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Effect of Enrichment-Bleaching and Low Oxygen Atmosphere Storage on All-Purpose Wheat Flour Quality

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ABSTRACT

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on All-Purpose Wheat Flour Quality

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All-purpose wheat flour is a useful long-term storage commodity, but is subject to off-odor formation. Although flour stored in a low oxygen atmosphere should inhibit rancid odor formation, it elicits consumer complaints about odor. The purpose of this study was to examine off-odor development in all-purpose wheat flour during ambient and elevated storage by determining the effect of low oxygen atmosphere and enrichment-bleaching on quality as measured by, free fatty acids (FFA), flour descriptive sensory analysis, conjugated dienes, headspace volatiles, bread consumer sensory analysis, color, loaf volume, and vitamin analysis. Enriched, bleached (EB) and unenriched, unbleached (UU) flour was stored in a low and normal oxygen atmosphere in no. 10 cans at 22, 30, and 40°C for 24 weeks. Moisture remained constant throughout the study. Headspace oxygen was <0.1% in flour stored in a low oxygen atmosphere and decreased in flour stored in a normal oxygen atmosphere. FFA increased with storage time and temperature. The "fresh flour" descriptive aroma of flour decreased during storage and decreased more rapidly in a low oxygen atmosphere. The "cardboard/stale" aroma increased in flour stored in a normal oxygen atmosphere. The "acid-metallic" aroma increased in flour stored in a low oxygen atmosphere and was determined to be the off-odor from consumer complaints. Conjugated dienes and volatiles generally increased more rapidly in flour stored in a normal oxygen atmosphere and in EB flour, suggesting that the acid-metallic odor did not result from lipid oxidation. Bread consumer sensory analysis identified EB flour stored in a normal oxygen atmosphere to have the lowest acceptance scores for aroma, overall acceptability, and flavor. The acid-metallic odor dissipated within 24 hours when the container was opened and was not detrimental to consumer acceptance of bread made from the flour. Oxygen absorbers prevented the darkening of flour but not the reddening or yellowing. A low oxygen atmosphere resulted in higher bread loaf volumes. Vitamin degradation is not a concern under normal storage conditions. Bleaching appears to increase flour oxidative rancidity more than enrichment. Although storage at a low oxygen atmosphere results in an off-odor present in newly opened cans, it gave higher quality flour and bread. A low oxygen atmosphere should continue to be used in flour stored long-term, and consumers should be made aware that the off-odor present in cans of flour dissipates after opening.

Keywords: oxygen absorbers, oxygen scavengers, volatiles, aroma, oxidative rancidity, bread, SPME-GC-MS, descriptive analysis

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Introduction

Grains can be stored long-term for purposes of disaster relief and military operations (Cuendet et al 1954; Rose et al 2011). White flour is a useful storage commodity because of its minimal processing needed for consumption, versatility of use, and long shelf-life. Many factors can affect the shelf-life of flour including storage temperature, moisture content, relative humidity, atmospheric oxygen, light, and microbial activity (Wang and Flores 1999). White flour shelf-life is limited by formation of off-odors (Greer et al 1954).

Storage in a low oxygen atmosphere reduces off-odor development. An observational study examining the effect of low oxygen atmosphere on flour aroma was performed by Greer et al (1954) who concluded that gas-tight containers could prevent rancid odor development for about 8 years. They also found that rancidity was minimal in stored flour that had final atmospheric oxygen levels less than 5%. Rose (2005), in another observational study, examined flour stored up to 11 years in no. 10 cans at low oxygen atmosphere. Flour samples maintained >60% consumer acceptance for all but one sample and did not significantly decrease with increasing flour storage time. Bread made from the stored flour maintained >60% acceptance and appeared to decrease slightly with increasing flour storage time. However, consumers have reported objectionable odors in newly opened cans of enriched, bleached all-purpose wheat flour stored in a low oxygen atmosphere after a short storage time at ambient conditions (*personal communication*, Joe Thompson, LDS Welfare Services, 2010).

Other factors besides the amount of headspace oxygen can affect the off-odor development in flour during storage. Moisture content affects off-odor formation during storage (Fine and Olsen 1928; Cuendet et al 1954; Greer et al 1954; Bothast et al 1981). Although findings were inconsistent, lowering flour moisture to about 6% apparently slowed rancid odor development. Bleaching via chlorine dioxide was determined by Cuendet et al (1954) to hasten rancid odor development. Bleaching using ozone gas may also increase rancid odors (Sandhu et al 2011). Salgueiro et al (2005) reported that enrichment of white flour using iron sulfate increased headspace pentane (a rancidity indicator) and changed sensory attributes during storage.

The purpose of this study was to examine off-odor development in all-purpose wheat flour during ambient and elevated temperature storage by determining the effect of low oxygen atmosphere and enrichment-bleaching on quality as measured by free fatty acids, flour descriptive sensory analysis, conjugated dienes, headspace volatiles, and bread consumer sensory acceptance.

Materials and Methods

Samples and Storage Conditions

Two lots each of enriched, bleached (EB) and unenriched, unbleached (UU) all-purpose flour, were obtained from a local mill. Enrichment was accomplished at the mill using a commercial premix to add 46.3 mg/kg niacin, 5.84 mg/kg thiamin (as thiamine mononitrate), 3.97 mg/kg riboflavin, 1.54 mg/kg folic acid, and 37.5 mg/kg iron (as reduced iron). Bleaching was accomplished at the mill using benzoyl peroxide added at 51 ppm of the finished flour. After milling, a qualitative iron test using AACC International Method 40.40.01 was performed on UU flour to ensure the absence of enrichment. Flour was stored in bulk storage tanks at the mill and then 11.3 kg was packaged in Kraft paper bags which were stacked on pallets and covered with a low density polyethylene plastic film. Within 2 weeks of milling, flour was removed from the Kraft bags and sealed into no. 10 cans (1.8 ± 0.05 kg) at normal and low oxygen atmospheres. A low oxygen atmosphere was achieved by placing 300 cc Ageless ZPT oxygen absorbers (Mitsubishi Gas Chemical America, Inc., New York, NY) inside the cans before sealing. Cans were stored at 22, 30, and 40°C, with controls at -18°C. Two cans (one from each flour lot) of each treatment combination were evaluated for moisture, water activity, headspace oxygen, flour descriptive sensory analysis, bread consumer sensory analysis, and volatiles every 4 weeks for 24 weeks. After opening the cans, flour was immediately repackaged in Mylar bags and held at -18°C until further analysis. All analyses, except flour descriptive sensory analysis and bread consumer sensory analysis, were performed in duplicate with the mean values reported.

Moisture and Water Activity

Moisture was measured gravimetrically using AOAC method 925.10 (AOAC 2006) by drying at 130°C for 1 hour. Water activity was measured using an Aqualab CX-2 (Decagon Devices, Inc., Pullman, WA) following manufacturer's instructions.

Headspace Oxygen

Can headspace oxygen was measured using a 6500-Series Headspace Oxygen Analyzer equipped with an activated carbon filter (Illinois Instruments, Inc., Johnsburg, IL). Cans were punctured using a can piercing station, and an air-tight syringe was used to extract 30 cc of headspace gas. To eliminate possible carry-over effects, the first of three headspace measurements from each can was discarded and the mean of the last two measurements was reported.

Free Fatty Acids (FFA) and Conjugated Dienes

FFA and conjugated dienes were tested on two cans (one from each lot) of each treatment combination at weeks 0, 12, and 24. Flour lipid fractions were extracted using the method of Rose et al (2008) with minor modifications. The amount of flour and hexane used for the extraction was 5 g and 50 mL, respectively. After redissolving lipid extracts into 10 mL isooctane, a 5 mL aliquot was used for FFA and a 1 mL aliquot was used for conjugated dienes.

FFA were quantified according to Kwon and Rhee (1986). Conjugated dienes were quantified according to Pegg (2001) using an extinction coefficient of $2.525 \times 10^4\ M^{-1}\ cm^{-1}$.

Descriptive Analysis of Flour Odor

Sensory testing was conducted in compliance with the Brigham Young University Institutional Review Board. Flour (7.0 g) was placed into 2 oz. plastic soufflé cups with lids (Solo Cup Company, Lake Forest, Illinois). Cups were labeled with randomly assigned three digit codes. Cups were kept at 22°C and served within 3 hours of preparation. Descriptive analysis of flour odor was performed using a trained panel (n = 11, 6 females, 5 males, ages 23 to 55 years). Training was accomplished by presenting panelists with a number of fresh and aged samples of flour that had been stored in no. 10 cans. An aroma lexicon was then established to identify common aromas related to flour stored in no. 10 cans at normal and low oxygen atmosphere. Panelists agreed on six aroma descriptors: fresh flour, acid-metallic, musty, play dough, cardboard/stale, and other (to account for aromas not matching other descriptors). Panelists evaluated flour aroma with a 0-15 universal intensity scale using the Spectrum method (Meilgaard et al 2007). Scale anchors were provided where 0 = “not detected,” 1-5 = “slight,” 6-10 = “moderate,” and 11-15 = “extreme.” Reference samples with related aromas were provided for each panel session. Samples were evaluated in duplicate sets with unique blinding codes for each set, and panelists were given a ten minute break between sample sets. Evaluations were conducted individually using paper ballots. Samples were evaluated in a random order for each panelist. Panelists were instructed to smell samples immediately after removing the lid. Overall means for each sample were reported.

SPME-GC-MS of Headspace Volatiles

Volatiles were analyzed using the extraction method of Kaseleht et al (2011), and the GC analysis method modified from Cramer et al (2005). Clear glass 20 mL headspace vials and magnetic screw caps with a 1.3 mm polytetrafluoroethylene/silicone septum (Supelco, Sigma Aldrich, St. Louis, MO) were baked in a forced draft oven overnight at 120°C to remove volatile contaminants. Flour (1.50 g) was placed in the headspace vials along with 10 µL internal standard (5.00 mg/L 1,2,3-trichloropropane in methanol; Supelco, Sigma Aldrich, St. Louis, MO).

Volatile extraction, separation, and detection was performed the following day by solid-phase microextraction-gas chromatography-mass spectroscopy (SPME-GC-MS) using a divinylbenzene/carboxen/polydimethylsiloxane StableFlex SPME fiber (Supelco, Sigma Aldrich, St. Louis, MO). Extraction was automated using an MPS 2XL Multipurpose Sampler (Gerstel, Mülheim, Germany). Vials were incubated at 40°C for 30 min. while being shaken at 250 rpm. The SPME fiber was then injected and volatiles were extracted at 40°C for 30 min. while being shaken at 250 rpm.

Volatiles were thermally desorbed at 200°C in the injector port of a HP6890 gas chromatograph (Agilent Technologies Inc., Santa Clara, CA) with a DB-5ms column (30.0m × 0.25mm with 0.5µm film thickness; Agilent Technologies Inc., Santa Clara, CA). Helium (0.8 mL/min) was used as the carrier gas. The oven temperature was programmed with an initial temperature of 33°C for 5 min., a ramp of 2°C/min up to 50°C, followed by a ramp of 5°C/min up to 77°C and 7 minute holding time, followed by another ramp of 5°C/min up to 125°C, and a final ramp of 10°C/min up to 225°C. The total run time was 45.5 min. Volatiles were detected using an HP5973 mass selective detector (Agilent Technologies Inc., Santa Clara, CA) and then identified by spectra comparison to a library using ChemStation software (Agilent Technologies Inc., Santa Clara, CA) and by retention index comparison to published retention indices in literature. Retention indicies for this method were calculated based on Kovats retention indicies for linear-temperature programmed settings (Vandendool and Kratz 1963), using a series of straight chain alkanes (C8-C20). Retention indicies from literature were found for DB-5 type columns. Semi-quantitative analysis was used for determination of volatile compounds, where concentrations were calculated relative to the internal standard, assuming a 1:1 response ratio.

Consumer Acceptance of Bread Made From Flour

Consumer acceptance was evaluated using six consumer panels (n = 101 to 105). Panelists were recruited from Brigham Young University and the surrounding communities. Consumer panels were conducted in compliance with the Brigham Young University Internal Review Board. Approximately equal numbers of panelists from both genders were represented with an approximately equal distribution among age groups, with ages ranging from 18 to 65. Each panel evaluated bread samples prepared from two flour lots which had been stored at one of the three storage temperatures.

Each panelist received five bread samples, served side by side, consisting of two EB and two UU treatments (each including a normal and low oxygen atmosphere sample), and a frozen EB flour control. Flour from each treatment combination at storage week 24 only, along with frozen EB flour controls, was used to make bread. Panelists evaluated bread for overall acceptability, appearance, aroma, flavor, and texture on a 9-point hedonic scale. The scale was labeled such that 9 = "like extremely", 5 = "neither like nor dislike", and 1 = "dislike extremely". Samples were presented under red light to prevent bias due to color difference attributable to bleaching. Product appearance scores were evaluated to confirm the absence of an effect due to appearance-related bias on the part of the panelists. All samples were randomly assigned a three digit blinding code. An electronic ballot was presented using Compusense *five* software (Compusense, Guelph, Ontario, Canada).

Bread was made using an optimized straight-dough bread-making method 10-10.03 (AACC International St. Paul). Briefly, flour (1500 g), yeast (27 g), sucrose (90 g), salt (22.5 g), shortening (45 g), and water were combined in a mixing bowl. The amount of water added (910-955 mL) was optimized for each flour treatment and lot, as determined by the baker who looked for dough clearing the mixing bowl walls. Flour from 30°C and 40°C storage required 15 mL and 30 mL more water than flour from 22°C storage, respectively. The dough was mixed in a Hobart mixer equipped with a dough hook (N50, Hobart Corp, Troy, OH) for 10 min. at speed 1. The dough was then scaled to 5 sections of equal weight. Punching and molding were accomplished using a laboratory sheet roll and molder (National Manufacturing Co., Lincoln, NE). After molding, the dough was placed in a 21.6 X 11.4 cm bread loaf pan. Baking was done using a laboratory reel type oven (Model 8/16, National Manufacturing Co., Lincoln, NE). An electric knife and guide were used to cut 1 cm thick slices of bread, and bread slices were then cut vertically in half and stored overnight in sealed low density polyethylene bags.

Data Analysis

Data was analyzed using SAS and JMP version 10 software (SAS Institute Inc., Cary, NC). Significance was set at $p<0.05$. Multiple linear regression models including significant two-way and three-way interactions were constructed. Reduced models were used wherever possible. Data from SPME analysis exhibited logarithmic increases and therefore required log transformations. The model for the consumer panel data required a panelist nested in temperature term using a random effect. The Tukey-Kramer procedure was used to establish significant differences between multiple comparisons. Principle component analysis was performed on flour descriptive sensory analysis data where eigenvectors of the first two principle components were used to identify correlations.

Results and Discussion

Moisture and Water Activity

The moisture of flour samples was between 12% and 13%. Water activity ranged from 0.46 to 0.62. Moisture and water activity in these ranges are similar to those of commercial all-purpose flour on the market. Moisture did not significantly change over time, which indicates the package provided a good moisture barrier. Moisture values less than 14% and water activity values less than 0.6 are needed to inhibit mold growth (Bothast et al 1981; Singh and Cadwallader 2002).

Headspace Oxygen

Flour stored in a low oxygen atmosphere had headspace oxygen levels <0.1%, which indicates the container provided a good oxygen barrier and that the oxygen absorbers were effective. In flour stored in a normal oxygen atmosphere, several significant effects on headspace oxygen were observed. All of these flour samples began with headspace oxygen at 20.9% and oxygen decreased during storage. Oxygen levels decreased significantly faster in EB flour compared to UU flour (4.8 percentage points lower over 24 weeks). Oxygen also decreased significantly faster in 40°C storage compared to both 22 and 30°C storage (8.5 and 6.5

percentage points lower over 24 weeks, respectively). Headspace oxygen levels had the lowest values and greatest declines in the EB treated flours and at the highest storage temperature. The decline in headspace oxygen can be attributed to oxidative reactions in the flour, which would be expected to proceed to a greater degree in these conditions (Halton and Fisher 1937).

Free Fatty Acids

FFA ranged from 0.87 to 1.56 µmols/g at time zero and increased steadily in flour as a function of time and temperature. FFA in flour stored at 22, 30, and 40°C increased, respectively, 2.96, 5.14, and 9.56 µmols/g over 24 weeks. FFA levels were not affected by enrichment-bleaching or a low headspace oxygen during storage, indicating that hydrolysis occurred independently of these factors.

Descriptive Analysis of Flour Odor

Principle component analysis of the six flour aroma descriptors accounted for 58% of the variation within two principle components (Fig. 1). The "acid-metallic" descriptor did not correlate with any other aroma descriptors. The "cardboard/stale" descriptor was correlated with "play dough," as was the "fresh flour" with "other." However, the "cardboard/stale" and "play dough" descriptors were both negatively correlated with the "fresh flour" and with "other" descriptors. The "musty" aroma descriptor showed no correlation because scores were low in all flour samples.

Consumers complaining about an objectionable odor in all-purpose wheat flour stored in no. 10 cans in a low oxygen atmosphere also stated they would not use the product (*personal communication*, Joe Thompson, LDS Welfare Services, 2010). In the present research, it is believed that this objectionable odor is the "acid-metallic" aroma descriptor. This objectionable odor was termed "acid-metallic" to describe an odor somewhat like an opened can of pineapple.

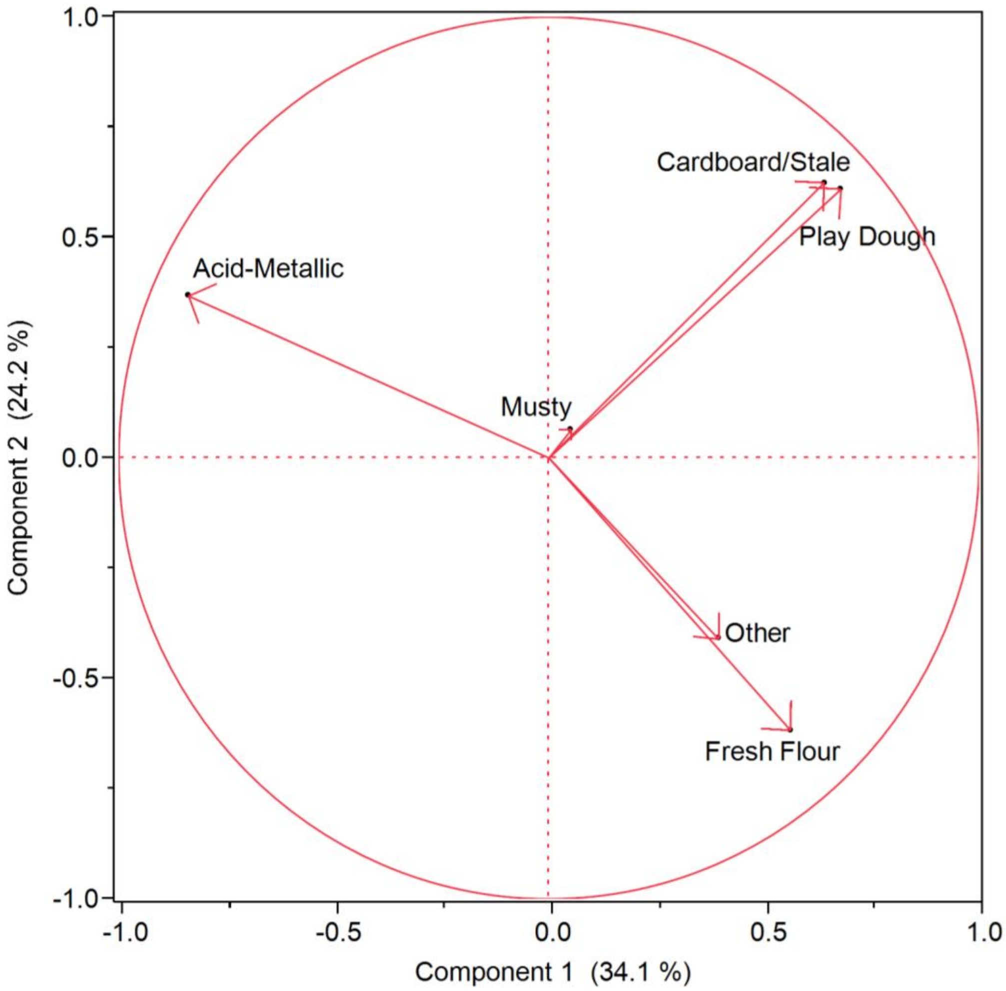

Fig. 1. Principle component biplot of aroma descriptor scores in flour samples.

The descriptors acid-metallic, fresh flour, and cardboard/stale, were examined for correlation with treatment variables. There was a significant decrease in fresh flour scores over time (Fig. 2A). A low oxygen atmosphere resulted in a significantly faster initial rate of decline in fresh flour aroma descriptive scores. Fresh flour scores in EB flour also decreased faster than UU flour (score was 0.3 lower at 24 weeks). These decreases are likely the result of an increase in the acid-metallic and cardboard/stale odors (discussed below), which masked the fresh flour odor.

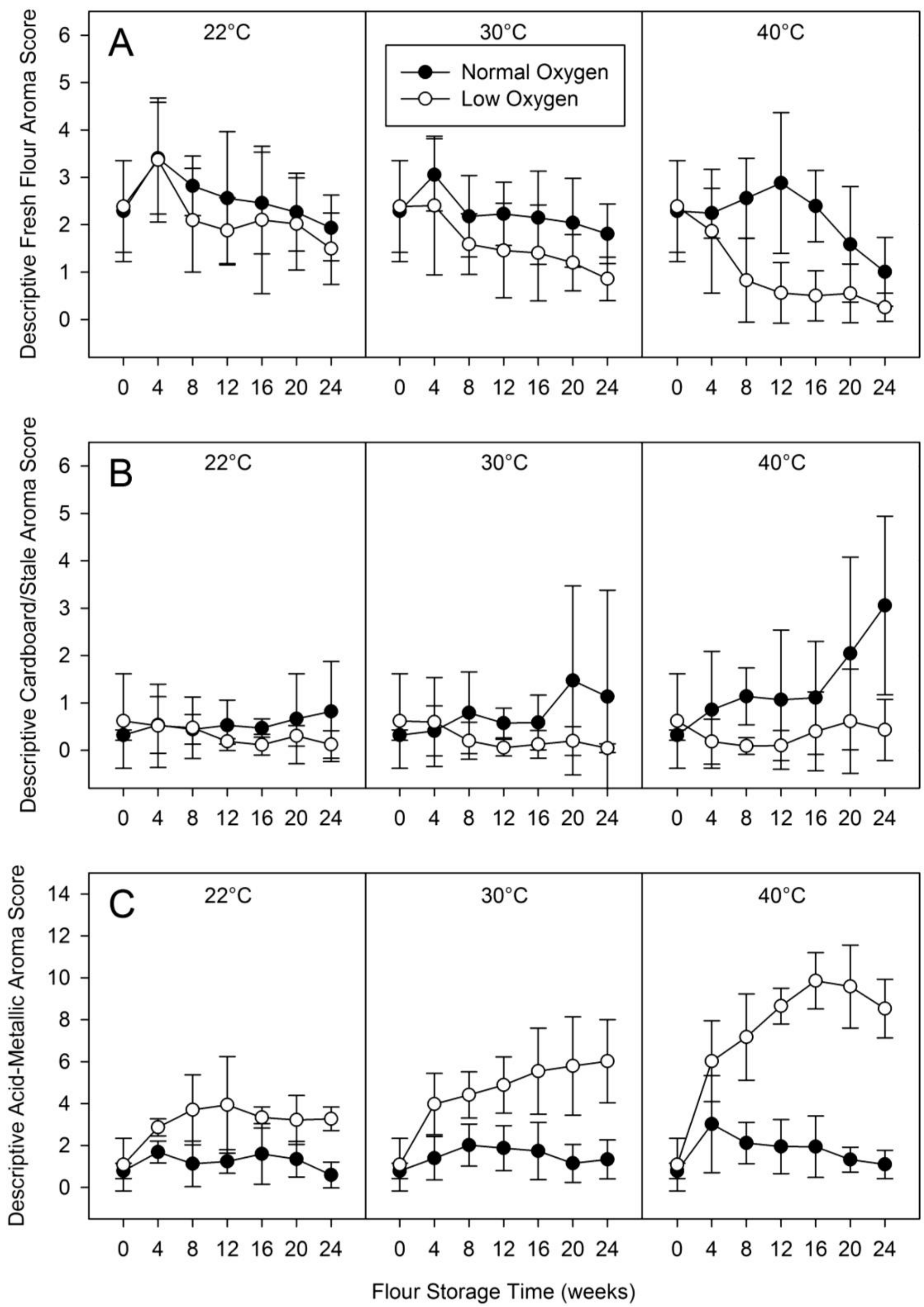

Fig. 2. Effect of low oxygen atmosphere on descriptive fresh flour (A), cardboard/stale (B), and acid-metallic (C) aroma scores in flour samples during storage at 22, 30, and 40°C. Each data point is the mean of enriched, bleached (EB) and unenriched, unbleached (UU) treatments. Error bars represent the 95% confidence interval. Note the difference in scales.

