5-6-2014

Analysis of Corn Distillers Dried Grains With Solubles (DDGS) / Flour Mixtures, and Subsequent Bread Baking Trials

J. A. Saunders
South Dakota State University

Kurt A. Rosentrater
Iowa State University, karosent@iastate.edu

P. G. Krishnan
South Dakota State University

Analysis of Corn Distillers Dried Grains With Solubles (DDGS) / Flour Mixtures, and Subsequent Bread Baking Trials

Abstract
Grains offer a variety of nutrients; it is thought that through the addition of distillers dried grains with solubles (DDGS) the fiber and protein in baked products may be improved. In this study, all-purpose flour and bread flour were tested with various DDGS substitution levels (0%, 25%, or 50% flour substitution) with the dough conditioner sodium stearoyl lactylate (SSL) (0%, 0.15%, or 0.3% flour weight basis). SSL is surfactant produced from reacting stearic acid with food grade lactic acid in the presence of sodium. Overall, as the substitution level of DDGS increased, so did protein, moisture, ash, and Hunter-a values. Peak height, side height, width, and length of baked loaves decreased as DDGS quantity increased, however. Baked bread containing SSL had enhanced quality, with increasing dough strength, rate of hydration, mixing tolerance, crumb strength, loaf volume, and shelf life. Overall, 25-50% DDGS substitution appeared to have a negative effect on physical features despite the fact that the nutrient content was enhanced. Less than 25% DDGS had minimal negative effects on bread properties.

Keywords
Bread, Baking, Ingredient, Protein, Fiber

Disciplines
Agriculture | Bioresource and Agricultural Engineering

Comments
This article is from *Journal of Food Research* 3 (2014): 78–104, doi:10.5539/jfr.v3n3p78.

Journal of Food Research; Vol. 3, No. 3; 2014
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Analysis of Corn Distillers Dried Grains With Solubles (DDGS) / Flour Mixtures, and Subsequent Bread Baking Trials

J. A. Saunders[1], Kurt A. Rosentrater[2] & P. G. Krishnan[1]

[1] Department of Nutrition, Food Science, and Hospitality, South Dakota State University, USA

[2] Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, IA, USA

Correspondence: Kurt A. Rosentrater, Department of Agricultural and Biosystems Engineering, Iowa State University, 3167 NSRIC Building, Ames, IA, 50011, USA. E-mail: karosent@iastate.edu

Received: August 14, 2013 Accepted: March 23, 2014 Online Published: May 6, 2014

doi:10.5539/jfr.v3n3p78 URL: http://dx.doi.org/10.5539/jfr.v3n3p78

Abstract

Grains offer a variety of nutrients; it is thought that through the addition of distillers dried grains with solubles (DDGS) the fiber and protein in baked products may be improved. In this study, all-purpose flour and bread flour were tested with various DDGS substitution levels (0%, 25%, or 50% flour substitution) with the dough conditioner sodium stearoyl lactylate (SSL) (0%, 0.15%, or 0.3% flour weight basis). SSL is surfactant produced from reacting stearic acid with food grade lactic acid in the presence of sodium. Overall, as the substitution level of DDGS increased, so did protein, moisture, ash, and Hunter-a values. Peak height, side height, width, and length of baked loaves decreased as DDGS quantity increased, however. Baked bread containing SSL had enhanced quality, with increasing dough strength, rate of hydration, mixing tolerance, crumb strength, loaf volume, and shelf life. Overall, 25-50% DDGS substitution appeared to have a negative effect on physical features despite the fact that the nutrient content was enhanced. Less than 25% DDGS had minimal negative effects on bread properties.

Keywords: bread, baking, ingredient, protein, fiber

1. Introduction

Grains such as wheat, corn, rice, and oats have traditionally been major constituents in the human diet. Historically this has been due to intrinsic nutrients as well as functionality. Wheat is very versatile, as it forms a complex known as gluten, which contains 80% - 90% glutenin and gliadin proteins (Lilliard, 2000; Wang et al., 2004). Many factors impact gluten structure and function in food systems. Gluten proteins must be hydrated and then physically manipulated to form both the gluten complex and ultimately the starch-protein matrix. Gluten offers structure to baked products, such as breads, by trapping fermentation gases thus allowing dough to rise. Weak gluten structure can result in excessive expansion and uneven texture, whereas too strong a gluten structure results in decreased expansion and low loaf volume (Schofield & Booth, 1983). Ultimately, protein content and quality impact bread quality and structure.

Not only does the preparation process affect the final product, but ingredients used and flour quality also have effects. To overcome potential variations and improve quality, a variety of dough conditioners, hydrocolloids, and enzyme treatments are often used in products to overcome differences and deficiencies in flour qualities (Azizi & Rao, 2004). These additives have a macroscopic effect on dough by inducing structural changes to the flour (Rosell et al., 2007). Hydrocolloids are water-soluble polysaccharides that provide various functional properties, including gelling, thickening, emulsifying, stabilizing, and foaming; they can also be used for syneresis inhibition, water retention, and improving textural properties. Guar gum is known for enhancing bread loaf volume and texture (Ribotta et al., 2004). Hydroxypropylmethylcellulose (HPMC) creates a softer bread crumb, increased loaf volume, enhanced shelf life, and improved sensory characteristics (Barcenas & Rosell, 2005; Collar et al., 1998; Rosell et al., 2001). Other examples include K-carrageenan, sodium alginate, and xanthan gum, all of which have been found to retard crumb hardening of bread (Guarda et al., 2004). Biochemical mechanisms allow enzymes to favor covalent bonding of proteins, thus improving functional properties of breads (Caballero et al., 2006). A dough conditioner commonly used in industry is sodium stearoyl lactylate (SSL). SSL is a surfactant that is produced by reacting stearic acid (50-90%) with food grade lactic acid in the presence of sodium (Krog & Lauridsen, 1976). Surfactants increase the quality of baked bread by

increasing dough strength, increasing rate of hydration, mixing tolerance, crumb strength, slicing behavior, loaf volume, shelf stability, and reducing shortening requirements (Azizi & Rao, 2004). The USDA has approved the use of SSL at 0.5% (w/w) of flour weight in bread (CFR, 1986).

Since flour quality variations greatly affect end products, it is important to analyze flour properties before use. This analysis can be completed using various processes, equipment, and systems. Alveograph tests determine parameters such as tenacity (*P*, or resistance to extension), dough extensibility (*L*), deformation energy (*W*), curve configuration ratio (*P/L*), and proteolytic degradation. Consistograph tests quantify the behavior of the flour during mixing. Parameters collected can include water absorption, dough development time, tolerance, and decay values (consistency difference between height at peak and its value at a specified level) (Caballero et al., 2006).

Dough can also be analyzed with a variety of instruments and procedures. Extensigraph tests determine a dough's resistance to being extended, which can impact gas retention during fermentation and oven rise. Fermentograph quantifies dough behavior during expansion of gas cells (Hrušková et al., 2006). Mixolab analyzes mixing and pasting properties of flours. The dough is mixed under controlled temperatures until a temperature of 90 °C is reached; this is followed by a cooling step. Torque (expressed in N-m) is measured in real time as it is produced between the dough and kneading arms (Rosell et al., 2007). Another commonly-used test is rapid visco-analysis (Rapid Visco Analyzer) (Shittu et al., 2007; Soulaka & Morrison 1985).

During testing, it is also important to use a consistent loaf preparation method. B. Hansen and A. Hansen (1992) concluded that the use of baking machines in scientific laboratories could result in high repeatability and reproducibility. Although these systems work well, they can be rather fixed and cannot substitute for a baker who can use various baking procedures. Test baking is highly-dependent upon the baker's competence and experience. Baking machines remove inconsistencies that can be found in manual methods. Advantages include rapid and easy implementation, low capital expense, and consistent mixing and baking. Bread baking trials can thus be completed using a consistent formula and procedure.

Once bread loaves are made, quality analyses can be completed by examining loaf weight, loaf volume, specific volume, and loaf height/width ratio of the central slice (Caballero et al., 2006; Shittu et al., 2007; Soulaka & Morrison, 1985). A texture analyzer can be used to quantify characteristics such as compression, penetration, three-point bending, and force-displacement. Other common tests for baked bread include crumb firmness, color indices (colorimeter), crumb moisture, crumb hardness (penetrometer), density, porosity, and softness (AACC, 2000). These tests give researchers quantifiable methods to quantify properties such as crispy, cracky, and hard (Pamies et al., 2000).

The aforementioned methods are essential for assessing the effectiveness of new ingredients. For example, distillers dried grains with solubles (DDGS) is an ingredient which can provide substantial protein and fiber to baked products, but to date has seen limited use in foods. DDGS is a cereal-based ingredient high in fiber but absent of gluten-forming proteins. Over the last few decades, many studies have been completed which have examined flour replacement with DDGS for a variety of food products. Tsen et al. (1982) used corn DDGS at 15% in molasses and spice cookies, and at 25% in chocolate chip cookies. Rasco et al. (1987) used wheat DDGS at 30% in chocolate chip cookies and banana bread. Abbot et al. (1991) used 36% untreated DDGS in oatmeal muffins. Reddy et al. (1986) used distillers dried grains (DDG) at 10% in wheat muffins and 15% in muffins that had blueberries or raisins added; DDG improved physical characteristics and increased consumer acceptance. Brochetti et al. (1991) used 5-10% DDG in bread, which resulted in acceptable sensory attributes; they also incorporated up to 15% DDG in bread, which was also acceptable, with the exception of differences in appearance compared to the control. Brewers' grains, similar to DDGS and DDG, have been successfully substituted at 6% (Finley & Hanamoto, 1980) and 10% (Tsen et al., 1982) into breads.

Depending on DDGS substitution levels, bread fermentation may be hindered due to dilution of gluten, thus altering the quality of final products. A study completed by Aamodt et al. (2005) which investigated bread characteristics as a function of protein quality indicated that flour blends with strengthened protein quality resulted in bread loaves with a larger loaf volume and bread slice area, and formed a higher height to width ratio. Rasco et al. (1990) hypothesized that wheat DDGS may have a reduced negative effect on gluten formation compared to other high-fiber ingredients. Wheat DDGS may have enhanced electrostatic interactions during protein hydration, which could improve gluten functionality. Additionally, high temperature post-fermentation treatments may soften the fiber in the DDGS, allowing faster hydration, which may decrease its inclination to puncture developing air cells.

Manufacturing processes also affect functionality of DDGS. Rasco et al. (1987) investigated drying systems and

found that DDGS produced using harsh heat treatments were less suitable for bakery products than DDGS produced using drum dryers at a lower temperatures. Food matrices using the harsh drying were of significant poorer quality. Temperatures during thermal processes such as extrusion (100-190 °C), flaking (30-150 °C), biscuit or cracker processing (100-140 °C), and commercial baking (190-260 °C) affect the resulting physical and functional properties (Hansen et al., 1975; Donovan, 1977; Li & Lee, 1996). Hydration levels and processing temperatures can determine whether gluten or starch will contribute to bread structure or hinder it. High moisture systems (45-50%) are commonly used for products such as breads, noodles, pasta, and biscuits, and they utilize gluten to provide the majority of the structure (MacRitchie, 1992). Low moisture systems (< 35%), often used for extruded products, generally depend on starch for structure (Chanvrier et al., 2006).

In recent years, the ethanol industry has evolved, and most DDGS is now produced from corn using the dry grind manufacturing process. But, very little research has examined use of this ingredient in human foods. And no studies have yet been conducted to improve DDGS functionality using dough conditioners. Perhaps these can improve DDGS use in bread systems. Therefore, the objectives of this study were to 1) examine the effects of corn-based DDGS on functionality of wheat flour/DDGS blends, 2) examine the impacts of wheat flour/DDGS blends on bread properties, and 3) determine if SSL can improve DDGS functionality and improve breads made with this ingredient.

