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Protein and Fiber Fortification of White Pan Bread Using Food-Grade Distiller's Dried Grains

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PROTEIN AND FIBER FORTIFICATION OF WHITE PAN BREAD USING FOOD-GRADE DISTILLER'S DRIED GRAINS

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science in Biological Science: Specialization Food Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the department.

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ABBREVIATIONS

µm	micrometers
AACC	American Association of Cereal Chemists
AOAC	Association of Official Analytical Chemists
APF	all-purpose flour
BSG	brewer's spent grains
cm	centimeters
DDG	distiller's dried grains
DDGS	distiller's dried grains with solubles
DON	Deoxynivalenol
ESCL	Experiment Station Chemical Laboratories (Missouri University)
FDA	Food and Drug Administration
g	grams
HP-DDG	high protein distiller's dried grains
HPLC	High Performance Liquid Chromatography
in	inches

kg	kilograms
L	liters
min	minutes
mm	millimeters
NM	not measured
ppb	parts per billion
ppm	parts per million
RACC	reference amount customarily consumed
sec	seconds
SSL	sodium stearoyl lactylate
TDF	total dietary fiber
TPA	texture profile analysis
USDA	United States Department of Agriculture

ABSTRACT

PROTEIN AND FIBER FORTIFICATION OF WHITE PAN BREAD USING FOOD-GRADE DISTILLER'S DRIED GRAINS

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Distiller's dried grains (DDG) are a coproduct of ethanol production. DDG has been used historically as animal feed. However, in the past decade, ethanol production has dramatically increased causing a surplus of distiller's grains and saturating the market. The use of DDG, which is high in both protein and fiber, to fortify baked goods is one option to reduce the excess of DDG while enhancing its economic value.

The purpose of this study was first, to evaluate the washing process for DDG to make it food grade, and second to evaluate the effects of incorporation of food-grade DDG from two different sources (DDGS and HP-DDG) on the quality of white pan bread (sandwich bread). HP-DDG was produced using a proprietary fractionation procedure prior to fermentation, while DDGS was produced using a conventional ethanol production procedure.

Distiller's dried grains with solubles (DDGS) and a high protein DDG (HP-DDG) were subjected to a washing process to make them food grade. Processing recovery (percent yield), color, and particle size were evaluated during the washing process. Substitutions of 5% and 10% of both DDGS and HP-DDG were used in all-purpose flour (APF). Dough rheology was tested using a Mixolab and a TA.XT*Plus* Texture Analyzer.

Bread was baked using a modified AACC straight dough process. Loaves were then analyzed for color, volume, density, internal crumb structure and texture profile. Sensory acceptance of breads was evaluated using a seven-point hedonic scale.

Yields from the washing process for the DDGS and HP-DDG, averaged 52.7% and 72%, respectively. While color of DDGS and HP-DDG was reduced through the washing process, finished products containing DDG were darker than the control. This led to visible color differences in the crumb of breads containing DDG. Significant differences were noted between washed DDG samples in relation to particle size distribution. Mycotoxins were not detected in either of the washed samples.

Incorporation of food-grade DDG into breads led to smaller, denser loaves with fewer air cells. Loaves with 10% food-grade DDG were found to be significantly more firm than the 5% loaves. Substantial increases in protein content were seen at all levels of DDG inclusion, however statistically significant increases in fiber were noted only in the 10% DDGS loaves. Sensory analysis showed that all bread treatments were acceptable to consumers, and that the only significant difference in acceptability of loaves was noted in the appearance scores.

1 INTRODUCTION & OBJECTIVES

1.1 Introduction

Distiller's grains are a coproduct of ethanol production from corn. They are the non-fermentable parts of the corn that are left over after ethanol production. One bushel of corn (56 lbs) yields 2.7 gallons of ethanol and 17.5 pounds of DDGS (Service, 2015). Since the majority of sugars and starch from the corn are converted to ethanol, DDG is generally high in protein and fiber (Weiss, 2007). DDG is typically considered as a low value waste product, the majority is sold to farmers to be used as animal feed. These sales help to off-set ethanol production costs. In the past decade production of DDGS has more than quadrupled, increasing from 7.0 million metric tons during the 2004/2005 season to 36.0 million metric tons in the 2014/2015 season (Service, 2015). Due to the recent increases in ethanol production the market for DDG(S) as feed is becoming saturated. There is a growing need to find additional uses for DDG (Rosentrater et al., 2005, Murthy, 2006). Food application is one such option. Current food trends are toward high protein and high fiber foods (Mintel, 2014b, Adams, 2015, Nachay, 2015). Food-grade DDG could therefore be an effective supplement to food products in order to increase their protein and fiber content (Rasco et al., 1987). This would not only benefit the consumer through increasing the nutritional quality of products, but also has the potential to benefit producers by increasing the value of DDG as a coproduct.

This study evaluated the quality and acceptability of white pan breads, also known as sandwich breads, which are fortified with DDG. In order for food to be acceptable to consumers it must conform to a particular set of quality standards. These

qualities depend on the identity of the food. While bread quality is generally difficult to define, the attributes of most importance in bread quality are freshness, appearance, and physical texture (Scanlon and Zghal, 2001, Heenan et al., 2008). In sandwich bread, consumers typically look for a soft spongy texture which gives a minimal amount of resistance to the tooth. Additionally, sandwich breads are typically light in both flavor and color serving as a platform for toppings such as meat and cheese. Cell structure is one of the determining factors in bread texture, and is determined by the combination of raw materials and the processing conditions (Scanlon and Zghal, 2001).

The high fiber content of DDG can interfere with the protein structure (mainly gluten) formed within the bread dough. Thus negatively affecting the final texture and density of the bread. In addition, unprocessed DDG has a distinct aroma and yellow color, both of which are undesirable in bread. It is the goal of this study to process the DDG in a way which will limit the negative effects on bread quality. This will be done through grinding to reduce particle size and minimization of color and flavor through washing with ethanol. Determining a maximum level of substitution in the dough which did not adversely impact texture but increased its nutritional content was an important aspect of this study.

1.2 Problem Statement

Sandwich bread is widely consumed among Americans. In addition, Americans need to consume more dietary fiber and are interested in eating foods higher in protein. DDG, which is a good source of both protein and fiber, is currently seen as a waste product of ethanol production. By processing DDG into a food-grade substance and

incorporating it in sandwich bread we will be able to address the needs of consumers while adding value to DDG.

While bread consumption was thought to be decreasing over previous years, according to Mintel's report Bread and Bread Products – U.S. (2014a), eight in ten adults reported buying the same amount or more packaged, branded, and sliced bread in the last six months. An increase has also been seen in the number of sandwiches consumers prepare at home (Sloan, 2014). The Food Marketing Institute's 2014 Shopping for Health Survey showed that in 2013 37% of customers exchanged their old bread for a heathier one. This was an increase of 3% over 2012. Similarly, Mintel reported that 37% of consumers chose one brand of bread over another due to high fiber claims, and 22% were influenced by all natural or added nutrient statements. Other factors which influenced brand choice to a lesser extent included: low sugar (20%), low carbohydrate (19%), perceived healthier flours (16%), and gluten free (11%)(Mintel, 2014a).

Proteins provide health benefits such as assisting weight management, promoting satiety, building and maintaining lean muscle, and enhancing skin health (Ohr, 2014). A study by Leidy et al. (2013) comparing the effects of high and normal protein breakfasts to skipped breakfasts among young people demonstrated that high protein breakfasts decreased hunger and increased the feeling of fullness over the regular protein and skipped breakfasts. Additionally, participants who ate high protein breakfasts voluntarily reduced their calorie intake by more than 400 calories per day while those who skipped breakfast showed significant increases in percent body fat mass over those who ate the regular or high protein breakfasts. This supports the role of protein in satiety and weight management.

The benefits of protein enrichment are recognized by many consumers. In NPD's report, The Market for Functional Foods, more than half the adults surveyed stated that adding protein to their diet was either somewhat or very important (Ohr, 2014). Similarly, Mintel's 2014 report *Protein Fever* indicated a global increase in protein consumption, with 25% of Americans and 26% of Chinese increasing their protein content in 2014. Such interest has made the protein content of foods a valuable selling point for manufacturers (Nachay, 2015).

In addition to paying attention to how much protein they consume, shoppers are also aware of where their protein is coming from, especially whether it is plant or animal based. Data from the NCC (2014) shows that the per capita consumption of meat has been decreasing since 2007. Conversely an increase of 8% has been seen in the sales of meat alternatives between 2010 and 2012 (Mintel, 2013). However, the popularity of plant based proteins does not come without challenges. Both flavor and texture can cause problems in the development of new food products.

Dietary fiber has been shown to have many health benefits including lowering blood pressure, improving blood glucose control in diabetics, promoting regularity, aiding in weight loss and improving immune function. A diet high in fiber has also been shown to reduce the risk of diseases such as stroke, hypertension, coronary heart disease, obesity and certain gastrointestinal disorders (Anderson et al., 2009, Adams, 2015). The 2010 Dietary Guidelines for American's suggest that individuals consume 14 g of fiber per day for every 1000 calories consumed, making the recommended intake at least 25 g per day for women and 38 g per day for men. It is estimated that Americans under consume fiber, averaging an intake of only 15 g per day (U.S. Department of Agriculture

(USDA), 2010). Despite the apparent discrepancy, consumers are making dietary choices in favor of fiber. A 2014 report by the International Food and Nutrition Council indicated that fiber and whole grain were the most sought after food ingredients in 2014. Similarly, in 2013, 37% of customers opted for healthier bread; 24% chose healthier pasta; and 22%, healthier crackers (FMI, 2014).

Previous research has investigated DDG and brewer's spent grains (BSG) as ingredients in baked products. A number of studies have shown their successful implementation in baked products in order to fortify their protein and fiber content. This has been shown for flat breads such as tortillas (Pourafshar et al., 2014a), chapatti, pita bread (Al Rayes, 2014), and barbari (Pourafshar et al., 2014b) as well as cookies and sandwich breads (Tsen et al., 1982, Rasco et al., 1987, Rasco et al., 1990, Ktenioudaki et al., 2012).

In general, most of these studies have found that DDG is a suitable ingredient for fiber and protein fortification when used at a low to moderate level. DDG addition has been shown to negatively impact appearance and texture, but these effects have not been seen to be detrimental to product quality.

Saunders et al. (2014) completed an analysis of DDGS in bread with and without the dough conditioner sodium stearoyl Lactylate (SSL). The findings of this research showed a significant discrepancy in quality of bread including DDGS as compared to the control. However, the level of DDGS substitution was 25%, which is quite high, and the DDG used was only minimally treated. Given this information, it is likely that the bread recipes were not optimized and that a product of acceptable quality and high nutritional

value could be obtained by improving the quality of DDG used and by reducing its level of addition.

The purpose of the current project was to compare the quality and sensory acceptance of bread products using flour supplemented with food grade DDG resulting from DDGS (after washing), and high-protein DDG (HP-DDG), a form of DDG resulting from ethanol production of fractionated corn. Bread samples including DDG were evaluated against a control made with All-purpose flour (APF) employing the same method as the other breads. In doing this, it was possible to determine which form of DDG is most acceptable for use in sandwich bread, and to determine the acceptability of DDG-fortified breads from a quality and sensory standpoint.

1.3 Research Objectives

The objectives of this study were to:

1. Test the quality of HP-DDG and DDGS for the manufacture of food-grade DDG through proximate analysis and testing for aflatoxins before and after washing.
2. Compare the effects of food-grade DDG from both HP-DDG and DDGS on dough and bread quality when using All-Purpose flour.
3. Compare the quality of dough and bread made from blends containing 5% DDG to those containing 10% DDG.
4. Evaluate the sensory acceptability of food-grade DDG fortified sandwich bread as compared to “white” sandwich bread (made with All-purpose flour).

1.4 Hypothesis

1. **H0:** There will be no significant difference between the effects of food-grade DDG produced from HP-DDG and DDGS on dough or bread quality.