Cardboard/stale scores increased significantly over time for flour stored in a normal oxygen atmosphere (Fig. 2B). Storage in a normal oxygen atmosphere gave significantly higher cardboard/stale scores for both EB flour (scores were 0.9 higher) and UU flour (scores were 0.5 higher). EB flour in normal oxygen atmosphere also had significantly higher scores than UU flour in normal oxygen atmosphere (scores were 0.6 higher; data not shown). This cardboard/stale odor is typical of oxidation reactions (StAngelo 1996). While it appears that a low oxygen atmosphere prevented the formation of the cardboard stale odor, it is possible that this odor was masked by the presence of other off-odors.

For acid-metallic scores (Fig. 2C), a low oxygen atmosphere resulted in significantly higher scores, with flour stored at 22, 30, and 40°C having mean scores 2.1, 3.5, and 6.4 higher, respectively. In flour stored in a low oxygen atmosphere at 40°C, the acid-metallic odor peaked at 16 weeks with a mean score of 9.9. No differences were found between EB flour and UU flour, suggesting the enrichment-bleaching treatment had no effect on the formation of the acid-metallic odor.

Because the acid-metallic odor resulted from storage in a low oxygen atmosphere, it is atypical of rancid odors resulting from oxidation reactions. There may be several possible mechanisms for the acid-metallic odor formation. The absence of oxygen may result in anaerobic microbial growth which produces volatile compounds. This, however, is not likely because the moisture and water activity observed in this study is sufficient to prevent microbial growth. It is also possible that the iron contained within the oxygen absorbers packet acts as a pro-oxidant, which initiates free radical reactions. Research by Ueno et al (2012) showed that iron (II) sulfate in powdered milk resulted in elevated oxidized odors and metallic tastes. Another possibility is that the removal of oxygen drives reactions leading to the reduction of certain compounds. For example, removal of oxygen bonds from carbon can convert carboxylic acids to aldehydes and ketones or aldehydes to alcohols. However, to react quickly enough, these reactions require a strong reducing agent, like sodium borohydride or lithium borohydride. Iron catalysts, however, have been found that will hydrogenate aldehydes and ketones under mild conditions (Casey and Guan 2007). It is possible that the iron in flour or within the oxygen absorber packet acted as a pro-oxidant or as a catalyst, and produced the compound(s) responsible for the acid-metallic odor during storage. Further exploration showed that cans which contained only oxygen absorbers, without the flour, did not result in the acid-metallic odor, suggesting that a reaction involving the flour is responsible for this odor.

Rose (2005) performed consumer sensory analysis on flour stored in no. 10 cans at low oxygen atmosphere, but did not mention any atypical odors. It is possible that this acid-metallic odor was not observed because the flour was allowed to air out before sensory evaluation occurred. The possible airing-out effect was further tested by the descriptive analysis panel, and it was found that the acid-metallic odor dropped to negligible levels in newly opened cans of flour within the first 24 hours.

Conjugated Dienes

Conjugated dienes (Fig. 3) increased significantly in flour stored in a normal oxygen atmosphere (values were 0.05 μmol/g higher at 24 weeks). No increases were observed for flour stored in a low oxygen atmosphere. No significant effects were observed due to storage temperatures. EB flour in normal oxygen atmosphere also had significantly lower conjugated diene values than UU flour in normal oxygen atmosphere.

An increase in conjugated dienes indicates the presence of primary lipid oxidation products. These results indicate that the primary lipid oxidation reactions are inhibited when flour is stored in a low oxygen atmosphere. This provides evidence that the acid-metallic odor in flour stored in a low oxygen atmosphere is not caused by typical primary lipid oxidative reactions common in foods. Additionally, the lower values in EB flour may indicate that the conjugated dienes were oxidizing more rapidly into secondary oxidation products.

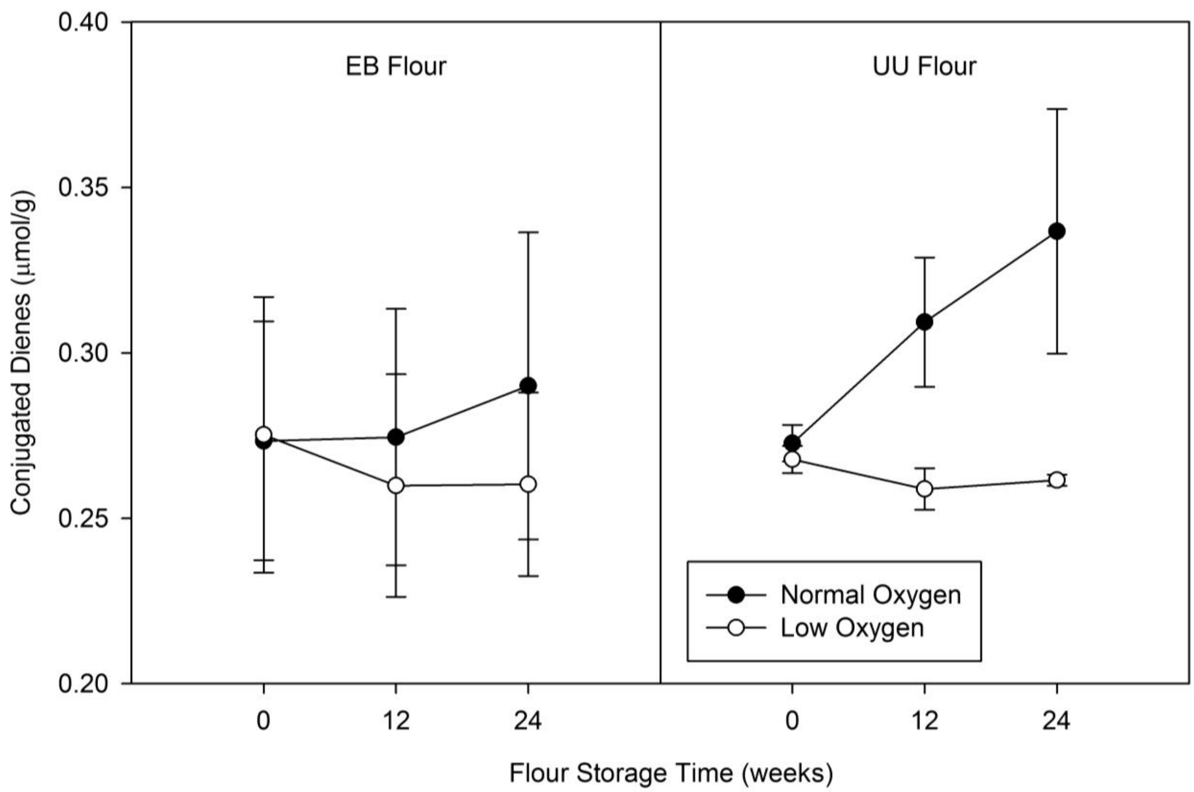

Fig. 3. Effect of low oxygen atmosphere on conjugated dienes in enriched, bleached (EB) and unenriched, unbleached (UU) flour during storage. Each data point is the mean of all storage temperature treatments. Error bars represent the 95% confidence interval.

SPME-GC-MS of Headspace Volatiles

Forty-three volatile compounds with odor impressions (data not shown) were identified using SPME-GC-MS. None of the compounds identified correlated with the acid-metallic odor. To explore potential reaction mechanisms, the three most abundant volatiles (2-pentyl furan, 1-hexanol, and hexanal) were examined for significant differences between treatments. For 2-pentyl furan (Fig. 4A) and 1-hexanol (Fig. 4B), a low oxygen atmosphere resulted in a significantly slower rate of formation. EB flour had significantly higher concentrations of these volatiles than UU flour.

Hexanal formation was significantly lower in UU flour than in EB flour (Fig. 4C). In storage at 22°C and 30°C, a low oxygen atmosphere resulted in a significantly decreased rate of hexanal formation. On the contrary, at 40°C, a low oxygen atmosphere resulted in a significantly increased rate of hexanal formation.

The acid-metallic aroma descriptive scores did not correlate with the amount of any volatile compounds that were identified using GC-MS. This suggests that the compound responsible for the acid-metallic odor is produced in very low concentrations and has a low sensory odor-threshold such that it is not detected by SPME-GC-MS but is detected by the human nose. Kaseleht et al (2011) also encountered a number of compounds in flour with low odor thresholds that were not detected using SPME-GC-MS.

The volatiles 2-pentyl furan, 1-hexanol, and hexanal are secondary products that result from the oxidation of linoleic acid, the most prevalent fatty acid in wheat flour (Huang et al 1994). Since a low oxygen atmosphere prevents the formation of these three volatiles, the acid-metallic odor is likely the result of a reaction pathway separate from lipid oxidation. More research is needed to determine the reaction mechanism responsible for this odor.

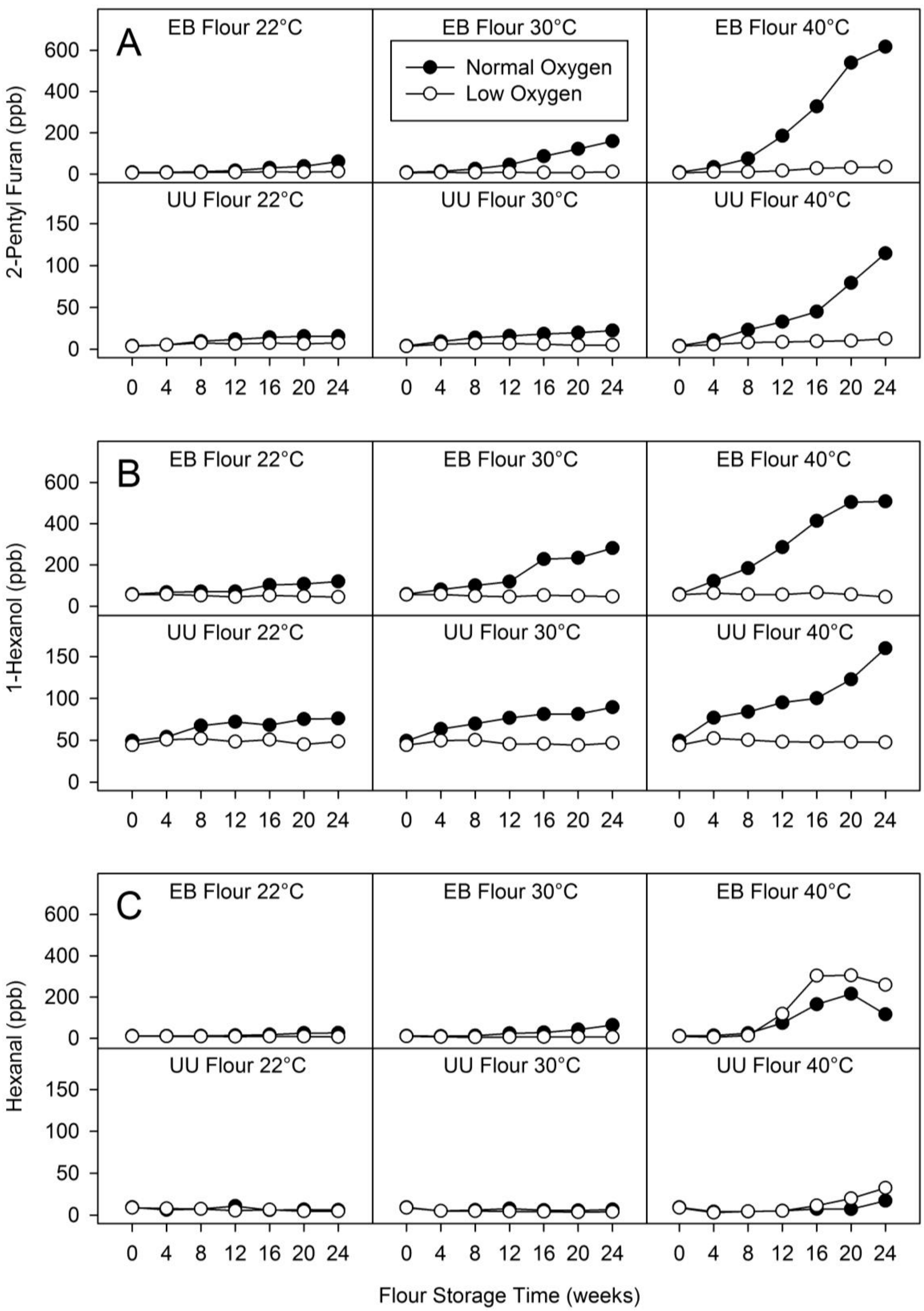

Fig. 4. Effect of a low oxygen atmosphere on headspace 2-pentyl furan (A), 1-hexanol (B), and hexanal (C) concentrations in enriched, bleached (EB) and unenriched, unbleached (UU) flour during storage at 22, 30, and 40°C. Note the difference in scales.

At 40°C storage, a low oxygen atmosphere resulted in increased hexanal concentrations. Apparently, there is a distinct hexanal-forming reaction occurring at 40°C that does not depend on atmospheric oxygen. Moreover, hexanal can continue to oxidize and form hexanoic acid, along with many other compounds (Palamand and Dieckman 1974). The lower concentration of hexanal in samples stored at 40°C in a normal oxygen atmosphere may be due to hexanal decomposition.

For all three of these compounds, EB flour had higher concentrations of volatiles than UU flour. This suggests that bleaching and/or enrichment is related to an increased amount of lipid oxidation reactions. Cuendet et al (1954) found that flour bleached using chlorine dioxide developed rancid odors sooner than unbleached flour. Marston (1972) reported complaints of flour with sweetish, slightly acrid, or rancid odors which were determined to be caused by excessive doses of benzoyl peroxide.

Consumer Acceptance of Bread Made From Flour

Overall mean acceptance scores for each sensory attribute examined ranged from 6.7 to 7.1, indicating a moderate liking of bread samples by consumers (data not shown). Appearance was not shown to have any significant differences between treatments, confirming there was no bias from sample appearance. Within each storage temperature, flours stored at 22 and 30°C did not have any significant differences between treatments.

The 40°C storage treatment mean acceptance scores are shown in Table 1. Bread made from EB flour stored in a normal oxygen atmosphere had aroma, overall acceptability, and flavor scores that were significantly lower than three or more other samples, including the control.

Table 1. Mean acceptance scores of bread made from flour stored at 40°C for 24 weeks

Flour Sample	Aroma	Overall Acceptability	Flavor	Texture
Control	6.6^{a}	6.7^{a}	6.7^{a}	6.7^{ab}
EB flour at low oxygen atmosphere	6.8^{a}	6.8^{a}	6.8^{a}	7.0^{a}
EB flour at normal oxygen atmosphere	6.3^{b}	6.3^{b}	6.3^{b}	6.9^{ab}
UU flour at low oxygen atmosphere	6.6^{ab}	6.6^{ab}	6.7^{a}	6.6^{b}
UU flour at normal oxygen atmosphere	6.8^{a}	6.9^{a}	6.9^{a}	7.0^{a}

In each column, values without the same superscript letter are significantly different ($p<0.05$). EB = enriched, bleached and UU = unenriched, unbleached. The control sample is EB flour stored at -18°C.

The acid-metallic aroma descriptive scores did not correlate with any consumer acceptance attributes in bread. This suggests that the acid metallic odor in flour does not carry through into bread made from the flour. The acid-metallic odor in newly opened cans of flour dissipated within the first 24 hours after opening. The bread making process also appears to allow the acid-metallic odor to dissipate and not impact bread acceptability.

The EB flour stored in a normal oxygen atmosphere at 40°C had the lowest aroma, flavor, and overall acceptability scores in the bread sensory panels and was also identified in the descriptive panel as having the highest cardboard/stale aroma descriptive scores. This suggests that the cardboard/stale odor in flour affects the sensory quality of baked products. Greer et al (1954) similarly reported that higher amounts of rancid odors in flour resulted in bread with higher rancid odors.

Conclusions

For all-purpose wheat flour, storage in a normal oxygen atmosphere resulted in higher primary and secondary lipid oxidation products, higher cardboard/stale descriptive aroma scores, and lower fresh flour descriptive aroma scores. These effects were greater in EB flour samples than UU flour samples. EB flour stored at 40°C in a normal oxygen atmosphere had the lowest bread consumer acceptance scores and the highest cardboard/stale descriptive aroma scores.

These results indicate that storing flour in a normal oxygen atmosphere results in increased oxidative rancidity in flour and bread. Additionally, the enrichment and/or bleaching treatments may result in increased oxidative rancidity during storage, but more research is needed.

On the other hand, storage in a low oxygen atmosphere resulted in an atypical off-odor present in newly opened cans of flour. This off-odor was termed “acid-metallic” in descriptive analysis. The compound responsible for the acid-metallic odor could not be detected using SPME-GC-MS, nor could the mechanism of its formation be determined. However, it apparently did not result from the formation of primary and secondary lipid oxidation products. The acid-metallic odor dissipated within 24 hours when the container was opened and was not detrimental to consumer acceptance of bread made from the flour. To ensure high sensory quality of baked goods, a low headspace oxygen atmosphere should be used for the long-term storage of all-purpose wheat flour, but consumers should be made aware of the off-odor present in newly opened cans.

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APPENDIX A: EXPANDED LITERATURE REVIEW

Background on Wheat Flour

Wheat Classification and Statistics

Wheat flour typically comes from the wheat species of *Triticum aestivum and Triticum durum*. In the United States, *Triticum aestivum* consists of the following classes: hard red winter (HRW), hard red spring (HRS), soft red winter (SRW), soft white (SWH), and hard white (HDWH). Red or white refers to the kernel color, hard and soft refers to the kernel hardness, and winter and spring refers to the time of the year it is planted, in the fall and spring, respectively (Atwell 2001). HRW is the most common variety in the United States and makes up about 40% of the wheat crop (Khan and Shewry 2009). HRW, HDWH, and HRS are usually used in making stiff doughs and breads, and SRW and SWH are usually used to make cakes. Wheat from *Triticum durum* is known as durum wheat and is mostly used to make pasta products (Atwell 2001).

Table 2 shows the total world and US production of different cereal grains in 2009. Wheat was the second highest produced cereal grain in the world and in the United States. The United States was the 4th largest producer of wheat in the world in 2011 (FAO 2013). In 2012, 420,336,000,000 pounds of wheat flour were produced in the US and 134.4 pounds of flour were consumed per capita (USDA 2013).

Composition of Wheat

The wheat kernel is divided up into three general components: the bran, germ, and endosperm. The bran, which makes up about 14% of the kernel and is the outside protective layer, is high in fiber and minerals. The germ, which makes up only 3% of the kernel, is high in lipids and other nutrients along with certain enzymes. The largest part of the kernel is the endosperm, which contains most of the protein and starch. The outermost layer of the endosperm, which separates the endosperm and the bran, is known as the aleurone layer. This layer is high in enzymes and is often removed along with the bran during milling (Atwell 2001).

Table 2. World and US production of cereal grains in 2011 (FAO 2013)

Cereal Grain	World Production (millions of tons)	US Production (millions of tons)
Maize	883	314
Rice, paddy	723	8
Wheat	704	54
Barley	134	3
Sorghum	54	5
Millet	28	>1
Oats	23	1
Rye	13	>1
Buckwheat	2	>1

Flour Milling and Processing

Dry milling of flour involves a number of steps. Wheat is first cleaned to remove any foreign particles, and then it is tempered, which uses water to increase the moisture content of the grain to 15.5-16.5% moisture in 12 to 18 hours (Atwell 2001). Tempering helps soften the endosperm and harden the bran, allowing for better separation of the bran and endosperm (Khan and Shewry 2009). The wheat is then ground and separated in a series of rollers and purifiers, and then the separated flour is further ground in reduction rollers. Flour can then be bleached, enriched, or treated with other additives (Atwell 2001).

Flour extraction rates refer to the amount of flour yield from milling (Khan and Shewry 2009). Whole wheat flour has essentially 100% extraction because it includes all components of the wheat kernel. Other types of white flour usually have between 45 and 72% extraction (Atwell

2001). Generally, the lower the flour's extraction rate, the better the purity and quality. There is an economical advantage to increasing the extraction rate; a better separation of the bran and endosperm results in more flour being produced (Khan and Shewry 2009).

White flour can be treated with bleaching and maturing agents to remove yellow pigment (mostly carotenoids) and/or aid in maturation (Pomeranz 1988). Maturation refers to time needed for flour to undergo oxidative reactions, which will make a stronger dough gluten (Belitz et al 2009). Maturation produces dough with better machining properties and an improved baked product (Pomeranz 1988). Flour bleaching and maturing agents that are approved for use in the United States include oxides of nitrogen, chlorine, nitrosyl chloride, chlorine dioxide, benzoyl peroxide, acetone peroxides, and azodicarbonamide (CFR 2010b). Benzoyl peroxide and azodicarbonamide are the most widely used. Benzoyl peroxide is the only bleaching agent that does not affect flour maturity, and azodicarbonamide is a flour maturing agent that has no bleaching action (Pomeranz 1988; Damodaran et al 2008).

Flour Types

In the United States, different types of white flour are marketed to consumers. These white flours are milled from the endosperm of the wheat kernel after removing the germ and the bran. Consumer flour types include cake flour, pastry flour, all-purpose flour, and bread flour. Cake flour and pastry flour are made from soft wheat and have protein contents of about 8% and 9%, respectively. Bread flour is made from hard wheat and has the highest protein content at 16%. All-purpose flour is made from a blend of hard and soft wheat and usually has a protein content around 12% (Bloom 2007). In the United States, all-purpose flour can vary by location. For instance, it can contain more hard wheat in the Midwest, and can contain more soft wheat in the Southeast (Pomeranz 1988).

There are other names in the milling industry to describe flour. Straight grade flour has most of the bran and germ removed and is has about 72% extraction (or 72% of the kernel remaining). Patent flour has even less bran than straight grade flour and has between 45% and 65% extraction. Clear flour, or low grade flour, is the flour remaining after straight grade flour has been refined to make patent flour. Cutoff flour is the flour omitted from between 45% and 65% extraction in patent flour (Atwell 2001).

Flour can also be distinguished based on protein quality. Weak flour, in comparison to strong flour, can either contain a lesser amount of protein, a lesser quality of protein, or protein that has been affected by proteolysis (Hui 2006). Strong flour requires a longer mixing time and makes a more elastic dough. Weak flour has lower mixing requirements and makes a less elastic dough (Khan and Shewry 2009).

Composition of Flour

White flour can have 7 to 15% protein, 63 to 72% starch, 4.5 to 5.0% nonstarch polysaccharides, and 1% lipids (Atwell 2001). All-purpose flour that is enriched and bleached has a composition similar to that shown in Table 3.

Table 3. Proximate composition of all-purpose flour, bleached and enriched (USDA 2010)

Nutrient	%
Water	11.92
Protein	10.33
Carbohydrate	76.31
Fat	0.98
Ash	0.47

Enzymes in wheat flour include amylases, proteases, lipases, lipoxygenases, pentosanases, phytase, and polyphenol oxidase. The enzymes that can cause rancid off-flavors are lipases and lipoxygenases (Atwell 2001). Lipases hydrolyze lipids creating free fatty acids (FFA) which can lead to increased oxidative rancidity. Lipoxygenase catalyzes lipid oxidation which causes oxidative rancidity, along with loss of unsaturated fatty acids and carotenoids. Many enzymes are located in the bran and germ portions of the wheat kernel, which makes whole grain flours spoil more quickly (Barnes 1983).