2. Materials and Methods

2.1 Flour Mixtures

DDGS for this experiment was acquired from a commercial fuel ethanol plant and was milled in a Glen Mills Inc. (Clifton, NJ, USA) mill, providing a mean particle diameter size of 0.384 mm. Using a factorial experimental design (Table 1), all-purpose flour and bread flour were tested with various DDGS substitution levels (0, 25, or 50% flour substitution) and various levels of the dough conditioner sodium stearoyl lactylate (SSL) (0, 0.15, or 0.3% flour weight basis). Independent variables were DDGS replacement (3 levels), SSL inclusion (3 levels), and type of flour (all-purpose vs. bread). Dependent variables included protein (% db, dry basis) using AACC method 46-30 (2000), lipid (% db) using method 920.39 (AOAC, 2003), ash (% db) using method 08-03 (AACC, 2000), and moisture (% wb, wet basis) using method 44-19 (AACC, 2000). All flours were acquired from a local grocery store. Mixtures of the various treatment combinations were stored at 20.5 ± °C until use.

Table 1. Experimental design for flour mixtures used in the study[1]

Treatment	Flour	DDGS Sub. Levels (%)	SSL (%)
1	AP	0	0
2	AP	0	0.3
3	AP	50	0
4	AP	50	0.3
5	B	0	0
6	B	0	0.3
7	B	50	0
8	B	50	0.3
9	AP	25	0.15
10	B	25	0.15

[1]AP indicates all-purpose flour; B indicates bread flour; this design was a 2× 2 × 2 factorial with 2 center points (treatments 9 and 10), thus 10 total treatment combinations. Each treatment was prepared in duplicate.

2.2 Mixolab Analysis

A Mixolab machine (Tripette & Renaud Chopin, Villeneuve La Garenne cedex, France) was used to examine the dough behavior of the flour blends. Manufacturer's instructions were followed in order to complete the Chopin Mixolab dough rheology tests (Anonymous, 2005). Water absorption levels, dough mixing time, dough stability, and mixing tolerance of the flour mixtures were determined from the Mixolab curves as a function of mixing and time. The torque (expressed in Nm) of the dough kneaded between two mixing blades was measured in real time.

Physical parameters of the flour were analyzed using the following Mixolab properties: amplitude (Nm), stability (s), water absorption, α (Nm/min), β (Nm/min), and γ (Nm/min). The Chopin+ protocol's total analysis time was 45 minutes and it utilized an 80-rpm mixing speed, 1.1 Nm target torque (for C1), 30 °C tank temperature, and 75 g dough weight.

2.3 Baking Trials

Two bread machines (OSTER® 2 lb. EXPRESSBAKE, Sunbeam Corporation, 1999) were used for the bread baking experiment to reduce baking differences and decrease error. The machines had the versatility of 1.5 lb or 2 lb loaves, 18 bread settings, 3 crust color selections, delay baker timer, cool touch exterior and Express Bake cycle options (60 min). The baking pan was a non-stick, oval aluminum bread pan with the following dimensions: 18 cm length, 13.6 cm width, and 13.2 cm height. A hook attached to the bottom mixed and kneaded the dough.

Ten flour mixtures were baked (replicated twice), for a total of 20 loaves of bread. For this experiment, the machines were adjusted to the "Basic" bread making setting (1.5 lb loaf), with a medium crust color, which allowed the breads to be baked in 3 hours. The following formulation was used to produce each 1.5 lb loaf: 240 g cold water (17 °C), 20 g vegetable (soybean) oil, 30 g sugar, 10 g salt, 400 g flour mixture, and 8 g quick acting dried yeast. Liquid ingredients were added first, dry ingredients second, and yeast last. Bread machine stages were as follows: 10 min dough was first kneaded, 20 min dough begins to rise, 15 min dough was kneaded a second time, 20 min dough continues to rise, 30 sec dough was "punched down", 55 min dough rises final time, 60 min bread bakes (Sunbeam Corporation, 1999). During the process, the bread loaves with 25% or 50% DDGS replacements were closely monitored to ensure the doughs formed appropriate balls.

Proximate composition of the baked breads included protein (%db) using method 46-30 (AACC, 2000), ash (%db) using method 08-03 (AACC, 2000), and lipid (%db) using method 920.39 (AOAC, 2003). Physical properties of the bread included side height (height of sides of loaves until the top starts to mound, cm), peak height (height of loaves to the top of the mound (i.e. at center), cm), width (cm), length (cm), mass (g). Moisture (%wb) was determined using method 44-19 (AACC, 2000). Water activity was determined using a water activity meter (AW Sprint). Strength (MPa) and stiffness (MPa) were determined using an Instron testing machine. Hunter L, a, and b values, for both internal crumb and exterior crust color, were measured with a Hunter spectrocolorimeter (LabScan XE, Hunter Associates, Reston, VA, USA).

Method 10-90 (AACC, 2000) was used to subjectively examine bread quality, and included uniformity, size, thickness of cell walls, grain, moistness, tenderness, softness, and crumb color.

2.4 Statistical Analysis

The completely randomized, factorial experimental design was a 2 × 2 × 2 with additional 2 center points for a total of 10 treatment combinations replicated three times to total 30 runs (Table 1). Statistical analyses on all collected data were performed via SAS v.8.0 (SAS Institute, Cary, NC) and Microsoft Excel v.2003 (Microsoft Corp., Redmond, WA) software, using a Type I error rate (α) of 0.05, and included summary statistics, general linear models to test for differences between experimental treatments (i.e., main, interaction, and treatment effects), and linear correlations.

3. Results and Discussion

3.1 Flour Mixtures

3.1.1 Chemical Properties

Table 2 shows the main effects on proximate composition of all ten flour mixtures. The quantity of DDGS replacement resulted in significant differences in protein, lipid, ash and moisture. The percentage of sodium stearyl lactylate (SSL) included resulted in significant differences for protein, lipid, and moisture. Finally, the type of flour used resulted in significant differences in protein, ash, and moisture. Treatment combination effects were significant as well (Figure 1).

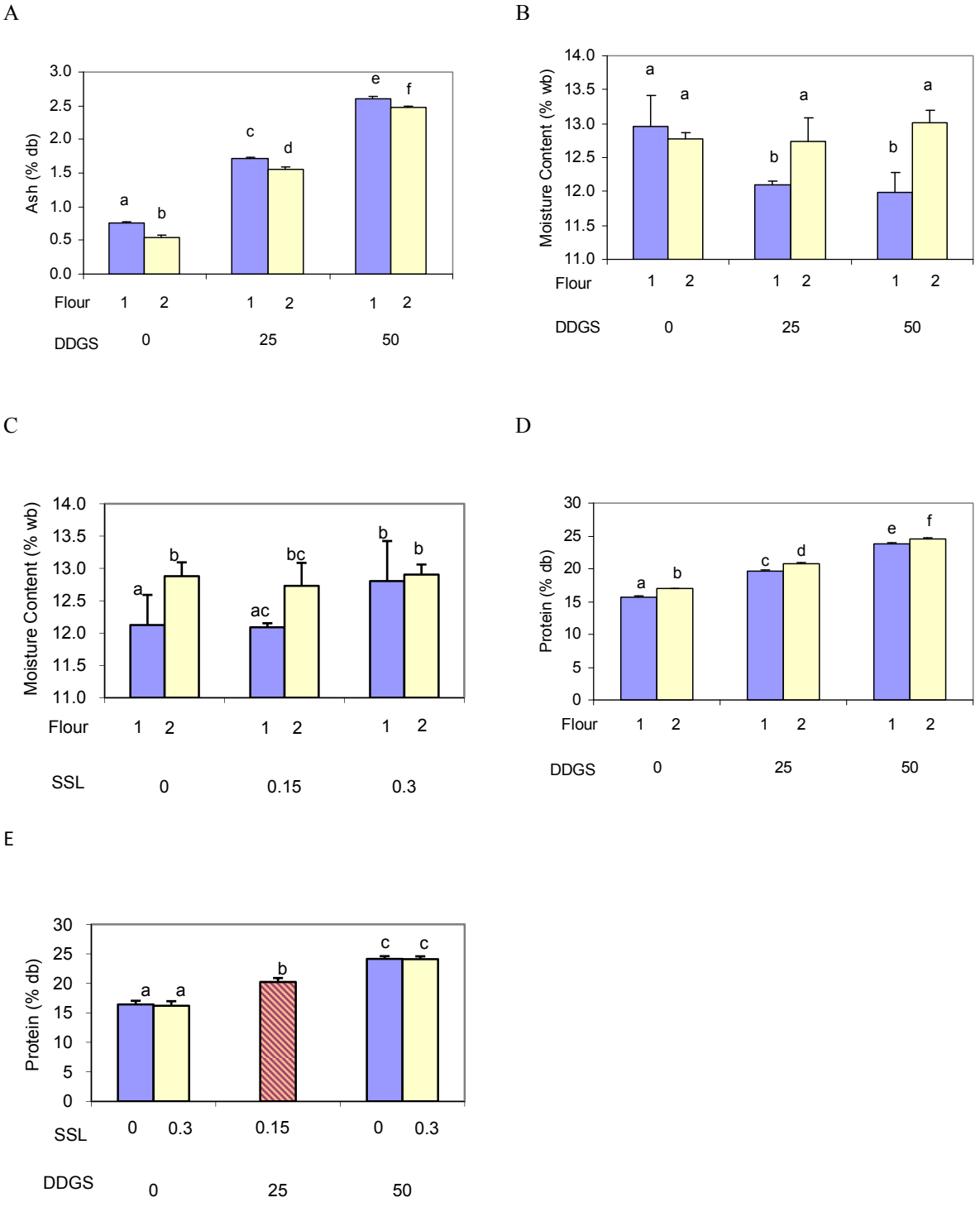

Figure 1. Treatment combination effects on ash, moisture, and protein due to flour type (1 is all-purpose flour, 2 is bread flour), dough conditioner levels, and DDGS levels. (A); between moisture content, flour, and DDGS levels (B); between moisture content, flour, and SSL levels (C); between protein, flour, and DDGS levels (D); and between protein, SSL, and DDGS levels (E). Overall, as DDGS levels increase the quantity of protein and ash increase while moisture levels decrease. Error bars represent ± 1 standard deviation

Table 2. Main effects for proximate composition of flour mixtures[1]

	Protein (% db)		Lipid (% db)		Ash (% db)		Moisture (% wb)	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
DDGS (%)								
0	16.31a	0.7	1.39a	0.23	0.66a	0.11	12.86a	0.33
25	20.22b	0.68	4.10b	0.31	1.63b	0.1	12.41b	0.42
50	24.15c	0.46	7.10c	0.35	2.54c	0.07	12.49b	0.59
SSL (%)								
0	20.27a	4.11	4.12a	3.01	1.6	0.98	12.50a	0.53
0.15	20.22ab	0.68	4.10a	0.31	1.63	0.1	12.41a	0.42
0.3	20.21b	4.25	4.39b	2.99	1.6	1	12.86b	0.43
Flour								
All-purpose	19.63a	3.76	4.33	2.71	1.69a	0.85	12.39a	0.58
Bread	20.84b	3.57	4.12	2.61	1.53b	0.9	12.86b	0.22

[1] for a given main effect, differing letters between levels for a given property signify significant differences ($p < 0.05$, LSD); St. Dev. is +/- 1 standard deviation.