   H1: There will be a significant difference between the effects of food-grade DDG produced from HP-DDG and DDGS on dough or bread quality.

2. **H0:** There will be a significant difference between the dough and bread quality of blends containing 5% food-grade DDG and 10% food-grade DDG.

   H1: There will not be a significant difference between the dough and bread quality of blends containing 5% food-grade DDG and 10% food-grade DDG.

3. **HO:** There will be no significant difference between sensory quality of conventional “white” sandwich bread and sandwich bread fortified with food-grade DDG.

   H1: There will be a significant difference between the sensory quality of conventional “white” sandwich bread and sandwich bread fortified with food-grade DDG.

1.5 Literature Review

The production of ethanol from corn can be done using one of two general methods: either a dry mill or a wet mill process. The dry grind process requires less initial capital and is more popular in the ethanol industry (Rosentrater et al., 2005). In this process the corn is ground up and mixed with water to form a "mash". This is then treated with enzymes to hydrolyze the sugar. Once exposed the sugars can then be fermented into ethanol by yeast. After the fermentation is completed the ethanol is distilled off leaving behind a fibrous slurry. This slurry is then typically centrifuged and dried to remove the excess water before disposal. The remaining protein and fiber are what is referred to as distiller's dried grains (DDG). Often the solubles are condensed after centrifugation then added back to the DDG before drying. This results in distiller's dried grains with solubles (DDGS) (RFA, 2015). There are three basic types of distillers grains: DDG, DDGS and fractionated DDG. The main difference between DDG and DDGS is that DDGS contains "solubles". These are composed mainly of sugars and starches which are water soluble and were removed during centrifugation of the DDG to remove excess moisture before drying (Weiss, 2007). The solubles can be condensed and added back to the DDG to reduce product losses.

Some producers have added a dry fractionation process prior to fermentation to increase production and yield efficiency. The process increases fermentation rate and final concentration of ethanol by reducing the amount of un-fermentable biomass in the fermentation tank (Singh et al., 2005, Wang et al., 2005), thus increasing the efficiency of the fermentation process by reducing the amount of un-fermentable biomass in the fermentation tank (Systems, 2006). Removal of the bran and germ prior to fermentation

also decreases the initial proportions of oils and fiber and results in DDG which is higher in protein than that made from conventional processes. Finally, the reduction in mass of un-fermentable material in the tank results in a reduction of up to 66% in the amount of DDGS produced (Singh et al., 2005). The germ and fiber portions of the kernel can then be diverted to other value added streams, while the DDG has increased in value due to its higher protein content.

Table 2.1 shows a comparison of the components of DDG, DDGS, and Fractionated DDG (HP-DDG). Since the majority of starches and sugars are removed during fermentation, the distiller's grains have a high protein and fiber content at approximately 40 and 38 percent, respectively. This enhances their potential as a source of protein and fiber fortification in baked products.

Table 1.1: Proximate Composition of DDG, DDGS, and HP-DDG in percent dry basis[1].

Proximate composition	Conventional DDG	Conventional DDGS	Hi protein DDG (HP-DDG)
Crude protein	41.64	36.00	47.40
Fat	8.89	16.59	3.23
ADF	23.23	12.32	26.12
NDF	38.13	24.72	29.40
Ash	1.98	4.81	1.09

[1] Data provided by National Corn to Ethanol Research Center.

1.5.1 Incorporation into Baked Products:

Numerous studies have evaluated the effects of both brewer's spent grain (BSG) and DDG in baked products. Initial research on this topic began in the 1980's and continued into the early 1990's. Research during this time focused mainly on DDG and BSG from wheat and barley. Within the past 10 years there has been a resurgence of research in this area, while research is still conducted on BSG from wheat and barley, corn based distillers' grains are now also a subject of many studies. The introduction of corn DDG to this field is likely due to the increased production of ethanol from corn as well as recent suggestions supporting the consumption of high fiber foods (Ktenioudaki et al., 2012, Service, 2015).

Cookies and pan breads were some of the first baked products tested with inclusion of DDG and BSG. Tsen et al. (1982) evaluated the quality of bar, spice, sugar, and chocolate chip cookies with DDG flour inclusion at a rate of 15%. It was found that while both DDG sources used produced acceptable cookies, those made without DDGS received significantly higher scores in sensory evaluation. Chocolate chip cookies including 30% DDG were also investigated by Rasco et al. (1987). In this study it was seen that there was no significant difference between sensory acceptability of chocolate chip cookies with DDGS and those with none. It should be noted that in both of these studies DDG samples were subjected to grinding before incorporation into products.

In addition, a study evaluating the quality of pan breads including 10% and 20% ground wheat DDG showed that incorporation of DDG lead to decreased development times and lower dough stability (Tsen et al., 1983). The same study compared white and whole wheat bread to those containing DDG. While the 20% DDG performed poorly, the

10% DDG formulation was shown to have superior nutritional content and shelf-life to white bread, and superior specific volume and color to whole wheat bread. Rosco et al. (1987) evaluated the sensory acceptance of whole wheat and white bread which included 30% replacement of All Purpose Flour (APF) with DDG from soft white winter wheat. Both breads received an average score of acceptable. The authors concluded that this rating along with data showing an increase in protein and fiber content among samples containing DDGS indicated a strong potential for DDGS as ingredient for fortification of baked goods in the future. A follow up study found that unwashed DDGS from white wheat resulted in higher loaf volume than the same DDGS which was washed prior to use. Incorporation of 8% DDGS also lead to lower loaf volume than 4% DDGS. Although no pattern was seen between grinding DDGS samples and loaf volume it was noted that the crumb of products was often improved when the DDGS included was ground rather than unground (Rasco et al., 1990).

A study on the inclusion of corn based DDGS in cornbread found that DDGS could be incorporated at levels up to 25% without causing a decrease in quality. Corn bread texture was seen to improve as DDGS incorporation increased, and product color darkened as DDGS incorporation increased (Liu et al., 2011). As seen in other studies DDGS addition caused increase in protein and fiber, the authors noted that this was beneficial nutritionally and could possibly be beneficial through lowering the product's glycemic index.

More recently the effect of incorporation of BSG into bread sticks was tested; 0, 15, 25, and 35% of flour was replaced with BSG to examine the potential for BSG as a source of fiber fortification in baked snack products. In this experiment Ktenioudaki et al.

(2012) found that while breadsticks with BSG had a significantly higher fiber content than the control, the addition of BSG also caused the breadsticks to be significantly darker, less crispy, and have lower volume. It was the conclusion of the authors that further experimentation on the incorporation of BSG into snack products would be successful in developing it as a source of fiber fortification.

A follow up study by Ktenioudaki et al. (2013a) incorporated BSG at levels of 0, 10, 15, and 25% into "crispy slices" in an effort to examine the effect of BSG inclusion on snack foods. These crispy slices were manufactured by baking bread, letting it cool, then thinly slicing it and drying the slices to obtain a thin crispy product. The researchers found that the 10% BSG formulation resulted in a product with higher fiber but a similar texture and structure to the control. However, an undesirable aroma was detected in the snacks by the sensory panel. This aroma was confirmed to have come from the BSG through mass spectrometry. As with the previous study the group concluded that further investigations must be performed in order to optimize the use of BSG in snack products.

In 2014, Saunders et al. evaluated the effect of corn DDGS and sodium stearoyl lactate (SSL), a dough conditioner, on bread quality. Formulations included 0, 25, and 50% DDGS and 0, 0.15, and 0.3% SSL. All combinations of DDGS and SSL were made with both bread and all-purpose flour. DDGS was found to have a negative effect on the color, shape and volume of the loaf. In this experiment DDGS was ground but not washed before incorporation. The authors of this paper concluded that while the inclusion of DDGS with and without SSL had severe negative effects on bread quality at the levels tested, there may be potential for DDGS inclusion in bread at lower levels.

Following this, studies at South Dakota State University focused on the incorporation of corn DDGS into flat breads. These studies processed DDGS into food-grade DDG through exhaustive washing with ethanol and water followed by drying and sterilization before incorporation into products (Arra, 2011, Al Rayes, 2014, Pourafshar et al., 2014b, 2015). The work completed by Arra (2011) evaluated the effect of food-grade DDG in Asian flatbreads including naan and chapathi and found that although fortified breads were regarded as acceptable by sensory panelists, they were still inferior to their respective control products. Studies evaluating the effect of DDGS in barbari and tortillas, two other ethic flat breads, were conducted by Pourafshar et al in 2014 and 2015, respectively. Results of these experiments concluded that doughs supplemented with DDGS produced breads that were significantly higher in protein, fiber and ash than controls. However, these differences in composition negatively affected the texture of tortillas causing a decrease in extensibility and increase in firmness of final products. While a statistically significant difference was seen both in the color and textural properties between the control and DDGS supplemented tortillas, no sensory analysis was done to determine whether tortillas supplemented with DDGS could be considered acceptable based on consumer opinion (Pourafshar et al., 2015). Similar results were found in the study on barbari breads. No significant difference was seen between center thickness, extensibility, or density of DDGS supplemented and control breads, however statistical differences were seen in edge thickness, firmness and color (L*a*b*). As in the tortilla study no sensory panel was conducted so no correlation between these properties can be made to differences in consumer desirability between the breads (Pourafshar et al., 2014b).

Most recently Al Rayes and Krishnan (2014) have investigated the nutritional properties of DDGS-supplemented pita bread. The preliminary findings of this research has found significant increases in protein and total dietary fiber (TDF) in breads supplemented with DDGS. A significant beneficial effect on the glycemic responses of persons who consumed the pita bread including DDGS as compared to control breads has also been found.

These studies have set the platform for experiments evaluating the effect of DDG in other baked products. While some success was seen in the past with incorporating BSG and wheat based DDGS into bread, studies which have investigated the incorporation of corn DDGS into sandwich breads reported varying degrees of success owing to the diversity of the starting materials. Previous studies on this topic included little to no pre-treatment of the DDGS. Pre-treatment of DDGS and BSG has been seen to be an integral part of developing high quality DDG and BSG fortified products (Arra, 2011, Ktenioudaki et al., 2013a, Al Rayes, 2014, Pourafshar et al., 2014b, 2015). Using the DDGS pre-treatment plan proposed in this study along with lower DDGS substitution levels (5% and 10%) sandwich breads fortified with DDGS are expected to be acceptable quality. The use of standardized tests and refined bread quality measurements will also permit ease of data interpretation.

2 MATERIALS AND METHODS

2.1 Experiment Design

Figure 2.1 depicts each step of food-grade DDG production and product analysis. All tests were performed in duplicate on the control and each of the two treatments

2.2 Methodology in detail:

2.2.1 DDG Collection and Analysis:

2.2.1.1 Sample Collection: DDGS was provided by Glacial Lakes Energy in Watertown, SD. HP-DDG was provided by a commercial source All samples were frozen upon receipt and thawed before use.