Enriched white flour is required to contain some nutrients that were removed during milling. The CFR (2010a) explains that each pound (453.6 g) of flour must contain 2.9 mg of thiamin, 1.8 mg of riboflavin, 24 mg of niacin, 0.7 mg of folic acid, and 20 mg of iron.

Flour Storage

Overview of Quality Factors

White flour in storage can have a limited shelf life for a number of reasons. Flour quality may deteriorate from mold growth, increased hydrolytic reactions, oxidative rancidity resulting in off-flavor development, loss of breadmaking ability, destruction of nutrients, and other factors. Quality may be influenced by factors such as packaging, moisture content, flour refinement, exposure to oxygen, or treatment with bleaching and maturing agents.

Mold Growth

Mold growth can be a concern in grain storage because of the potential production of mycotoxins, which are toxic secondary metabolites produced by fungi and can be harmful to humans (Chassy 2010). Flour with moisture over 14% can develop mold growth during storage (Sharp 1924; Fisher et al 1937; Greer et al 1954; Franz 1968; Arya and Parihar 1981; Bothast et al 1981). Mold counts were significantly reduced in flour at 14% moisture or less (Cuendet et al 1954). Mold is not normally a problem in all-purpose flour since it has around 12% moisture. However, it is possible that lower-moisture all-purpose flour can gain moisture from the environment (especially in very humid climates) and be in danger of spoilage by mold. Water activity can also be used to predict mold growth. Clayton and Morrison (1972) did not see mold growth in their samples with water activity under 0.66. Mold can develop in flour samples with water activity over 0.75 to 0.80 (Clayton and Morrison 1972). Akhtar (2008) suggested that mold can be prevented during storage by using packaging with good moisture barrier properties and by avoiding storage in hot and humid conditions. Mold can be measured using an agar which selects for molds and yeasts (Tariq et al 2006; Akhtar et al 2008).

FFA Formation

One of the most dramatic changes in flour is the increase of free fatty acids (FFA) caused by hydrolysis of triacylglycerides. Clayton and Morrison (1972) found an increase in FFA, which was probably caused by both lipase enzymes and non-enzymatic hydrolysis reactions. They did not find evidence of any other lipid degradation enzymes, like lipoxygenases. In other studies, higher moisture increased the rate of FFA formation (Gracza 1965; Murray and Moss 1990). FFA increased fastest in the order of weak, medium, and strong flours (Warwick et al 1979). Other studies have also shown a time dependent increase in FFA during storage (McCalla et al 1939; Bellenger and Godon 1972; Galliard 1986; Rose 2005; Maraschin et al 2008). Hansen and Rose (1996) found that sensory acceptability of bread made from stored flour was inversely related to FFA content. FFA formation increases with increasing storage temperature (Murray and Moss 1990; Salman and Copeland 2007; Maraschin et al 2008). Shellenberger et al (1958) found that FFA increased greatly at 38°C, while storage at 4°C had FFA that increased slightly or not at all over one year. Flour stored at 15°C was shown to have greatly slowed FFA formation (Barton-Wright 1938). Arya and Parihar (1981) found that moisture levels of 11.6% and above allow for hydrolysis of phospholipids and galactolipids. They also found that at moisture levels of 10.4% and below, hydrolysis of neutral lipids was predominant. Flour at very low moisture levels (3%) had no noticeable increase, or very slight increases, in FFA (Cuendet et al 1954). FFA formation may increase lipid-starch complexes, which can affect the pasting properties, or the gluten development, in dough (Salman and Copeland 2007). FFA concentration can be measured by solvent extraction of the fat, followed by titration with KOH (Hansen and Rose 1996) or NaOH (Warwick et al 1979).

Oxidative Rancidity and Off-Flavor Development

Rose (2005) examined ten samples of flour stored up to 11 years under low oxygen conditions in no. 10 cans. He discovered that aroma sensory acceptance scores of stored flour were not dependent on flour storage time. Aroma, however, could be predicted by moisture content, hexanal, and lipid hydrolysis. This concurs with the findings of Bothast et al (1981). Greer et al (1954) found that gas-tight containers could prevent rancid odor development for about 8 years. They also found that rancidity was minimal in samples that had final oxygen levels less than 5%. Flours with greater than 5% oxygen developed rancid or musty odors if moisture was around 14%. In another study, unpleasant musty odors were noticed in bread made from stored flour (between 12 and 14% moisture) at 25°C after 7 months and at 12°C after 21 months (Bell et al 1979). A musty odor was also identified in 6 month old flour at 14.7% moisture stored at room temperature, but it was unknown whether microorganisms might have been responsible (Pomeranz et al 1968). Musty off-odors may be related to the musty smelling geosmin and 2-methylisoborneol compounds caused by microbial growth in wheat grain (Jelen et al 2003). Other off odors may be caused by volatile compounds related to lipid oxidation, like hexanal (Rose 2005; Shearer 2010). Oxidative rancidity can be evaluated using sensory evaluation (Hansen and Rose 1996), and headspace hexanal by gas chromatography (Rose 2005).

Use of reduced-oxygen atmospheres in storage of flour has not been well studied. Greer et al (1954) studied flour packaged and stored in air-tight tins, which resulted in oxygen levels less than 5% of some samples after 6 to 8 years of storage. They explained that this was due to conversion of oxygen to carbon dioxide and uptake of oxygen by the flour in oxidation reactions. Rose (2005) studied how flour quality was affected by oxygen absorbers in sealed containers that resulted in <1% oxygen. Bell et al (1979) and Warwick et al (1979) both stored control flour samples that were flushed with nitrogen and hydrogen and then exposed to a palladium catalyst to remove traces of oxygen. Generally, in all these studies, flour samples with low headspace oxygen had better quality after storage than other flours. This suggests that low-oxygen storage increases the shelf-life. Oxygen is typically removed from storage containers using oxygen absorbers, or oxygen scavengers, which are based on oxidation of iron powder to iron oxide (Ozdemir and Floros 2004). Oxygen can also be displaced by flushing the container's headspace with nitrogen gas before sealing (Cuendet et al 1954), but this technique is not as effective (Warmbier 1976).

Enzyme activity is lower in white flour than wheat grain or bran, except for some enzymes including glutamate dehydrogenase, isocitrate dehydrogenase, and malate dehydrogenase (Honold et al 1967). The lower enzyme activity is attributed to removal of the more enzyme dense germ and aleurone layer. It was found that the amount of germ in flour can affect the amount of enzyme activity in flour, and that flours with less germ contamination will deteriorate more slowly (Wang and Flores 1999). Lipoxygenase can play a part in oxidative rancidity and development of volatile compounds. Clayton and Morrison (1972) concluded that there was negligible activity of lipoxygenase in their flour samples due to the low water activity.

However, Warwick and shearer (1980) found evidence that lipoxygenase reactions still can occur in stored flour.

Another reaction that can affect flour quality is protein co-oxidation. Lipid and protein radicals can be formed in food from processing and storage. Protein oxidation is now receiving more attention as a source for texture changes, off-flavors, and potential toxic compounds in food (Schaich 2009). Protein co-oxidation has been studied in wheat flour foods. In flour extrudates, nitrogen-centered radicals were found, originating in part from lipid reactions with side-chain amino groups (Schaich and Rebello 1999). A strong correlation was also observed in flour extrudates between the protein free-radical content and structure of dough products (Rebello and Schaich 1999). This suggests that protein co-oxidation may have an impact on flour quality, and can be correlated with volatiles developed in the flour.

Breadmaking Quality

Short-term storage of flour immediately after milling helps to improve the breadmaking characteristics of bread. Bellenger and Godon (1972) discovered that flour stored in sealed containers only showed small changes in baking quality after 4 weeks. A large improvement, however, was shown in flour that was exposed to air. They concluded that aerated flour will lead to rapid maturation, but long-term stored flour should be in a closed container to prevent oxidation. Short term storage in air helps oxidize sulfhydryl groups, allowing the formation of disulfide bonds (Ewart 1988). Johnson and Hoseney (1980) found that defatted flours that are stored in air for 2 months have greatly improved baking characteristics and that improvement can be accelerated with heat treatment. Other studies found that aging of flour exposed to oxygen for about 10 days improves breadmaking quality (Shelke et al 1992; Chen and Schofield 1996).

Storage of flour up to 3-6 months was found to have peak baking quality when stored in air-tight containers (Fisher et al 1937; Franz 1968; Srivastava and Rao 1991). Cenkowski et al (2000) found that storage of flour for one year at high temperatures (40°C) resulted in a tight inextensible dough, which would cause problems for bakers. A lower temperature (30°C), on the other hand, resulted in moderate strengthening of the dough, which is not necessarily detrimental. In other studies, treatment with chlorine dioxide showed a decrease in loaf volume after extended storage (Cuendet et al 1954; Franz 1968). These studies also showed that loaf volume decreased faster with higher moisture. However, very low moisture (3%), while initially showing a higher loaf volume, had a lower quality after 52 weeks of storage compared to the 6% moisture sample. This suggests that oxidative degradation can be accelerated at very low moisture contents. Storage in sacks, and storage at high temperatures decreased breadmaking quality, while storage in sealed containers and at refrigerated temperature significantly increased shelf life (McCalla et al 1939).

When trying to determine the cause of loss of breadmaking quality during storage, gluten was found to decrease in quality when unsaturated fatty acids had been added to flour (Barton-Wright 1938). The gluten had even larger decreases in quality with increasing number of double bonds in the fatty acids or decreasing chain length. This, however, did not have as large of an effect on the baking quality as it had on the gluten quality. Cuendet et al (1954) saw a decrease in loaf volume with an increase in FFA. The levels of FFA in flour, however, do not seem to cause loss of breadmaking quality, although some correlation between these may exist (Bell et al 1979). Breadmaking ability is commonly measured by loaf volume using rapeseed displacement (Cuendet et al 1954; Shellenberger et al 1958; Bell et al 1979; Chen and Schofield 1996).

Nutrient Loss and Other Factors

In whole wheat flour stored at 37.8° over 52 weeks, thiamin loss was not observed in flour having 3-10% moisture, but was observed at 14% moisture (Cuendet et al 1954). Franz (1968) did not observe thiamin loss in flour between 11 and 12% moisture during 12 months of storage. Carotenoid destruction was found to be highest at increased moisture contents and had losses as high as 54% in 20 weeks at higher temperatures (Arya and Parihar 1981). Maraschin et al (2008), citing Bellenger and Godon (1972), explained that losses of carotenoids occurred with increased moisture because of the increased activity of lipoxygenase enzymes. Farrington et al (1981) found that lutein, a carotenoid pigment beneficial to eye health, decreased steadily over five years in flour that was exposed to air, but did not decrease in oxygen-free storage. Thiamin loss can be analyzed by thiochrome fluorescence (Franz 1968). Carotenoids can be measured by absorbance at 436 nm (Arya and Parihar 1981).

Discoloring of the flour was observed at low temperatures (–4 °C) after 6 months storage (Ortolan et al 2010). An "objectionable color" was found in flour stored at room temperature for 6 months in polyethylene bags (Pomeranz et al 1968). In another study, no color changes were noticed in flour stored in sealed mason jars or cotton bags for two years (Jones and Gersdorrf 1941). No changes were observed after 3 months in green or blue Agtron values, a color test for flour that excludes any breakdown of carotenoids (Watson and Shuey 1977). Rose (2005) found that consumers disliked the darkening of flour, but did not dislike bread made from the flour.

Shelf Life

The shelf life of food can depend on many factors, including air and temperature (Norseth 1986). If properly packaged and stored, white flour can last up to ten years before there is a loss of breadmaking ability (Greer et al 1954). However, flour may start developing odors after about 5 years (Greer et al 1954). The shelf-life of intact wheat kernels, on the other hand, is considerably longer than flour. One study showed that wheat grain can still be acceptable in its breadmaking ability and nutrition quality when stored for 32 years in no. 10 cans (Rose et al 2011).

The type of packaging is important for maximizing shelf life. No. 10 cans that were properly sealed worked well for storage of wheat flour, wheat grain, and dehydrated mashed potatoes, (Farnsworth et al 2003; Rose 2005; Rose et al 2011). However, the wheat flour samples were stored at ambient conditions which ranged in actual storage temperature. A controlled storage study of flour in no. 10 cans is needed to better determine the effect of temperature on flour quality.

Use in Food Storage

The United States Department of Homeland Security recommends preparing for emergencies by storing foods with a long shelf-life (USDHS 2010). White flour can be considered to have a long shelf-life, but the shelf-life is often limited because of off-odor development. In order for white flour to be a more valuable part of emergency food storage, more research should be conducted to determine the causes of off-odors and how to prevent them.

Summary

Previous research has extensively studied effects of sealed and open containers, storage temperature, flour moisture content, and breadmaking quality. Much research has also looked at formation of FFA and mold in flour. There are relatively few studies, however, looking at how flour aroma is affected by storage conditions. There are also very few studies attempting to identify and characterize specific volatile compounds that develop during storage.

APPENDIX B: EXPANDED AND ADDITIONAL METHODS

Samples and Storage Conditions

Two lots each of enriched, bleached (EB) and unenriched, unbleached (UU) all-purpose flour, was obtained from Deseret Mills in Kaysville, UT. The flour was milled from a blend of 80% hard red winter, 15% hard red spring, and 5% soft white wheat. Enrichment was accomplished at the mill using a premix to add 46.3 mg/kg niacin, 5.84 mg/kg thiamin (as thiamine mononitrate), 3.97 mg/kg riboflavin, 1.54 mg/kg folic acid, and 37.5 mg/kg iron (as reduced iron). Bleaching was accomplished at the mill using benzoyl peroxide added at 51 ppm of the finished flour. After milling, a qualitative iron test using AACC International Method 40.40.01 was performed on UU flour to ensure the absence of enrichment. Flour was stored in bulk storage tanks at the mill and then 11.3 kg was packaged in Kraft paper bags which were stacked on pallets and covered with a low density polyethylene plastic film. Within 2 weeks of milling, flour was removed from the Kraft bags and 1.8 ± 0.05 kg was sealed into no. 10 cans at normal and low oxygen atmospheres. A low oxygen atmosphere was achieved by placing 300 cc Ageless ZPT oxygen absorbers (Mitsubishi Gas Chemical America, Inc., New York, NY), inside the cans before sealing. Cans were sealed using a semi-automatic seamer (LDS Field Support Services, Salt Lake City, UT). Cans were randomly assigned to a storage temperature and storage duration. Cans were stored at 22, 30, and 40°C, with controls at -18°C. Two cans (one from each flour lot) of each treatment combination were evaluated for moisture, water activity, headspace oxygen, flour descriptive sensory analysis, bread consumer sensory analysis, and volatiles every 4 weeks for 24 weeks. After opening the cans, flour was immediately repackaged in Mylar bags and held at -18°C until further analysis. Cans were allowed to equilibrate to room temperature for 24 hours before opening. Prior to opening, headspace oxygen was analyzed;

immediately following opening, water activity, and color were analyzed. Samples for solid-phase microextraction (SPME), thiamin, riboflavin, folate, and moisture analyses were repackaged in 118 mL Whirl-Pak bags and held at -18°C. All analyses, except flour descriptive sensory analysis and bread consumer sensory analysis, were performed in duplicate with the mean values reported.

Headspace Oxygen

The oxygen analyzer was set to take readings over a 7 second time period, with the last value being recorded.

Free Fatty Acids (FFA) and Conjugated Dienes

FFA and conjugated dienes were tested on two cans (one from each lot) of each treatment combination at weeks 0, 12, and 24. The lipid fraction was extracted using the method of Rose et al (2008). Flour (5 g) was accurately weighed into a 125 mL Erlenmeyer flask. Hexane (50 mL, Sigma Aldrich, St. Louis, MO) was added and the flask was shaken at 140 rpm on an orbital shaker (Model 980001, VWR International, Radnor, Pennsylvania) for 30 min. Hexane was decanted into a 500 mL round bottom flask through a Whatman no. 1 filter paper. The extraction was repeated twice, and pooled hexane was evaporated in a rotavapor (R-215; BUCHI, Flawil, Switzerland) at 40°C. Lipid extracts were re-dissolved in 10 mL of iso-octane (Sigma Aldrich, St. Louis, MO), with a 5 mL aliquot used for free fatty acids and a 1 mL aliquot used for conjugated dienes.

FFA were measured using a method by Kwon and Rhee (1986). A 5% (w/v) aqueous solution of cupric acetate (Sigma Aldrich, St. Louis, MO), with pH adjusted to 6.1 with pyridine (Sigma Aldrich, St. Louis, MO) was prepared and stored in a glass bottle with a Teflon lid. The lipid extract dissolved in iso-octane (5 mL aliquot) was added to a 15 mL polypropylene centrifuge tube (SARSTEDT AG & Co., Nümbrecht, Germany). One mL of the cupric acetate solution was added. The centrifuge tube was vigorously shaken for 1 minute and then centrifuged (Physician Compact Centrifuge, Clay Adams, Parsippany, NJ) at 1200g for 1 minute. The organic layer was transferred to a cuvette and absorbance was read at 715 nm, using an iso-octane blank, in a spectrophotometer (Spectronic 1001 Plus, Milton Roy, Ivyland, PA). Free fatty acid concentration was quantified using oleic acid (Sigma Aldrich, St. Louis, MO) as a standard (Fig. 5).

Conjugated dienes were measured according to Pegg (2001) using absorbance at 233 nm and an extinction coefficient of 2.525 X 10^4 M^{-1} cm^{-1}. The 1 mL aliquot of the lipid extract was diluted 1:10 in iso-octane. This was then transferred to a quartz cuvette and absorbance was read at 233 nm, using an iso-octane blank.

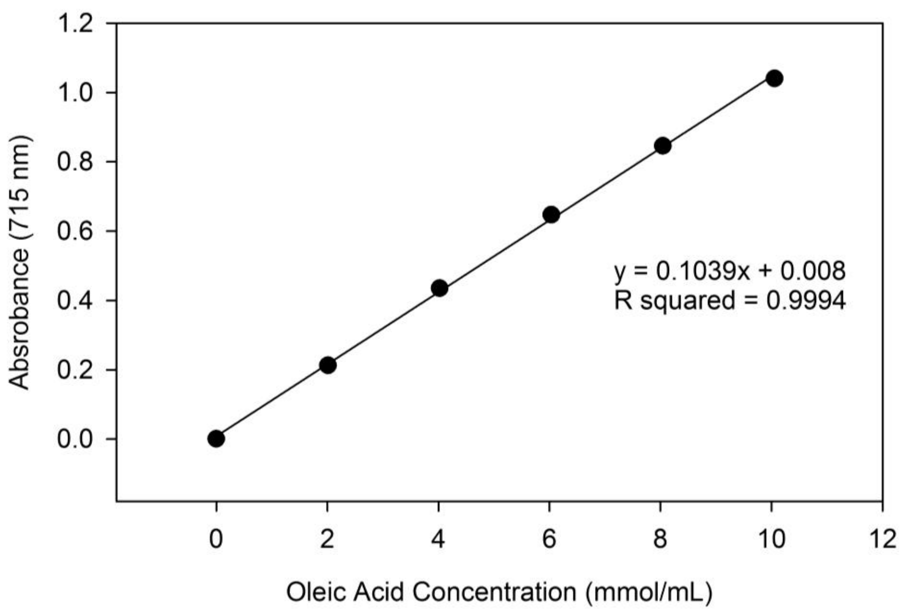

Fig. 5. Free fatty acid (FFA) external standard curve.

SPME-GC-MS of Headspace Volatiles

An alkane standard solution (C8-C20, Fluka, Sigma Aldrich) was run using GC parameters. The retention time for each alkane is listed below (Table 4).

Table 4. Alkane standards and retention times.

Number of Carbons	Alkane	Retention Time
8	n-octane	12.533
9	n-nonane	18.721
10	n-decane	25.326
11	n-undecane	32.032
12	n-dodecane	36.343
13	n-tridecane	39.055
14	n-tetradecane	41.008
15	n-pentadecane	42.608
16	n-hexadecane	44.002
17	n-hepadecane	45.260
18	n-octadecane	Not detected
19	n-nonadecane	Not detected
20	n-icosane	Not detected

Auto Sampler, Gas Chromatograph, and Mass Spec Detector Parameters

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                       INSTRUMENT CONTROL PARAMETERS:    5973N GC MSD
                       ----------------------------------------------

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      Thu Aug 23 21:55:34 2012

Control Information
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Sample Inlet      :  GC
Injection Source  :  External Device
Mass Spectrometer :  Enabled

Injection Location:  Front

=============================================================================
                                6890 GC METHOD
=============================================================================


OVEN
   Initial temp:  33 'C (On)                 Maximum temp:  325 'C
   Initial time:  5.00 min                   Equilibration time:  0.50 min
   Ramps:
      #  Rate  Final temp  Final time
      1  2.00       50        0.00
      2  5.00       77        7.00
      3  5.00      125        0.00
      4 10.00      225        0.00
      5   0.0(Off)
   Post temp:  0 'C
   Post time:  0.00 min
   Run time:  45.50 min


FRONT INLET (SPLIT/SPLITLESS)             BACK INLET (SPLIT/SPLITLESS)
   Mode:  Splitless                          Mode:  Split
   Initial temp:  200 'C (On)                Initial temp:  50 'C (Off)
   Pressure:  11.17 psi (On)                 Pressure:  0.00 psi (Off)
   Purge flow:  14.7 mL/min                  Total flow:  45.0 mL/min
   Purge time:  1.00 min                     Gas saver:  Off
   Total flow:  18.1 mL/min                  Gas type:  Helium
   Gas saver:  On
   Saver flow:  20.0 mL/min
   Saver time:  2.00 min
   Gas type:  Helium


COLUMN 1                                  COLUMN 2
   Capillary Column                          (not installed)
   Model Number:  J&W 122-5536
   DB-5ms
   Max temperature:  325 'C
   Nominal length:  30.0 m
```

Nominal diameter: 250.00 um
Nominal film thickness: 0.50 um
Mode: constant flow
Initial flow: 1.0 mL/min
Nominal init pressure: 11.18 psi
Average velocity: 25 cm/sec
Inlet: Front Inlet
Outlet: MSD
Outlet pressure: ambient

FRONT DETECTOR (NO DET)

BACK DETECTOR (FID)
Temperature: 250 'C (Off)
Hydrogen flow: 40.0 mL/min (Off)
Air flow: 450.0 mL/min (Off)
Mode: Constant makeup flow
Makeup flow: 45.0 mL/min (Off)
Makeup Gas Type: Helium
Flame: Off
Electrometer: Off
Lit offset: 2.0

SIGNAL 1
Data rate: 20 Hz
Type: col comp 1
Save Data: Off
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

SIGNAL 2
Data rate: 20 Hz
Type: test plot
Save Data: Off
Zero: 0.0 (Off)
Range: 0
Fast Peaks: Off
Attenuation: 0

COLUMN COMP 1
(No Detectors Installed)

COLUMN COMP 2
(No Detectors Installed)

THERMAL AUX 2
Use: MSD Transfer Line Heater
Description: MSD transfer line
Initial temp: 250 'C (On)
Initial time: 0.00 min

#	Rate	Final temp	Final time
1	0.0(Off)		

POST RUN
Post Time: 0.00 min

TIME TABLE

Time	Specifier	Parameter & Setpoint

GC Injector

```
     Front Injector:
          Injector not conFig.d, use these parameters if it becomes conFig.d
        Sample Washes                 0
        Sample Pumps                  6
        Injection Volume           2.00 microliters
        Syringe Size               10.0 microliters
        PostInj Solvent A Washes      0
        PostInj Solvent B Washes      0
        Viscosity Delay               0 seconds
        Plunger Speed              Fast

     Back Injector:
No parameters specified

 Column 1 Inventory Number : 5
 Column 2 Inventory Number :
```

GERSTEL MPS SPME Injection

```
SAMPLE PREPARATION
   SPME                     : from Incubator

   Incubator                : Agitator
   Incubation Temperature   : 40 'C
   Incubation Time          : 30.00 min
   Agitator On Time         : 20 s
   Agitator Off Time        : 1 s
   Agitator Speed           : 250 rpm

SAMPLE PARAMETERS
   Sample Tray Type         : VT32-20
   Vial Penetration         : 31.00 mm
   Extraction Time          : 30.00 min
   Inj. Penetration         : 50.00 mm
   Desorption Time          : 300 s

FIBER BAKEOUT
   Bakeout                  : not used  -  use these parameters if it becomes
'used'
   Bakeout at               : -
   Pre Bakeout Time         : 0.00 min
   Post Bakeout Time        : 0.00 min
   Bakeout Penetration      : 43.00 mm

DERIVATISATION
   Derivatisation           : not used  -  use these parameters if it becomes
'used'
   Deriv. Time              : 1.00 min
   Deriv. Penetration       : 24.00 mm
```

```
                              MS ACQUISITION PARAMETERS


General Information
------- -----------

Tune File                : stune.u
Acquistion Mode          : Scan


MS Information
-- -----------

Solvent Delay            : 1.00 min

EMV Mode                 : Relative
Relative Voltage         : 0
Resulting EM Voltage     : 1506

[Scan Parameters]

Low Mass                 : 35.0
High Mass                : 550.0
Threshold                : 150
Sample #                 : 2       A/D Samples    4

[MSZones]

MS Source                : 230 C   maximum 250 C
MS Quad                  : 150 C   maximum 200 C

                           END OF MS ACQUISITION PARAMETERS


                            TUNE PARAMETERS for SN: US82322085
                      -----------------------------

 Trace Ion Detection is OFF.