There was a statistically significant increasing trend in ash content as the percentage of DDGS substitution increased. Tsen et al. (1983) also found that the addition of DDG into flour formulations increased ash content compared to white flour. Ash content is heavily impacted by environmental factors such as the fertility of the soil and genetics of the plant (Fennema, 1996). Other constituents may include quantity of sunlight received, access to water, and stage of growth during harvest. Flour mixtures that had 0% DDGS had a mean ash content of 0.66%, 25% DDGS mean ash content of 1.63%, and 50% DDGS had a mean ash content of 2.54%. This was reasonable, as the addition of DDGS, a fibrous material, to flour resulted in more nutritive and non-nutritive residues left after ashing.

As the quantity of DDGS increased, moisture levels decreased. In fact, the initial moisture content of DDGS (12.9%) had a higher percentage of water compared to the all-purpose (7.9%) or bread flours (7.7%). Therefore, it may be logical to assume that as the percentage of DDGS (which contained greater initial moisture), replaced more all-purpose or bread flour (which were drier than the DDGS), the flour mixture moisture content would increase. DDGS also contains a high level of fiber, which can readily absorb free water. Dreese and Hoseney (1982) concluded that products high in fiber also had increased quantity of water absorption. Therefore, in order to achieve an ideal product, the quantity of liquid included in the formulation may need to be increased. Once the fiber molecules are softened, the fiber should incorporate more easily into the dough system. Fiber plays many roles in food systems, such as providing structure and bulk, modification of rheological properties, as well as other functions (Fennema, 1996). Several studies have advocated the idea that DDGS would provide an excellent fiber supplement for baked products (Brochetti et al., 1991; Waelti & Ebeling, 1982; Wu et al., 1984; Rasco et al., 1987).

The quantity of moisture slightly increased as the SSL in bread flour mixtures increased. SSL is a food additive, which is categorized as a surfactant. It is amphiphilic, and exhibits both hydrophilic and hydrophobic properties. Its major function is to reduce interfacial tension between two fluids. Other functional properties of surfactants include emulsifying, foaming, solubilizing, wetting, and dispersing (Lilliard, 2000). The bakery industry uses surfactants to improve dough-mixing qualities and to enhance loaf volume. Azizi and Rao (2004) completed a study that analyzed the combination of surfactant gels and gums on dough and bread systems. Farinograph results revealed that surfactant gels actually decrease a flours ability to absorb water by 0.4-1.2%. Also, alveograph characteristics showed a 0.4-1.6 mL increase in the swelling index of dough's using surfactants. Less variation in moisture content was shown by all-purpose flour mixtures and likely due to a decrease in water absorption. Moisture content increased with increased SSL levels. The bread flours slightly increased swelling ability may explain this slight increase in moisture content. Perhaps additional water was captured into the food matrix and was not able to be dissipated as steam during baking. For the most part, the function of the surfactants is to reduce interfacial tension in food systems, decreasing the amount of water that flour can absorb. The protein

content increased as the quantity of DDGS included increased. Reddy et al. (1986) observed an increase in amino acid content of muffins containing DDGS. When 10, 15, or 20% of DDGS was added to the muffins the following amino acid contents greatly increased: threonine, serine, glutamic acid, alanine, methionine, leucine, and histidine. Of these, threonine, methionine, leucine, and histidine are essential amino acids, which cannot be made in the body and must be obtained through the diet (Wardlaw & Kessel, 2002).

The addition of proteins to a food system offers additional functionality such as dispersibility, swelling, water-holding capacity, gelation, and viscosity (Fennema, 1996). DDGS contains a fairly high amount of protein; therefore, higher percentages of replacement should result in increased protein quantities. Similarly, protein content increased as the quantity of SSL increased. Perhaps SSL alters the mechanism in which molecules interact, and the process by which chemical or physical reactions occur. For example, maybe the reduced interfacial surface tension allowed more proteins to adsorb or condense onto the surface of the flour (Fennema, 1996).

3.1.2 Mixolab

The Mixolab instrument has capabilities to measure physical properties of dough such as stability, strength, and pasting. In this study, dough was subjected to dual mixing of two kneading arms with an applied temperature constraint. All torque measurements were completed in real time (Kahraman et al., 2008). The Mixolab curve is typically divided into five main parameters (Figure 2): development (C1), protein reduction (C2), starch gelatinization (C3), amylase activity (C4), and starch gelling (C5).

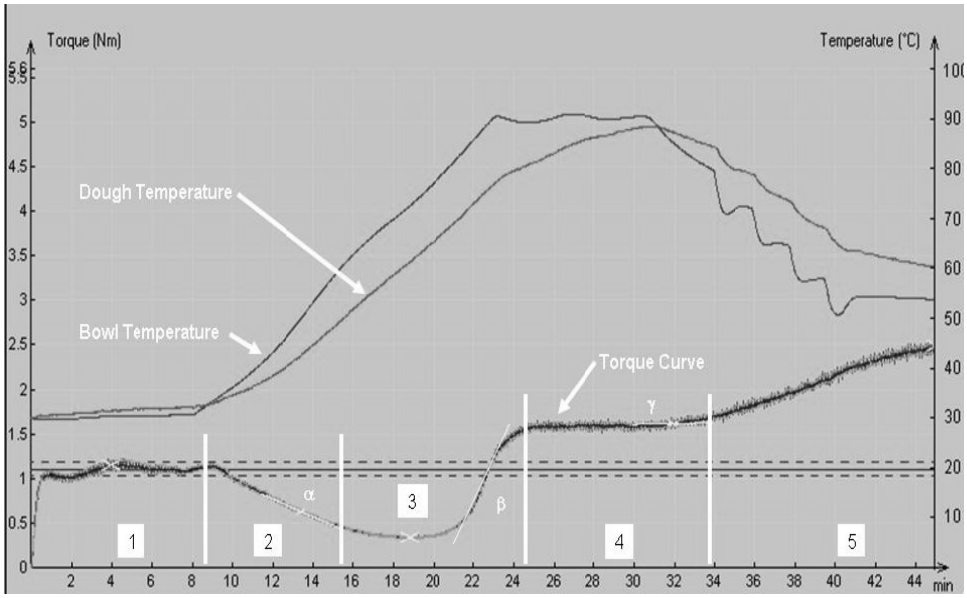

Figure 2. Typical output produced when running a Mixolab test (for Treatment 10). The primary output includes temperature curves for the bowl and the dough, as well as reporting torque exerted on the rotary blade in the instrument. The main phases of the resulting Mixolab torque curve are denoted on 1 through 5

Development, stage C1, occurs when maximum torque is reached or time elapsed during heating results in torque remaining at 1.1 N-m (Collar et al., 2007). Flour water absorption capacities are determined while at a constant temperature. Properties such as stability, elasticity, and water absorption are also measured. Torque increases until a maximum value is reached, which indicates that the dough is capable of resisting deformation for a period of time (Kahraman et al., 2008). Mixing conditions that are excessive can cause dough properties to change from smooth and elastic to slack and sticky (Rosell et al., 2007).

In stage C2, protein reduction occurs and the minimum torque produced is subjected to mechanical stress and thermal constraints (Collar et al., 2007). Protein weakening occurs when the dough is over-manipulated. Depending on flour composition, lower protein qualities are signified by greater decreases in consistency. Another decrease in consistency can be viewed on the Mixolab curve, in section C2, when temperatures begin to increase. After the initial decrease from rising temperatures, dough consistency will then begin to increase

(Kahraman et al., 2008).

However, this eventual increase in consistency is mostly contributed to the beginning of stage C3, starch gelatinization. The quality of starch determines the rise in consistency. Starch granules absorb water and swell, while viscosity increases due to leaching of amylose (Kahraman et al., 2008). Starch gelatinization results in peak torque during the heating stages, comprising initial and final pasting (Collar et al., 2007). Gelatinization occurs when amylose and/or amylopectin (insoluble in cold water) molecules are suspended in water, producing a starch slurry. Once heating begins, water becomes absorbed and granules hydrate. Continued heating weakens hydrogen bonds as molecules swell resulting in irreversible changes to the starch structure and eventually some granules will burst (White & Johnson, 2003).

In stage C4, endogenous or amylastic activity results in minimum torque during the cooling stage when reaching stability (Collar et al., 2007). This activity ultimately determines how large the decrease in consistency will be. Larger decreases are proportional to a greater quantity of amylastic activity (Anonymous, 2005; Kahraman et al., 2008). Two types of amylases include α-amylase and β-amylase. Alpha amylase hydrolyzes interior α-1,4-glucosidic bonds of starch, glycogen, and cyclodextrins. These enzymes are *endo*-splitting which acts to increase viscosity. Beta-amylase hydrolyze the α-1,4-glucosidic bonds of starch beginning at the non-reducing end to result in β-maltose. Since they are *exo*-splitting, many bonds need to be hydrolyzed before a significant impact on viscosity can be seen (Fennema, 1996).

Finally, stage C5, gel formation, which is related to retrogradation, causes an increase in consistency among the dough as the temperature decreases for the cooling stage (Kahraman et al., 2008). This cooling stage allows the starch to retreat and thus increase product consistency. Staling may be delayed through the addition of certain chemicals that enhance product flexibility (Anonymous, 2005). Retrogradation occurs after maximum viscosity is reached, in which some granules have broken or burst. Upon cooling of the starch solution, some starch granules will partially reassociate to form a gel. The retrogradation of amylopectin requires more time than amylose, giving it the distinction for causing part of staling (Fennema, 1996).

The Mixolab collects additional properties, comparable to the Farinograph (Brabender Instruments, New Jersey). A Farinograph measures flour and dough indices from the first 8 min of a sample run, whereas the Mixolab further analyzes flour's performance throughout the entire bread making process, including the heating and cooling phases. These extra phases have the potential to tie research and industry together, as the curve can relate differences between flours and traditional industrial baking conditions. The Mixolab instrument aids in explaining baking performance differences due to starch-protein interactions, enzyme activity, gelatinization, gelling of starch, and environmental factors (Saunders et al., 2007). A study completed by Kahraman et al. (2008) tested the possibility of using the Mixolab versus Zeleny sedimentation or Alveoconsistograph values to predict cake-baking quality of various flours. Results showed that Mixolab analysis proved to be a useful tool whereas the Alveoconsistograph results and cake characteristics were not significant. Overall, the Mixolab technique has been viewed as comparable to classical instruments such as the Farinograph, Mixograph, Extensograph, or Alveograph (Bloskma & Bushuk, 1988; Chiotelli et al., 2004).

Table 3 illustrates the main effects for the Mixolab analysis of the flour mixtures. The percentage of DDGS substitution resulted in significant differences among amplitude, stability, water absorption, protein breakdown (α), and cooking stability rate (γ). The percentage of SSL resulted in significant differences among water absorption, α, and gelatinization (β). Finally, the type of flour included in the flour mixtures resulted in significant differences among stability, water absorption and γ.

Treatment combinations were also significant (Figure 3). As the percentage of DDGS increased, the stability of the dough decreased. Since DDGS material contains no gluten forming proteins, it makes sense that the dough system would have less stability. A slight increasing trend showed that as the quantity of DDGS increased, water absorption also increased. An increased amount of water was absorbed due to the increased levels of fiber provided from the DDGS. Fiber required additional water in order to soften and to be incorporated into a dough ball. Also, as the quantity of DDGS increased, so did γ values, except for the 0.15% whose values were not available through data analysis. γ values are indicative of the rate in which a dough system reaches cooking stability. As DDGS increased, it took less time to stabilize the cooking process, which caused γ rate values to increase. This might influence the acceptance of DDGS in industry, as less time is required to reach stability

Collar et al. (2007) defined α, β and γ on a Mixolab curve as ascending and descending curves to be protein breakdown, gelatinization, and cooking stability rate, respectively. Table 3 illustrates these values found in the treatments, and Figure 4 shows respective Mixolab curves. The bowl temperature was greater than dough temperature because a heating element was applied to the mixing bowl, which simulates the baking process, thus

the bowl temperature was always higher than the dough temperature.