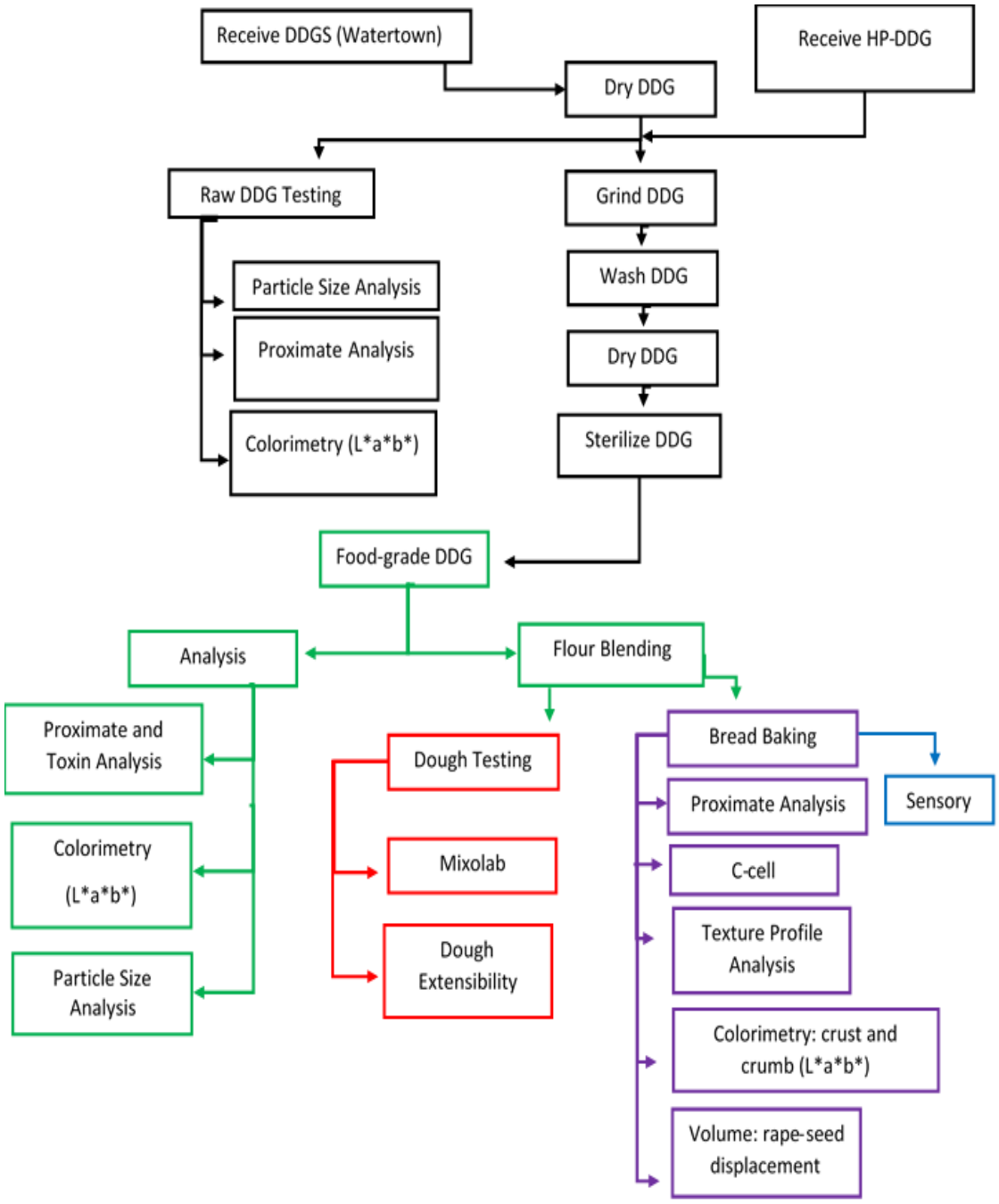

Figure 2.1: Process flow chart for treatment of DDG and its evaluation in baked bread.

2.2.1.2 Particle Size Analysis: The particle size distribution of Dry Raw DDG samples was analyzed using a Ro-Tap device and a series of sieves with mesh sizes: 40, 60, 80, 100, and 200.

2.2.1.3 Proximate Analysis: Raw DDG samples were sent to Missouri University Agriculture Experiment Station Chemical Laboratories (ESCL) to be evaluated for protein (AOAC Official Method 990.03), fat (AOAC Official Method Number 920.39), moisture (AOAC Official Method 934.01), and ash content (AOAC Official Method 942.05). Total dietary fiber (TDF) (method number) and Amino Acid profile (AOAC Official Method 982.30) were also measured (Horwitz et al., 2006). Carbohydrates were determined by difference (Kraisid et al., 2003).

2.2.1.4 Colorimetery (L*a*b*): A Minolta Colorimeter was used to evaluate the color profile of DDG samples using the L*a*b* scale for color (Figure 2.2). On this scale “L” refers to the “brightness” of the sample and is scored from 0 being pure black to 100 being pure white. Parameters “a” and “b” are scored on positive and negative scales with negative and positive “a” signifying green and red, respectively, and negative and positive “b” indicating blue and yellow. An evaluation of these three parameters was performed before and after washing to compare product color and characterize changes. The same color evaluation system was also used to compare color differences between the control and DDG breads.

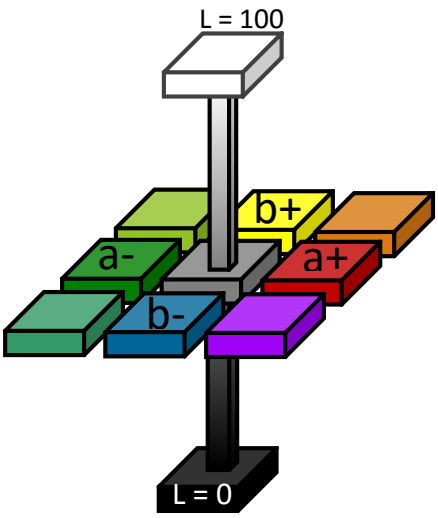

Figure 2.2: L*a*b* Color Scale.

2.2.2 Food-grade DDG preparation (Fig. 2.3):

2.2.2.1 Drying: DDGS was thawed and placed on a foil lined freeze-dryer tray. Samples were then frozen for at least 12 hours to crystallize water which was present in the sample. Once thoroughly frozen, samples were placed in the freeze dryer for 3 days until they were dry. HP-DDG arrived pre-dried and was not dried further before grinding.

2.2.2.2 Grinding: Dry DDG was ground using a centrifugal mill and a 1 mm sieve.

2.2.2.3 DDG was manually washed in an excess of food-grade solvents employing a protocol developed at South Dakota State University.

2.2.2.4 Drying: DDG was spread onto foil-lined freeze dryer trays. The trays were then covered with plastic wrap and frozen for at least 12 hours to crystallize all remaining water. Once thoroughly frozen, samples were placed in the freeze dryer for 3 days until they were dry.

2.2.2.5 Sterilization: The ground DDG was then sterilized to prepare it for addition to food. This was done by autoclaving the samples in hermetically sealed Mason Jars at 121°C for 15 minutes.

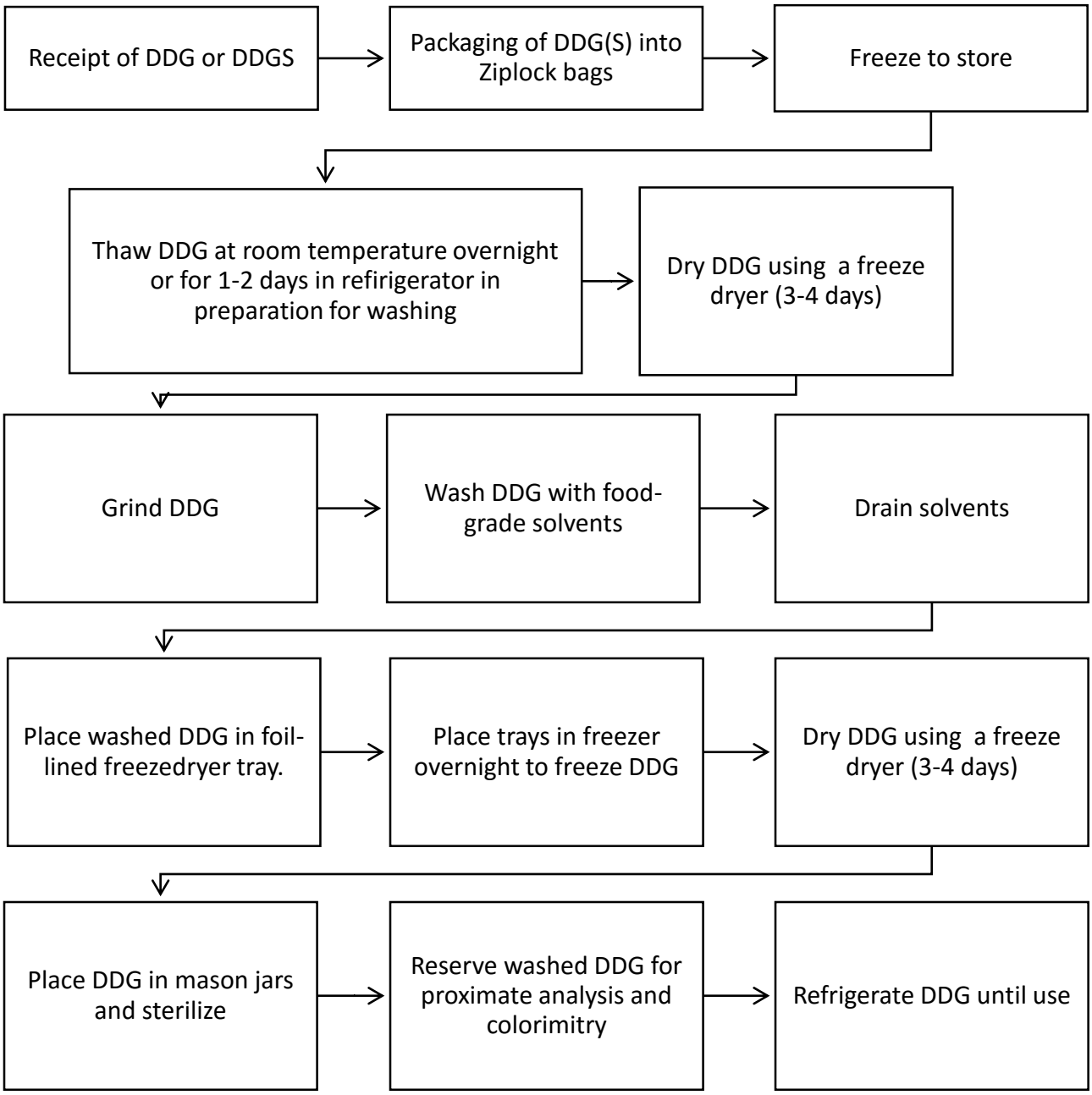

Figure 2.3: Production flow chart for processing of distiller's dried grains.

2.2.3 Analysis of Food-grade DDG:

2.2.3.1 Proximate Analysis: food-grade DDG samples were sent to Missouri University ESCL to be evaluated for protein (AOAC Official Method 990.03), fat (AOAC Official Method Number 920.39), moisture (AOAC Official Method 934.01), and ash content (AOAC Official Method 942.05). Total dietary fiber (TDF) (method number) and Amino Acid profile (AOAC Official Method 982.30) were also tested (Horwitz et al., 2006). Carbohydrates were determined by difference (Kraisid et al., 2003).

2.2.3.2 Particle Size Analysis: The particle size distribution of washed DDG samples was analyzed using a Ro-Tap device and a series of sieves with mesh sizes: 40, 60, 80, 100, and 200. Each sample was mixed and 100 grams were placed in the top sieve of the machine. The machine was run for five minutes, then the sieves were separated and the contents remaining on each sieve was weighed.

2.2.3.3 Toxin Analysis: Washed DDG samples were sent to Missouri University Veterinary Medical Diagnostic Laboratory to be evaluated for mycotoxins: Fumonisin B1, Aflatoxin B1, Ochratoxin A, Zearalenone, and Deoxynivalenol (DON) using High Performance Liquid Chromatography (HPLC).

2.2.3.4 Colorimetry (L*a*b*): A Minolta Colorimeter was again used to evaluate the color profile of washed DDG samples using the L*a*b* scale for color (Figure 2.2).

2.2.3.5 Flour Blending: Flour blends were prepared using a Cross Flow Blend Master Model B Lab Blender (Peterson Kelly Co. Inc., Stroudsburg, PA). Blends were

made in 1.5 kg batches using 5% and 10% substitution factors for both DDGS and HP-DDG. Great Value All Purpose Flour (APF) was used for the control and the base of the blends. All blends were mixed for 60 minutes using the "Shell Drive" setting. Blends were then stored in the refrigerator in zip-lock freezer bags until they were used.

2.2.4 Dough Analysis:

2.2.4.1 Dough Rheological properties: A Mixolab (Chopin Technologies, Villeneuve La Garenne, France) was used to evaluate the dough rheological properties of blends. This machine uses two mixer blades to mix flour samples with water, and subsequently measures the resistance of dough to mixing. This resistance directly correlates to the strength of the dough. The Chopin S test setting was used to determine absorption and the development time of the dough.