 EMISSION    :      34.610
 ENERGY      :      69.922
 REPELLER    :      28.280
 IONFOCUS    :      77.820
 ENTRANCE_LE :       0.000
 EMVOLTS     :    1505.882
                                Actual EMV  :    1505.88
                                GAIN FACTOR :       0.83
 AMUGAIN     :    2294.000
 AMUOFFSET   :     126.000
 FILAMENT    :       1.000
 DCPOLARITY  :       0.000
 ENTLENSOFFS :      19.075
 MASSGAIN    :     -61.000
 MASSOFFSET  :     -13.000
```

Descriptive Analysis of Flour Odor

Sample Paper Ballot

Flour Descriptive Analysis **Name:** ____________________ Date:______________
ROUND A

Scale:	0 = None detected 1-5 =Slight 6-10 = Moderate 11-15= Extreme

Sample	Fresh Flour	Acid-Metallic	Musty	Playdough	Cardboard/ Stale	Other: ________
793	________	________	________	________	________	________
851	________	________	________	________	________	________
972	________	________	________	________	________	________
287	________	________	________	________	________	________
163	________	________	________	________	________	________
587	________	________	________	________	________	________
320	________	________	________	________	________	________
693	________	________	________	________	________	________
412	________	________	________	________	________	________

Consumer Acceptance of Bread Made From Flour

Bread was made using an optimized straight-dough bread-making method (AACC International St. Paul). Flour (1500 g), yeast (27 g), sucrose (90 g), salt (22.5 g), shortening (45 g), and water were combined in a mixing bowl. The amount of water added (910-955 mL) was optimized for each flour treatment and lot, as determined by the baker who looked for dough clearing the mixing bowl walls. Flour from 30°C and 40°C storage required 15 mL and 30 mL more water than flour from 22°C storage, respectively. The dough was mixed in a Hobart mixer equipped with a dough hook (N50, Hobart Corp, Troy, OH) for 10 min. at speed 1. The dough was then scaled to 5 sections of equal weight (510 g). Punching and molding were accomplished using a laboratory sheet roll and molder (National Manufacturing Co., Lincoln, NE). Punching occurred after 52 and 77 min., and molding occurred after 90 min. After molding, the dough was placed in a 21.6 X 11.4 cm bread loaf pan and proofed for 33 min. Baking was done using a laboratory reel type oven (Model 8/16, National Manufacturing Co., Lincoln, NE) at 215°C for 24 min. An electric knife and guide (Presto, Eau Claire, WI) were used to cut 1 cm thick slices of bread, and bread slices were then cut vertically in half and stored overnight in sealed low density polyethylene bags.

Consumer Sensory Analysis Survey

WHITE BREAD CONSUMER TEST **2186**

Welcome to the Food Science Sensory Laboratory. A copy of the form titled "Consent to Be a Research Subject" is posted in each booth. Please read it carefully before continuing. By signing your name below, you acknowledge that you have read and understand the consent form, and desire of your own free will and volition to participate in this study. You may withdraw at any time without penalty. Please inform the receptionist if you wish to withdraw.

Name___________________________ Signature___________________________

In this session, you will evaluate five samples of White Bread side by side. Please read all instructions and questions carefully. Before you receive your samples, please answer these questions by checking the appropriate circles.

* What is your age category?

- O Under 20 years
- O 20 – 29 years
- O 30 – 39 years
- O 40 – 49 years
- O 50 – 60 years
- O Over 60 years

* What is your gender?

- O Female
- O Male

* What is your attitude about WHITE BREAD?

- O I like it
- O I neither like nor dislike it
- O I dislike it

* How often do you typically eat WHITE BREAD?

- O More than once a week
- O Once a week to every two weeks
- O Once every two weeks to once a month
- O Once a month to once every three months
- O Less than every three months

…Turn the page to continue…

WHITE BREAD CONSUMER TEST 2186

Press the green READY light to call for your samples. If at any time during the test you need help, press the button by the HELP light to the right of the screen.

Please fill in the code numbers on the top of the columns in the same order left to right as they are arranged in front of you.

DO NOT taste the samples yet.

* How much do you like or dislike the **APPEARANCE** of each sample?

Sample Number (please fill in)

	______	______	______	______	______
Like extremely	O	O	O	O	O
Like very much	O	O	O	O	O
Like moderately	O	O	O	O	O
Like slightly	O	O	O	O	O
Neither like nor dislike	O	O	O	O	O
Dislike slightly	O	O	O	O	O
Dislike moderately	O	O	O	O	O
Dislike very much	O	O	O	O	O
Dislike extremely	O	O	O	O	O

Please smell the samples, but do not taste.

* How much do you like or dislike the **AROMA** of each sample?

Like extremely	O	O	O	O	O
Like very much	O	O	O	O	O
Like moderately	O	O	O	O	O
Like slightly	O	O	O	O	O
Neither like nor dislike	O	O	O	O	O
Dislike slightly	O	O	O	O	O
Dislike moderately	O	O	O	O	O
Dislike very much	O	O	O	O	O
Dislike extremely	O	O	O	O	O

…Turn the page to continue…

WHITE BREAD CONSUMER TEST **2186**

Now taste the samples from left to right as they are arranged for you on the tray. Take a sip of water and take a bite of cracker between samples to refresh your sense of taste.

* What is your FIRST IMPRESSION of the **OVERALL ACCEPTABILITY** of each sample?

Sample Number (please fill in)

	______	______	______	______	______
Like extremely	O	O	O	O	O
Like very much	O	O	O	O	O
Like moderately	O	O	O	O	O
Like slightly	O	O	O	O	O
Neither like nor dislike	O	O	O	O	O
Dislike slightly	O	O	O	O	O
Dislike moderately	O	O	O	O	O
Dislike very much	O	O	O	O	O
Dislike extremely	O	O	O	O	O

* How much do you like or dislike the **FLAVOR** of each sample?

Like extremely	O	O	O	O	O
Like very much	O	O	O	O	O
Like moderately	O	O	O	O	O
Like slightly	O	O	O	O	O
Neither like nor dislike	O	O	O	O	O
Dislike slightly	O	O	O	O	O
Dislike moderately	O	O	O	O	O
Dislike very much	O	O	O	O	O
Dislike extremely	O	O	O	O	O

* How much do you like or dislike the **TEXTURE** of each sample?

Sample Number (please fill in)

	______	______	______	______	______
Like extremely	O	O	O	O	O
Like very much	O	O	O	O	O
Like moderately	O	O	O	O	O
Like slightly	O	O	O	O	O
Neither like nor dislike	O	O	O	O	O
Dislike slightly	O	O	O	O	O
Dislike moderately	O	O	O	O	O
Dislike very much	O	O	O	O	O
Dislike extremely	O	O	O	O	O

…Turn the page to continue…

While comments are not required, you may write any COMMENTS you have in the space below. Please refer to sample numbers in your comments. If you choose to make a comment, be brief. If you don't have any comments you are finished.

____

____

____

____

You are finished. Please place the samples and tray in the pass-through compartment and **PRESS THE BUTTON BY THE "FINISHED" LIGHT**. Please give this questionnaire to the receptionist. **THANK YOU!**

IRB Approval for Flour Descriptive Sensory Analysis and Bread Consumer Sensory Analysis

Institutional Review Board
for Human Subjects

Brigham Young University
A-285 ASB Provo, Utah 84602
(801) 422-3841 / Fax: (801) 422-0620

October 29, 2012

Laura Jefferies
S-103 ESC
Campus Mail

Re: X 000199
General Sensory Evaluation of Foods

Dear Laura Jefferies

This is to inform you Brigham Young University's IRB has renewed its approval of the above noted research study.

The approval period is from 10-29-2012 to 11-9-2013. Your study number is X000199. Please be sure to reference either this number and/or the study title in any correspondence with the IRB.

All conditions for continued approval during the prior approval period remain in effect. These include, but are not necessarily limited to the following requirements:

A copy of the Informed Consent Document, approved as of 10-29-2012 is enclosed. No other consent form should be used. It must be signed by each subject prior to initiation of any protocol procedures. In addition, each subject must be given a copy of the signed consent form.

All Protocol amendments and changes to approved research must be submitted to the IRB and not be implemented until approved by the IRB.

Please note that this renewal approval is for an adult population only.

Sincerely,

Lane Fischer, PhD, Chair
Sandee M.P. Munoz, Administrator
Institutional Review Board for Human Subjects

Color Analysis

Flour color was measured using a Hunterlab ColorFlex Spectrophotometer with glass sample cups (Hunter Associates Laboratory, Inc., Reston, VA). Flour was filled to a 50 mm sample thickness and compacted slightly by gently tapping the sample cup on a hard surface (HunterLab 2008). Color was measured using the CIE L*a*b* color scale with illuminant/observer settings of D65/10°. Each flour sample was measured in duplicate.

Bread Loaf Volume

Loaf volume was measured in duplicate using bread made from a straight-dough bread-making method (AACC International St. Paul). Flour (100 g) was weighed out into a mixing bowl. Yeast (3 g), sucrose (5g), salt (1 g), and water (66 mL) were added to the bowl. Dough was mixed for 35 seconds in a 100-200g micro-mixer (National Manufacturing Co., Lincoln, NE). Dough was then proofed at 30°C and 85% RH. Punching occurred after 105 and 155 min. and molding occurred after 180 min. Punching and molding was accomplished using a laboratory sheet roll and molder (National Manufacturing Co. Lincoln, NE). After molding, the dough was placed in 14.6 X 7.2 cm baking pans and proofed for 55 min. Baking was done at 425°C for 25 min. in a laboratory reel type oven (Model 8/16, National Manufacturing Co. Lincoln, NE).

Loaf volume was measured via rapeseed displacement (AACC International St. Paul) using a pup loaf volumeter (National Manufacturing Co., Lincoln, NE). Volume was measured within 10 min. of being removed from the oven.

Vitamin Analysis

The vitamins folate, thiamin, and riboflavin were analyzed on all flour samples. Vitamin results are reported as means of 2 - 4 measurements. Folate was analyzed using the AOAC trienzyme extraction method 2004.05 (AOAC 2006) using minor changes from Chapman et al (2010).

Riboflavin and thiamin were analyzed using the method by Arella et al (1996) with modifications from AOAC method 953.17 (AOAC 2006) and El-Arab et al (2004). To a 250 mL Erlenmeyer flask containing 5.0 g of flour, 50 mL of 0.1 N HCL was added. This was then autoclaved at 121°C for 30 min., cooled, and adjusted to pH 4.5 with 2.5 M sodium acetate. Next, 500 mg takadiastase (100 U/mg, Sigma Aldrich, St. Louis, MO) was added, and the flour suspension was incubated at 37°C for 18 hours. The flour suspension was filtered through a Whatman no. 541 filter, and the filtrate was diluted to 200 mL. A 1 mL aliquot of this solution was filtered through a 0.2 μm cellulose acetate filter and used for riboflavin determination. A 10 mL aliquot of the first filtrate, along with 2.5 g NaCl, was mixed in a 50 mL conical centrifuge tube. Three milliliters of the oxidizing reagent (1 mL of 1% potassium ferricyanide (Sigma Aldrich, St. Louis, MO) and 24 mL of 3.75 M NaOH), along with 15 mL isobutanol, were added to the centrifuge tube. The tube was shaken for 2.5 min., and then centrifuged at 1200g for 4 min. The organic layer (1 mL) was filtered through a 0.2 μm cellulose acetate filter and used for thiamin determination (as thiochrome). Separation and quantification was performed isocratically using an Agilent 1100 Series HPLC (Agilent Technologies Inc., Santa Clara, CA), equipped with an octadecylsilyl column (150 mm x 4.60 mm, 5 μm particle size, Phenomenex Inc., Torrance, CA). The sample injection volume was 10 μL, and the mobile phase was methanol-0.05 M sodium acetate (30:70 v/v) with a flow rate of 1 mL/min. The analytes were detected using a fluorometric detector with excitation and emission wavelengths at 422 nm and 522 nm, respectively, for riboflavin, and 366 nm and 435 nm, respectively, for thiochrome. Quantification was performed using external standard curves (Fig. 6).

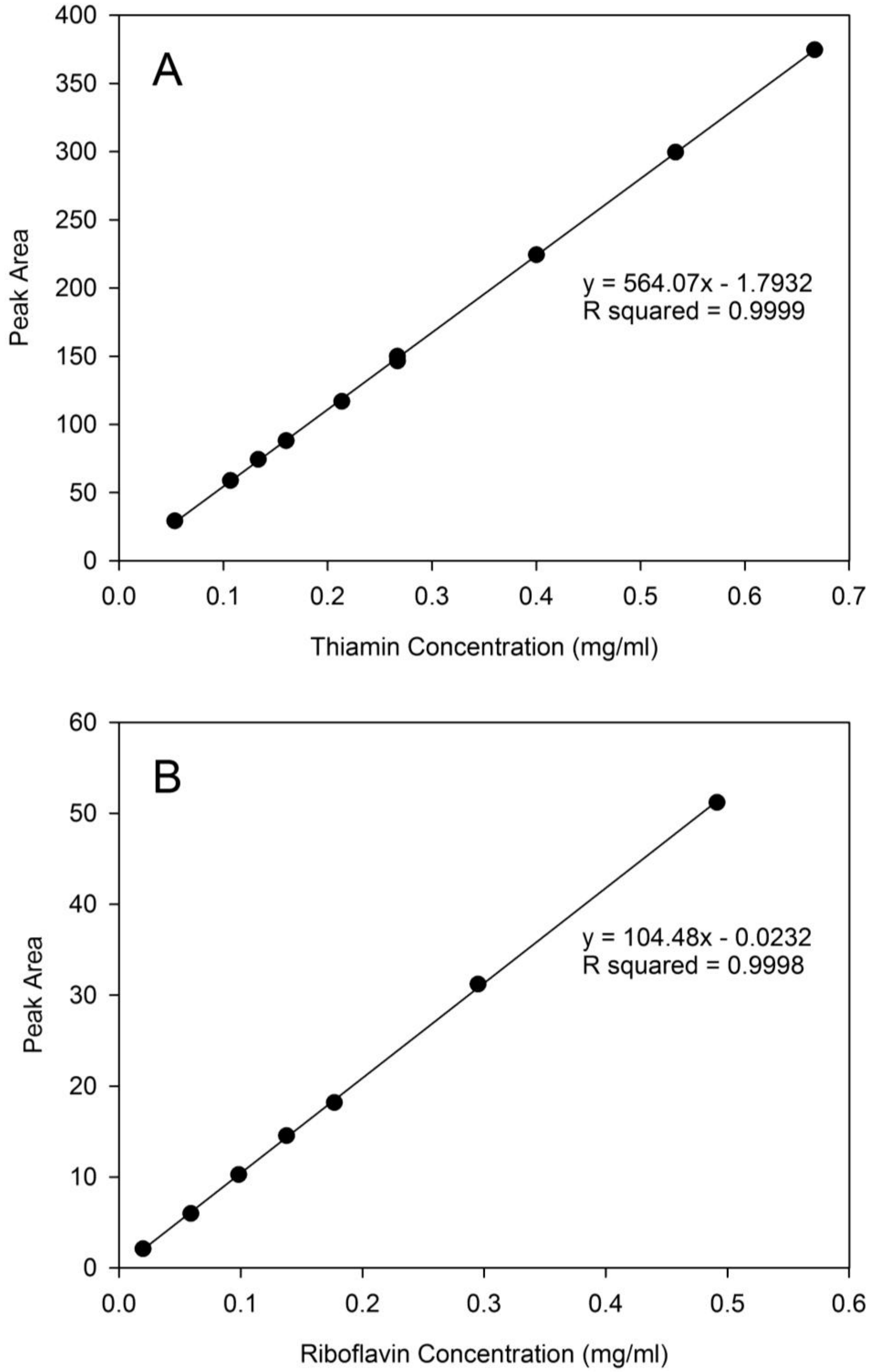

Fig. 6. Thiamin (A) and riboflavin (B) external standard curves.

Protein

The protein of one time zero flour sample from each lot was measured in triplicate by Dumas nitrogen combustion using a nitrogen analyzer, an EDTA standard, and a protein-to-nitrogen ratio of 5.7 (AACC International St. Paul).

Iron

Iron was quantified in time zero flour samples using AOAC method 985.01, inductively coupled plasma (ICP) spectroscopy (AOAC 2006).

Effect of Enrichment and Bleaching on Oxidative Rancidity

A second set of all-purpose wheat flour was collected and analyzed. This set was obtained during the same time period and from the same mill as the first set. This second set consisted of two lots each of over-enriched, bleached (OEB) and under-enriched, unbleached (UEU) all-purpose wheat flour. Storage treatments and storage times were identical to the first set of data. Bleaching was confirmed using b* color values, and the amount of enrichment was quantified using flour iron content. Oxidative rancidity was assessed using the three most abundant volatiles (2-pentyl furan, 1-hexanol, and hexanal). A log transformation was used on volatile data because of logarithmic increases. Multiple linear regression models including significant two-way interactions were created. Iron content was used as an indicator for enrichment level. Effects were reported following a back-transformation. Effects on volatile concentration by iron content were based on the minimum iron content required in all-purpose flour (4.4 mg/100g flour).

Data Analysis

Data was analyzed using SAS and JMP version 10 software (SAS Institute Inc., Cary, NC). Significance was set at $p<0.05$. Multiple linear regression models including significant two-way and three-way interactions were constructed. Reduced models were used wherever possible. Data from SPME analysis exhibited logarithmic increases and therefore required log transformations. The model for the consumer panel data required a panelist nested in temperature term using a random effect. The Tukey-Kramer procedure was used to establish significant differences between multiple comparisons. Principle component analysis was performed on flour descriptive sensory analysis data where eigenvectors of the first two principle components were used to identify correlations.

APPENDIX C: STATISTICAL MODEL PARAMETERS

Model parameters were constructed using "enrich" to represent the enrichment-bleached variable with "EB" standing for enriched, bleached flour and "UU" standing for unenriched, unbleached flour. "Oxygen" represents the oxygen atmosphere storage variable with "N" standing for storage in a normal oxygen atmosphere and "O" standing for storage in a low oxygen atmosphere. "Temp" represents the storage temperature variables, with temperatures expressed as °C. "Time" represents the storage time measured in weeks. "Bleached" represents the bleaching variable with "B" standing for bleached and "U" standing for unbleached. "Enriched Lev" represents the level of enrichment variable measured in mg/100g of iron.

Moisture Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	0.1244485	0.000394	150.00	316.15	<.0001*	0.1236707	0.1252263
Enrich[EB]	0.0026555	0.000557	150.00	4.77	<.0001*	0.0015555	0.0037555

Water Activity Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	0.567559	0.005752	139.00	98.68	<.0001*	0.5561873	0.5789308
Enrich[EB]	0.0158819	0.004017	139.00	3.95	0.0001*	0.0079396	0.0238243
Temp[22]	-0.028469	0.00492	139.00	-5.79	<.0001*	-0.038196	-0.018741
Temp[30]	-0.016781	0.00492	139.00	-3.41	0.0008*	-0.026509	-0.007054
Time	0.0006466	0.000294	139.00	2.20	0.0295*	6.5256e-5	0.0012279

Oxygen (Normal Oxygen Atmosphere) Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	21.762025	1.385133	62.00	15.71	<.0001*	18.993184	24.530866
Enrich[EB]	-1.383017	1.556755	62.00	-0.89	0.3778	-4.494926	1.7288922
Temp[22]	-2.381579	1.769276	62.00	-1.35	0.1832	-5.918311	1.1551524
Temp[30]	-1.751163	1.769276	62.00	-0.99	0.3261	-5.287894	1.7855691
Time	-0.300264	0.084928	62.00	-3.54	0.0008*	-0.470032	-0.130495
Enrich[EB]*Temp[22]	3.9354583	1.421116	62.00	2.77	0.0074*	1.0946872	6.7762295
Enrich[EB]*Temp[30]	2.8979583	1.421116	62.00	2.04	0.0457*	0.0571872	5.7387295
Enrich[EB]*Time	-0.200174	0.084928	62.00	-2.36	0.0216*	-0.369943	-0.030406
Temp[22]*Time	0.3555295	0.104015	62.00	3.42	0.0011*	0.1476063	0.5634526
Temp[30]*Time	0.2697259	0.104015	62.00	2.59	0.0118*	0.0618027	0.4776491

Oxygen (Low Oxygen Atmosphere) Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	-2.6e-18	0.002067	66.00	-0.00	1.0000	-0.004128	0.0041279
Temp[22]	0.0131917	0.002924	66.00	4.51	<.0001*	0.0073539	0.0190294
Temp[30]	0.001225	0.002924	66.00	0.42	0.6766	-0.004613	0.0070627
Time	2.982e-19	0.000133	66.00	0.00	1.0000	-0.000265	0.000265
Temp[22]*Time	-0.000631	0.000188	66.00	-3.36	0.0013*	-0.001006	-0.000257
Temp[30]*Time	-6.518e-5	0.000188	66.00	-0.35	0.7295	-0.00044	0.0003096

Free Fatty Acids Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	1.4858684	0.279293	66.00	5.32	<.0001*	0.928242	2.0434949
Temp[22]	-0.286871	0.39498	66.00	-0.73	0.4702	-1.075474	0.5017319
Temp[30]	-0.231931	0.39498	66.00	-0.59	0.5591	-1.020534	0.5566716
Time	0.398141	0.018028	66.00	22.08	<.0001*	0.3621463	0.4341356
Time*Temp[22]	-0.274932	0.025496	66.00	-10.78	<.0001*	-0.325837	-0.224028
Time*Temp[30]	-0.184083	0.025496	66.00	-7.22	<.0001*	-0.234988	-0.133179

Conjugated Dienes Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	0.2680252	0.008716	66.00	30.75	<.0001*	0.2506223	0.285428
Enrich[EB]	0.0023867	0.009318	66.00	0.26	0.7986	-0.016218	0.0209911
Oxygen[N]	0.0180336	0.012327	66.00	1.46	0.1482	-0.006578	0.0426449
Time	-0.000442	0.000476	66.00	-0.93	0.3560	-0.001391	0.0005074
Oxygen[N]*Enrich[EB]	-0.029321	0.013178	66.00	-2.22	0.0295*	-0.055631	-0.00301
Oxygen[N]*Time	0.0021228	0.000672	66.00	3.16	0.0024*	0.0007802	0.0034655

2-Pentyl Furan Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	1.4096009	0.157787	153.00	8.93	<.0001*	1.0978783	1.7213235
Enrich[EB]	0.3927297	0.171144	153.00	2.29	0.0231*	0.0546191	0.7308403
Oxygen[N]	0.3973595	0.171144	153.00	2.32	0.0216*	0.0592489	0.7354701
Temp[22]	0.4766162	0.192109	153.00	2.48	0.0142*	0.097088	0.8561444
Temp[30]	0.271363	0.192109	153.00	1.41	0.1598	-0.108165	0.6508912
Time	0.0526919	0.009567	153.00	5.51	<.0001*	0.0337909	0.0715928
Enrich[EB]*Oxygen[N]	0.5235727	0.136915	153.00	3.82	0.0002*	0.2530842	0.7940612
Enrich[EB]*Temp[22]	-0.55552	0.167686	153.00	-3.31	0.0012*	-0.8868	-0.224241
Enrich[EB]*Temp[30]	-0.354082	0.167686	153.00	-2.11	0.0363*	-0.685362	-0.022803
Enrich[EB]*Time	0.0278975	0.008557	153.00	3.26	0.0014*	0.010992	0.044803
Oxygen[N]*Temp[22]	-0.88854	0.167686	153.00	-5.30	<.0001*	-1.219819	-0.55726
Oxygen[N]*Temp[30]	-0.317442	0.167686	153.00	-1.89	0.0602	-0.648721	0.0138379
Oxygen[N]*Time	0.0699838	0.008557	153.00	8.18	<.0001*	0.0530782	0.0868893
Temp[22]*Time	-0.05303	0.01048	153.00	-5.06	<.0001*	-0.073735	-0.032325
Temp[30]*Time	-0.052111	0.01048	153.00	-4.97	<.0001*	-0.072816	-0.031406

1-Hexanol Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	3.9391665	0.127281	132.00	30.95	<.0001*	3.6873928	4.1909402
Enrich[EB]	0.2373815	0.11869	132.00	2.00	0.0476*	0.0026013	0.4721616
Oxygen[N]	0.2532645	0.169938	132.00	1.49	0.1385	-0.08289	0.5894186
Temp[22]	0.1257977	0.12589	132.00	1.00	0.3195	-0.123224	0.3748197
Temp[30]	0.0024132	0.12589	132.00	0.02	0.9847	-0.246609	0.2514352
Time	-0.007451	0.006143	132.00	-1.21	0.2273	-0.019602	0.0046996
Enrich[EB]*Oxygen[N]	0.505694	0.11869	132.00	4.26	<.0001*	0.2709138	0.7404742
Enrich[EB]*Temp[22]	-0.392792	0.145365	132.00	-2.70	0.0078*	-0.680338	-0.105247
Enrich[EB]*Temp[30]	-0.178909	0.145365	132.00	-1.23	0.2206	-0.466454	0.1086373
Oxygen[N]*Temp[22]	-0.628815	0.145365	132.00	-4.33	<.0001*	-0.91636	-0.341269
Oxygen[N]*Temp[30]	-0.311704	0.145365	132.00	-2.14	0.0338*	-0.59925	-0.024158
Oxygen[N]*Time	0.0400428	0.008687	132.00	4.61	<.0001*	0.0228586	0.0572269

Hexanal Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	0.9717752	0.242387	152.00	4.01	<.0001*	0.4928931	1.4506573
Enrich[EB]	1.0541518	0.22327	152.00	4.72	<.0001*	0.613038	1.4952656
Oxygen[N]	0.4901068	0.304266	152.00	1.61	0.1093	-0.11103	1.0912435
Temp[22]	1.3029675	0.326834	152.00	3.99	0.0001*	0.6572431	1.948692
Temp[30]	0.9689148	0.326834	152.00	2.96	0.0035*	0.3231903	1.6146393
Time	0.1006504	0.016113	152.00	6.25	<.0001*	0.0688157	0.132485
Enrich[EB]*Temp[22]	-1.171687	0.238686	152.00	-4.91	<.0001*	-1.643257	-0.700116
Enrich[EB]*Temp[30]	-1.041059	0.238686	152.00	-4.36	<.0001*	-1.512629	-0.569488
Enrich[EB]*Time	0.0518365	0.01218	152.00	4.26	<.0001*	0.0277718	0.0759012
Oxygen[N]*Temp[22]	-0.474887	0.430297	152.00	-1.10	0.2715	-1.325023	0.375249
Oxygen[N]*Temp[30]	-0.31789	0.430297	152.00	-0.74	0.4612	-1.168026	0.5322456
Oxygen[N]*Time	-0.072298	0.021097	152.00	-3.43	0.0008*	-0.113979	-0.030617
Temp[22]*Time	-0.14736	0.021097	152.00	-6.98	<.0001*	-0.189042	-0.105679
Temp[30]*Time	-0.148663	0.021097	152.00	-7.05	<.0001*	-0.190344	-0.106982
Oxygen[N]*Temp[22]*Time	0.1012871	0.029836	152.00	3.39	0.0009*	0.0423408	0.1602334
Oxygen[N]*Temp[30]*Time	0.113864	0.029836	152.00	3.82	0.0002*	0.0549177	0.1728103

Flour Descriptive Sensory Analysis Eigenvectors

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6
Fresh Flour	0.39263	-0.51288	0.29132	-0.38650	0.25643	0.53178
Acid Metallic	-0.58562	0.30493	0.03694	0.14575	0.38908	0.62456
Musty	0.03653	0.05331	0.88623	0.44067	-0.04435	-0.11939
Play Dough	0.44731	0.51824	0.00338	-0.10162	0.68241	-0.23521
Cardboard/Stale	0.47474	0.50615	-0.06170	0.10612	-0.50915	0.49409
Other	0.27585	-0.34113	-0.35290	0.78332	0.23658	0.11589

Fresh Flour Aroma Descriptive Scores Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	3.1831289	0.488375	131.00	6.52	<.0001*	2.2170067	4.149251
Enrich[EB]	0.2361696	0.215931	131.00	1.09	0.2761	-0.190993	0.6633319
Oxygen[N]	0.3891988	0.435994	131.00	0.89	0.3737	-0.473301	1.2516989
Temp[22]	1.3988343	0.164011	131.00	8.53	<.0001*	1.0743813	1.7232874
Temp[30]	0.7256035	0.164011	131.00	4.42	<.0001*	0.4011505	1.0500565
Time	-0.483934	0.124043	131.00	-3.90	0.0002*	-0.72932	-0.238548
Enrich[EB]*Time	-0.028592	0.013861	131.00	-2.06	0.0411*	-0.056013	-0.001171
Oxygen[N]*Temp[22]	-0.938091	0.231947	131.00	-4.04	<.0001*	-1.396937	-0.479245
Oxygen[N]*Temp[30]	-0.595754	0.231947	131.00	-2.57	0.0113*	-1.0546	-0.136908
Oxygen[N]*Time	0.1735427	0.067863	131.00	2.56	0.0117*	0.0392938	0.3077915
Time*Time	0.0303186	0.009602	131.00	3.16	0.0020*	0.0113232	0.0493141
Oxygen[N]*Time*Time	-0.006043	0.002373	131.00	-2.55	0.0120*	-0.010736	-0.001349
Time*Time*Time	-0.000621	0.000225	131.00	-2.76	0.0066*	-0.001067	-0.000176

Acid Metallic Aroma Descriptive Scores Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	3.8069162	0.61725	124.00	6.17	<.0001*	2.5852048	5.0286276
Enrich[EB]	1.9845615	0.644697	124.00	3.08	0.0026*	0.7085257	3.2605974
Oxygen[N]	-1.685399	0.756902	124.00	-2.23	0.0278*	-3.18352	-0.187277
Temp[22]	-2.55407	0.565819	124.00	-4.51	<.0001*	-3.673983	-1.434156
Temp[30]	-2.19065	0.565819	124.00	-3.87	0.0002*	-3.310564	-1.070737
Time	0.5390485	0.087254	124.00	6.18	<.0001*	0.3663487	0.7117483
Enrich[EB]*Oxygen[N]	-0.551193	0.273659	124.00	-2.01	0.0462*	-1.09284	-0.009546
Enrich[EB]*Temp[22]	-0.520775	0.335162	124.00	-1.55	0.1228	-1.184155	0.142604
Enrich[EB]*Temp[30]	-0.825988	0.335162	124.00	-2.46	0.0151*	-1.489367	-0.162608
Enrich[EB]*Time	-0.251383	0.098061	124.00	-2.56	0.0116*	-0.445474	-0.057291
Oxygen[N]*Temp[22]	1.4140906	0.764287	124.00	1.85	0.0667	-0.098647	2.9268286
Oxygen[N]*Temp[30]	1.3866656	0.764287	124.00	1.81	0.0720	-0.126072	2.8994036
Oxygen[N]*Time	-0.448461	0.102071	124.00	-4.39	<.0001*	-0.650488	-0.246435
Temp[22]*Time	-0.149942	0.034693	124.00	-4.32	<.0001*	-0.218609	-0.081276
Temp[30]*Time	-0.042387	0.034693	124.00	-1.22	0.2241	-0.111053	0.0262797
Time*Time	-0.013781	0.002969	124.00	-4.64	<.0001*	-0.019658	-0.007905
Enrich[EB]*Time*Time	0.0087367	0.003428	124.00	2.55	0.0120*	0.001951	0.0155223
Oxygen[N]*Temp[22]*Time	0.2040466	0.049063	124.00	4.16	<.0001*	0.1069377	0.3011554
Oxygen[N]*Temp[30]*Time	0.1062686	0.049063	124.00	2.17	0.0322*	0.0091598	0.2033774
Oxygen[N]*Time*Time	0.0076069	0.003428	124.00	2.22	0.0283*	0.0008213	0.0143926

Cardboard/Stale Aroma Descriptive Scores Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	0.0251202	0.272482	129.00	0.09	0.9267	-0.513992	0.5642326
Enrich[EB]	0.4251623	0.170635	129.00	2.49	0.0140*	0.087557	0.7627676
Oxygen[N]	0.2299528	0.244312	129.00	0.94	0.3483	-0.253424	0.71333
Temp[22]	1.115873	0.280382	129.00	3.98	0.0001*	0.5611309	1.6706151
Temp[30]	0.7228856	0.280382	129.00	2.58	0.0111*	0.1681435	1.2776277
Time	-0.039783	0.032429	129.00	-1.23	0.2221	-0.103945	0.0243788
Oxygen[N]*Enrich[EB]	0.4493778	0.170635	129.00	2.63	0.0095*	0.1117725	0.7869831
Oxygen[N]*Temp[22]	-0.953695	0.208984	129.00	-4.56	<.0001*	-1.367176	-0.540215
Oxygen[N]*Temp[30]	-0.617741	0.208984	129.00	-2.96	0.0037*	-1.031221	-0.20426
Oxygen[N]*Time	0.0562692	0.012489	129.00	4.51	<.0001*	0.031559	0.0809794
Temp[22]*Enrich[EB]	-0.524215	0.208984	129.00	-2.51	0.0134*	-0.937695	-0.110734
Temp[30]*Enrich[EB]	-0.257539	0.208984	129.00	-1.23	0.2201	-0.671019	0.1559415
Temp[22]*Time	-0.062044	0.015296	129.00	-4.06	<.0001*	-0.092307	-0.03178
Temp[30]*Time	-0.049551	0.015296	129.00	-3.24	0.0015*	-0.079814	-0.019287
Time*Time	0.0025645	0.001069	129.00	2.40	0.0179*	0.0004498	0.0046792

Musty Aroma Descriptive Scores Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	0.0453538	0.025124	137.00	1.81	0.0732	-0.004327	0.0950351
Enrich[EB]	0.0451389	0.027251	137.00	1.66	0.0999	-0.008748	0.0990258
Temp[22]	0.0440972	0.027251	137.00	1.62	0.1079	-0.00979	0.0979841
Temp[30]	0.0478671	0.027251	137.00	1.76	0.0812	-0.00602	0.101754
Time	-0.002537	0.001152	137.00	-2.20	0.0293*	-0.004814	-0.00026
Enrich[EB]*Temp[22]	-0.094444	0.038539	137.00	-2.45	0.0155*	-0.170652	-0.018237
Enrich[EB]*Temp[30]	-0.085483	0.038539	137.00	-2.22	0.0282*	-0.16169	-0.009275

Play Dough Aroma Descriptive Scores Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	-0.082104	0.225632	132.00	-0.36	0.7165	-0.528426	0.3642178
Enrich[EB]	0.1424162	0.260537	132.00	0.55	0.5856	-0.372952	0.6577845
Temp[22]	0.3094378	0.225632	132.00	1.37	0.1726	-0.136884	0.7557599
Temp[30]	0.2596263	0.225632	132.00	1.15	0.2520	-0.186696	0.7059484
Time	0.018569	0.014484	132.00	1.28	0.2021	-0.010082	0.0472202
Oxygen[N]	0.0867945	0.260537	132.00	0.33	0.7396	-0.428574	0.6021628
Enrich[EB]*Oxygen[N]	-0.271486	0.368455	132.00	-0.74	0.4625	-1.000327	0.457355
Enrich[EB]*Time	-0.009767	0.016725	132.00	-0.58	0.5602	-0.042851	0.0233165
Temp[22]*Time	-0.035482	0.014484	132.00	-2.45	0.0156*	-0.064133	-0.00683
Temp[30]*Time	-0.028906	0.014484	132.00	-2.00	0.0480*	-0.057557	-0.000255
Time*Oxygen[N]	0.0019463	0.016725	132.00	0.12	0.9075	-0.031137	0.0350299
Enrich[EB]*Oxygen[N]*Time	0.0483085	0.023653	132.00	2.04	0.0431*	0.0015213	0.0950958

Other Aroma Descriptive Scores Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	0.037004	0.040249	140.00	0.92	0.3595	-0.042571	0.1165787
Enrich[EB]	0.0151701	0.056921	140.00	0.27	0.7902	-0.097365	0.1277057
Oxygen[N]	0.2918541	0.056921	140.00	5.13	<.0001*	0.1793185	0.4043897
Enrich[EB]*Oxygen[N]	-0.174849	0.080498	140.00	-2.17	0.0315*	-0.333998	-0.015699

Acid-Metallic Dissipation Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	7.5609797	0.405686	15.00	18.64	<.0001*	6.6962796	8.4256798
Treatment[FC Nov06]	0.9297297	0.564048	15.00	1.65	0.1201	-0.27251	2.1319694
Treatment[SEO206]	-2.998649	0.564048	15.00	-5.32	<.0001*	-4.200888	-1.796409
Treatment[SEO306]	-1.075	0.564048	15.00	-1.91	0.0760	-2.27724	0.1272396
Time	-0.416505	0.028647	15.00	-14.54	<.0001*	-0.477564	-0.355446
Treatment[FC Nov06]*Time	-0.026351	0.020196	15.00	-1.30	0.2116	-0.069398	0.0166957
Treatment[SEO206]*Time	0.0817568	0.020196	15.00	4.05	0.0011*	0.0387097	0.1248038
Treatment[SEO306]*Time	0.0291667	0.020196	15.00	1.44	0.1693	-0.01388	0.0722138
Time*Time	0.005601	0.000605	15.00	9.25	<.0001*	0.0043109	0.0068912

Bread Consumer Sensory Analysis SAS Code