α values that are higher are indicative of protein breakdown, or weakening of the flour mixture. C2 curves that decrease less could be good indicators of protein quality. As the quantity of DDGS increased, the slope at which protein breakdown occurred also increased by the α values becoming more negative. Even though gluten forming proteins decreased as DDGS increased, the DDGS significantly adds other proteins to the flour mixture, thus there is more protein in the system to potentially breakdown. The bread flour mixtures had a higher initial protein content than the all-purpose mixtures, thus showing slightly increased protein weakening. Bread flour curves demonstrate a slightly lower decreased slope and curve than all-purpose flour.

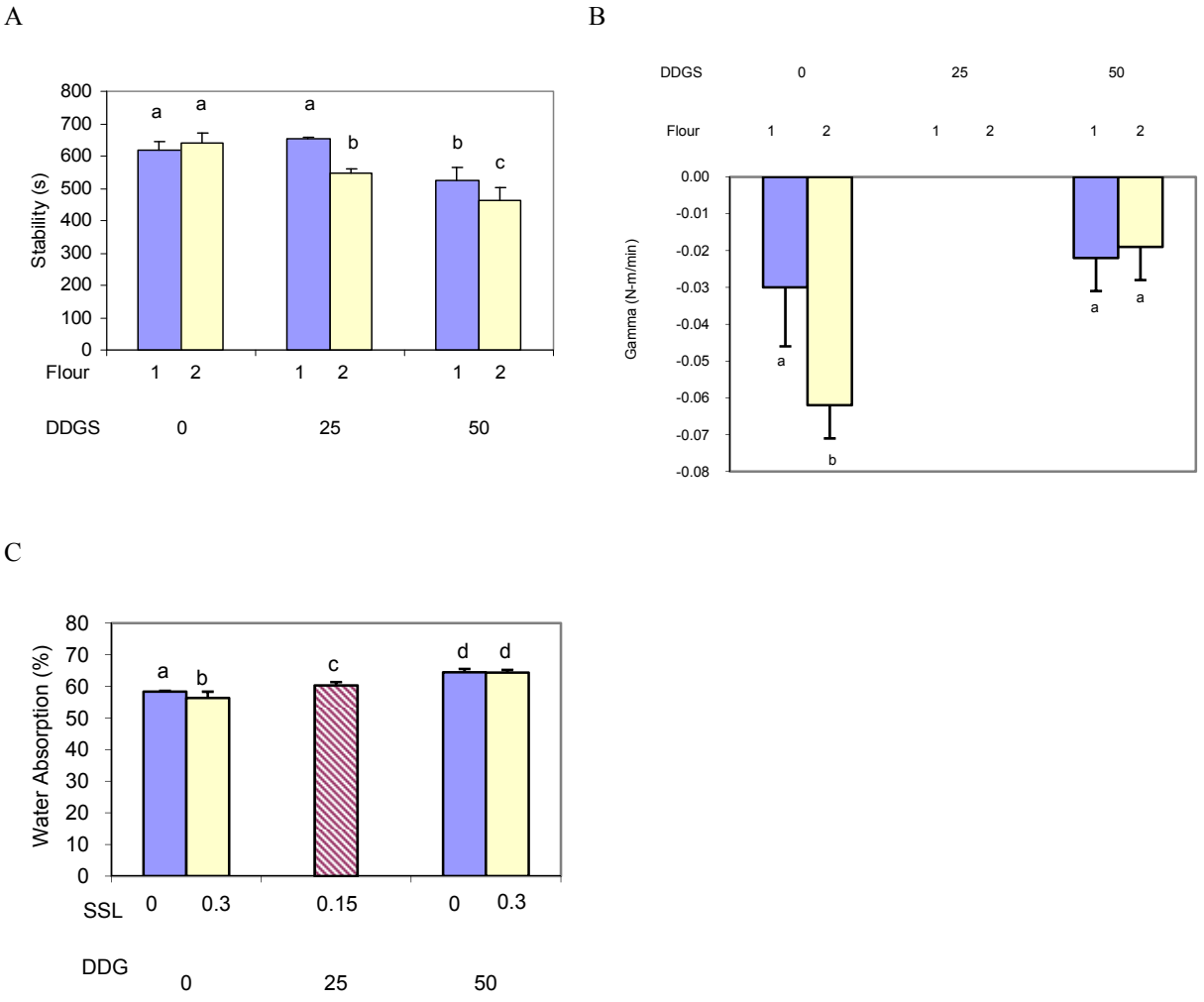

Figure 3. Treatment combination effects on stability, gamma, and water absorption between due to flour (1=all-purpose & 2=bread) and DDGS levels (A); between γ, flour, and DDGS levels (B); between water absorption, SSL, and DDGS levels (C). γ values were not produced for the center points (25% DDGS) during the Mixolab analysis. Overall, as the quantity of DDGS increases water absorption and γ values increase while time to reach stability decreases. Error bars represent ± 1 standard deviation

Table 3. Main effects for Mixolab operational parameters[1]

	Amplitude (Nm)		Stability (s)		Water Absorption (%)	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
DDGS (%)						
0	0.09a	0.02	629.08a	30.48	57.33a	1.73
25	0.11b	0.01	601.33a	58.52	60.33b	1.06
50	0.16c	0.02	494.67b	48.92	64.43c	0.92
SSL (%)						
0	0.13	0.05	570.42	73.13	61.41a	3.30
0.15	0.11	0.01	601.33	58.52	60.33b	1.06
0.3	0.13	0.04	553.33	87.58	60.36b	4.46
Flour						
All-purpose	0.12	0.03	588.67a	60.93	60.29a	3.89
Bread	0.12	0.05	550.87b	87.36	61.26b	3.09

	α (N-m/min)		β (N-m/min)		γ (N-m/min)	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
DDGS (%)						
0	-0.07a	0.04	0.38	0.17	0.05a	0.02
25	-0.12a	0.01	0.02	0.01	N/A	N/A
50	-0.08a	0.04	0.43	0.06	0.02b	0.01
SSL (%)						
0	-0.10a	0.03	0.44a	0.05	-0.03	0.02
0.15	-0.12a	0.01	0.02b	0.01	N/A	N/A
0.3	-0.06b	0.04	0.36c	0.16	-0.03	0.02
Flour						
All-purpose	-0.09	0.04	0.29	0.20	0.03a	0.01
Bread	-0.08	0.04	0.35	0.18	0.04b	0.02

[1] for a given main effect, differing letters between levels for a given property signify significant differences ($p < 0.05$, LSD); St. Dev. is +/- 1 standard deviation.

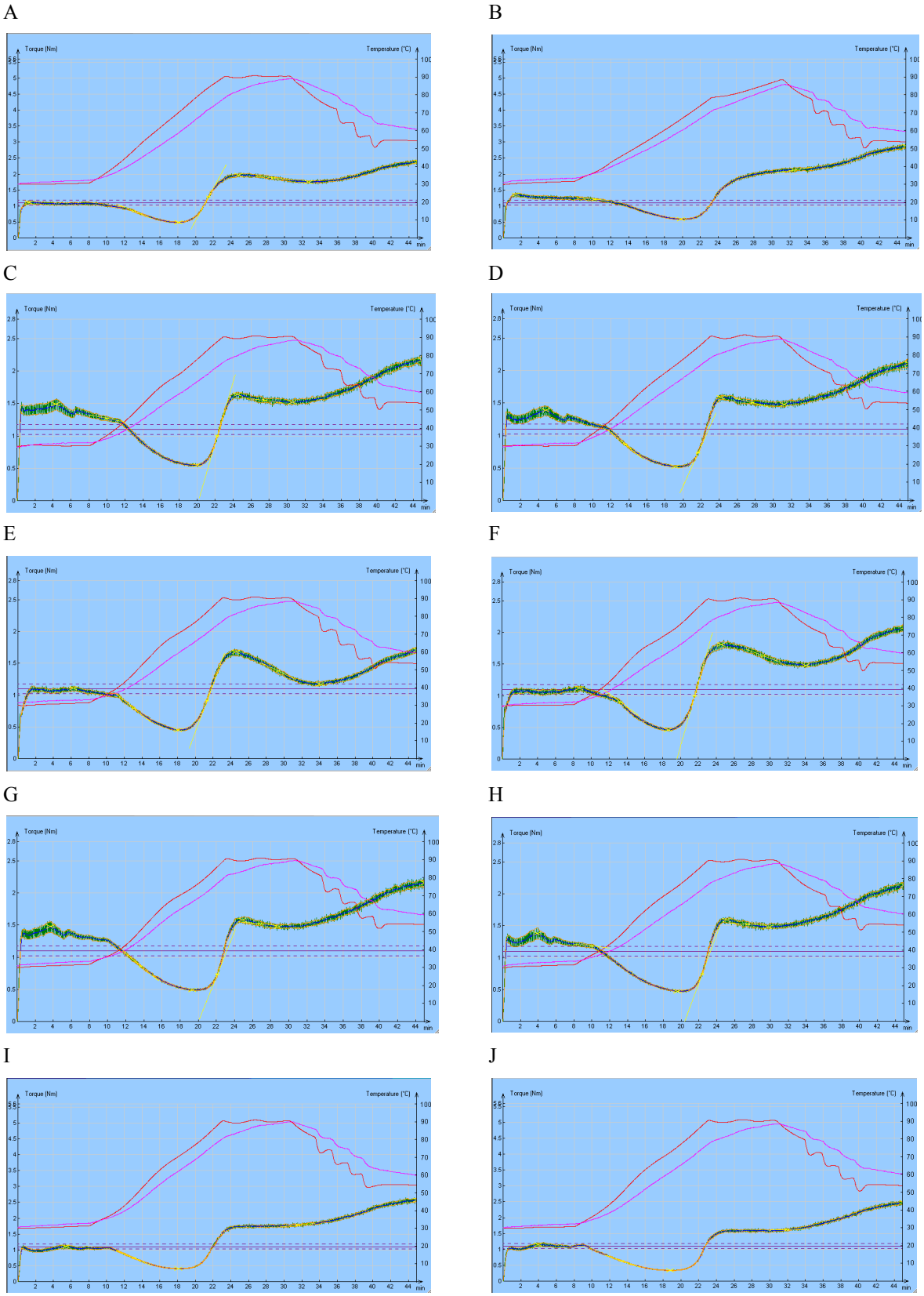

Figure 4. Mixolab curves for treatments 1-10. A) 100% all-purpose flour (Trt. 1); B) All-purpose flour and 0.3% SSL (Trt. 2); C) All-purpose flour and 50% DDGS (Trt. 3); D) All-purpose flour, 50% DDGS, and 0.3% SSL (Trt. 4); E) 100% bread flour (Trt. 5); F) Bread flour and 0.3% SSL (Trt. 6); G) Bread flour and 50% DDGS (Trt. 7); H) Bread flour, 50% DDGS, and 0.3% SSL (Trt. 8); I) All-purpose flour, 25% DDGS, and 0.15% SSL (Trt. 9); J) Bread flour, 25% DDGS, and 0.15% SSL (Trt. 10)

Positive β values are good indicators of starch gelatinization. However, if the β values become too high it is possible that the starch slurry may become over-gelatinized. Overcooked starch granules tend to swell and burst resulting in a complete loss of crystallinity and birefringence. Such starch molecules are increasingly susceptible to hydrolysis via enzymes (Nielsen, 2003). On the other hand, lower β values indicate little to no starch gelatinization or slower rate. This would then indicate that the flour does not contain enough reducing sugars. Not all flour mixtures will complete sufficient starch gelatinization to become viscous. Even though they may not be used in some baked products, industry can find other uses for thin starch slurries (e.g., in pie fillings). Figure 4 shows that as the percentage of DDGS increased, the quantity of starch gelatinization decreased. DDGS is low in sugar and starch, as these were used for ethanol production. DDGS is low in reducing sugars necessary for starch gelatinization. γ values indicate the slope and the rate that cooking stability is reached. As the quantity of DDGS increased, γ values decreased, indicating less time needed to reach cooking stability. This may be an industrial advantage, in that less time needed to reach cooking stability could be favorable in a bakery setting.