2.2.4.2 Dough Extensibility: A TA.XT*Plus* Texture Analyzer (Texture Technologies Corp., Hamilton, MA/Stable Micro Systems, Goldaming, Surrey, UK) was used to test dough extensibility. Ten grams of flour were mixed with the necessary amount of water in a 10-gram pin mixer. The amount of water and the mixing time was determined by the results of the Mixolab. Doughs were then pressed into a pre-oiled form and allowed to rest for 40 min. After the resting period was over dough strips were removed one at a time and placed into the Kieffer dough extensibility rig for testing. The test was run in the tension mode using a test speed of 3.30 mm/seconds. The target mode was set to distance and the distance used was 75.000 mm. All strips that were fully formed were used for testing,

any incomplete or misshapen dough strips were discarded. A pre-designed macro was then used to determine the resistance to extension, and extensibility.

2.2.5 Bread Preparation and Analysis

2.2.5.1 Bread Baking: Loaves were made using a modified AACC straight dough method 10-10B using 100g of flour (or flour substitute) as a basis for the dough (D'Appolonia and Youngs, 1978, Krishnan et al., 1987, Approved methods of the American Association of Cereal Chemists, 2000). Flour, salt, shortening, yeast, sugar and water (Table 2.1) were combined in a 100g pin mixer and mixed for 4 minutes. Dough was then placed directly into a lightly greased bowl and covered with plastic wrap. Doughs were proofed for 55 min in a proofing cabinet set at 30°C. A single punch was then performed by rolling the dough through a sheeter set to a roll width of 3 inches and a spacing of 5/16 inches. The dough was formed by rolling tightly by hand and placed in a greased pan (top inside: 4 ½ in x 2 $^{5}/_{8}$ in; bottom outside: 3 ¾ in x 2 in). Moulded loaves were again covered with cling wrap and placed in the proofing cabinet for 55 min. Upon completion of the second proofing loaves were removed and placed in a rotating oven set to 230°C for 20 min. Loaves were cooled for 1-2 hours before weighing and measurement of volume.

Table 2.1: Ingredient formulations used in the production of 5% and 10% DDG breads.

Ingredient	Control (APF)	5% DDGS* in APF	10% DDGS* in APF	5% HP-DDG in APF	10% HP-DDG in APF
APF**	100g	95g	90g	95g	90g
DDGS**	-	5g	10g	-	-
HP-DDG**	-	-	-	5g	10g
Salt (NaCl)	2.0g	2.0g	2.0g	2.0g	2.0g
Sucrose	5.0g	5.0g	5.0g	5.0g	5.0g
Dry Active Yeast	3.0g	3.0g	3.0g	3.0g	3.0g
Shortening	3.0g	3.0g	3.0g	3.0g	3.0g
Water	53.2g	59.0g	62.9g	55.2g	58.0g

*DDGS = DDGS with solubles removed as part of washing process.
**APF and food-grade DDG types (DDGS and HP-DDG) were blended together in appropriate proportions prior to baking and were not added individually.

2.2.5.2 C-cell: Digital image analysis of the bread cell structure was done using a C-cell machine (CC.300.06, Calibre Control International Ltd, Warrington, UK). Bread was sliced to 0.5 inches thick and images of corresponding slices were compared across bread formulations. This machine gave information on the cell size, number of cells, cell wall thickness, and the overall shape of the bread slices. This information was used in conjunction with the texture analysis information to evaluate the role of DDG in cell structure and bread texture.

2.2.5.3 Texture Analysis: A TA.XT*Plus* Texture Analyzer was used to test bread texture through Texture Profile Analysis (TPA). A cylinder 1 ¼ in tall and 7/8 in. in diameter was cut out of bread using a cutter provided with the machine. Cylinders were cut out along the y axis (Fig 2.4) The test was run with a pre-test speed of 1.00 mm/sec, a test speed of 5.00 mm/sec, and a posttest speed of 5.00 mm/sec. Additionally, the probe was set to compress to 10.000 mm. time was set to 5.00 seconds, and A trigger force of 5.0g was used (Crowley et al., 2002, Miñarro et al., 2010).

2.2.5.4 Loaf Weight & Volume: Samples were allowed to cool completely (1-2 hours) prior to measurement of weight and volume. Weight of each samples was taken by weighing on a scale with a maximum weight limit of 200.00 g. The rapeseed displacement test was used to determine the volume of each of the loaves of bread and compare them. Each loaf was placed in a container; rapeseeds were then added until a volume of 2000ml was reached. The volume of rapeseeds was measured in a graduated cylinder to determine loaf volume by difference.

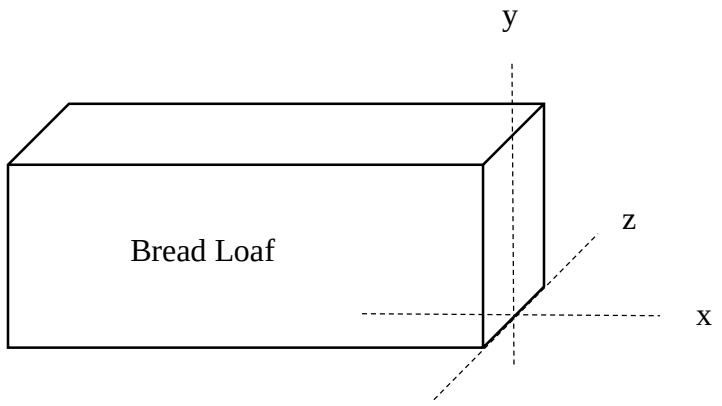

Figure 2.4: Description of sample cutting for texture analysis.

2.2.5.5 Proximate Analysis: Bread samples were sent to the University of Missouri ESCL to be analyzed for protein (AOAC Official Method 990.03), fat (AOAC Official Method Number 920.39) and ash content (AOAC Official Method 942.05). Total dietary fiber (TDF) (method number) and Amino Acid profile (AOAC Official Method 982.30) were also tested (Horwitz et al., 2006). Carbohydrates were determined by difference (Kraisid et al., 2003). Moisture content was determined at SDSU by oven drying (AOAC Official Method 930.15)(Horwitz et al., 2006).

2.2.5.6 Sensory: A seven-point hedonic scale was used to evaluate the acceptance of each of the samples on qualities including appearance, taste, texture, aroma, and overall acceptance. Descriptors which corresponded to the points ranged from "dislike very much" (1) to "like very much" (7). Ten participants were used in the study. Each participant tasted a half-slice of 5 samples (one of each treatment and the APF control). The study was conducted once.

2.2.5.7 Data Analysis: SPSS was used to run a One-way Analysis of Variance Test (ANOVA) and Duncan's multiple range test on the data to determine significance between means at $p < 0.05$. The effect of variables (DDG type, flour type, and level of substitution) on quality of flour, composition, rheology, and bread quality was determined.

3 RESULTS & DISCUSSION

3.1 Experimental Definition of "DDGS"

Prior to evaluating the results of this study, it is necessary to explain that during the washing process the solubles from distiller's dried grains with solubles (DDGS) were removed. Due to this the most accurate name for the product resulting from the washing of DDGS would be food-grade distiller's dried grains. However, for the purpose of clarity all treatments using washed materials resulting from DDGS were labeled as containing 5% or 10% "DDGS".

3.2 Comparison of Raw and Washed DDG

3.2.1 Yield

As in all food processing it is ideal to have as high a yield as possible from a process. The yields in preparation of HP-DDG samples and DDGS samples were relatively consistent across batches (Table 3.1). Yields of HP-DDG samples after washing ranged from 71% to 73% while those of DDGS samples ranged from 51% to 54%. The most likely explanation of the difference in yield between the two would be that the "solubles" portion was washed away from the DDGS. However, since the HP-DDG did not contain "solubles", a higher proportion of the sample was retained. A survey of US fuel ethanol plants in 2007 indicated that 62% of ethanol plant managers were interested in creating a food-grade co-product from DDG(S) (Saunders and Rosentrater, 2009). However, in order for this to happen an acceptable processing

procedure for DDGS must be in place. The yield of the DDG washing processes was quite low in this study. Even the yield for HP-DDG, which was considerably higher than that of DDGS, was likely not high enough to be accepted in its current state. While it is impossible to prevent all product loss, further improvements will need to be made to the process to limit product loss before it will be suitable for industry.

Table 3.1: Yield of batches of HP-DDG and DDGS after washing.

Batch number	Type	Initial weight (g)	Final weight (g)	Yield (%)
1	HP-DDG	989.5	717.0	72%
2	HP-DDG	986.5	705.1	71%
3	HP-DDG	860.0	631.7	73%
1	DDGS*	1062.0	543.9	51%
2	DDGS*	390.0	204.9	53%
3	DDGS*	878.0	478.4	54%

*DDGS = DDGS with solubles removed as part of washing process.

3.2.2 Proximate Analysis

Proximate analysis testing was conducted through the University of Missouri Agricultural Experiment Station Chemical Laboratory. Tests conducted included total dietary fiber (TDF), crude protein, moisture, fat, and ash. These results can be seen in Table 3.2. and Fig. 3.1. Prior to washing, all HP-DDG and DDGS samples were significantly different in TDF, crude protein, moisture, fat, and ash content. After washing the samples were found to be significantly different in only crude protein, fat, and ash.

It was found that the washing and drying procedure resulted in a relative increase in TDF and crude protein over the raw DDGS and HP-DDG samples. Conversely, the moisture, fat, and ash content of the washed and dried samples were lower than the raw samples. The amount of sugars and starches present in the samples was not tested but can be determined by difference using the sum of TDF, crude protein, fat, and ash and subtracting from 100%. In this manner we would find that carbohydrates in HP-DDG were reduced from 7.21% to nearly 0% and in DDGS they were reduced from 15.62% to 4.78%. Since we did not test the carbohydrate content of samples we cannot confirm the composition of particles which were lost. It is likely that product lost was not entirely carbohydrate, but also contained fines of protein and fiber as well as other components. Fats were likely extracted from the ground samples during ethanol washing, and water soluble minerals which were present in the sample were likely washed away in the fines that escaped through the mesh.

The findings on the proximate composition of raw and washed DDG were supported by the findings of Roth et. al (2015) and Tsen et al. (1983). The increases in

protein and fiber and the decrease in fat increased the nutritional and monetary value of food-grade DDG produced from DDGS and HP-DDG as fortification ingredients. Interestingly the lipids in the washed DDGS were reduced to a level which was close to that of the washed HP-DDG. In order to fortify foods with food-grade DDG we desire it to have a high protein and fiber content, but low fat and carbohydrate content. The large loss of fat from DDGS during washing and the insignificant difference between TDF content in washed HP-DDG, which made from fractionated corn, and DDGS samples shows that it would be beneficial to fractionate corn prior to fermentation. This not only results in a higher protein content, but also allows the oils to be recovered and sold rather than lost in the washing process.

Table 3.2: Average proximate composition of raw and washed DDGS and HP-DDG*.

Component	DDGS (raw)	DDGS** (washed)	HP-DDG (raw)	HP-DDG (washed)
TDF	38.40a	53.04c	44.86b	51.70c
	(± 0.07)	(± 0.51)	(± 0.69)	(± 0.40)
Crude Protein***	32.11a	38.72b	42.29c	47.62d
	(± 0.43)	(± 0.23)	(± 0.19)	(± 0.45)
Moisture	5.90b	0.89a	9.26c	0.45a
	(±0.04)	(± 0.89)	(± 0.17)	(± 0.39)
Fat	10.03d	1.71b	4.43c	0.75a
	(± 0.19)	(± 0.09)	(± 0.26)	(± 0.10)
Ash	3.85c	1.75b	1.21ab	0.76a
	(± 0.01)	(± 0.36)	(± 0.02)	(± 0.09)

* Results expressed on dry weight basis, mean values for dependent variables with differing letters within rows are significantly different across treatments ($p<0.05$).
**DDGS = DDGS with solubles removed as part of washing process.
*** Percentage N X 6.25. W/W%= grams per 100 grams of sample.