```
PROC IMPORT OUT= WORK.in
        DATAFILE= "C:\SAS\bioag\fsn\pike\swindler\Consumer Sensory Panel Data.xlsx"
        DBMS=EXCEL REPLACE;
    RANGE="'Combined Raw Data$'";
    GETNAMES=YES;
    MIXED=NO;
    SCANTEXT=YES;
    USEDATE=YES;
    SCANTIME=YES;
RUN;

*Enrich Oxygen Temp Proj_Name Panelist Appearance Aroma Overall_Acceptability Flavor
Texture;

proc sort data=in;
by Proj_Name;
run;

data good;set in;
retain dumnum;
if _n_=1 then dumnum=0;
if first.Proj_Name then dumnum=dumnum+1;
panelist=200*(dumnum-1)+panelist;
if enrich='Control' then treatment='Control';
if enrich='EB' and oxygen='N' then treatment='EB_N';
if enrich='EB' and oxygen='O' then treatment='EB_O';
if enrich='UU' and oxygen='N' then treatment='UU_N';
if enrich='UU' and oxygen='O' then treatment='UU_O';
by proj_name;
run;
proc print data=good;
var dumnum panelist;
run;
```

```
%macro ddd(dvar=temp);
title2 "Analysis for &dvar";

proc mixed data=good;
class treatment panelist temp;
model &dvar=temp treatment temp*treatment;
random panelist(temp);
lsmeans treatment temp treatment*temp/pdiff adjust=tukey;
estimate 'interaction' treatment -1 1 1 -1;
ods output diffs=temp;
run;

data temp;set temp;
if treatment="" then output;
*if temp=_;
if temp=_temp then output;
run;
proc sort data=temp;
by descending adjp;
proc print data=temp;run;

%mend;