3.2 Baking Analysis

3.2.1 Chemical Properties

Table 4 showed the main effects of the independent variables on the proximate composition of the bread loaves. As the percentage of DDGS replacement increased, significant differences were found among protein, ash, and lipid values. A significant difference was also found between type of flour and protein. As DDGS substitution increased, so did protein content. This reaffirms the study completed by Reddy et al. (1986), which showed an increase in protein content of muffins as DDGS substitution increased. The addition of proteins may have strengthened the food system by offering extra proteins to promote functional tasks such dispersibility, swelling, water-holding capacity, gelation, and viscosity (Fennema, 1996).

3.2.2 Physical Properties

Table 5 shows the main effects of the independent variables on the physical properties of the bread loaves. As the quantity of DDGS increased, significant differences could be found in strength, water activity, a-crust, b-crust, L-crumb, a-crumb, b-crumb, side height, peak height, width, length, and mass. As the percentage of SSL increased, significant interactions between strength, a-crust, L-crumb, a-crumb, peak height, width, and mass were found. Finally, the type of flour showed significant differences among strength, water activity, a-crumb, side height, peak height, and length.

Treatment combination effects were also significant (Figure 5). Hunter a crust values decreased as the quantity of DDGS and SSL increased. As the quantity of red pigments decrease, the product becomes more brown. As the level of DDGS increases, it appears that the Maillard reactions and carmelization during baking are factors that may contribute to browning. Hunter a values decreased as did Hunter L values for the crust color, also indicating the presence of Maillard browning. SSL may also contribute to the decrease in Hunter a values as the dough conditioner may alter the way molecules interact during baking. Hunter a crumb values significantly increased from 0% to 25% DDGS, which indicated more red pigments. All in all, DDGS has more initial red pigments than found in all-purpose or bread flour. Therefore, as the level of DDGS increased, the Hunter a value of the interior crumb should become redder as well. These values did not decrease as the Hunter crust values do, because the interior crumb was protected and encountered a decreased amount of Maillard browning compared to the crust. Rasco et al. (1990) determined how the addition of a variety of distillers grain products from wheat and barley would affect mixing and baking properties of breads and cookies. Breads included 4 or 8% of various types of distillers grains, while the cookies substituted 2, 4, or 8%. Color analysis of bread loaves crust showed that almost all loaves had decreased Hunter a values. Loaves were found to become greener as the level of DDGS substitution increased. Color analysis of interior structure of these loaves revealed that all loaves exhibited increased Hunter a values. The interior structure became notably darker and redder as the level of DDGS increased. These trends found by Rasco et al. (1990) parallel the trends found in our study.

An increase in dark pigments (due to the addition of DDGS) has also been found in a study completed by Brochetti et al. (1991). Breadcrumb color darkened and Hunter L values decreased as DDG level increased. Our breadcrumb and crust Hunter L values also showed a decreasing trend as DDGS substitution increased. Hunter L values are indicative of product brightness, as a value of 0 indicates black and 100 indicates white. The DDGS added was “golden yellow” from dark pigments mostly located in the corn lipids. Darker colors may also have been due to heat (during drying), which caused undesirable Maillard browning. This type of browning is a chemical reaction between reducing sugars (i.e. D-glucose) and a free amino acid or amino group (Fennema, 1996).

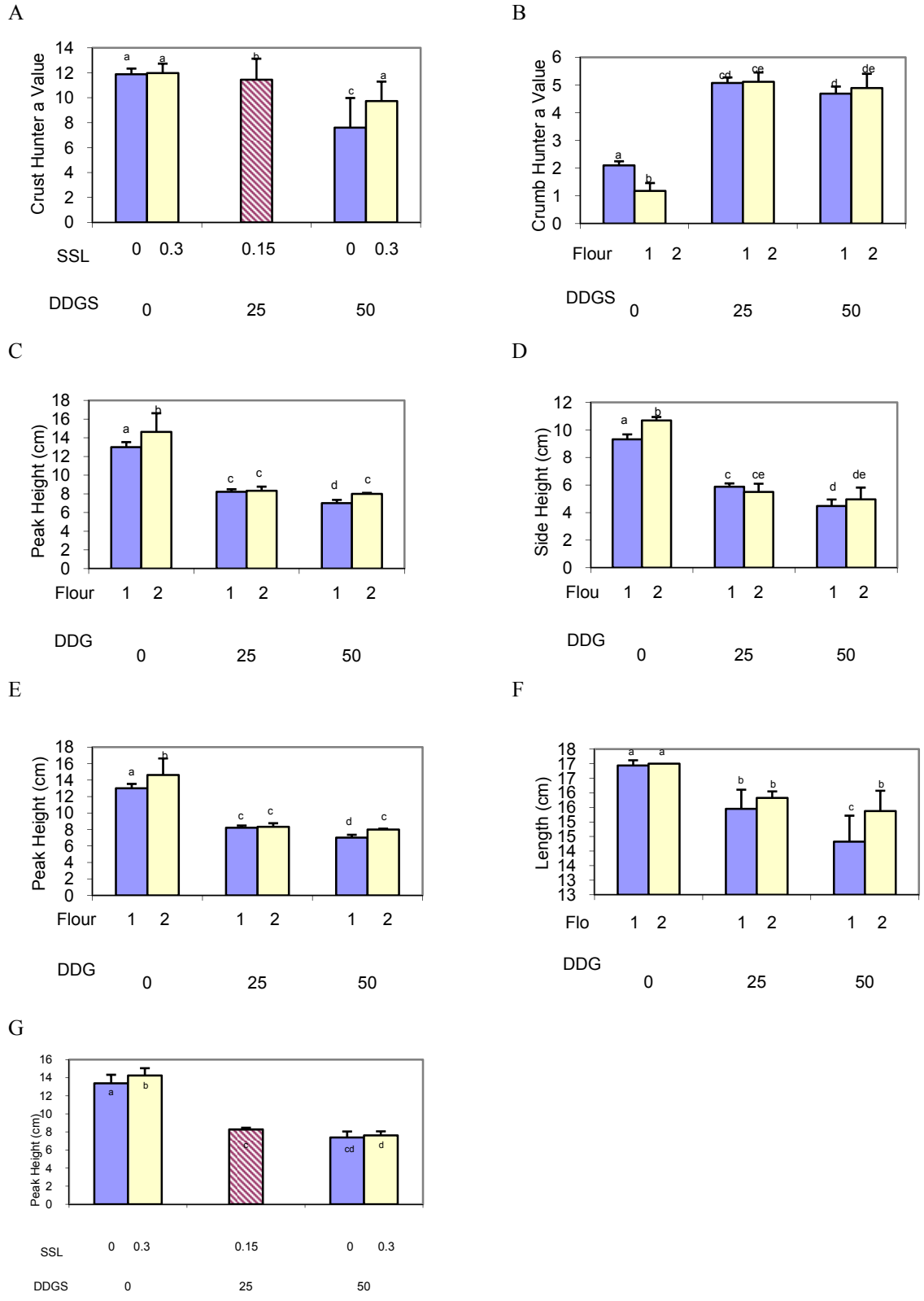

Figure 5. Treatment combination effects on physical properties due to flour type, SSL, and DDGS levels (A); between crumb Hunter a values, flour (1=all-purpose & 2=bread), and DDGS levels (B); between peak height, Flour, and DDGS levels (C); between side height, flour, and DDGS levels (D); between width, SSL, and DDGS levels (E); between length, flour, and DDGS levels (F); and between peak height, SSL, and DDGS levels (G). Overall, as DDGS levels increase red pigments in the crumb also increase while peak height, side height, and length decrease. As SSL increases red pigments in the crust, width, and peak height decrease. Error bars represent ± 1 standard deviation

Table 4. Main effects for proximate composition of prepared breads[1]

	Protein (% db)		Ash (% db)		Lipid (% db)	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
DDGS (%)						
0	13.28a	0.74	3.09a	0.08	8.05a	0.56
25	16.48b	0.40	3.95b	0.06	10.20b	0.71
50	19.58c	0.52	4.78c	0.10	13.26c	0.46
SSL (%)						
0	16.53	3.53	3.95	0.94	10.50	3.05
0.15	16.48	0.40	3.95	0.06	10.20	0.71
0.3	16.33	3.32	3.91	0.87	10.81	2.58
Flour						
All-purpose	15.95a	3.08	3.96	0.76	10.59	2.41
Bread	16.92b	1.31	3.91	0.84	10.54	2.63

[1] for a given main effect, differing letters between levels for a given property signify significant differences ($p < 0.05$, LSD); St. Dev. is +/- 1 standard deviation.

Table 5. Main effects for physical properties of prepared breads[1]

	Strength (MPa)		Stiffness (MPa)		Moisture (% wb)		Water Activity	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
DDGS (%)								
0	0.004a	0.001	0.04	0.03	32.66	2.80	0.870a	0.023
25	0.005b	0.000	0.07	0.03	35.88	2.64	0.871a	0.012
50	0.005b	0.000	0.17	0.24	35.98	3.21	0.855b	0.017
SSL (%)								
0	0.005a	0.000	0.06	0.03	34.19	3.72	0.868	0.021
0.15	0.005a	0.000	0.07	0.03	35.88	2.64	0.871	0.014
0.3	0.004b	0.001	0.15	0.25	34.45	3.18	0.858	0.022
Flour								
All-purpose	0.005a	0.000	0.08	0.02	34.85	3.76	0.873a	0.019
Bread	0.004b	0.001	0.12	0.23	34.42	2.85	0.856b	0.018

[1] for a given main effect, differing letters between levels for a given property signify significant differences ($p < 0.05$, LSD); St. Dev. is +/- 1 standard deviation.

Table 5. Main effects for physical properties of prepared breads[1] (Cont.)

	L-Crust		a-Crust		b-Crust		L-Crumb	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
DDGS (%)								
0	35.86	2.32	11.93a	0.61	14.06a	1.34	69.27a	2.35
25	33.18	5.44	11.44a	1.56	13.48a	3.78	53.71b	1.25
50	30.77	5.49	8.66b	2.27	11.21b	3.83	55.58b	4.77
SSL (%)								
0	33.56	5.24	9.74a	2.51	12.32	3.14	61.16a	8.39
0.15	33.18	5.44	11.44b	1.56	13.48	3.78	53.71b	1.25
0.3	33.07	4.67	10.85b	2.06	12.95	3.28	63.69c	7.37
Flour								
All-purpose	32.63	5.91	10.26	2.85	12.13	4.20	60.12	6.46
Bread	33.95	3.75	10.79	1.39	13.48	1.82	61.24	9.22

[1] for a given main effect, differing letters between levels for a given property signify significant differences ($p < 0.05$, LSD); St. Dev. is +/- 1 standard deviation.