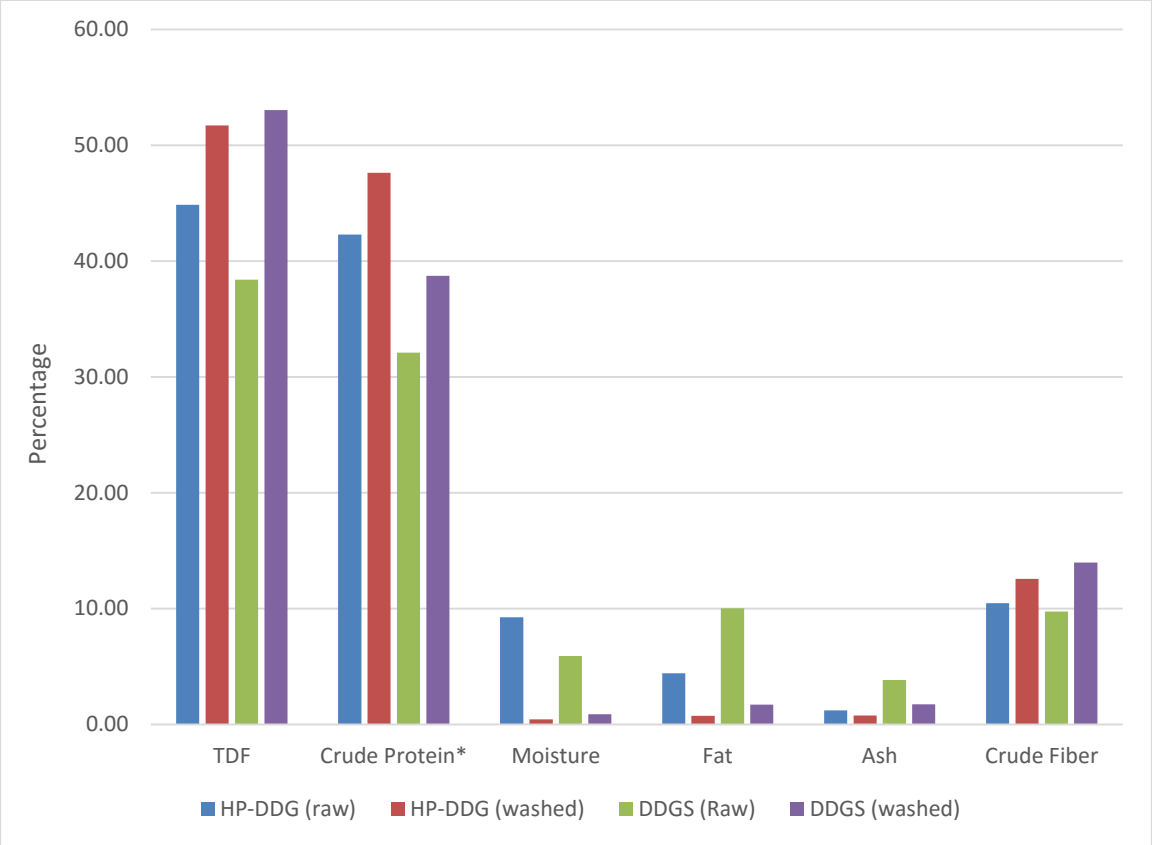

Figure 3.1: Comparison of proximate composition of raw and washed DDGS* and HP-DDG samples.
*DDGS = DDGS with solubles removed as part of washing process.

Corresponding to the increase in protein levels the respective amounts of amino acids in the washed samples increased over the raw samples. HP-DDG samples were noted as having significantly higher total amino acids than DDGS samples, and both types of DDGS were seen to be significantly higher in percent individual amino acids after washing. Table 3.3 displays the percent weight of amino acids in each sample for all 22 of the amino acids tested. With the exception of Lysine, both raw and washed HP-DDG samples were seen to be higher in all amino acids (present at >0.5%) than DDGS (Fig. 3.2). Glutamic Acid, Leucine and Proline were seen to be the three most prevalent amino acids in all samples. Tryptophan was the limiting essential amino acid in both samples. Washed HP-DDG was significantly higher than Washed DDGS in all amino acids including Tryptophan. This makes the protein supplied by HP-DDG more complete than that supplied by DDGS. Results of the amino acid analysis were supported by similar results by Spiehs et al (2002) and Gold (2005) who also analyzed amino acid content of DDGS from Minnesota and South Dakota (Rosentrater et al., 2005). In a protein assessment of corn distiller's grains with solubles, Dong et al. (1987) also found Alanine, Glutamic Acid, Leucine, and Proline to be the most prevalent amino acids in DDGS. Data from the study also showed that the amino acid profile of DDGS is very similar to that of corn (Dong et al., 1987).

Table 3.3: Amino Acid analysis of raw and washed DDGS and HP-DDG*.

Amino Acid	DDGS (Raw)	DDGS* (washed)	HP-DDG (raw mix)	HP-DDG (washed)
Taurine	0.05a	0.07a	0.06a	0.04a
Hydroxyproline	0.19b	0.10a	0.07a	0.07a
Aspartic Acid	1.98a	2.42b	2.58c	2.91d
Threonine	1.24a	1.44b	1.55c	1.72d
Serine	1.40a	1.64b	1.82c	2.04c
Glutamic Acid	4.25a	6.58b	7.09c	8.84d
Proline	2.48a	3.18b	3.61c	4.32d
Lanthionine	0.00	0.00	0.00	0.00
Glycine	1.35a	1.49b	1.54b	1.68c
Alanine	2.19a	2.84b	3.12c	3.63d
Cysteine	0.58a	0.76b	0.79c	0.91d
Valine	1.62a	1.92b	2.11c	2.38d
Methionine	0.65a	0.82b	0.88c	1.01d
Isoleucine	1.29a	1.53b	1.73c	1.96d
Leucine	3.83a	4.95b	5.69c	6.65d
Tyrosine	1.19a	1.40b	1.55c	1.85d
Phenylalanine	1.91a	1.96a	2.19b	2.61c
Hydroxylysine	0.07c	0.02b	0.00a	0.02b
Ornithine	0.03b	0.015a	0.02ab	0.015a
Lysine	1.18b	1.23c	1.14a	1.24c
Histidine	0.85a	1.02b	1.12c	1.28d
Arginine	1.39a	1.53b	1.50b	1.67c
Tryptophan	0.29a	0.31a	0.31a	0.34b
Total	29.97a (± 0.25)	37.18b (± 0.54)	40.44c (± 0.47)	47.15d (± 0.16)

*Results are expressed on a dry weight basis, mean values for dependent variables with differing letters are significantly different across treatments ($p<0.05$).
*DDGS = DDGS with solubles removed as part of washing process.

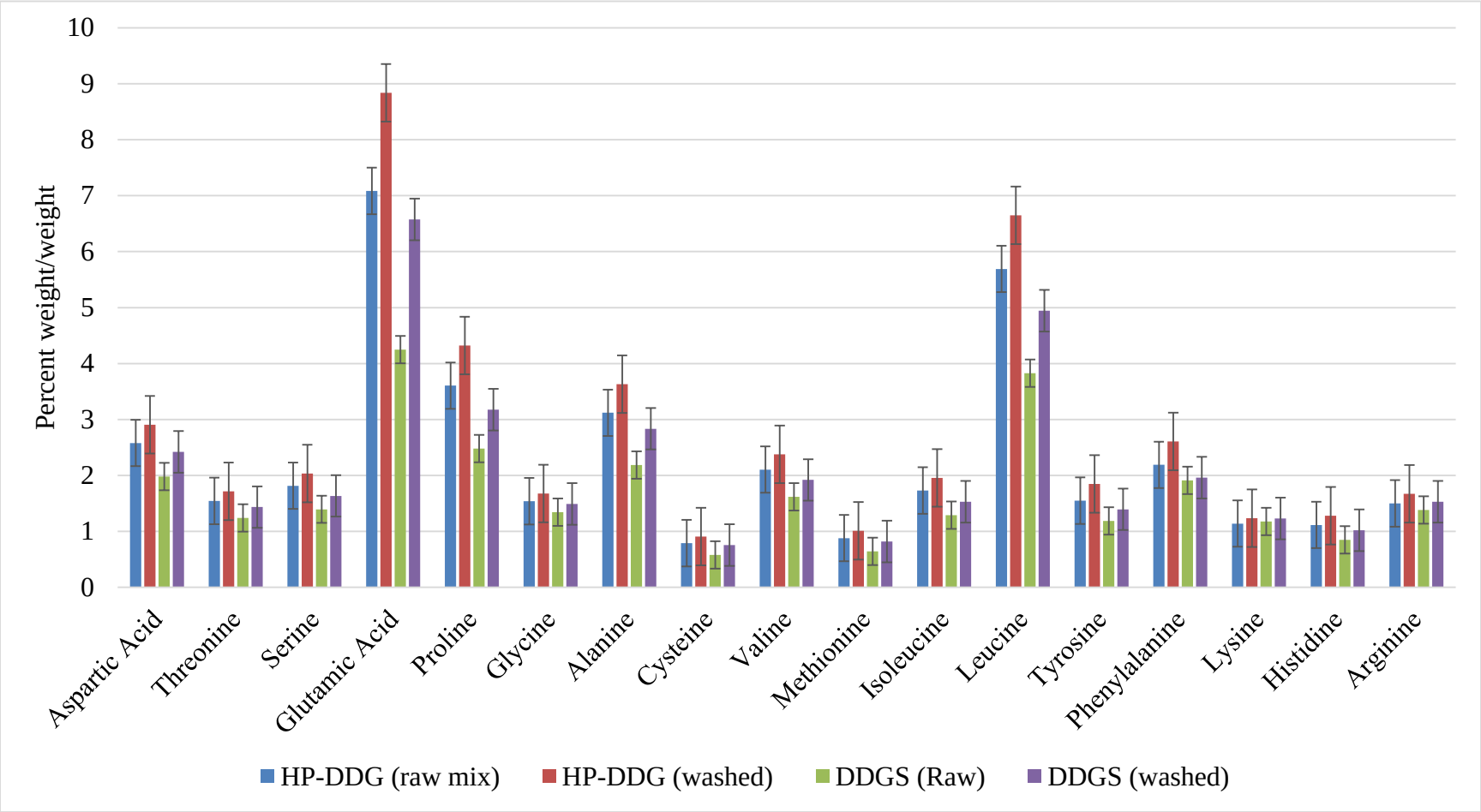

Figure 3.2: Percent weight of amino acids* per weight of sample in raw and washed HP-DDG and DDGS**
*Amino acids which comprised less than 0.5% of the total amino acid content were excluded from the graph.
*DDGS = DDGS with solubles removed as part of washing process.

3.2.3 Particle Size Analysis

Particle size of both types of DDG samples (DDGS and HP-DDG) were measured before grinding, after grinding, and after washing the ground samples (Table 3.4, Fig. 3.3 A&B). Significant differences were seen after grinding and washing, however similar trends were seen for both DDG types (DDGS and HP-DDG). Prior to grinding, the majority of raw HP-DDG and DDGS samples were larger than 400 μm in particle size with the percentage of sample retained on each of the following sieves decreasing along with the particle size retained. In comparison, the trend seen for the ground and washed samples were more "bell-shaped" with the majority of particles being around 250 μm. This outcome was expected as the object of grinding was to reduce particle size.

When comparing the ground and washed samples, a slight shift was seen in particle size with the ground samples having a slightly higher percentage of particles in the >400, 400, and 250 μm groups while the washed samples had higher percentages in the 180, 150, and ≤75 μm groups. It was expected that the smallest particles would be lost during washing and therefore lead to a reduction rather than an increase in the percentage of small particles. As seen in Fig. 3.4 A & B static interactions caused clumping of particles when evaluating particle size for the ground but unwashed samples. The washing and drying procedures decreased the ability of the particles to interact with each other, preventing them from aggregating and allowing them to flow more freely through the sieves.