%ddd(dvar=Appearance);
%ddd(dvar=Aroma);
%ddd(dvar=Overall_Acceptability);
%ddd(dvar=Flavor);
%ddd(dvar=Texture);
```

L* (Lightness; % initial) Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	1.0041752	0.001016	128.00	988.24	<.0001*	1.0021646	1.0061858
Oxygen[N]	-0.003841	0.000818	128.00	-4.70	<.0001*	-0.005459	-0.002224
Temp[22]	-0.002237	0.001417	128.00	-1.58	0.1168	-0.005041	0.0005659
Temp[30]	-0.001586	0.001426	128.00	-1.11	0.2680	-0.004407	0.0012347
Time	-0.000192	6.064e-5	128.00	-3.16	0.0020*	-0.000312	-7.164e-5
Oxygen[N]*Temp[22]	0.003582	0.001149	128.00	3.12	0.0022*	0.0013092	0.0058547
Oxygen[N]*Temp[30]	0.0023848	0.001149	128.00	2.08	0.0400*	0.0001107	0.004659
Temp[22]*Time	0.0002172	8.385e-5	128.00	2.59	0.0107*	5.1249e-5	0.0003831
Temp[30]*Time	0.0001915	8.477e-5	128.00	2.26	0.0256*	2.3744e-5	0.0003592

a* (Redness; % initial) Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	1.1073689	0.043357	124.00	25.54	<.0001*	1.0215527	1.193185
Enrich[EB]	-0.058615	0.053473	124.00	-1.10	0.2751	-0.164454	0.0472237
Oxygen[N]	-0.058521	0.011495	124.00	-5.09	<.0001*	-0.081273	-0.035769
Temp[22]	0.0229874	0.035294	124.00	0.65	0.5160	-0.046869	0.0928434
Temp[30]	-0.001223	0.035242	124.00	-0.03	0.9724	-0.070978	0.0685317
Time	0.00315	0.006237	124.00	0.51	0.6144	-0.009195	0.015495
Enrich[EB]*Temp[22]	-0.1049	0.028231	124.00	-3.72	0.0003*	-0.160777	-0.049022
Enrich[EB]*Temp[30]	-0.055209	0.028191	124.00	-1.96	0.0524	-0.111008	0.0005892
Enrich[EB]*Time	0.0247355	0.008314	124.00	2.98	0.0035*	0.0082794	0.0411915
Temp[22]*Time	-0.011974	0.002058	124.00	-5.82	<.0001*	-0.016047	-0.007901
Temp[30]*Time	-0.008693	0.00208	124.00	-4.18	<.0001*	-0.012811	-0.004575
Time*Time	0.0002016	0.000214	124.00	0.94	0.3485	-0.000222	0.0006257
Enrich[EB]*Time*Time	-0.000718	0.000292	124.00	-2.46	0.0153*	-0.001295	-0.00014

b* (Yellowness; % initial) Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	1.0310692	0.008546	119.00	120.65	<.0001*	1.0141479	1.0479904
Enrich[EB]	-0.003729	0.011644	119.00	-0.32	0.7493	-0.026786	0.0193269
Oxygen[N]	-0.029734	0.009281	119.00	-3.20	0.0017*	-0.04811	-0.011357
Temp[22]	-0.023787	0.010853	119.00	-2.19	0.0303*	-0.045277	-0.002298
Temp[30]	-0.011331	0.010947	119.00	-1.04	0.3027	-0.033007	0.0103444
Time	0.0013438	0.000541	119.00	2.48	0.0144*	0.0002723	0.0024152
Enrich[EB]*Oxygen[N]	-0.000265	0.011718	119.00	-0.02	0.9820	-0.023467	0.0229375
Enrich[EB]*Temp[22]	0.00103	0.0144	119.00	0.07	0.9431	-0.027484	0.0295442
Enrich[EB]*Temp[30]	-0.002831	0.014447	119.00	-0.20	0.8450	-0.031438	0.025775
Enrich[EB]*Time	0.0030185	0.00075	119.00	4.03	0.0001*	0.001534	0.0045031
Oxygen[N]*Temp[22]	0.0341781	0.006323	119.00	5.40	<.0001*	0.021657	0.0466992
Oxygen[N]*Temp[30]	0.0205667	0.006332	119.00	3.25	0.0015*	0.0080289	0.0331046
Oxygen[N]*Time	-0.001963	0.000551	119.00	-3.56	0.0005*	-0.003054	-0.000872
Temp[22]*Time	-0.000654	0.000674	119.00	-0.97	0.3337	-0.001989	0.0006805
Temp[30]*Time	-0.000918	0.000687	119.00	-1.34	0.1841	-0.002278	0.0004424
Enrich[EB]*Oxygen[N]*Time	0.0016817	0.000757	119.00	2.22	0.0282*	0.000183	0.0031805
Enrich[EB]*Temp[22]*Time	-0.003811	0.000926	119.00	-4.11	<.0001*	-0.005645	-0.001977
Enrich[EB]*Temp[30]*Time	-0.002959	0.000936	119.00	-3.16	0.0020*	-0.004812	-0.001106

Loaf Volume Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	485.34685	6.356816	134.00	76.35	<.0001*	472.77417	497.91952
Enrich[EB]	43.164306	2.871717	134.00	15.03	<.0001*	37.48455	48.844061
Oxygen[N]	-18.025	4.97396	134.00	-3.62	0.0004*	-27.86263	-8.187375
Temp[22]	38.038333	8.757559	134.00	4.34	<.0001*	20.717407	55.35926
Temp[30]	18.516458	8.757559	134.00	2.11	0.0363*	1.1955316	35.837385
Time	-5.821625	0.364057	134.00	-15.99	<.0001*	-6.541665	-5.101585
Oxygen[N]*Temp[22]	19.825	7.034241	134.00	2.82	0.0056*	5.9124968	33.737503
Oxygen[N]*Temp[30]	11.18125	7.034241	134.00	1.59	0.1143	-2.731253	25.093753
Temp[22]*Time	4.755	0.514854	134.00	9.24	<.0001*	3.736709	5.773291
Temp[30]*Time	4.1945089	0.514854	134.00	8.15	<.0001*	3.176218	5.2127999

Thiamin Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	0.9864134	0.022607	131.00	43.63	<.0001*	0.9416921	1.0311348
Temp[22]	-0.025127	0.031401	131.00	-0.80	0.4250	-0.087245	0.0369912
Temp[30]	-0.00351	0.031521	131.00	-0.11	0.9115	-0.065865	0.0588456
Time	-0.005683	0.001459	131.00	-3.89	0.0002*	-0.008569	-0.002796
Temp[22]*Time	0.006144	0.002018	131.00	3.04	0.0028*	0.0021522	0.0101358
Temp[30]*Time	0.0040422	0.00204	131.00	1.98	0.0496*	7.1517e-6	0.0080772

Riboflavin Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	1.0911875	0.022487	134.00	48.53	<.0001*	1.0467129	1.1356621
Enrich[EB]	-0.080794	0.018072	134.00	-4.47	<.0001*	-0.116537	-0.045051
Time	-0.000844	0.00132	134.00	-0.64	0.5238	-0.003454	0.0017671

Folate Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	1.2122545	0.053379	133.00	22.71	<.0001*	1.1066735	1.3178356
Enrich[EB]	-0.105758	0.073987	133.00	-1.43	0.1552	-0.252102	0.0405864
Time	-0.009901	0.003456	133.00	-2.86	0.0049*	-0.016737	-0.003065
Enrich[EB]*Time	0.009889	0.004771	133.00	2.07	0.0401*	0.0004522	0.0193258

Protein Tukey-Kramer Ordered Difference Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
FE	MU	1.192630	0.1246370	0.793500	1.591760	<.0001*
FE	FU	1.014790	0.1246370	0.615660	1.413920	0.0002*
FE	SE	0.980210	0.1246370	0.581080	1.379340	0.0002*
SE	MU	0.212420	0.1246370	-0.186710	0.611550	0.3811
FU	MU	0.177840	0.1246370	-0.221290	0.576970	0.5185
SE	FU	0.034580	0.1246370	-0.364550	0.433710	0.9920

Iron Tukey-Kramer Ordered Difference Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
SE	FU	5.435000	0.0665207	5.164204	5.705796	<.0001*
SE	MU	4.515000	0.0665207	4.244204	4.785796	<.0001*
FE	FU	3.345000	0.0665207	3.074204	3.615796	<.0001*
FE	MU	2.425000	0.0665207	2.154204	2.695796	<.0001*
SE	FE	2.090000	0.0665207	1.819204	2.360796	<.0001*
MU	FU	0.920000	0.0665207	0.649204	1.190796	0.0006*

Second Set 2-Pentyl Furan Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	1.0084145	0.189586	271.00	5.32	<.0001*	0.6351662	1.3816629
Bleached[B]	0.6967819	0.125568	271.00	5.55	<.0001*	0.4495687	0.9439951
Enrich Lev	-0.037459	0.009429	271.00	-3.97	<.0001*	-0.056022	-0.018897
Oxygen[N]	0.6390224	0.161577	271.00	3.95	<.0001*	0.3209171	0.9571276
Temp[22]	0.6371264	0.185432	271.00	3.44	0.0007*	0.2720567	1.0021961
Temp[30]	0.4008936	0.185432	271.00	2.16	0.0315*	0.0358239	0.7659633
Time	0.1274708	0.021839	271.00	5.84	<.0001*	0.0844742	0.1704674
Bleached[B]*Temp[22]	-0.562422	0.138213	271.00	-4.07	<.0001*	-0.834529	-0.290315
Bleached[B]*Temp[30]	-0.326798	0.138213	271.00	-2.36	0.0188*	-0.598904	-0.054691
Enrich Lev*Time	0.0024336	0.000556	271.00	4.37	<.0001*	0.0013381	0.003529
Oxygen[N]*Temp[22]	-1.274818	0.138213	271.00	-9.22	<.0001*	-1.546925	-1.002712
Oxygen[N]*Temp[30]	-0.504666	0.138213	271.00	-3.65	0.0003*	-0.776773	-0.232559
Oxygen[N]*Time	0.0733348	0.00826	271.00	8.88	<.0001*	0.0570733	0.0895963
Temp[22]*Time	-0.06355	0.010116	271.00	-6.28	<.0001*	-0.083466	-0.043634
Temp[30]*Time	-0.06529	0.010116	271.00	-6.45	<.0001*	-0.085206	-0.045373
Bleached[B]*Oxygen[N]	0.4466568	0.11285	271.00	3.96	<.0001*	0.2244825	0.6688312
Time*Time	-0.002161	0.000707	271.00	-3.06	0.0025*	-0.003553	-0.00077

Second Set 1-Hexanal Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	3.7302148	0.113597	271.00	32.84	<.0001*	3.5065699	3.9538597
Bleached[B]	0.5402235	0.140214	271.00	3.85	0.0001*	0.2641758	0.8162711
Enrich Lev	-0.035164	0.009049	271.00	-3.89	0.0001*	-0.052979	-0.017349
Oxygen[N]	0.4116588	0.115148	271.00	3.58	0.0004*	0.1849613	0.6383563
Temp[22]	0.4052124	0.132182	271.00	3.07	0.0024*	0.1449786	0.6654462
Temp[30]	0.0827734	0.132182	271.00	0.63	0.5317	-0.17746	0.3430072
Time	0.0047449	0.006697	271.00	0.71	0.4792	-0.00844	0.0179298
Bleached[B]*Temp[22]	-0.275084	0.098523	271.00	-2.79	0.0056*	-0.469051	-0.081117
Bleached[B]*Temp[30]	-0.048942	0.098523	271.00	-0.50	0.6198	-0.242909	0.1450249
Enrich Lev*Oxygen[N]	0.0346288	0.005419	271.00	6.39	<.0001*	0.0239595	0.045298
Enrich Lev*Time	0.0012135	0.000554	271.00	2.19	0.0294*	0.0001224	0.0023047
Oxygen[N]*Temp[22]	-0.855707	0.098523	271.00	-8.69	<.0001*	-1.049673	-0.66174
Oxygen[N]*Temp[30]	-0.447892	0.098523	271.00	-4.55	<.0001*	-0.641859	-0.253925
Oxygen[N]*Time	0.0514279	0.005888	271.00	8.73	<.0001*	0.0398362	0.0630197
Temp[22]*Time	-0.023972	0.007211	271.00	-3.32	0.0010*	-0.038169	-0.009775
Temp[30]*Time	-0.014055	0.007211	271.00	-1.95	0.0523	-0.028252	0.000142
Bleached[B]*Time	-0.006804	0.008227	271.00	-0.83	0.4090	-0.023001	0.0093933

Second Set Hexanal Indicator Function Parameterization

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|	Lower 95%	Upper 95%
Intercept	0.2680231	0.260027	270.00	1.03	0.3036	-0.243915	0.7799614
Bleached[B]	1.4230725	0.19584	270.00	7.27	<.0001*	1.0375049	1.80864
Enrich Lev	-0.019673	0.018127	270.00	-1.09	0.2788	-0.055361	0.0160159
Oxygen[N]	-0.246772	0.161755	270.00	-1.53	0.1283	-0.565233	0.0716888
Temp[22]	1.2423416	0.269144	270.00	4.62	<.0001*	0.7124532	1.7722301
Temp[30]	0.5029784	0.269144	270.00	1.87	0.0627	-0.02691	1.0328669
Time	0.1812152	0.030755	270.00	5.89	<.0001*	0.1206646	0.2417659
Bleached[B]*Temp[22]	-0.730453	0.27696	270.00	-2.64	0.0088*	-1.275728	-0.185179
Bleached[B]*Temp[30]	-0.665399	0.27696	270.00	-2.40	0.0170*	-1.210674	-0.120125
Enrich Lev*Oxygen[N]	0.0235481	0.010903	270.00	2.16	0.0317*	0.0020829	0.0450134
Enrich Lev*Temp[22]	-0.045492	0.018658	270.00	-2.44	0.0154*	-0.082226	-0.008758
Enrich Lev*Temp[30]	-0.034442	0.018658	270.00	-1.85	0.0660	-0.071176	0.002292
Enrich Lev*Time	0.0035806	0.000798	270.00	4.49	<.0001*	0.0020095	0.0051517
Oxygen[N]*Temp[22]	0.6963736	0.198213	270.00	3.51	0.0005*	0.306134	1.0866132
Oxygen[N]*Temp[30]	1.0888793	0.198213	270.00	5.49	<.0001*	0.6986397	1.4791189
Temp[22]*Time	-0.141275	0.014508	270.00	-9.74	<.0001*	-0.169837	-0.112712
Temp[30]*Time	-0.116997	0.014508	270.00	-8.06	<.0001*	-0.14556	-0.088435
Time*Time	-0.002079	0.001014	270.00	-2.05	0.0412*	-0.004075	-8.348e-5

APPENDIX D: EXPANDED AND ADDITIONAL RESULTS

Moisture and Water Activity

Flour moisture during storage is shown in Fig. 7. Moisture did not significantly change over time. EB flour had small but significantly higher moisture values than UU flour (0.3%).

The water activity of flour during storage is shown in Fig. 8. Water activity had significant effects from storage temperature and time. Water activity increased slightly over time (increase of 0.02 over 24 weeks). Water activity also showed slightly higher values as the storage temperature increased, with 30°C and 40°C storage having values 0.01 and 0.03 higher than 22°C storage, respectively. This effect on water activity may be due to an equilibration of the flour in the can over time.

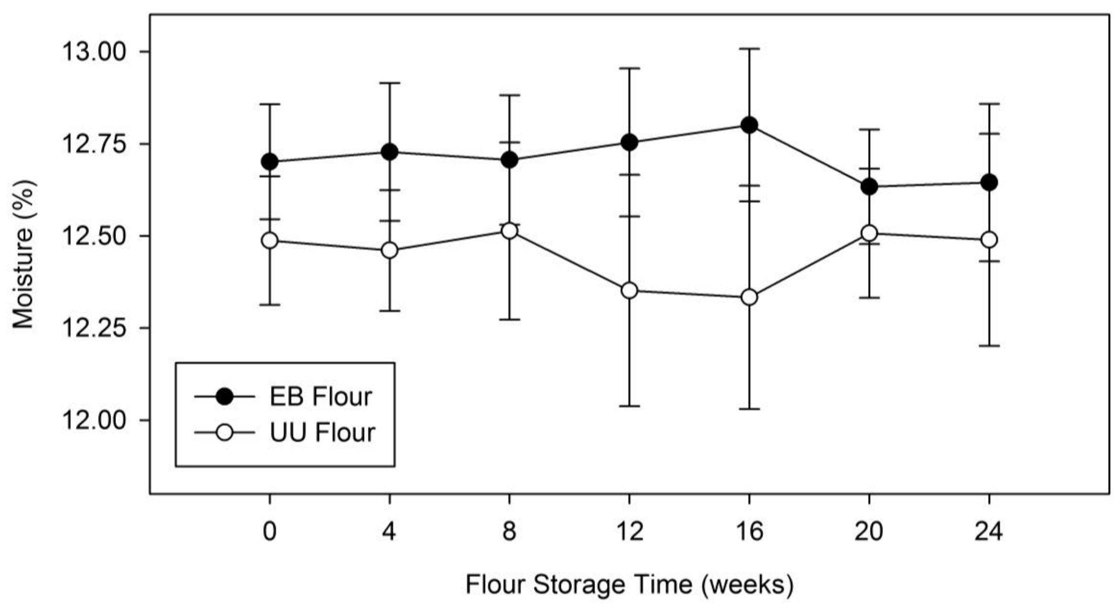

Fig. 7. Moisture of enriched, bleached (EB) and unenriched, unbleached (UU) flour during storage. Error bars are constructed using the standard error.

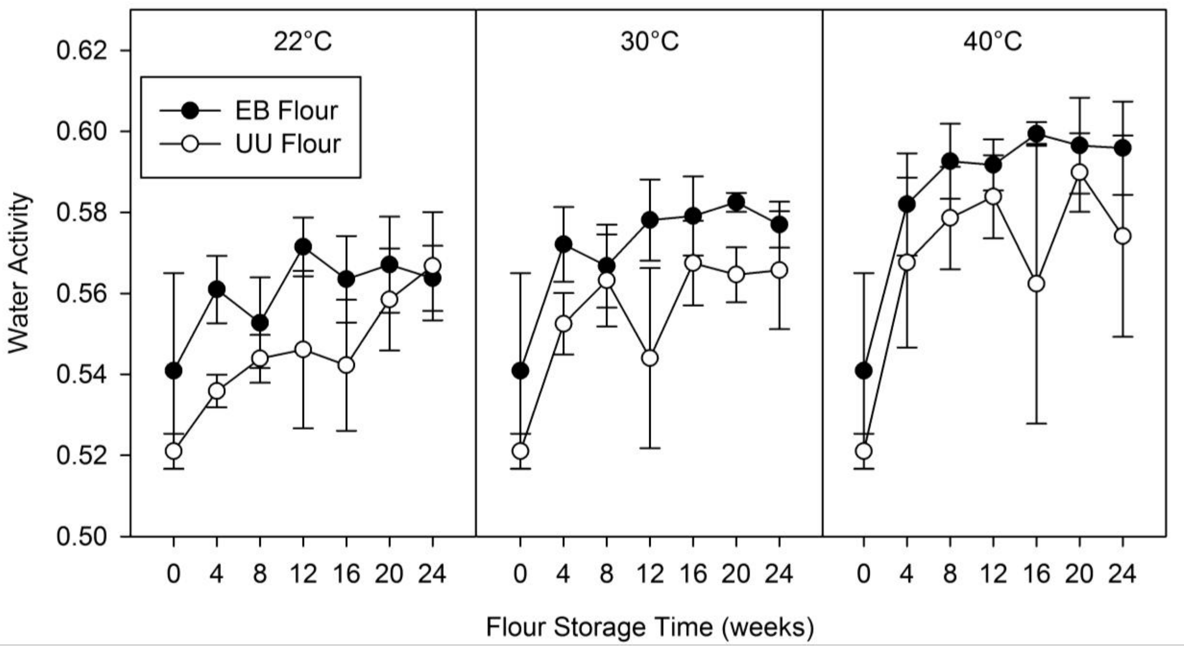

Fig. 8. Water activity of enriched, bleached (EB) and unenriched, unbleached (UU) flour during storage. Error bars are constructed using the standard error.

Headspace Oxygen

The headspace oxygen in flour samples stored at a normal oxygen atmosphere is shown in Fig. 9. One of the lots of EB flour had a more rapid decrease of headspace oxygen with samples stored at 40°C reaching 0% oxygen. It is unclear why this occurred. It is possible that the benzoyl peroxide used to bleach the flour had not fully reacted before the flour was canned.

The headspace oxygen of flour samples stored in a low oxygen atmosphere is shown in Fig. 10. All time zero flour samples were tested for headspace oxygen within two days of canning. All of these time zero samples reached <0.5% oxygen within two days at ambient conditions. This indicates a rapid removal of oxygen by the oxygen absorbers.

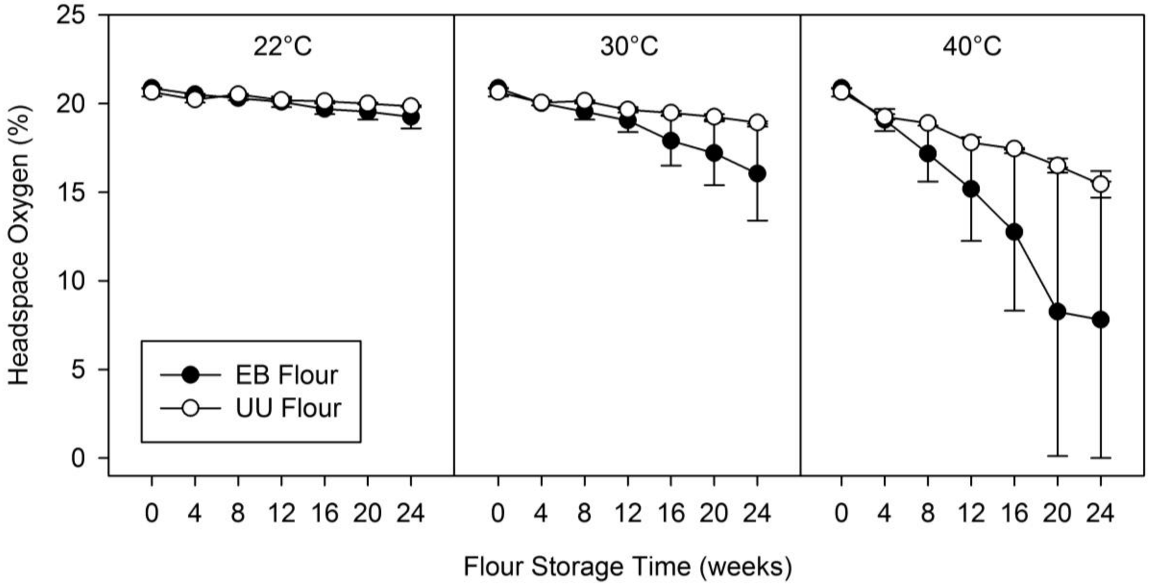

Fig. 9. Headspace oxygen in enriched, bleached (EB) and unenriched, unbleached (UU) flour stored in a normal oxygen atmosphere. Error bars are constructed using the standard error.

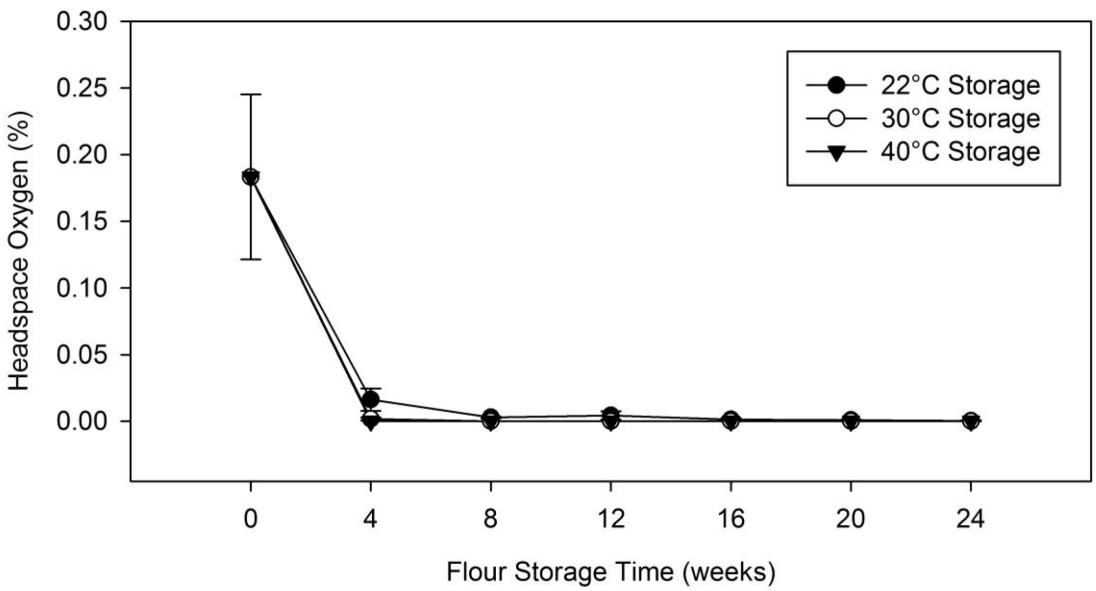