Table 5. Main effects for physical properties of prepared breads[1] (Cont.)

	a-Crumb		b-Crumb		Side Ht. (cm)		Peak Ht. (cm)	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
DDGS (%)								
0	1.64a	0.52	16.71a	0.37	10.00a	0.78	13.81a	0.96
25	5.10b	0.26	20.29b	0.45	5.69b	0.47	8.28b	0.19
50	4.79c	0.41	20.32b	1.22	4.72c	0.72	7.51c	0.56
SSL (%)								
0	3.38a	1.73	18.38	2.09	7.34	2.70	10.38a	3.19
0.15	5.10b	0.26	20.29	0.45	5.69	0.47	8.28b	0.19
0.3	3.05c	1.64	18.65	2.04	7.38	2.95	10.94c	3.48
Flour								
All-purpose	3.73a	1.39	18.87	1.94	6.69a	2.29	9.65a	2.87
Bread	3.45b	1.94	18.87	2.03	7.36b	2.86	10.72b	3.29

[1] for a given main effect, differing letters between levels for a given property signify significant differences ($p < 0.05$, LSD); St. Dev. is +/- 1 standard deviation.

Table 5. Main effects for physical properties of prepared breads[1] (Cont.)

	Width (cm)		Length (cm)		Mass (g)	
	Mean	St Dev.	Mean	St. Dev.	Mean	St. Dev.
DDGS (%)						
0	12.50a	0.00	16.97a	0.13	602.77a	3.79
25	11.84b	0.44	15.64b	0.50	629.26b	4.35
50	12.19c	0.71	14.85c	0.94	629.15b	4.81
SSL (%)						
0	12.09a	0.62	15.73	1.34	617.19a	15.16
0.15	11.84b	0.44	15.64	0.50	629.26b	4.35
0.3	12.59c	0.20	16.09	1.20	614.72c	13.25
Flour						
All-purpose	12.22	0.64	15.60a	1.35	619.15	12.00
Bread	12.27	0.42	16.12b	0.87	618.09	15.64

[1] for a given main effect, differing letters between levels for a given property signify significant differences ($p < 0.05$, LSD); St. Dev. is +/- 1 standard deviation.

Peak height and side height decreased as the percentage of DDGS increased. Rasco et al. (1990), also found a decreased loaf volume on the majority of bread loaves with 4 or 8% distillers grains substitution. O'Palka et al. (1989) completed a study of baked products containing sour mash corn distillers dried grains. Three of four baked products with various distillers substitution levels resulted in decreased loaf volumes: dinner rolls (17 or 33%), nut rolls (33%), and carrot coconut bread (40%).

The decrease in volume was thought to have been due to the dilution of gluten, particularly in yeast-leavened products, along with other factors that accompany the addition of DDGS to wheat dough systems. Increased concentrations of fiber results often absorb more water (Dreese & Hoseney, 1982). Fiber molecules can be incorporated more easily into a food system once the fiber molecules are softened. Fiber plays many roles in a food matrix, such as providing structure and bulk, as well as modifying rheological properties (Fennema, 1996). If fibrous materials are not softened via hydration, the structure may be compromised due to the cutting of gluten strands, thus diminishing inflation and structure. In this study, width decreased as the percentage of SSL and DDGS increased. The SSL's purpose was to condition and facilitate dough quality; however, increased quantity of DDGS had an opposite effect, altering bread functionality. Length of bread loaves decreased as the quantity of DDGS increased, which reinforced that higher replacement rates of DDGS impacted size and shape. Peak height decreased as SSL and DDGS substitution increased. This showed how bread loaves had a higher peak height when 0% SSL was used compared to 0.15% SSL, which indicated that 0.15% was not a significant quantity to make much difference in enhancing dough and loaf properties. Brochetti et al. (1991) analyzed bread with 5, 10, and 15% DDG substitution. Results indicated that at a 15% DDG substitution level, bread loaf volume also decreased. This volume decrease is reflected in decreased peak height, side height, length, and width parameters. Tsen et al. (1983) and Morad et al. (1984) have also found similar results. The more DDGS included into the bread, the less wheat flour, which dilutes the amount of gluten proteins available. It is also possible that increased water absorption can negatively affect the ratio of wheat flour-to-water (3:1), thus preventing ideal gluten formation (Fennema, 1996). Additional water may disrupt interactions that can bond the structure together. Bread loaves with higher DDGS content may have been manipulated past maximum resistance, thus resulting in a decrease in resistance, which breaks down the gluten structure. Finally, the addition of albumin- and globulin-type proteins adversely affects bread volume and gluten structure. Therefore it is important to monitor the addition of these proteins into baked products (Fennema, 1996).

Height, shape, and color differed among the flour treatment combinations (Figure 6). Additional images showed the grain quality, color, size, and structure of center cut slices (Figure 7). As the quantity of DDGS replaced increased, cell structure of loaves became increasingly compact, dense, and thicker.

3.2.3 Subjective Measurements

Table 6 shows results for the subjective baking quality analysis. As the percentage of DDGS included in the bread formation increased, significant differences could be found among uniformity, size, thickness, grain, tenderness, softness, and crumb color. No significant differences were found between the percentage of SSL or the other independent variables. The type of flour had a significant effect on softness.

Figure 8 illustrates the effects on softness and DDGS substitution. As the percentage of DDGS increased, softness decreased. This was expected as the DDGS was highly fibrous, which has previously been shown to alter the grain structure of the food matrix. Brochetti et al. (1991) tested sensory characteristics of breads with DDG replacement. Breads with 10% and more DDG substitution had a less uniform cell distribution and harsher texture than bread that was considered “ideal”. Their findings indicated adverse effects of increased DDG concentrations that affected sensory and textural characteristics. They also found that as DDGS replacement increased, the several properties were negatively affected, including cell uniformity, cell thickness, grain condition, texture tenderness, texture softness, and crumb color. This issue could possibly be fixed if DDGS refinement processes could result in extremely fine DDGS flour.

Figure 6. Initial loaf profiles show differences in shape, color, and texture between experimental treatments. As the quantity of DDGS increased physical dimensions such as loaf volume, height, and length decreased. DDGS substitution resulted in a loaf color that was darker as well

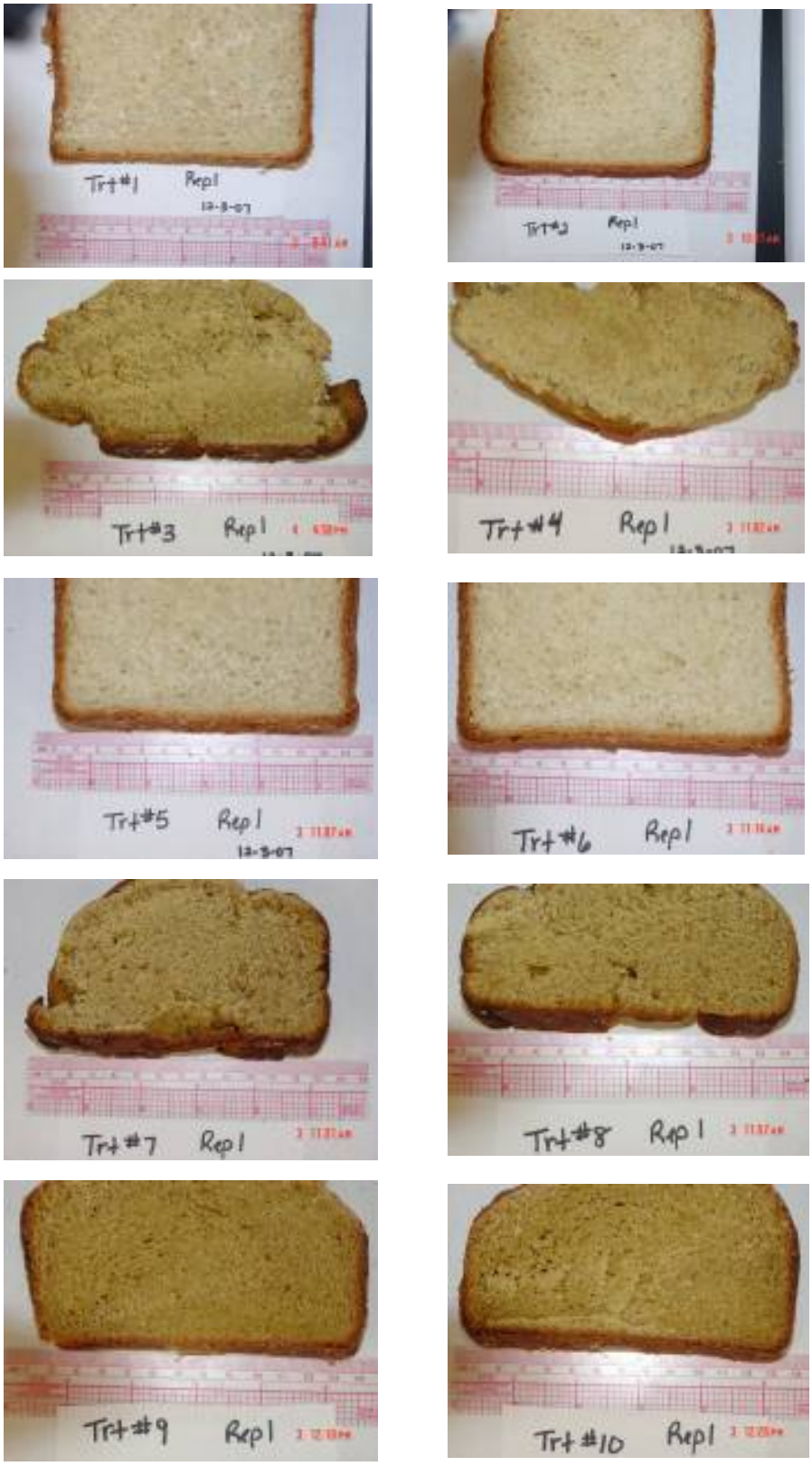

Figure 7. Center slices show differences in texture and cell structure. Loaves with high DDGS levels tended to have small, closed cells with thick cell walls compared to the open cells for loaves without DDGS substitution

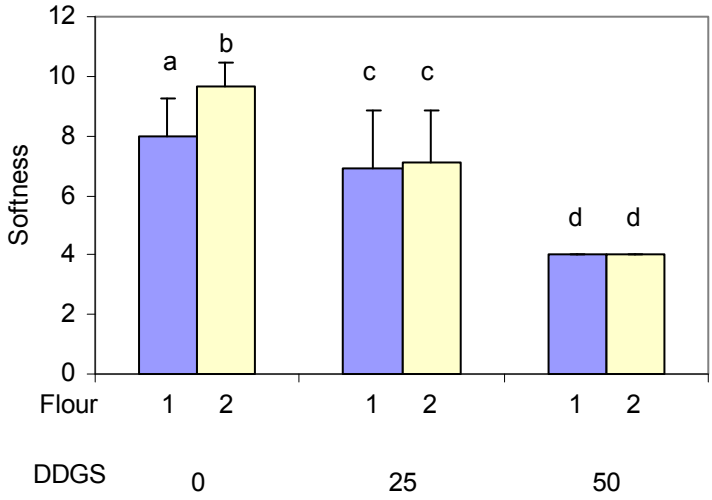

Figure 8. Treatment combination effects between, flour (1=all-purpose & 2=bread) and DDGS levels on bread softness. Overall, as the quantity of DDGS increased the softness qualities exhibited by the bread loaves decreased. Error bars represent ± 1 standard deviation. Differing letters indicate significant differences between treatment combinations ($p < 0.05$, LSD)

Table 6. Main effects for subjective quality tests for prepared breads[1]

	Uniformity		Size		Thickness		Grain	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
DDGS (%)								
0	8.0a	2.0	6.1a	2.3	9.8a	1.0	16.0a	0.0
25	6.5ab	1.4	7.1ab	1.5	4.8b	1.9	10.0b	0.0
50	4.9b	3.7	8.2b	2.3	2.0c	0.0	8.2c	0.6
SSL (%)								
0	6.4	3.3	6.9	2.6	5.9	4.0	12.1	4.0
0.15	6.5	1.4	7.1	1.5	4.8	1.9	10.0	0.0
0.3	6.5	3.5	7.3	2.5	5.9	4.0	12.1	4.0
Flour								
All-purpose	6.7	3.3	7.0	2.3	5.5	3.6	11.7	3.7
Bread	6.2	2.9	7.3	2.4	5.8	3.7	11.7	3.6

[1] for a given main effect, differing letters between levels for a given property signify significant differences ($p < 0.05$, LSD); St. Dev. is +/- 1 standard deviation.