Small particles were desirable in order to increase the textural and visual uniformity in bread and dough samples. Flour particles are typically smaller on average than DDG. Research done by Li (2014) showed that the majority of flour particles were

between 150 and 250 µm in size with 0% of particles > 400 µm, 41.40% between 400 and 250 µm, and 39.86% between 180 and 150 µm. Similarly, a study conducted by Hareland (1994) on the particle size distribution of flours from both hard and soft wheat found that 89-98% of flour particles were between 10 µm and 300 µm, while 2-11% of particles were < 10 µm.

In a study using wheat based DDG in bread, Roth et al. (2015) used samples with particle sizes ranging from <1250 µm to <250 µm and found no significant difference on bread quality. Given this, it may not be necessary to reduce particle size further to improve the structural quality of bread. However, it may be beneficial to eliminate large particles to prevent structural damage to air cells as well as particles from being identified within slices.

Table 3.4: Particle size analysis of un-ground, ground, and washed DDGS and HP-DDG using 40, 60, 80, 100, and 200 mesh sieves in a Ro-tap sieve shaker*.

Particle Size (µm)	Un-Ground DDGS	Ground DDGS	Washed DDGS**	Un-Ground HP-DDG	Ground HP-DDG	Washed HP-DDG
>400	76.3e	3.58a	7.82b	60.8d	21.8c	10.7b
	(± 0.464)	(± 0.108)	(± 3.61)	(± 0.641)	(± 0.349)	(± 0.330)
>250 - 400	13.9a	22.1bc	19.0ab	17.0ab	24.7c	21.7bc
	(± 0.265)	(± 5.52)	(± 5.73)	(± 0.056)	(± 0.302)	(± 0.455)
>180 - 250	6.32a	64.7d	50.1c	9.99a	35.9b	35.1b
	(± 0.257)	(± 3.29)	(± 2.64)	(± 0.396)	(± 3.78)	(± 1.89)
>150 - 180	2.11a	6.90b	15.3c	6.38b	15.3c	25.4d
	(± 0.010)	(± 2.91)	(± 2.72)	(± 1.24)	(± 0.080)	(± 2.51)
>75 - 150	1.41a	0.647a	4.73b	3.86b	1.06a	3.96b
	(± 0.064)	(± 0.251)	(± 3.29)	(± 0.993)	(± 3.81)	(± 0.653)
≤ 75	0.457ab	0.18a	1.87cd	1.35bc	0.03a	2.67d
	(± 0.099)	(± 0.227)	(± 0.910)	(± 0.601)	(± 0.038)	(± 0.309)

*Mean values for dependent variables with differing letters within rows are significantly different across treatments ($p<0.05$).
*DDGS = DDGS with solubles removed as part of washing process.

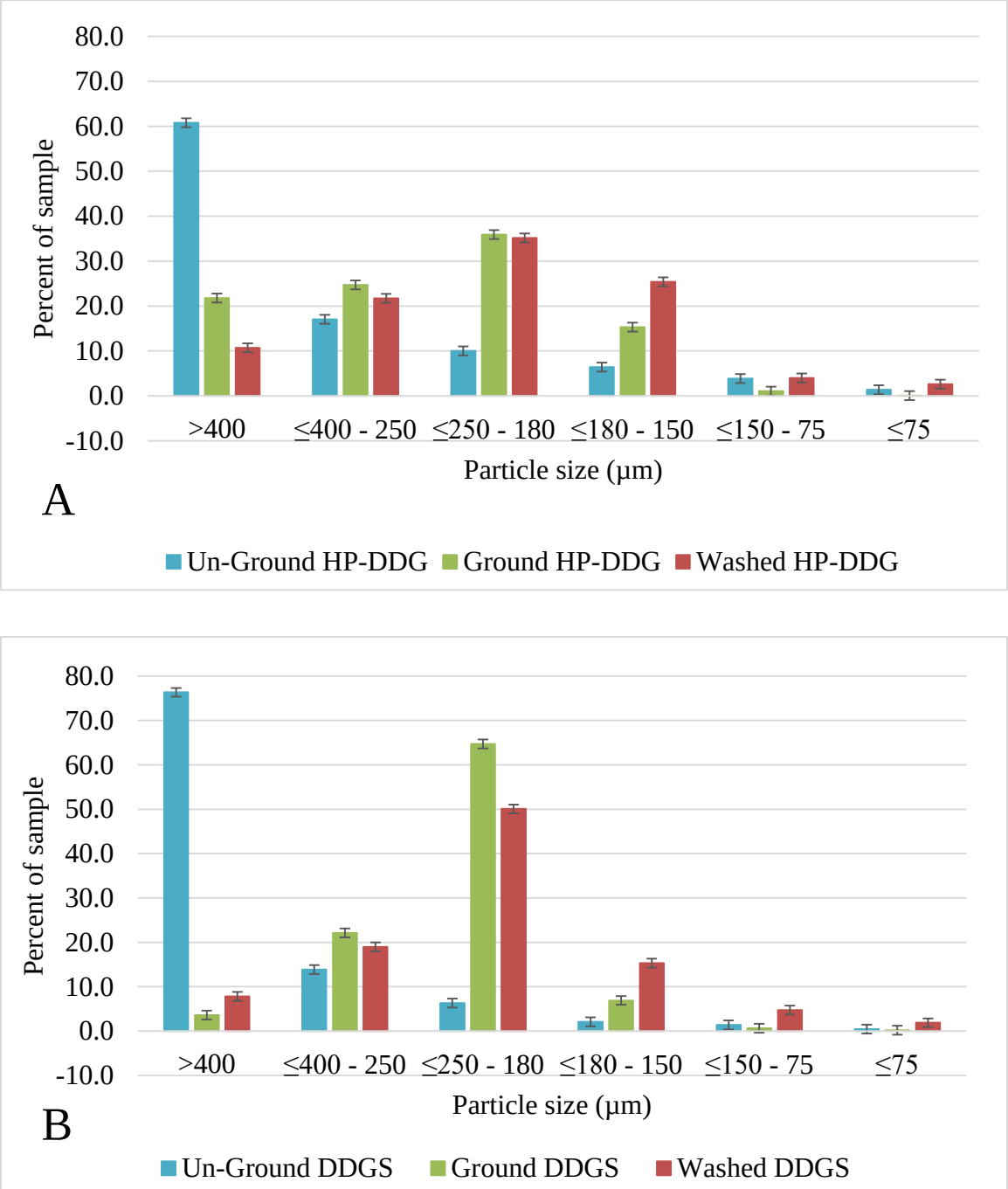

Figure 3.3: Particle size of unground, ground, and washed* HP-DDG and DDGS** samples.
A) HP-DDG; B) DDGS
*refers to samples which were washed after grinding
**DDGS = DDGS with solubles removed as part of washing process.

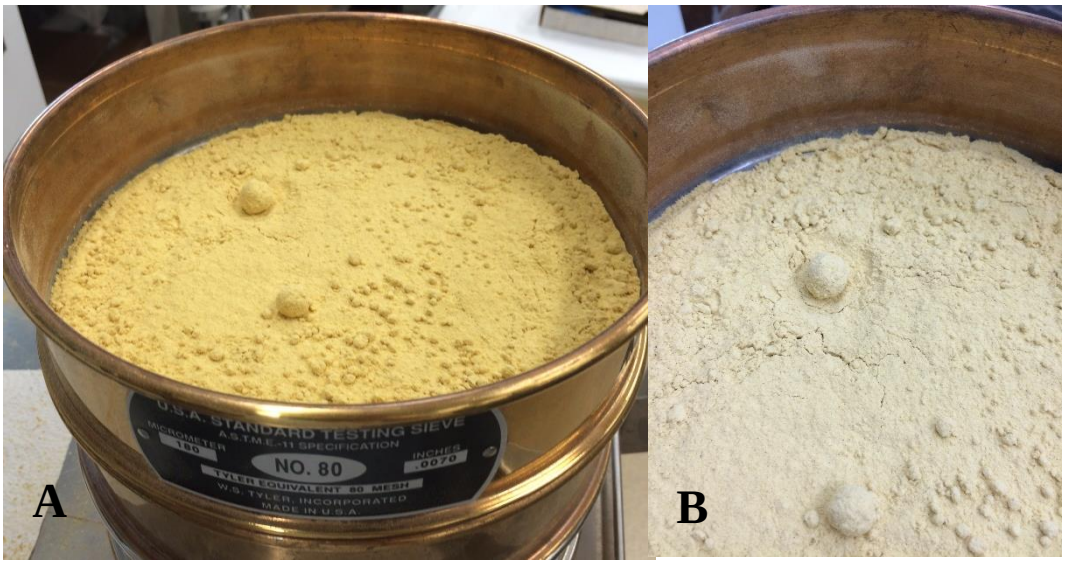

Figure 3.4: Ground DDGS on 80 mesh sieve after 5 minutes of agitation in the Ro-tap machine. A) Entire pan B) close-up

3.2.4 Colorimetry

Changes in color of both processed DDG samples were seen after the washing protocol (Table 3.5). The decrease of color parameter a* and b* values toward zero in both samples shows the decrease in redness and yellowness, respectively. The increase of the L* parameter toward 100 indicates that both HP-DDG and DDGS increased in brightness through washing. All changes in color between raw and unwashed samples were seen to be significantly different. With the exception of the b* value (yellowness) all washed and unwashed DDGS were seen to be brighter and had a lower color intensity than the corresponding HP-DDG sample. These differences were determined to be significant. While there was not a significant difference in yellowness of raw samples, DDGS samples were noted as being significantly less yellow than HP-DDG samples after washing.

The nature of the initial samples is the likely cause of the initial differences in color that was carried through washing. For example, HP-DDG had high redness (6.38) prior to washing, and after washing (5.88). The perception of flour color depends on the product in which it will be used. In white bread, bleached flours are typically used so that the bread interior (crumb) is practically pure white with little hint of yellow. However, the color standard for whole wheat bread or chocolate cake is very different. In the first example, the natural color of the grain is allowed to come through and the final color is an identifying factor of whole wheat bread. In the second, chocolate gives a distinct color to the cake and makes the color of the added flour less noticeable. In our study, the degree of yellowness (b value) was still quite high after washing (27-23). If food-grade DDG is to be incorporated into white bread the washing process will need to be improved

to enhance the reduction of yellowness. At the same time, further testing must be done to determine the acceptability of the color imparted to sandwich bread by food-grade DDG.

Table 3.5: Color evaluation of raw and washed DDGS and HP-DDG[1].

Parameter[2]	DDGS[3]		HP-DDG	
	raw	washed	Raw	washed
L*	74.51c	78.85d	64.83a	71.13b
	(± 0.31)	(± 0.97)	(± 0.85)	(± 0.99)
a*	2.24b	1.81a	6.38d	5.88c
	(± 0.10)	(± 0.43)	(± 0.58)	(± 0.34)
b*	33.83c	23.51a	33.22c	27.83b
	(± 0.64)	(± 1.07)	(± 1.15)	(± 0.43)

[1] Mean values for dependent variables with differing letters are significantly different across treatments ($p<0.05$).

[2] L: (0 = black, 100 = white); a: (positive = red, negative = green); b: (positive = yellow, negative = blue)

[3]DDGS = DDGS with solubles removed as part of washing process.