Fig. 10. Headspace oxygen in flour stored in a low oxygen atmosphere at 22, 30, and 40°C. Error bars are constructed using the standard error.

Free Fatty Acids (FFA)

Free fatty acid formation in flour during storage is shown in Fig. 11. Within this study, fatty acids showed a linear increase during storage. A higher storage temperature resulted in a higher rate of formation. Similar increases are seen in Maraschin et al (2008).

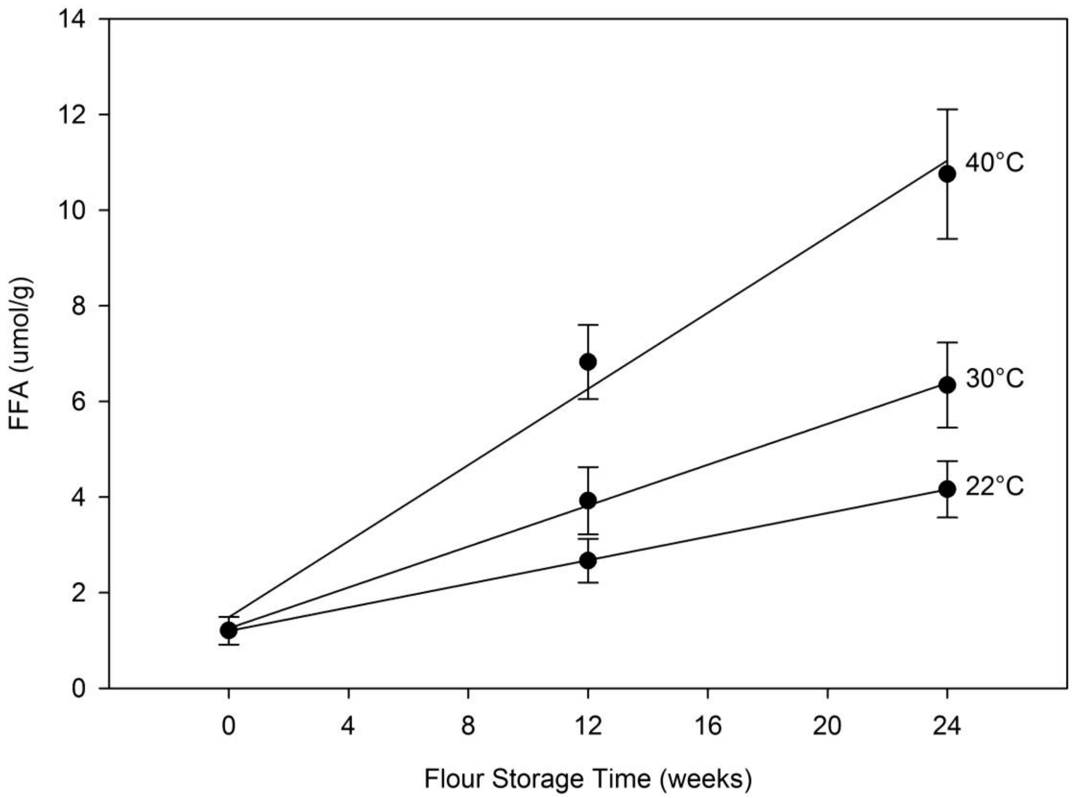

Fig. 11. Free fatty acids (FFA) in flour stored at 22, 30, and 40°C. Error bars represent the 95% confidence interval.

Volatile Profile of Flour During Storage

Forty three compounds with odor impressions were identified in flour samples (Table 5). Other detected compounds were not listed because they were either not identified or, according to the literature, had no odor. Compounds were identified by comparison to a mass spectrum library and also by retention index (RI) comparison to values found in literature. Most of the odorous compounds appeared to be lipid oxidation products, made up of alcohols, ketones, aldehydes, and 2-pentyl furan (Shahidi and Pegg 1994). Some compounds, including the furans, may be derived from the Maillard reaction during milling and storage. Eleven of the compounds were aldehydes, eight were alcohols, and seven were ketones. Nine of the forty three compounds were methyl esters or acetates.

Table 5. Volatile compounds identified in stored flour

No.	Compound Name	Kovats RI	Literature RI[ab]	Odor Description[ac]
1	Acetone	—	477	Solvent, ethereal, apple, pear
2	Methyl acetate	—	515	Ether, sweet, fruity
3	Acetic Acid	—	600	Sharp, pungent, sour, vinegar
4	2-Butanone	—	597	Acetone-like, ethereal, fruity, camphor
5	1-Butanol	—	675	Fusel oil, sweet, balsam
6	2-pentanone	—	711	Sweet, fruity, ethereal, wine, banana, woody
7[d]	2-ethyl furan	—	705, 728	Sweet, burnt, earthy, malty
8	Pentanal	—	732	Fermented, bready, fruity, nutty, berry
9	Methyl butrate	—	724, 873	Fruity, apple, sweet, banana, pineapple
10	3-methyl-1-butanol	—	735	Fusel oil, alcoholic, whiskey, fruity, banana
11	2-methyl-1-butanol	—	739, 744, 755	Roasted, wine, onion, fruity
12	Toluene	—	770	Sweet
13	1-Pentanol	—	766, 768	Balsamic
14	Methyl-2-methyl butyrate	—	776, 777	Sweet, fruity, tutti frutti, fatty, green apple
15	Hexanal	801.1	801	Fresh, green, fatty, aldehydic, grass, leafy, fruity, sweaty
16	2-methyl-2-pentenal	818.4	808	Powerful green grass, somewhat fruity, gassy
17	Methyl pentanoate	829.0	821, 825	Sweet, green, fruity, apple, pineapple, nutty
18	E-2-hexenal	858.0	854	Green banana, aldehydic, fatty, cheesy
19	1-Hexanol	874.0	851, 867, 884	Ethereal, fusel oil, fruity, alcoholic, sweet, green
20	2-Heptanone	890.9	888, 889, 895	Fruity, spicy, sweet, herbal, coconut, woody
21	o-xylene	892.2	888, 892, 896	Geranium
22[d]	2-butyl furan	892.2	-	Mild fruity, wine, sweet, spicy
23	2-Heptanol	901.2	905	Fresh, lemon, grass, herbal, sweet, floral, fruity, green
24[d]	Heptanal	901.2	899, 900, 903	Fresh, aldehydic, fatty, green, herbal, wine-lee, ozone
25	Gamma butyrolactone	905.1	891	Creamy, oily, fatty, caramel

No.	Compound Name	Kovats RI	Literature RI[ab]	Odor Description[ac]
26	Amyl acetate	911.6	915	Ethereal, fruity, banana, pear, apple
27	Methyl Hexanoate	919.5	934, 1000	Fruity, pineapple, ether
28	D- (+)-alpha-pinene	927.2	937, 939	Harsh terpene, minty
29	2-Heptenal	949.3	951, 957	Green, fatty
30	1-Heptanol	963.4	969	Musty, leafy, violet, herbal, green, sweet, woody, peony
31	Beta Pinene	971.9	980, 981, 990	Dry woody, resinous pine, hay, green
32	1-Octen-3-ol	975.1	942, 978, 980, 982	Mushroom, earthy, green, oily, fungal, raw chicken
33	2-pentyl-furan	987.2	992, 993	Fruity, green, earthy, beany, vegetable, metallic
34	Octanal	1004.4	999, 1001, 1004, 1006	Aldehydic, waxy, citrus, orange, peel, green, fatty
35	Hexyl Acetate	1015.1	1014, 1015	Fruity, green, apple, banana, sweet
36	Methyl heptanoate	1028.5	1006, 1021	Sweet fruit, green, orris, waxy, floral, berry
37	3 octen-2-one	1043.5	1036	Earthy, spicy, herbal, sweet, mushroom, hay, blueberry
38	Gamma-hexalactone	1056.0	1055, 1323	Herbal, coconut, sweet, coumarin, tobacco
39	2-octenal	1063.7	1060	Fatty, green, herbal
40	Nonanal	1105.3	1098, 1103, 1104, 1108	Waxy, aldehydic, rose, fresh, orris, orange peel, fatty, peely
41	Methyl octanoate	1125.5	1126, 1041	Waxy, green, sweet, orange, aldehydic, vegetable, herbal
42	2-nonenal	1163.5	1142, 1149, 1162	Fatty, green, waxy, cucumber, melon
43	Decanal	1207.3	1204, 1209, 1211, 1229	Sweet, aldehydic, waxy, orange peel, citrus, floral

[a] www.flavornet.org
[b] www.pherobase.com
[c] www.thegoodscentscompany.com
[d] this compound co-eluted with the previous compound

Descriptive Analysis of Flour Odor

Descriptive results for the musty (Table 6), play dough (Table 7), and other (Table 8) aroma descriptive scores are shown below. The musty odor showed no significant differences between treatments but showed significant effects in the enriched, bleached treatment at different temperatures. The enriched, bleached treatment also had a very small but significant decrease over time (0.1 lower scores over 24 weeks). The play dough odor showed significantly higher increases during storage at 40°C compared to both 22 and 30°C. EB flour stored in a normal oxygen atmosphere also had significantly higher increases during storage compared to UU flour stored in a normal oxygen atmosphere (average of 1.2 higher scores over 24 weeks). The other aroma descriptive scores did not show significant effects from storage temperature or time. There were significantly higher scores for UU flour stored in a normal oxygen atmosphere compared to other treatment combinations (averages of 0.1 to 0.3 higher). The odor descriptions suggested by two or more panelists for each treatment combination is shown on Table 9. UU flour stored at a normal oxygen atmosphere had the most suggested descriptors, including multiple "sweet" and "fruity" odors.

Table 6. Mean musty aroma descriptive scores for enriched-bleached (EB) and unenriched, unbleached (UU) flour stored at 22, 30, and 40°C

Storage Temperature and Treatment*	Flour Storage Time (weeks)						
	0**	4	8	12	16	20	24
EB flour 22°C storage	0.0	0.0	0.0	0.0	0.0	0.0	0.0
UU flour 22°C storage	0.0	0.1	0.0	0.0	0.0	0.0	0.0
EB flour 30°C storage		0.2	0.0	0.1	0.0	0.0	0.0
UU flour 30°C storage		0.1	0.0	0.1	0.0	0.1	0.0
EB flour 40°C storage		0.1	0.2	0.1	0.0	0.0	0.0
UU flour 40°C storage		0.0	0.0	0.0	0.0	0.0	0.0

* EB = enriched, bleached and UU = unenriched, unbleached.
** Samples tested at 0 weeks were not associated with a storage temperature.

Table 7. Mean play dough aroma descriptive scores for flour stored in a normal and low oxygen atmosphere at 22, 30, and 40°C

Storage Temperature and Treatment	Flour Storage Time (weeks)						
	0*	4	8	12	16	20	24
22°C and normal oxygen atmosphere	0.2	0.4	0.4	0.3	0.2	0.2	0.2
22°C and low oxygen atmosphere	0.1	0.1	0.2	0.0	0.0	0.0	0.0
30°C and normal oxygen atmosphere		0.2	0.2	0.3	0.4	0.3	0.4
30°C and low oxygen atmosphere		0.2	0.2	0.1	0.0	0.0	0.0
40°C and normal oxygen atmosphere		0.2	0.2	0.2	0.9	0.9	1.3
40°C and low oxygen atmosphere		0.3	0.0	0.0	0.0	0.0	0.2

* Samples tested at 0 weeks were not associated with a storage temperature.

Table 8. Mean other aromas descriptive scores for flour stored in a normal and low oxygen atmosphere at 22, 30, and 40°C

	Flour Storage Time (weeks)						
Storage Temperature and Treatment	0*	4	8	12	16	20	24
22°C and normal oxygen atmosphere	0.1	0.2	0.1	0.2	0.2	0.3	0.2
22°C and low oxygen atmosphere	0.3	0.0	0.1	0.0	0.1	0.2	0.1
30°C and normal oxygen atmosphere		0.1	0.2	0.5	0.2	0.3	0.2
30°C and low oxygen atmosphere		0.0	0.1	0.1	0.0	0.1	0.0
40°C and normal oxygen atmosphere		0.1	0.1	0.2	0.6	0.5	0.2
40°C and low oxygen atmosphere		0.1	0.0	0.0	0.0	0.0	0.0

* Samples tested at 0 weeks were not associated with a storage temperature.

Table 9. Suggested aroma descriptor(s) for other aromas by two or more panelists for enrichment-bleaching and oxygen atmosphere storage treatment combinations

Treatment Combination*	Suggested Aroma Descriptor(s)
EB flour and normal oxygen	Menthol, Plastic, Sweet, Sweet Plastic
EB flour and low oxygen	Fruity
UU flour and normal oxygen	Fruity, Fruity Plastic, Pine, Menthol, Sawdust, Solvent, Sweet, Sweet Plastic
UU flour and low oxygen	Menthol, Soapy

*EB = enriched, bleached and UU = unenriched, unbleached.

Acid-Metallic Dissipation

To evaluate the ability of the acid-metallic odor to dissipate from an opened container, cans of flour (n = 2) at 24 weeks storage time (storage temperatures 22, 30, and 40°C), along with a 6 year sample held at 22°C storage, were tested. Cans were opened and immediately evaluated by the descriptive analysis panel for the acid-metallic odor. The acid-metallic aroma scores decreased to zero within 48 hours for all samples (Fig. 12).

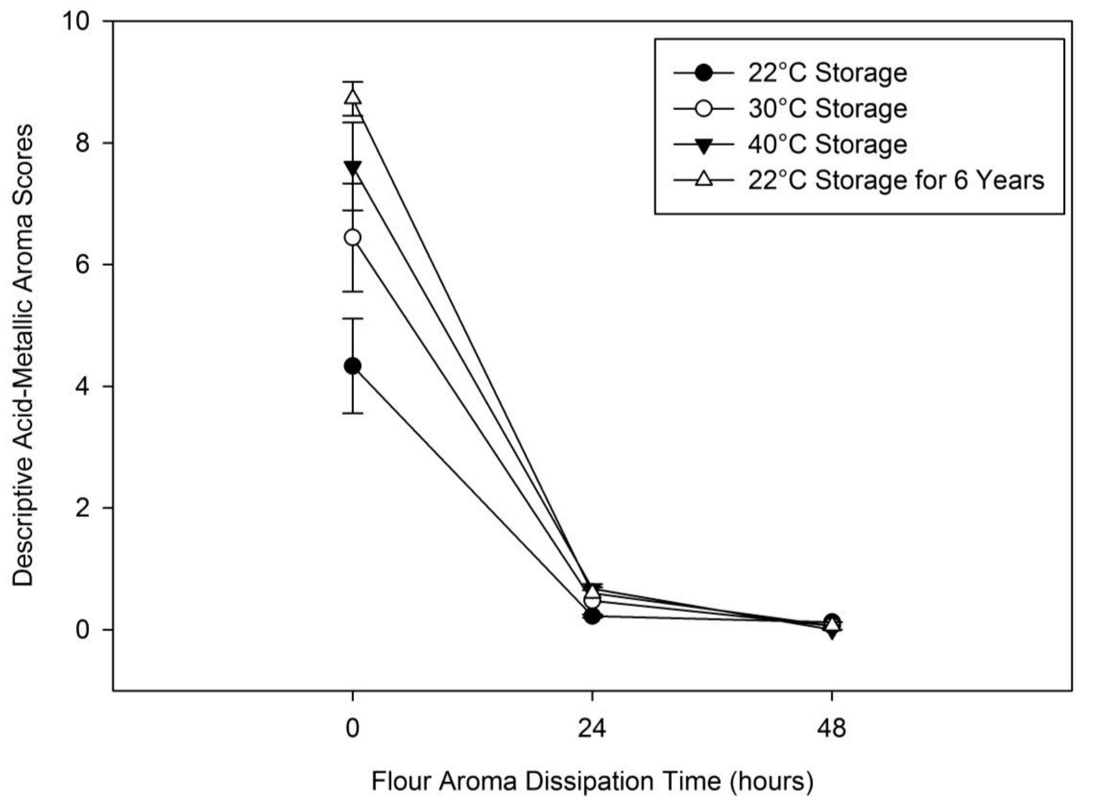

Fig. 12. Acid-metallic dissipation in a newly opened container over time in 24 week samples at 22, 30 and 40°C storage, and in a 6 year-old sample at 22°C storage. Error bars are constructed using the standard error.

Bread Consumer Sensory Analysis

Consumer acceptance of bread made from flour samples stored at 22 (Table 10) and 30°C (Table 11) are shown below. No significant differences were found between samples made from flour stored at these temperatures.

Table 10. Mean acceptance scores of bread made from flour stored at 22°C for 24 weeks

Flour Sample	Appearance	Aroma	Overall Acceptability	Flavor	Texture
Control*	7.0[a]	6.8[a]	6.7[a]	6.8[a]	6.9[a]
EB flour at low oxygen atmosphere	7.3[a]	6.8[a]	6.8[a]	6.8[a]	7.0[a]
EB flour at normal oxygen atmosphere	7.2[a]	6.8[a]	6.8[a]	6.9[a]	7.0[a]
UU flour at low oxygen atmosphere	7.1[a]	6.6[a]	6.7[a]	6.7[a]	6.7[a]
UU flour at normal oxygen atmosphere	7.1[a]	6.6[a]	6.6[a]	6.7[a]	6.8[a]

In each attribute, values without the same superscript letter are significantly different ($p<0.05$).
*EB = enriched, bleached and UU = unenriched, unbleached.

Table 11. Mean acceptance scores of bread made from flour stored at 30°C for 24 weeks

Flour Sample	Appearance	Aroma	Overall Acceptability	Flavor	Texture
Control*	7.1[a]	6.8[a]	6.8[a]	6.9[a]	6.9[a]
EB flour at low oxygen atmosphere	7.1[a]	6.9[a]	6.8[a]	6.9[a]	7.0[a]
EB flour at normal oxygen atmosphere	7.2[a]	6.7[a]	6.8[a]	6.8[a]	7.0[a]
UU flour at low oxygen atmosphere	7.2[a]	6.9[a]	6.9[a]	6.8[a]	7.0[a]
UU flour at normal oxygen atmosphere	7.3[a]	6.8[a]	6.7[a]	6.8[a]	6.9[a]

In each attribute, values without the same superscript letter are significantly different ($p<0.05$).
*EB = enriched, bleached and UU = unenriched, unbleached.

Color Analysis

L* (lightness), a* (redness), and b* (yellowness) values were examined for significant effects during storage due to enrichment-bleaching, headspace oxygen, and storage temperatures (Fig. 13). Seven UU flour samples from one lot were determined, through vitamin and color analysis, to have received a partial enrichment-bleaching treatment and thus were removed from color analysis. Color results are reported in percent initial values of time zero flour sample lots.

The L* values in time zero flour lots had means of 92.6 and 92.0 for EB and UU flour, respectively. There was a significant decrease in L* values during storage at 40°C, but no significant decreases during storage at 20 and 30°C (Fig. 13A). A normal headspace oxygen resulted in significantly lower L* values in flour stored at 40°C. There was no effect of the enrichment-bleaching treatment on changes in L* values.

Time zero flour lots had a* means of 0.5 and 0.6 for EB and UU flour, respectively. Storage at a low oxygen atmosphere resulted in a significantly higher a* values for all treatments. EB flour had a higher increase in a* values during storage at 40°C compared to UU flour (Fig. 13B).

Time zero flour lots had b* means of 8.5 and 11.6 for EB and UU flour, respectively. Generally, b* values increased during flour storage. A normal headspace oxygen resulted in significantly lower increases in b* values during storage for UU flour but not for EB flour (Fig. 13C). EB flour had significantly higher increases in b* values than UU flour. At 30 and 40°C storage, a normal oxygen atmosphere resulted in significantly lower b* values in flour samples.

