD Test performed on significant results. The rank order tests for chewiness and preference were analyzed using one-way $\chi^2$ tests.

**Results**
Figure 4 shows the results from the descriptive tests for flavor, color, and texture. All three flours were thought to have fairly desirable flavor, texture, and color as indicated by the means of each test being close to 5 (Figure 2 shows that 5 is the most desirable characteristic).

**Figure 4.** Mean Sensory Evaluation Scores for Flavor, Color, and Texture in Bread made from Fresh versus Aged Flour

For the flavor, the means of each sample were relatively close to 5 and a one-way, between-subjects ANOVA found no significant difference between means: $F(2, 72)=0.414$, $p>0.05$. This indicates that there was not a significant difference in flavor in any of the bread types.

For the color, the means of each sample were also relatively close to 5; however, one significant relationship was found using a one-way, between-subjects ANOVA: $F(2,$
\( F(2, 72) = 7.166, p < 0.05. \) When Tukey's HSD post-hoc test was performed, the significant difference found was between the 3-week and 10-week sample, and the mean for the 0-week sample was found in between the 3-week and 10-week sample.

**Table 1.** Panelists' Opinion of the Chewiest Bread

<table>
<thead>
<tr>
<th>Age of flour</th>
<th>Chewiest (f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-week</td>
<td>6</td>
</tr>
<tr>
<td>3-Week</td>
<td>9</td>
</tr>
<tr>
<td>10-Week</td>
<td>10</td>
</tr>
</tbody>
</table>

The texture of the bread was analyzed in two ways: the panel evaluated the softness versus the chewiness of the bread and the researchers evaluated the volume and cell structure of the bread. The means of the panel’s scores of each sample were all relatively close to 5 and no significant difference was found between means: \( F(2, 72) = 1.77, p > 0.05. \) The panel also indicated which bread-type was the chewiest when compared to the other two bread types (see Table 1). These data were analyzed using a one-way chi square test: \( \chi^2(2) = 1.0404, p > .05. \) The results of these two tests indicate that no significant differences existed between the perceived textures of the three bread types.

The researchers’ evaluation of volume and cell structure is displayed in Table 2. Three different researchers evaluated and scored the crumb structure based on pore uniformity and pore size (Figure 4). Note that the color differences between samples seen in Figure 4 were due to changes in lighting conditions, and do not accurately represent each sample color. It was determined that the freshest flour produced the most uniform crumb structure and the flour that was aged 10 weeks produced the largest pore size. The volume of the flour aged 10 weeks, as indicated by the height of all products baked in pans of the same dimensions, was also the largest.

**Table 2.** Analysis of Bread Height and Crumb Structure

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baked Height (in)</th>
<th>Crumb Structure (Pore uniformity)</th>
<th>Crumb Structure (Pore size)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1-non-uniform</td>
<td>1-Extremely small</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3-slightly uniform</td>
<td>3-Slightly small</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-somewhat uniform</td>
<td>5-Medium</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7-almost uniform</td>
<td>7-Slightly large</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9-uniform</td>
<td>9-Large</td>
</tr>
<tr>
<td>Flour aged 0 weeks</td>
<td>1.94</td>
<td>6.33</td>
<td>3.33</td>
</tr>
<tr>
<td>Flour aged 3 weeks</td>
<td>2.17</td>
<td>5.67</td>
<td>3.00</td>
</tr>
<tr>
<td>Flour aged 10 weeks</td>
<td>2.19</td>
<td>4.67</td>
<td>4.00</td>
</tr>
</tbody>
</table>

**Figure 4.** Pore uniformity and size of each bread sample. Pictured from top to bottom: 0-week-old flour, 3-week-old flour, and 10-week-old flour.

Finally, the panel was asked which bread type was their favorite. The panel was almost exactly evenly divided between the three bread types (see Table 3). Still, the results were analyzed with a one-way chi square test which indicated that bread preference was not dependent on the age of the flour: $\chi^2(2)=0.080$, $p>.05$.

**Table 3.** Panelists’ Favorite Bread Type

<table>
<thead>
<tr>
<th></th>
<th>Favorite (f)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>8</td>
</tr>
<tr>
<td>3-Week</td>
<td>9</td>
</tr>
<tr>
<td>10-Week</td>
<td>8</td>
</tr>
</tbody>
</table>
Discussion

The purpose of this study was to determine whether the length of aging whole wheat flour affects flavor and/or texture of its finished baked product. The hypothesis was that while aging flour may improve the texture of flatbread, the flavor would be less desirable. As this study revealed, these results were not obtained.

The significant results found in the color between the 3-week and 10-week samples do not make practical sense because the mean of the rating for color of the 0-week sample was 4.96, which is between the means of the 3-week and 10-week sample. Therefore, these significant results most likely reflect a type 1 experimental error that was most likely caused by one of the confounding variables discussed below.

The results of the study were not significant because too much variability existed within the subjects and not enough variability existed between subjects. One explanation for the large amount of variability within the subjects is that an untrained panel was used for descriptive sensory analysis. Since the panel was not made up of trained professionals, the nuances in texture, color, and flavor between the bread samples were difficult to detect. Perhaps a trained panel would have detected a chewier texture in the aged flour and a more pleasing taste in the fresh flour.

Also, there were some confounding variables that affected the characteristics of the flour. The fresh flour and the flour that was aged three weeks were from the same wheat and milled on the same mill; although the flour that was aged ten weeks was from the same type of wheat, it was a bought from a different company and milled in a different location with a different mill. Also, the flour that was aged three weeks was used first and while the dough was rising the house was still cold. The house was warmer while the dough from the other two flours was rising. This could affect the volume and texture of the final bread product.

Most likely, however, significant results were not found because the difference in texture, color, and flavor in the different bread samples were so small or non-existent. These results suggest that aging does not significantly affect the texture, and therefore, perhaps it is better to use freshly milled flour for its nutrient content.1,7

Possibilities for future studies include making all variation with flour from the same grain and ground in the same mill to reduce variation between conditions; adding dough conditioners to improve the texture and gluten development of un-aged flour; aging the flour longer than 10 weeks to determine if detectable changes occur; making more loaves per condition to improve the power of the statistical tests to detect small changes; using specialized equipment to objectively measure the elasticity of the dough and texture of the
final product; using a trained sensory panel to better describe and discriminate small differences; measuring changes in the nutrient profile over time to determine the extent of nutrient loss; and testing different bread recipes that may require additional gluten development such as a chewy Italian bread.

References

11) Department of Nutrition and Exercise Science, Food Science Laboratory TR5115L. *Food Science Lab 5: Gluten and Bread*. Kenmore, WA: Bastyr University; Fall Quarter 2012.