Table 6. Main effects for subjective quality tests for prepared breads[1] (Cont.)

	Moistness		Tenderness		Softness		Crumb Color	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
DDGS (%)								
0	8.9	1.0	12.1a	1.0	8.8a	1.3	8.5a	0.9
25	8.5	2.0	11.4a	1.0	7.0b	1.8	7.8b	0.7
50	8.9	2.0	6.1b	3.0	4.0c	0.0	5.1c	1.6
SSL (%)								
0	8.9	1.0	9.1	4.0	6.3	2.5	6.6	2.2
0.15	8	2.0	11.4	1.0	7.0	1.8	7.8	0.7
0.3	8.9	1.0	9.0	4.0	6.6	2.7	7.0	2.1
Flour								
All-purpose	8.8	1.0	9.4	3.0	6.2a	2.2	7.0	2.0
Bread	8.9	1.0	9.7	4.0	6.9b	2.7	7.0	1.9

[1] for a given main effect, differing letters between levels for a given property signify significant differences ($p < 0.05$, LSD); St. Dev. is +/- 1 standard deviation.

3.3 Correlations and Multivariate Analysis

Linear correlations were determined (Table 7), these are not indicative of causation, nor do they represent relationships found in non-linear behavior. As bread mass increased so did bread protein (r=0.863), flour protein (r=0.864), bread ash (r=0.901), flour ash (r=0.893), bread lipid (r=0.856) and flour lipid (r=0.866). These chemical properties obviously impacted the final physical condition of bread loaves. Flours that are rich in nutrients may produce increasingly dense loaves of bread. Water absorption (r=0.804) of the flour also increased as bread mass increased. This may indicate an increased quantity of water retained and not released as steam during baking processes. Also, water absorption increased as bread protein (r=0.953) and flour protein (r=0.967) increased, indicating protein molecules may be increased building to hydration. Results also show Hunter a and b values were positively impacted by chemical properties as well. These values increased as the quantity of bread protein (a values r=0.835 and b values r=0.878), bread ash (a values r=0.878 and b values r=0.886), and bread lipid (a values r=0.820 and b values r=0.844) increased.

As bread protein content increased, stability in the dough system decreased (r=-0.855). As the replacement rate of DDGS increases, more protein is added to the food matrix. DDGS has many factors, particularly fiber, that may prevent the coproducts from being easily incorporated into dough. Also, as the content of flour ash increases, softness decreases (r=-0.973). This decrease in softness may be due to the increased quantity of nutrients and non-nutrient residues, mostly increasing from rising substitution of DDGS.

In terms of multidimensional space, Principal Components Analysis was used to examine the data distribution when considering all independent and dependent variables simultaneously (Figure 9). Overall, the covariance matrix appeared to be better suited to examine this data set, as it required fewer components (2 vs. 10) to summarize the variance in the data. As shown, three variables were most influential, and thus had the greatest influence on the data, including DDGS level, bread mass, and stability. It also appeared that when considering the first two principal components there was clustering in the data, and this was specifically due to the DDGS level.

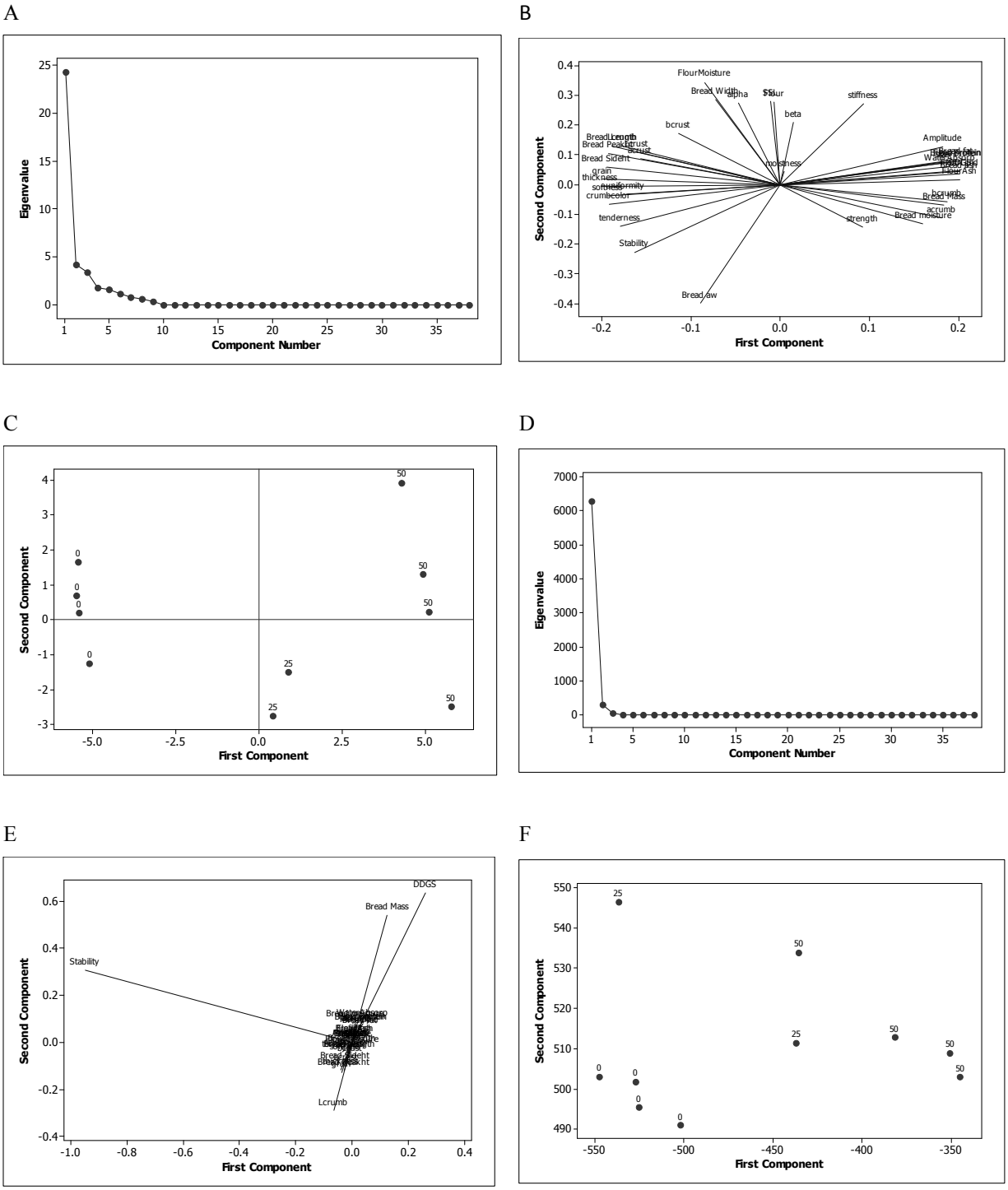

Figure 9. Principal components analysis of data indicates that, using the correlation matrix, A) less than 10 variables had significant effects in multi-dimensional space, B) many components were interrelated and influenced the data, and C) clustering according to DDGS level occurred. Using the covariance matrix, on the other hand, indicated that D) very few factors were required to explain the variance in the data, E) the data could be explained by DDGS, bread mass, and stability, and F) clustering according to DDGS level was observed in multivariate data space

Table 7. Selected significant Pearson product moment linear correlation (r > 0.9) results

	DDGS	Bread Side Ht	Bread Peak Ht	Bread Length	Bread Mass	L crumb	a crumb	b crumb	L crust	Bread Protein	Bread Ash
Bread Peak Ht		0.994	1.000								
Bread Length			0.903	1.000							
L crumb		0.937	0.956			1.000					
a crumb					0.982		1.000				
b crumb					0.934		0.938				
b crust								0.915	0.909		
Bread Protein	0.983									1.000	
Bread Ash	0.996				0.901					0.973	1.000
Bread Lipid	0.988									0.970	0.991
Amplitude	0.923									0.903	0.935
Water Absorption	0.948									0.953	0.934
Flour Ash	0.995									0.960	0.995
Flour Lipid	0.997									0.970	0.992
Flour Protein	0.987									0.996	0.977
Size	0.905									0.913	
Thickness		0.974	0.963	0.909							
Grain		0.980	0.972			0.920					
Softness		0.923	0.903								

	Bread Lipid	Amplitude	Water Absorption	Flour Ash	Flour Lipid	Flour Protein	Thickness	Grain	Tenderness	Softness
Amplitude	0.941	1.000								
Water Absorption	0.919		1.000							
Flour Ash	0.983	0.919	0.923	1.000						
Flour Lipid	0.988	0.918	0.938	0.995	1.000					
Flour Protein	0.970	0.906	0.967	0.966	0.978	1.000				
Size	0.900		0.905		0.909	0.918				
Grain							0.992	1.000		
Softness							0.949	0.910	0.957	1.000
Crumb Color							0.912		0.944	0.943

Table 7. Selected significant Pearson product moment linear correlation (r < - 0.9) results (continued)

	DDGS	Bread Side Ht	Bread Peak Ht	Bread Length	Bread Mass	L crumb	a crumb	b crumb	Bread Protein
Bread Side Ht	-0.942								
Bread Peak Ht	-0.924								
Bread Length	-0.901								
Bread Mass		-0.950	-0.956						
L crumb					-0.971	1.000			
a crumb		-0.960	-0.972			-0.993			
b crumb		-0.965	-0.957			-0.907			
Bread Ash		-0.944	-0.930	-0.911					
Bread Lipid		-0.902							
Flour Ash		-0.956	-0.943	-0.916					
Flour Lipid		-0.940	-0.920	-0.904					
Flour Protein		-0.911							
Thickness	-0.990				-0.936		-0.921	-0.937	-0.966
Grain	-0.972				-0.950		-0.943	-0.971	-0.955
Moistness									
Tenderness	-0.918								
Toftness	-0.962								-0.911
Crumb Color	-0.955								-0.941

	Bread Ash	Bread Lipid	Amplitude	Water Absorption	Flour Ash	Flour Lipid	Flour Protein	Uniformity
Stability			-0.910					
Size								-0.921
Thickness	-0.991	-0.971		-0.922	-0.992	-0.985	-0.971	
Grain	-0.968	-0.940		-0.907	-0.971	-0.965	-0.960	
Tenderness	-0.912	-0.927	-0.944		-0.917	-0.925		
Toftness	-0.969	-0.955	-0.951	-0.904	-0.973	-0.964	-0.926	
Crumb Color	-0.950	-0.955	-0.933	-0.923	-0.944	-0.956	-0.944	

4. Conclusions

This research investigated the effects of inclusion of distillers dried grains with solubles (DDGS) and sodium sterol lactate (SSL) on baking performance of bread using all-purpose and bread flours. This research bridges lab findings and industrial practice. Knowledge of both flour functionality and baking performance was necessary for researchers to completely understand predicted and actual performance. As the substitution of DDGS increased, so did protein, moisture, ash, and Hunter-a color values. Peak height, side height, width, and length decreased as DDGS quantity increased, which was not unexpected. Overall, 25-50% DDGS substitution appeared to have negative effects on physical features, despite the fact that the chemical content was enhanced. DDGS incorporation less than 25% replacement may be ideal for future research, because the performance of these breads was actually enhanced.