3.2.5 Toxicology

The possible presence of toxins including Fumonisin, Aflatoxin, Ochratoxin, Zearalneone, and Deoxynivalenol (DON) in grains pose a potential safety concern to both animals and humans (Miller, 2001). These toxins are produced by microscopic filamentous fungi commonly found in corn and other cereal grains, and occur naturally in crops based on seasonal weather conditions (FDA, 1992, Oplatowska-Stachowiak et al., 2015). Due to their natural occurrence, it is impossible to totally prevent their existence in food products, rather their incidence must be limited to the lowest practical levels attainable using modern processing technology. Although they are naturally occurring substances, mycotoxins are regulated as adulterants in food due to their ability to be prevented (Wood, 1992, Price et al., 1993). A variety of monitoring programs and government regulations are in place to assure that levels do not exceed limits (FDA, 1992, Services, 2001, 2010). Studies to determine the fate of mycotoxins in contaminated corn used for ethanol production have generally supported that the toxins are not destroyed during ethanol production, that the resulting ethanol contains no toxins, and that toxins collect in the distiller's grains (Bothast et al., 1992). During ethanol production one ton of grain produces approximately 0.33 tons DDG. Therefore, it is estimated that mycotoxins in DDG become concentrated up to 3 times the concentration of toxins in the starting material (Oplatowska-Stachowiak et al., 2015). Based on this information there was a concern that the DDG used in this study would have high amounts of mycotoxins. In order to assure samples were safe for human consumption they were tested for toxin content before and after washing.

Toxicology testing was performed by the Veterinary Medical Diagnostic Laboratory at Missouri University using High Performance Liquid Chromatography (HPLC). As seen in Table 3.6, of the 5 mycotoxins tested for, none of them were detected in either of the washed samples. While the sample used was judged to be representative of the DDG supplied to us, there is potential for large variability of toxin concentration across samples (Outreach, 2012). Thus it is important to consider that other DDG samples which have been processed in the same manner may have different toxin levels due to initial corn contamination.

Due to the inability to fully prevent the presence of mycotoxins in grains and other products the FDA has set specific acceptable limits for products used for humans or for animal feed. In general, the acceptable limit for human foods is lower than that of animal feed. These limits are as follows: 20 ppb Aflatoxin B1 (Services, 2005), 1 ppm DON (also known as Vomitoxin) in finished wheat products (Services, 2010), 4 ppm total Fumonisins (FB1 + FB2 + FB3) in whole or partially degermed dry milled corn products (Fat content >2.25%), and 2 ppm total Fumonisins (FB1 + FB2 + FB3) in De-germed dry milled corn products (Fat content <2.25%)(Services, 2001). There are currently no FDA limits for Ochratoxin or Zearalneone in corn or grain products. Thus, based on the results of our testing, both samples can be deemed safe for human consumption.

Currently there are no approved methods for reduction of mycotoxins in corn. Blending multiple batches of corn in order to reduce the overall mycotoxin concentration is not allowed by the FDA and is considered a form of adulteration according to FDA section 402(a)(2)(A). During the 1980's, numerous research studies were done to test the

effectiveness of ammonification of grains in the reduction of aflatoxins; this was done in part to encourage FDA approval of the method for animal feeds (Anderson, 1983). While the FDA has approved methods for reduction of aflatoxins in products such as cottonseed and rice hulls for animal feed, the process has not been approved for corn (U.S. Food and Drug Administration (FDA), 2015b). It is not currently known whether the reduction of mycotoxins through washing DDG(S) would be a process accepted by the FDA. In order for this to be accepted the process would have to be proven safe to consumers. We see the likelihood of this process receiving approval in the future as high given that the process is a food-safe one.

Table 3.6: Toxin content of washed HP-DDG and DDGS* samples using HPLC**.

Mycotoxin	Washed DDGS	Washed HP-DDG
Fumonisin B1[a]	ND***	ND
Aflatoxin [b]	ND	ND
Ochratoxin [c]	ND	ND
Zearalenone [d]	ND	ND
Vomitoxin [e]	ND	ND

*DDGS = DDGS with solubles removed as part of washing process.
**High Performance Liquid Chromatography method
***Not Detected
a: Fumonisin B1 detection limit 500 ppb (0.5 ppm)
b: Aflatoxin detection limit 10 ppb (0.01ppm)
c: Ochratoxin A detection limit 50 ppb (0.05 ppm)
d: Zearalenone detection limit 250 ppb (0.25 ppm)
e: Vomitoxin detection limit 500 ppb (0.5 ppm)

3.3 Dough Testing

3.3.1 Mixolab

The Mixolab was used to evaluate dough development and water absorption of each blend. Blends were tested until a dough consistency resulting in a machine torque of 1.0 – 1.2 Nm was obtained. The mixing time and water absorption was then documented and used in the evaluation of dough extensibility. Table 3.7 shows the data obtained from the Mixolab. Blends containing 10% DDGS or HP-DDG had higher water absorption values than the respective 5% blends. This was likely due in part to a lower initial moisture content of the DDG incorporated into those samples. Research completed by Saunders et al. (2014) in which DDGS was substituted for flour at levels of 25% and 50% also showed significantly higher water absorption in blends containing DDGS.

Development times for the HP-DDG blends were much longer than the APF (control) and DDGS blends. Other research has suggested that this is due to the increase in time necessary for particle hydration (Dreese and Hoseney, 1982, Tsen et al., 1983, Ktenioudaki et al., 2013b). The addition of BSG was found by Ktenioudaki et al. (2013b) and Dreese and Hoseney (1982) to also increase mixing time. In comparison Tsen et al. (1983) found that incorporation of DDG decreased dough development time, while Rasco et al. (1990) found no differences from the control. Test baking of bread made with all-purpose flour was done using the Mixolab-suggested development time. From this test it was determined that 1 minute of mixing was insufficient for dough development so mixing time was increased until the dough could be stretched to form a thin film without tearing. A mixing time of 4 minutes was found to be sufficient for all dough types.

Table 3.7: Mixolab analysis of APF and APF/DDG blends containing 5% and 10% DDGS* and 5% and 10% HP-DDG.

Parameter	APF (control)	5% DDGS in APF	10% DDGS in APF	5% HP-DDG in APF	10% HP-DDG in APF
Percent moisture**	10.6	11.0	10.7	10.4	10.1
Percent water absorption	53.2	59.0	62.9	55.2	57.0
Development time (min)	1.07	1.3	1.15	4.0	4.23

*DDGS = DDGS with solubles removed as part of washing process.
**Moisture determined prior to Mixolab testing using a forced air oven.

3.3.2 Dough Extensibility

A kieffer dough rig was used on a TA.XT*Plus* texture analyzer to evaluate the extensibility and resistance to expansion of each type of dough. Both of these parameters are important factors in the expansion and gas retention of doughs (Ktenioudaki et al., 2013b). A significant difference in the resistance to extension was found between all samples tested. APF samples took the greatest force to extend followed by 5% DDGS, 10% DDGS, 10% HP-DDG, and 5% HP-DDG, respectively. While there were differences between the average extensibility of the dough types, only the extensibility of dough from the 5% HP-DDG blends was found to be significantly different from the other samples. Among the samples which were not significantly different, APF was least extensible and 10% HP-DDG was most extensible. Table 3.8 shows the results of this testing. Gluten is a native wheat protein which is responsible for the extensibility of doughs (Damodaran et al., 2007). As flour was substituted with DDG the amount of gluten in the dough was diluted, this reduced the strength of the doughs and caused a decrease in resistance to extension. As the resistance to extension to decreased it became easier for the doughs to be stretched, this trend was noted in the increase in measured extensibility of treatments.

In contrast to the this study, Arra (2011) found a negative correlation between DDGS inclusion and extensibility when used in chapathi doughs made with whole wheat and bread flour. Levels of DDGS inclusion used in this study were 0%, 10%, and 20% DDGS. The differences in dough formulation, amount of gluten contributed by the flour, and the degree of gluten development in the dough likely account for the observed differences between the doughs. Chapathies are similar to pizza dough in that they have

highly developed gluten and are relatively tough and chewy after baking. This requires a high level of gluten development and results in a high dough extensibility. In comparison sandwich bread is less chewy and requires a balance between attaining the necessary amount of gluten to retain leavening gases, but not too much to compromise the tenderness of the final product.

Ktenioudaki et al. (2013b) found similar results to Arra (2011) for the extensibility and resistance to extension of doughs supplemented with 15%, 25%, and 35% BSG. Extensibility decreased from 71 mm for the control to less than 30 mm for the 15% inclusion and less than 10 mm for the 35% inclusion. Maximum force to extend slightly increased for 15% BSG samples but decreased for 25% and 35% samples. The dough formulation used was not specified in this study so comparisons cannot be made between the types of dough. Since the Mixolab recommended development times were used it is possible that the doughs used for the extensibility measurements in my experiment were not fully developed. Further testing comparing dough development time and extensibility would be necessary to clarify this.

Table 3.8: Average resistance to extension, extensibility for APF, 5% HP-DDG[1], 10% HP-DDG, 5% DDGS[2], and 10% DDGS* blends**.

Sample Treatment	Resistance to Extension (g)	Extensibility (mm)
APF (Control)	73.78e	15.72a
	(± 5.17)	(± 0.96)
5% DDGS in APF	64.46d	17.56a
	(± 4.86)	(± 0.71)
10% DDGS in APF	58.35c	16.59a
	(± 2.36)	(± 0.73)
5% HP-DDG in APF	35.00a	35.64b
	(± 3.23)	(± 8.29)
10% HP-DDG in APF	43.23b	21.13a
	(± 1.55)	(± 4.45)

*DDGS refers to the food grade product resulting from distiller's dried grains with solubles after washing and does not contain solubles from the ethanol production process
**Mean values for dependent variables within columns with differing letters are significantly different across treatments ($p<0.05$).
[1]HP-DDG = high protein distiller's dried grains
[2]DDGS = DDGS with solubles removed as part of washing process.

3.4 Bread Testing

3.4.1 Weight and Volume

Bread volume is a key quality parameter for determining the efficiency of the proofing process as well as evaluating loaf density which is directly correlated to the structural quality of the bread crumb (Scanlon and Zghal, 2001). Weights and volumes of each loaf were taken after loaves had fully cooled (1-2 hours after removal from oven). Densities were then calculated based upon these measurements. Means for each bread type are shown in Table 3.9. Significant differences were seen in loaf weight and volume across treatments. APF loaves had the lowest average weight and were significantly lighter than all other loaves. 10% DDGS loaves were significantly heavier than the other samples including 5% DDGS or 5% and 10% HP-DDG. This trend could be due to the DDG having a higher moisture retention during baking.

An opposite trend was seen with loaf volume. APF loaves were seen to have significantly higher volume than other samples while 10% DDGS were significantly smaller. Together these resulted in APF loaves having the lowest densities, followed by 5% DDGS and HP-DDG, 10% HP-DDG and 10% DDGS. While 5% DDGS and 5% HP-DDG were not significantly different from one another all other bread types were significantly different in density. A similar trend was found in the weight and loaf volume of bread including 10% and 20% DDG by Tsen et al. (1983). Loaf weight increased with the addition of DDG, while loaf volume decreased. The same study by Tsen et al. (1983) also found that breads made with 10% DDG were denser than white bread, but less dense than whole wheat bread.

Table 3.9: Mean weight, volume, and density of bread loaves made from APF, 5% DDGS, 10% DDGS, 5% HP-DDG, and 10% HP-DDG*.

Parameter	APF	DDGS**		HP-DDG	
		5% in APF	10% in APF	5% in APF	10% in APF
Weight (g)	141.19a (± 0.78)	147.48c (± 1.64)	152.13d (± 1.86)	143.46b (± 1.14)	146.70c (± 2.02)
Volume ($cm^{3)}$	507d (± 26.81)	440c (± 30.19)	311a (± 19.09)	421c (± 25.05)	346b (± 23.72)
Density (g/cm^3)	0.279a (± 0.02)	0.335b (± 0.03)	0.489d (± 0.03)	0.341b (± 0.02)	0.424c (± 0.03)

*Mean values for dependent variables within rows with differing letters are significantly different across treatments ($p<0.05$).
**DDGS = DDGS with solubles removed as part of washing process.

3.4.2 Proximate Analysis

Since the main reason for the addition of food-grade DDG to bread was to increase its protein and fiber content, the proximate analysis of bread samples was one of the more important aspects of this study. As seen in Table 3.10 significant differences were seen in TDF, crude protein, moisture, and ash. However, there was not a significant difference between fat content of samples. While total mineral analysis was not done on the ash from bread samples, breads containing either type of food-grade DDG had significantly higher ash contents than the control. Of the two types, bread with DDGS had significantly higher ash content than bread with HP-DDG.