A low headspace oxygen appeared to prevent the darkening reaction which occurred during flour storage in a normal oxygen atmosphere, as indicated by L* values. However, a low headspace oxygen appeared to increase the reddening (a*) and yellowing (b*) of flour during storage. Thus, storage in a low oxygen atmosphere prevents the darkening of flour, but does not prevent the discoloration in redness and yellowness of flour. However, these color changes were small and unlikely to affect consumer acceptance of the flour. Research by Rose (2005) showed that while consumers disliked flour with lower L* values, they did not dislike bread made from this flour. Khan et al (2002) found that the use of oxygen-limiting packaging prevents discoloration of flour during storage. More research is needed to determine how consumer acceptance is affected by the different color changes in flour.

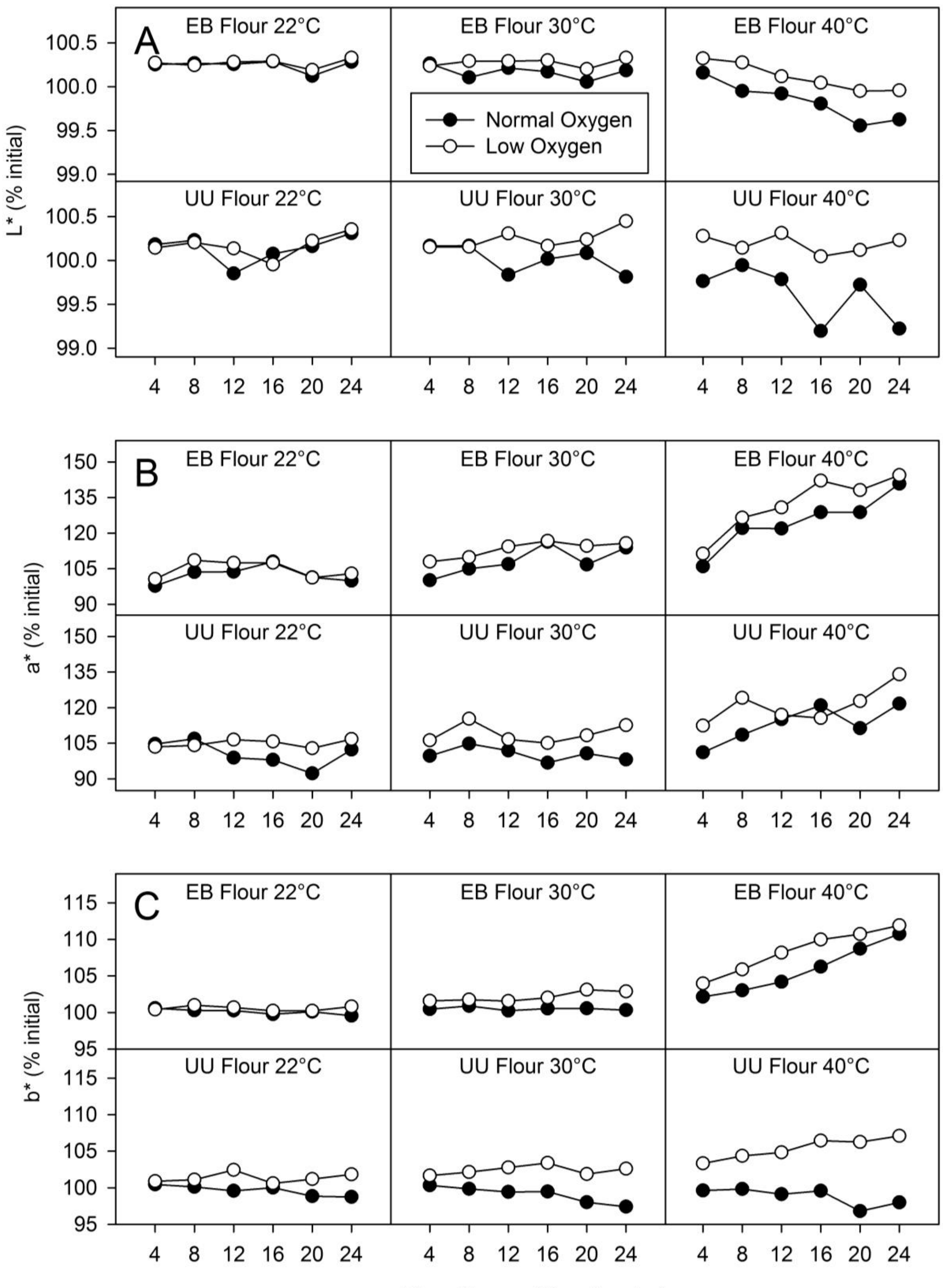

Fig. 13. Effect of headspace oxygen on L* (A), a* (B), and b* (C) values in enriched, bleached (EB) and unenriched, unbleached (UU) flour during storage at 22, 30, and 40°C.

Loaf Volume

Bread loaf volume is a good indicator of bread baking quality. Bread loaf volume results are summarized in Fig. 14. Results are adjusted so that mean EB flour loaf volumes at time zero represents 100% loaf volume. A significant decrease in loaf volume over time was observed for all samples. Flour at 22, 30, and 40°C storage lost, respectively, 6.4, 13.0, and 34.6% of loaf volume after 24 weeks. Other studies have observed a reduction in breadmaking ability with increased storage temperatures. Franz (1968) saw a similar rate of decrease in loaf volume for flour stored at room temperature and a lesser rate of decrease in flour stored at 10°C. Moreover, Cenkowski (2000) observed dough becoming more bulky or inextensible as storage temperatures increased from 20°C to 30°C and 40°C. This can cause problems in breadmaking by increasing required mixing time and making dough more difficult to sheet.

The UU flour had significantly lower loaf volumes (7.4% lower) than EB flour. However, the rates of loaf volume reduction between EB and UU flour at each temperature were not significantly different. These results suggest that bleaching using benzoyl peroxide does not impair loaf volume during storage. Other studies found that bleaching using chlorine dioxide impaired the breadmaking quality of stored flour (Cuendet et al 1954).

A low oxygen atmosphere resulted in a significantly larger loaf volume (3.2% larger) for flour held at 40°C. No significant differences were observed from storage in a low oxygen atmosphere for flour stored at 22°C and 30°C. The lower loaf volume in samples stored in a normal oxygen atmosphere may be due to an over-oxidation of flour proteins. It is theorized that an excess of disulfide bonds, with a loss of sulfhydryl groups, hinders the disulfide bond interchange and results in a rapid breakdown of dough (Sandhu et al 2011).

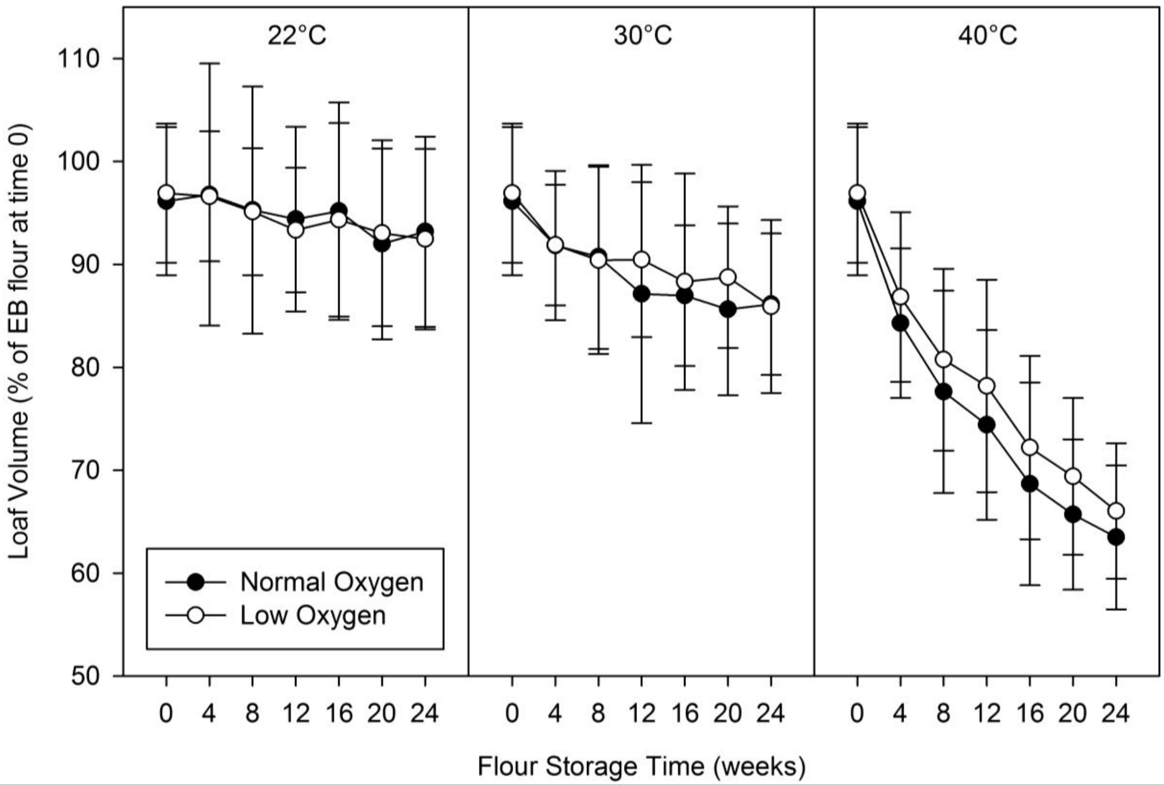

Fig. 14. Effect of a low oxygen atmosphere on bread loaf volume of flour stored at 22, 30, and 40°C. Error bars represent the 95% confidence interval.

It is interesting to note that the short-term improvement in volume resulting from storage time and aeration, as seen by Bellenger and Godon (1972), did not occur in this study. This may be the result of the package's hermetic seal, which prevented further aeration. Also, since this flour was packaged in cans within two weeks of milling, the flour may have already matured before storage started. This is evident in work by Nishio (2004), who found a large increase in loaf volume after an aeration time of only two weeks.

Vitamin Analysis

Thiamin, riboflavin, and folate were examined for significant effects during storage due to enrichment-bleaching, headspace oxygen, and storage temperatures. Seven UU flour samples from one lot were determined, through vitamin and color analysis, to have received a partial enrichment-bleaching treatment and thus were removed from vitamin analysis. Vitamin results (dry weight basis) are reported in percent initial vitamin concentration of time zero flour sample lots.

Thiamin in time zero flour lots had means of 1.36 and 0.45 mg/100g for EB and UU flour, respectively. The United States Food and Drug Administration (FDA) requires enriched all-purpose flour to have a minimum thiamin content of 0.73 mg/100g (dwb; based on 12% moisture). Flour stored at 40°C had a significant loss in thiamin over time (Fig. 15), with a mean loss of 13% after 24 weeks. There were no significant losses in thiamin in flour stored at 20°C and 30°C. There were no significant effects from the enrichment-bleaching treatment or the low oxygen atmosphere treatment.

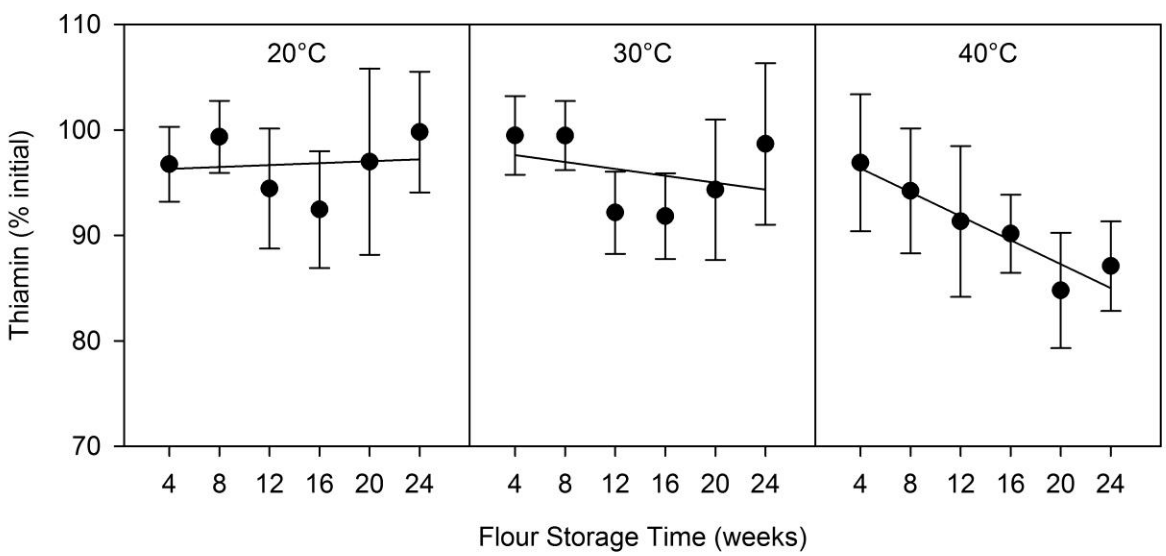

Fig. 15. Thiamin retention in flour during storage at 22, 30, and 40°C. Error bars represent the 95% confidence interval.

These results concur with findings by Hollenbeck et al (1952) who reported a thiamin loss of 5% in white flour at 14.5% moisture stored at 38°C for 4 months and no thiamin loss in the same flour at 25°C storage. Franz (1968) also reported no thiamin loss in white flour between 11 and 12% moisture stored at ambient conditions for 12 months. It appears that thiamin loss increases at high temperatures and isn't affected by thiamin source or the headspace oxygen. Thus, thiamin in all-purpose wheat flour is probably stable under normal long-term storage conditions. However, more research is needed to confirm these findings.

Riboflavin in time zero flour lots had means of 0.59 and 0.12 mg/100g for EB and UU flour, respectively. The FDA requires enriched all-purpose flour to have a minimum riboflavin content of 0.45 mg/100g (dwb; based on 12% moisture). There was no significant riboflavin loss in flour during storage (Fig. 16), nor were there losses due to storage temperature, headspace oxygen, and riboflavin source (enriched or native riboflavin). This suggests that riboflavin degradation is not a concern in flour stored long-term.

Folate in time zero flour lots had means of 0.27 and 0.05 mg/100g for EB and UU flour, respectively. The FDA requires enriched all-purpose flour to have a minimum folate content of 0.18 mg/100g (dwb; based on 12% moisture). There was a significant loss of folate in UU flour but not in EB flour (Fig. 17). Folate in UU flour exhibited a 24% loss over 24 weeks. There were no significant effects from storage temperature or headspace oxygen on folate.

These results concur with Keagy et al (1975), who reported a loss in native folate of all-purpose flour of 17% and 25% when stored for 52 weeks at 28.9 and 37.8°C, respectively. It was also reported that enriched folic acid had little or no loss at these storage temperatures. Since EB flour showed no significant effects from the headspace oxygen or the storage temperature, it appears that degradation of added folic acid is not a concern in enriched all-purpose wheat flour during long-term storage.

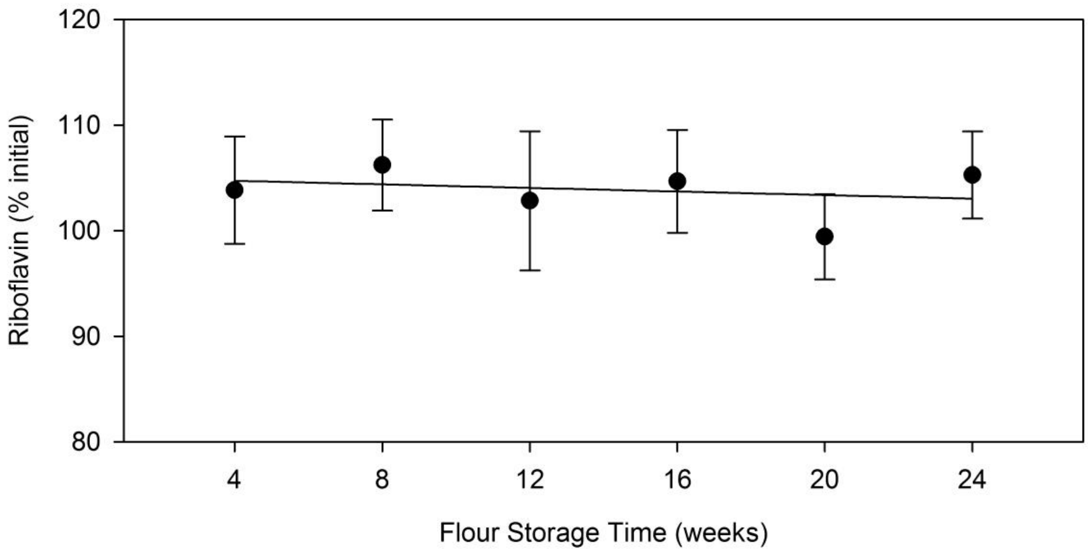

Fig. 16. Riboflavin retention in enriched flour during storage. Error bars represent the 95% confidence interval.

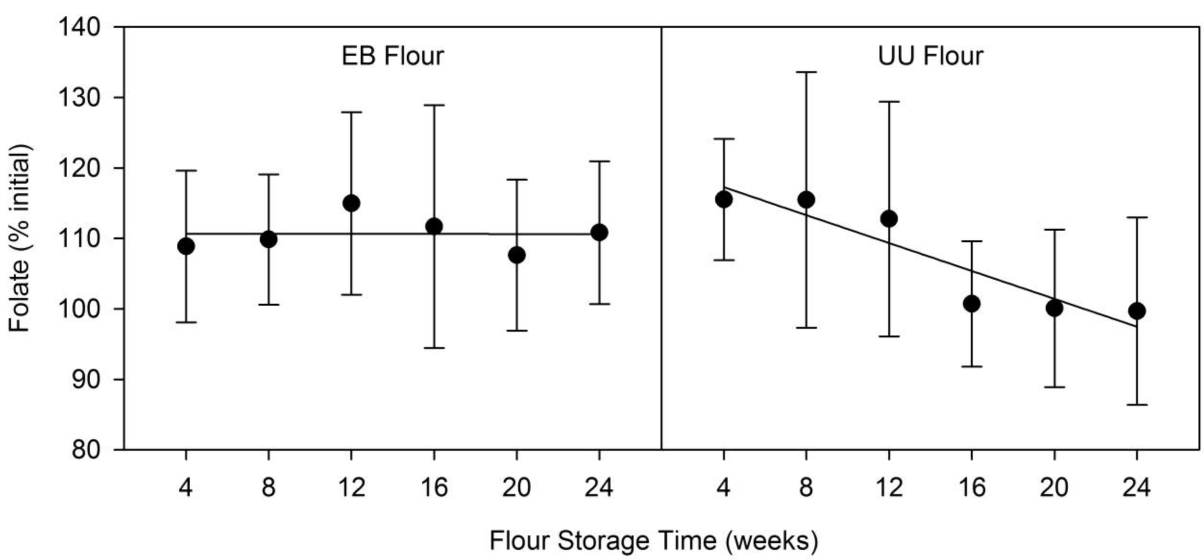

Fig. 17. Folate retention in enriched, bleached (EB) and unenriched, unbleached (UU) flour during storage. Error bars represent the 95% confidence interval.

Protein

Flour protein of samples tested ranged from 9.8% to 11.0%. The sample from EB lot 1 had a significantly higher protein than the other lots (Fig. 18). There were no other significant differences.

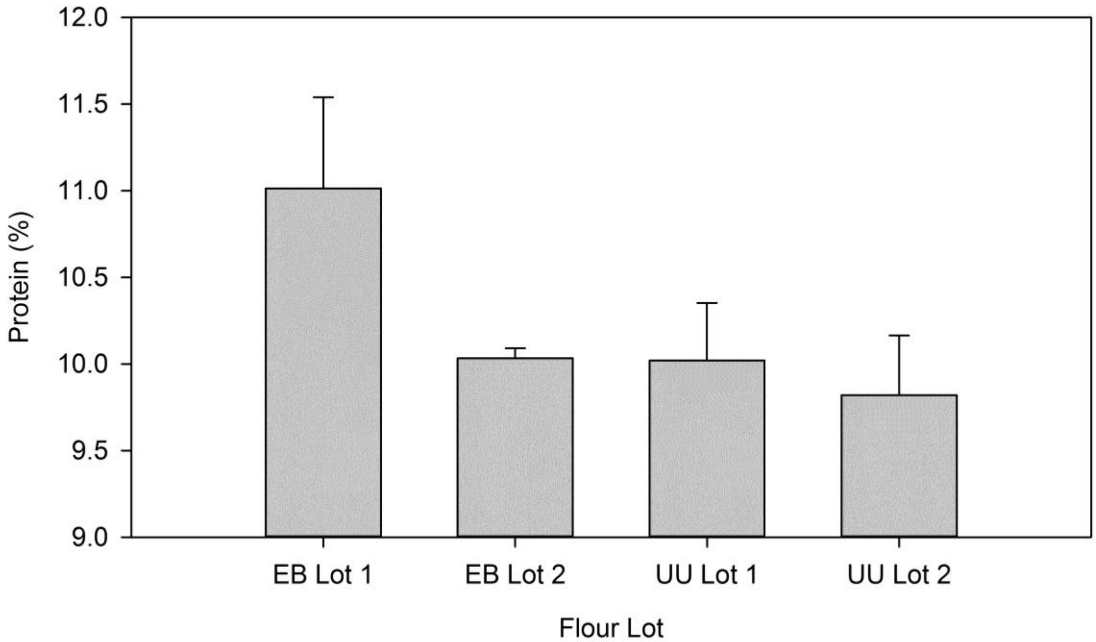

Fig. 18. Mean protein content (wwb) in one initial flour sample from each lot (*n* = 3). EB = enriched, bleached and UU = unenriched, unbleached. Error bars represent the 95% confidence interval.

Iron

Iron was shown to have significant differences between all lots (Fig. 19). As expected, enriched flour had significantly higher iron values than unenriched flour. The differences between lots of the same enrichment treatment were likely due to variations in the enrichment amount.

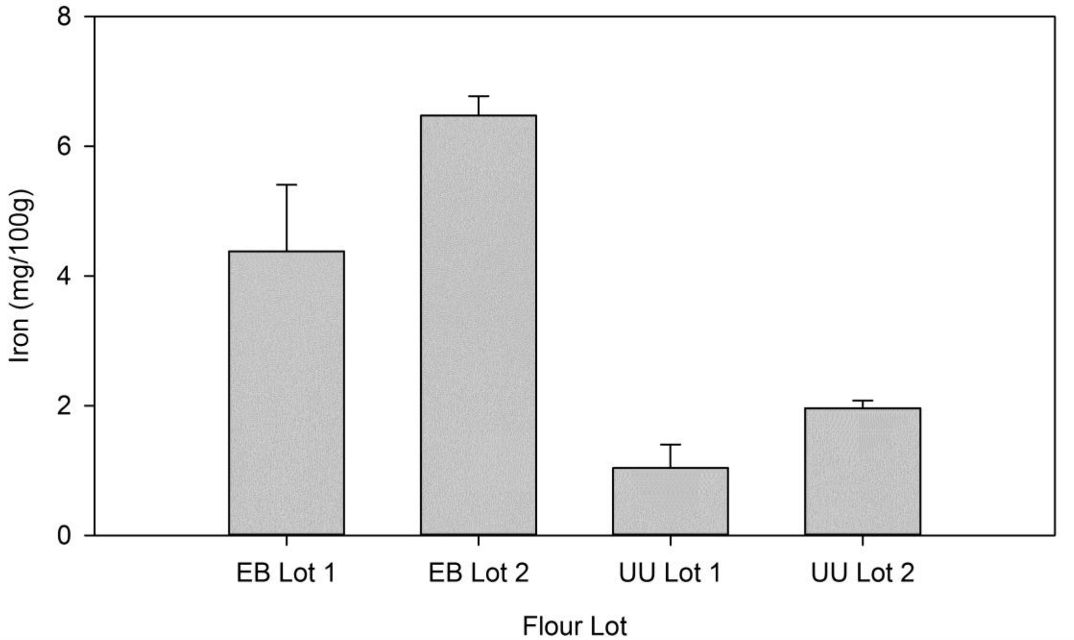

Fig. 19. Mean iron of initial samples (n = 2) in flour lots. EB = enriched, bleached flour and UU = unenriched, unbleached flour. Error bars represent the 95% confidence interval.

Effects of Bleaching and Enrichment on Oxidative Rancidity

The second set of flour samples, consisting of over-enriched, bleached (OEB) flour and under-enriched, unbleached (UEU) flour, showed enrichment levels (as indicated by iron content) higher than corresponding samples in the first set (Fig. 20B). Bleached and unbleached flour was well-distinguished in flour lots based on b* values (Fig. 20A). Thus, this data allows for an analysis which differentiates between the enrichment treatment and the bleaching treatment. Results of the second set of flour are summarized in Fig. 21. Statistical models showed significant differences from both enrichment level and bleaching in the three most abundant volatiles (2-pentyl furan, 1-hexanol, and hexanal).

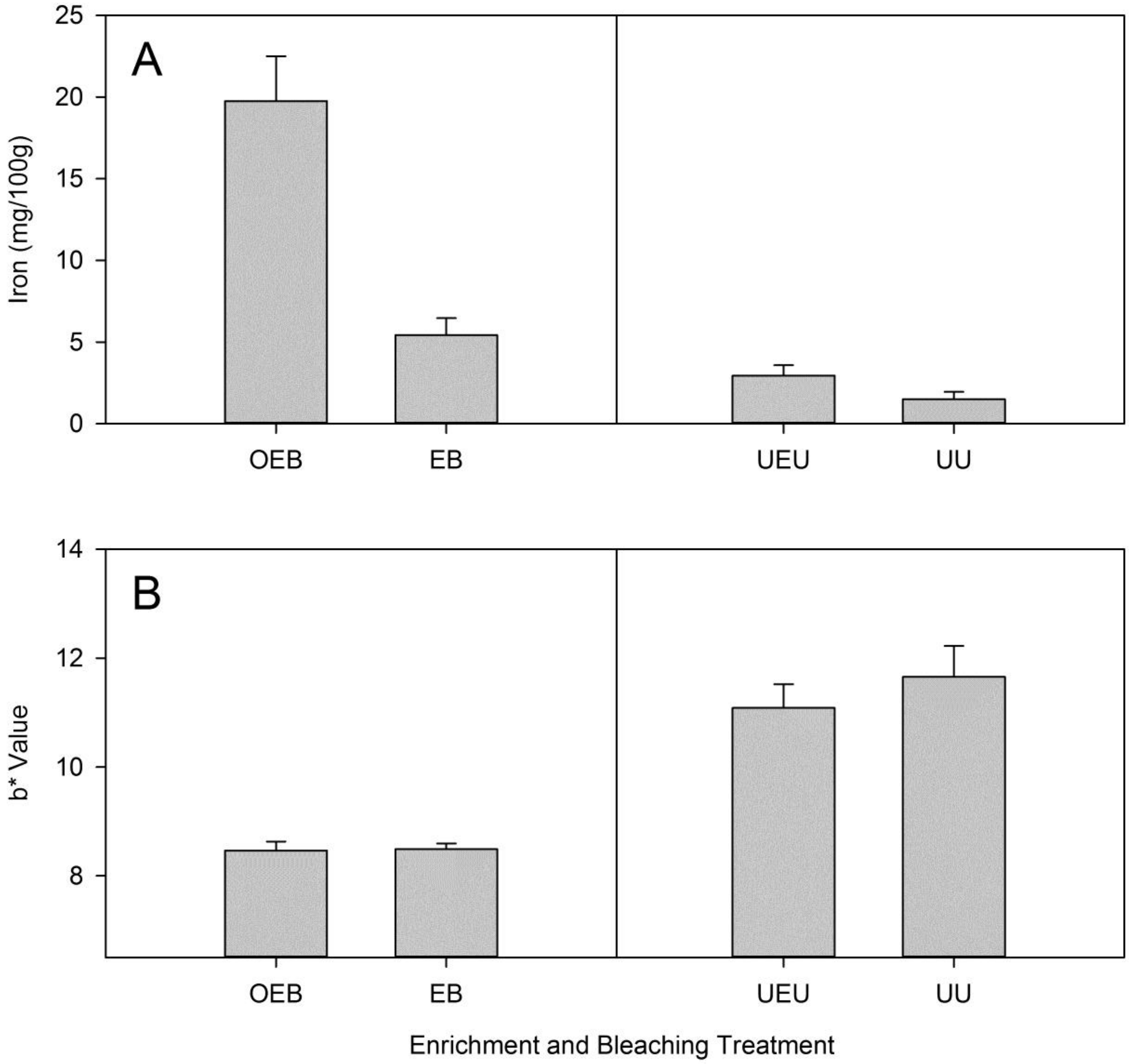

Fig. 20. Iron (A) and b* values (B) of initial over-enriched, bleached (OEB), enriched, bleached (EB), under-enriched, unbleached (UEU), and unenriched, unbleached (UU) flour. Error bars are constructed using the standard error (n = 2).

Bleached samples had a significantly greater amount of headspace volatiles after adjusting for other significant treatment variables including enrichment, time, temperature, and headspace oxygen. For 2-pentyl-furan, flour stored in a normal oxygen atmosphere and flour stored in a low oxygen atmosphere, had 2.3 and 1.5 times greater values, respectively, with bleaching. With bleaching, 2-pentyl furan also had values 1.4, 1.8, and 2.5 times greater in flour stored in 22, 30, and 40°C storage, respectively. There were higher values of 1-hexanol in bleached samples stored at 40 and 30°C (1.6 and 1.5 times greater values, respectively) compared to unbleached samples. Hexanal quantified in flour stored at 22, 30, and 40°C were, respectively, 2.0, 2.1, and 4.1 times greater with bleaching.

Enrichment also had significant effects on volatiles after accounting for other significant treatment variables including bleaching, time, temperature, and headspace oxygen. The volatiles 2-pentyl furan, 1-hexanol, and hexanal had 1.3, 1.7, and 1.5 times greater slopes (over 24 weeks) with increased enrichment (4.4 mg/100g iron). For 1-hexanol, increasing enrichment (4.4 mg/100g iron) gave 1.2 times greater values in flour stored in a normal oxygen atmosphere, but had 86% lower values in flour stored in a low oxygen atmosphere. Hexanal had 1.1 times greater values in flour stored in a normal oxygen atmosphere but had 92% lower values in flour store in a low oxygen atmosphere as the enrichment was increased (4.4 mg/100g iron). Hexanal also had 82%, 86%, and 92% lower values with increasing enrichment (4.4 mg/100g iron) in flour stored at 22°C, 30°C, and 40°C, respectively.

From these results, it appears that both bleaching and enrichment affect the amount of lipid oxidation products and oxidative rancidity. It also appears that bleaching resulted in the greatest increase. However, since flour lots were not randomly assigned to a bleaching and enrichment treatment, cause and effect cannot be inferred. Further research is needed to determine the extent to which bleaching and enrichment cause oxidative rancidity in flour.

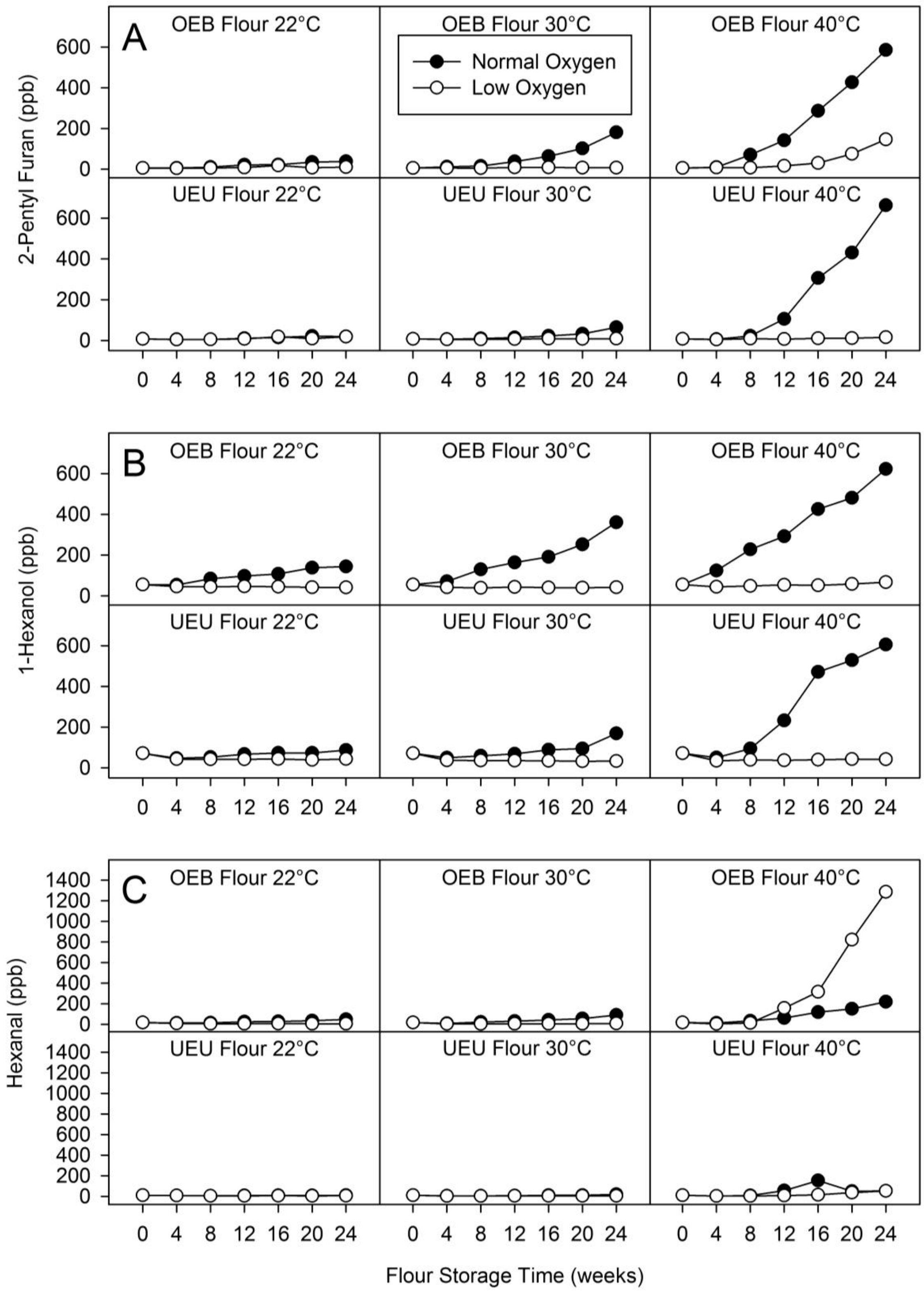

Fig. 21. Effect of a low oxygen atmosphere and enrichment-bleaching on 2-pentyl furan (A), 1-Hexanol (B), and Hexanal (C) in over-enriched, bleached (OEB) and under-enriched, unbleached (UEU) flour stored at 22, 30, and 40°C.

APPENDIX E: BIBLIOGRAPHY

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