References

AACC. (2000). *Approved Methods* (10th ed.). St. Paul, MN: American Association of Cereal Chemistry.

Aamodt, A., Magnus, E. M., & Faergestad, E. M. (2005). Hearth bread characteristics: Effect of protein quality, protein content, whole meal flour, DATEM, proving time, and their interactions. *Cereal Chemistry, 82*(3), 290-301. http://dx.doi.org/10.1094/CC-82-0290

Abbot, J., O'Palka, J., & McGuire, C. F. (1991). Dried distillers' grains with solubles: Particle size effects on

volume and acceptability of baked products. *Journal of Food Science, 56*(5), 1323-1326. http://dx.doi.org/10.1111/j.1365-2621.1991.tb04763.x

Anonymous. (2005). *Mixolab® User's Manual*. Tripette-Renaud Chopin, France.

AOAC International. (2003). *Official Methods of Analysis of the Association of Analytical Chemists* (17th ed.) Method 920.39. The Association: Gaithersburg, MA.

Azizi, M. H., & Rao, G. V. (2004). Effect of surfactant gel and gum combinations on dough rheological characteristics and quality of bread. *Journal of Food Quality, 27*(5), 320-336. http://dx.doi.org/10.1111/j.1745-4557.2004.00600.x

Barcenas, M. E., & Rosell, C. M. (2005). Effect of HPMC addition on the micostructure, quality and aging of wheat bread. *Food Hydrocolloids, 19*(6), 1037-1043. http://dx.doi.org/10.1016/j.foodhyd.2005.01.005

Brochetti, D., Penfield, M. P., & Heim-Edelman, M. R. (1991). Yeast bread containing distillers' dried grain: Dough development and bread quality. *Journal of Food Quality, 14*, 331-344. http://dx.doi.org/10.1111/j.1745-4557.1991.tb00074.x

Caballero, P. A., Gomez, M., & Rosell, C. M. (2006). Bread quality and dough rheology of enzyme-supplemented wheat flour. *Zeitschrift fur Lebensmittel-Untersuchung und-Forschung A, 224*(5), 525-534.

CFR (Code of Federal Regulations). (1986). Food and Drugs, Title 21, Parts 170-199. Office of the Federal Register, National Archives and Records Administration, US Government Printing Office, Washington, D.C.

Chanvrier, H., Uthayakumaran, S., & Lillford, P. (2006). Rheological properties of wheat flour processed at low levels of hydration: Influence of starch and gluten. *Journal of Cereal Science, 45*, 263-274. http://dx.doi.org/10.1016/j.jcs.2006.09.006

Chiotelli, E., Rolee, A., & Le Meste, M. (2004). Rheological properties of soft wheat flour doughs: effect of salt and triglicerides. *Cereal Chemistry, 81*, 459-468. http://dx.doi.org/10.1094/CCHEM.2004.81.4.459

Collar, C., Armero, E., & Martinez, J.C. (1998). Lipid binding of formula bread doughs. *Zeitschrift fur Lebensmittel-Untersuchung und Forschung A, 207*(2), 110-121. http://dx.doi.org/10.1007/s002170050304

Collar, C., Bollaín, C., & Rosell, C. M. (2007). Rheological behaviour of formulated bread doughs during mixing and heating. *Food Sci Tech Int., 13*(2), 99-107. http://dx.doi.org/10.1177/1082013207078341

Donovan, J. W. (1977). A study of the baking process by differential scanning calorimetry. *J. Sci. Food Agric., 28*, 571-578. http://dx.doi.org/10.1002/jsfa.2740280616

Dreese, V. G., & Hoseney, R. C. (1982). Baking properties of the bran fraction from brewer's spent grains. *Cereal Chemistry, 59*(2), 89-91.

Fennema, O. R. (1996). *Food Chemistry* (3rd ed., O. R. Fennema, ed.) New York, NY: Marcel Dekker, Inc., pp. 218-220, 394-434.

Finley, L. W., & Hanamoto, M. M. (1980). Milling and baking properties of dried brewer's spent grains. *Cereal Chemistry, 57*(3), 166-168.

Guarda, A., Rosell, C. M., Benedito, C., & Galotto, M. J. (2004). Different hydrocolloids as bread improvers and antistaling agents. *Food Hydrocolloids, 18*, 241-247. http://dx.doi.org/10.1016/S0268-005X(03)00080-8

Hansen, B., & Hansen, Å. (1992). Test baking of bread by household baking machine. *Lebensm.-Wiss. u.-Technol., 25*(6), 585-587.

Hansen, L. P., Johnston, P. H., & Ferrel, R. E. (1975). Heat-moisture effects on wheat flour. I. Physical-chemical changes of flour proteins resulting from thermal processing. *Cereal Chemistry, 52*, 459-472.

Hrušková, M., Švec, I., & Jirsa, O. (2006). Correlation between milling and baking parameters of wheat varieties. *Journal of Food Engineering, 77*(3), 439-444. http://dx.doi.org/10.1016/j.jfoodeng.2005.07.011

Kahraman, K., Sakiyan, O., Ozturk, S., Koksel, H., Sumnu, G., & Dubat, A. (2008). Utilization of Mixolab to predict the suitability of flours in terms of cake quality. *Eur Food Res Technol., 227*, 565-570. http://dx.doi.org/10.1007/s00217-007-0757-y

Krog, N., & Lauridsen, J. B. (1976). *Food emulsifiers and their association with water*. Food Emulsions. In S. Friberg (Ed.) (pp. 67-139). New York: Marcel Dekker.

Li, M., & Lee, T. C. (1996). Effect of extrusion temperature on solubility and molecular weight distribution of wheat flour proteins. *J. Agric. Food Chem., 44*(3), 763-768. http://dx.doi.org/10.1021/jf950582h

Lilliard, D. A., Christen, G. L., & Smith, J. S. (2000). *Food Chemistry: Principles and Applications.* West Sacramento, CA: Science Technology System, pp. 131-148.

MacRitchie, F. (1992). Physico-chemical properties of wheat proteins in relation to functionality. *Advances in Food and Nutrition Research, 36*, 1-87. http://dx.doi.org/10.1016/S1043-4526(08)60104-7

Morad, M. M., Doherty, C. A., & Rooney, L. W. (1984). Utilization of dried distillers grains from sorghum in baked food systems. *Cereal Chemistry, 61*(5), 409-414.

Nielsen, S .S. (2003). Food Analysis, 3rd ed. Springer Science + Business Media Inc. New York, NY. pp. 143-174.

O'Palka, J., Eidet, L, & Abbott, J. (1989). Use of sodium bicarbonate and increased liquid levels in baked products containing sour mash corn dried distillers' grains. *J. Food Sci., 54*, 1507. http://dx.doi.org/10.1111/j.1365-2621.1989.tb05147.x

Pamies, B. V., Roudaut, G., Dacremont, C., Le Meste, M., & Mitchell, J. R. (2000). Understanding the texture of low moisture cereal products: mechanical and sensory measurements of crispness. *J Sci. Food. Agric., 80*(11), 1679-1685. http://dx.doi.org/10.1002/1097-0010(20000901)80:11<1679::AID-JSFA697>3.0.CO;2-P

Rasco, B. A., Downey, S. E., & Dong, F. M. (1987). Consumer acceptability of baked goods containing distillers' dried grains with solubles from soft white winter wheat. *Cereal Chemistry, 64*(3), 139-143.

Rasco, B. A., Rubenthaler, G., Borhan, M., & Dong, F. M. (1990). Baking Properties of Bread and Cookies Incorporating Distillers' or Brewer's Grain from Wheat or Barley. *Journal of Food Science, 55*(2), 424-429. http://dx.doi.org/10.1111/j.1365-2621.1990.tb06778.x

Reddy, N. R., Pierson, M. D., & Cooler, F. W. (1986). Supplementation of wheat muffins with dried distillers grain flour. *Journal of Food Quality, 9*(4), 243-249. http://dx.doi.org/10.1111/j.1745-4557.1986.tb00794.x

Ribotta, P. D., Perez, G. T., Leon, A. E., & Anon, M. C. (2004). Effect of emulsifier and guar gum on micro structural, rheological and baking performance of frozen bread dough. *Food Hydrocolloids, 19*, 93-99. http://dx.doi.org/10.1016/j.foodhyd.2004.04.018

Rosell, C. M., Rojas, J. A., & Benedito, C. (2001). Influence of hydrocolloids on dough rheology and bread quality. *Food Hydrocolloids, 15*, 75-81. http://dx.doi.org/10.1016/S0268-005X(00)00054-0

Rosell, C. M., Collar, C., & Haros, M. (2007). Assessment of hydrocolloid effects on the thermo-mechanical properties of wheat using the Mixolab. *Food Hydrocolloids, 21*, 452-462. http://dx.doi.org/10.1016/j.foodhyd.2006.05.004

Saunders, J, Stauffer, S., & Krishnan, P. (2007). Low-sugar bread formulations using Alice, a hard white winter wheat. *South Dakota State Journal of Undergraduate Research, 5*, 1-9.

Schofield, J. D., & Booth, M. R. (1983). *Wheat proteins and their technological significance*. Developments in Food Proteins-2. B. J. F. Hudson, ed. London: Applied Science Publishers.

Shittu, T. A., Raji, A. O., & Sanni, L. O. (2007). Bread from composite cassava-wheat flour: I. Effect of baking time and temperature on some physical properties of bread loaf. *Food Research International, 40*(2), 280-290. http://dx.doi.org/10.1016/j.foodres.2006.10.012

Soulaka, A. B., & Morrison, W. R. (1985). The Bread baking quality of six wheat starches differing in composition and physical properties. *J. Sci. Food Agric., 36*, 719-727. http://dx.doi.org/10.1002/jsfa.2740360812

Sunbeam Corporation. (1999). User Manual and Bread Recipes. Boca Raton, FL: Sunbeam Corporation.

Tsen, C. C., Eyestone, W., & Weber, J. L. (1982). Evaluation of the quality of cookies supplemented with distillers' dried grain flour. *Journal of Food Science, 47*(2), 684-685. http://dx.doi.org/10.1111/j.1365-2621.1982.tb10156.x

Tsen, C. C., Weber, J. L., & Eyestone, W. (1983). Evaluation of distillers' dried grain flour as a bread ingredient. *Cereal Chemistry, 60*(4), 295-297.

Waelti, H., & Ebeling, J. M. (1982). *Fuel alcohol. Distillers' dried grain nutritional value*. AE 108. Cooperative Extension Services, Washington State University: Pullman, W.A.

Wang, X., Choi, S., & Kerr, W. L. (2004). Water dynamics in white bread and starch gels as affected by water and gluten content. *Lebensm.-Wiss. U.-Technol., 37*(3), 377-384. http://dx.doi.org/10.1016/j.lwt.2003.10.008

Wardlaw, G. M., & Kessel, M. (2002). *Perspectives in Nutrition* (5th ed, p. 259). Von Hoffmann Press, Inc.: Boston.

White, P. J., & Johnson, L. A. (2003). *Corn Chemistry and Technology* (2nd ed, p. 607). American Association of Cereal Chemists, Inc. St. Paul, MN.

Wu, Y. V., Sexton, K. R., & Lagoda, A. A. (1984). Protein-rich residue from wheat alcohol distillation: Fractionation and characterization. *Cereal Chemistry, 61*(5), 423-427.