The trend for moisture content of each type of bread matches the Mixolab water absorption percentages required for dough formation. While the amount of water used in the doughs was based on the Mixolab values, the amount of moisture added was expected to bring the total moisture of the dough equivalent to 60%. Since the moisture content of the dough was not measured, it is unclear whether the optimum moisture content of 60% was achieved.

While the 10% DDGS sample was the only one found to be significantly different in TDF than all other samples, some numerical differences were noted between the control and the other food-grade DDG containing samples. APF samples were seen to have approximately half as much TDF as 5% and 10% HP-DDG samples and slightly less than three-quarters the amount found in 5% DDGS samples. Unfortunately, there were some inconsistencies between the replicate values which likely affected the significance of the results. It is possible that the small loaf size and small sample size

contributed to inconsistent results. Better results would likely be obtained if samples were tested in triplicate or quadruplicate.

Of all the proximate components tested crude protein content exhibited a trend closest to what was hypothesized. All types of bread were seen to be significantly different from one another. Breads containing 10% food-grade DDG had significantly higher protein content than those containing 5%, and breads containing HP-DDG had significantly higher protein than the respective DDGS breads. This is logical due to HP-DDG's significantly higher protein content over DDGS (Table 3.2).

The reference amount customarily consumed (RACC) for bread is 50 grams (U.S. Food and Drug Administration (FDA), 2015a). This equates to approximately 2 slices of sandwich bread. Table 3.11 shows the protein, TDF, moisture and fat values for all breads used in this study as well as commercial white bread. Differences were seen between the compositions of breads made in this study and the store-bought bread. On a 50-gram as consumed basis, breads made with the 10% DDGS blends, and the 5% and 10% HP-DDG blends were higher in TDF than the store-bought bread. The crude protein of all samples was seen to be higher than the protein value calculated for 50g of store-bought white bread. Moisture was not tested for the store-bought bread, however Ranhotra and Gelroth (1988) found similar results for TDF content of commercial white bread (2.51g/100g) which had a moisture content of 38%.

According to the FDA labeling regulations, in order to claim a food is a good source of a particular nutrient it must contain between 10% and 19% of the daily recommended value for that nutrient (U.S. Food and Drug Administration (FDA),

2015c). The daily recommended value for protein by adults is 50g (U.S. Food and Drug Administration (FDA), 2016). Breads containing DDGS and HP-DDG at the 5% and 10% levels would qualify to be labeled as a “good source” of protein.

A 50g serving of the 10% DDGS bread would surpass 2.5g of fiber, the value needed to equal 10% of the recommended intake for women. However, it would not be sufficient to meet the corresponding value for men of 3.8g. Since nutritional labels are typically based on a 2,000 calorie diet it may be possible to use a “good source” of fiber claim on the bread package.

Table 3.10: Proximate analysis of bread made with blends containing 5% and 10% food-grade DDG*.

Component	APF	DDGS**		HP-DDG	
		5% in APF	10% in APF	5% HP-DDG	5% in APF
TDF	2.77a (± 0.767)	4.29a (± 0.258)	11.6b (± 2.17)	6.25a (± 1.48)	6.28a (± 0.680)
Crude Protein***	14.3a (± 0.044)	15.8b (± 0.049)	17.0d (± 0.036)	16.1c (± 0.021)	17.9e (± 0.029)
Moisture	39.0a (± 1.11)	49.5d (± 0.139)	49.7d (± 1.00)	41.3b (± 0.517)	44.5c (± 1.52)
Fat	2.57 (± 0.202)	2.86 (± 0.001)	3.55 (± 0.545)	3.04 (± 0.117)	3.26 (± 0.112)
Ash	2.64a (± 0.008)	2.75c (± 0.004)	2.77c (± 0.003)	2.67b (± 0.001)	2.67b (± 0.011)

* Results expressed on dry weight basis, mean values for dependent variables with differing letters within rows are significantly different across treatments ($p<0.05$).
**DDGS = DDGS with solubles removed as part of washing process.
*** Percentage N X 6.25. W/W%= grams per 100 grams of sample.

Table 3.11: Comparsion of average proximate composition of food-grade DDG containing breads to whole wheat and white sandwich breads.

Component	APF	DDGS		HP-DDG		Great Value White Bread**	
		5% in APF	10% in APF	5% in APF	10% in APF		
Sample Size	50g	50g	50g	50g	50g	50g	28g
TDF (g)	0.98	1.40	3.77	2.16	2.12	<1.78	<1
Crude Protein* (g)	5.05	5.18	5.55	5.57	6.03	-	-
Protein (g)	-	-	-	-	-	3.75	2
Moisture	28%	33%	33%	29%	31%	NM	NM
Fat (g)	0.91	0.93	1.16	1.05	1.10	0.89	0.50

* Percentage N X 6.25

** Information collected from the nutritional label of Great Value White Sandwich Bread (24 oz) at Walmart in Brookings, SD.

TDF = Total Dietary Fiber

NM = Not measured

3.4.3 C-Cell

The c-cell machine uses a digital imaging system to capture a picture of the sample and then evaluates it based on a number of parameters. For the bread, parameters of particular interest were loaf size and shape, and air cell size and uniformity. The four center slices of bread were used for loaf analysis. Table 3.12 includes the dimensions of slices of each type of bread tested. The data shows significant differences between the slice height, width, and area. Bread made with all-purpose flour was the largest followed by 5% DDGS and 5% HP-DDG samples which were not significantly different from each other. Interestingly, the 10% HP-DDG samples were found to be 267mm^2 larger than the 10% DDGS samples. The higher concentration of protein present in HP-DDG may have facilitated a stronger dough matrix and enabled more dough expansion during proofing as well as retention of gas during baking. The size difference in slices can be seen in Fig. 3.5.

Table 3.12: Dimensions of bread slices taken from loaves made with APF, and blends including 5% and 10% DDGS* and 5% and 10% HP-DDG. *

	slice area (mm^2)	max height (mm)	width (mm)
APF	5537d	93.6d	78.0d
	(± 88.49)	(± 1.89)	(± 0.778)
5% DDGS** in APF	5112c	88.8c	74.2c
	(± 138.2)	(± 1.87)	(± 1.79)
10% DDGS in APF	4097a	76.1a	65.7a
	(± 105.2)	(± 1.75)	(± 0.862)
5% HP-DDG in APF	5092c	89.9c	73.3c
	(± 130.7)	(± 1.70)	(± 1.51)
10% HP-DDG in APF	4364b	79.9b	67.8b
	(± 66.91)	(± 0.900)	(± 1.06)

*Mean values for dependent variables with differing letters within rows are significantly different across treatments ($p<0.05$).
**DDGS = DDGS with solubles removed as part of washing process.

C-cell imaging also aids in the evaluation of internal structure of breads. A key aspect of bread texture is its foam-like structure. Gas bubbles are formed in the dough during mixing. When the dough is fermented yeast produce CO_2 and cause these bubbles to expand during proofing. Heat applied during baking then gelatinizes the starch in the dough and solidifies them into a stable structure. The number and size of cells present in a bread sample are determined by the procedure used to make the dough and form the loaf. Ideally, bread will have a large number of small and uniform air cells; cell walls should be thin to give a light spongy texture to the interior (Pyler, 1988, Scanlon and Zghal, 2001).

The parameters used by the c-cell to describe bread structure were: number of cells, percent area of cells, wall thickness, number of holes, percent area of holes and non-uniformity (Table 3.13). Slices of bread made with APF and 5% DDGS blends had significantly more air cells than breads made from 5% and 10% HP-DDG blends. Slices of the 10% DDGS bread had the lowest number of air cells and were found to be significantly different from all other groups.

A similar trend was seen in respect to percent area of air cells. The percent area of cells was significantly higher in slices from APF and 5% HP-DDG bread. This was followed by the 5% DDGS group. Air cells in slices from 10% DDGS and 10% HP-DDG comprised a significantly lower percentage of the slice than the other 3 groups. A higher percent area of air cells accompanied by a large number of air cells is desirable in sandwich bread because this correlates with a light and voluminous bread. Differences in number and size of air cells was likely due to differences in the ability of the doughs to retain gas bubbles, but during mixing when bubbles are initially formed and during

proofing when air cells expand. Since incorporation of DDG decreases dough strength and resistance to extension it is easier for the air-cells to break or collapse during the bread making process, leading to fewer and smaller air cells.

Interestingly an opposite trend was seen in relation to cell wall thickness. While it was expected that bread made with APF would have many small air cells with thin cell walls it was seen that the air cells in the 10% HP-DDG samples had the thinnest cell walls. This was followed by 10% DDGS, 5% DDGS, APF and 5% HP-DDG, respectively. This could possibly be explained by the cell walls being too thin to hold the gas inside or by large DDG particles compromising the structure of the thin cell walls and causing reduced slice area.

Holes were seen in all bread samples evaluated. Those made with 5% HP-DDG and 10% DDGS had significantly fewer holes than the other three types. As expected the samples with the most holes had the highest percent area of holes. The degree of non-uniformity followed the same trend as the number of holes. This is because uniformity largely reflects the presence and size of any holes. The mechanical processes used in dough formation and development (punching, sheeting, and moulding), impact the final dough structure through the redistribution of gas and leavening agents within the dough (Tipples, 1975). Since moulding was done by hand, it is possible that there were slight inconsistencies across formulations which caused differences in final loaf quality. While every effort was made to keep the process the same, it is impossible to remove any chance of human error or inconsistency. Holes are also formed due to the coalescence of cells. As air cells within the dough expand, pressure is put on air cell walls by the gasses in the surrounding cells. If the cell wall is not strong enough to withstand the pressure of

the air inside, the cells the wall will break causing a hole to form (Scanlon and Zghal, 2001). Research by Bloksma (1981) and Vliet et al. (1992) indicates that the force of air cells on a shared cell wall becomes important for hole formation when gas cells expand beyond a volume fraction of roughly 0.74. At this point expansion of an air cell changes from independent expansion to being dependent on the expansion of surrounding air cells. In this experiment, the sample which had the most holes on average was the bread made with APF. While this was not expected, it is logical when compared to the relationship of bread volume to number of air-cells. In the APF samples, both bread volume and air cell number was high, however as food-grade DDG was added both the volume and the air-cell number decreased. While the volume ratio of air cells was not measured in this study it is possible that more air cells were found in APF samples than any other samples because this was the only sample which retained gasses well enough to reach a point where forces between expanding cells caused coalescence.

Table 3.13: C-Cell evaluation of cells and holes in bread slices taken from loaves made with APF, and blends including 5% and 10% DDGS and 5% and 10% HP-DDG.

	number of cells	percent area of cells	cell wall thickness (mm)	number of holes	percent area of holes	non-uniformity
APF	4220c	51.0c	0.416cd	2.69c	2.85c	5.116c
	(± 92.74)	(± 0.427)	(± 0.00371)	(± 1.10)	(± 1.43)	(± 2.266)
5% DDGS in APF	4122c	50.1b	0.409bc	1.71b	1.37b	3.018b
	(± 241.4)	(± 0.578)	(± 0.0110)	(± 1.05)	(± 0.730)	(± 2.088)
10% DDGS in APF	3530a	48.9a	0.402b	0.73a	0.48ab	0.913a
	(± 125.1)	(± 0.612)	(± 0.00639)	(± 0.45)	(± 0.45)	(± 0.420)
5% HP-DDG in APF	3790b	51.2c	0.419d	0.17a	0.16a	0.908a
	(± 133.3)	(± 0.625)	(± 0.00819)	(± 0.290)	(± 0.30)	(± 0.401)
10% HP-DDG in APF	3840b	49.3a	0.396a	1.01ab	0.60ab	1.027a
	(± 125.1)	(± 0.458)	(± 0.00604)	(± 0.947)	(± 0.669)	(± 0.359)

* Mean values for dependent variables with differing letters within columns are significantly different across treatments ($p<0.05$).

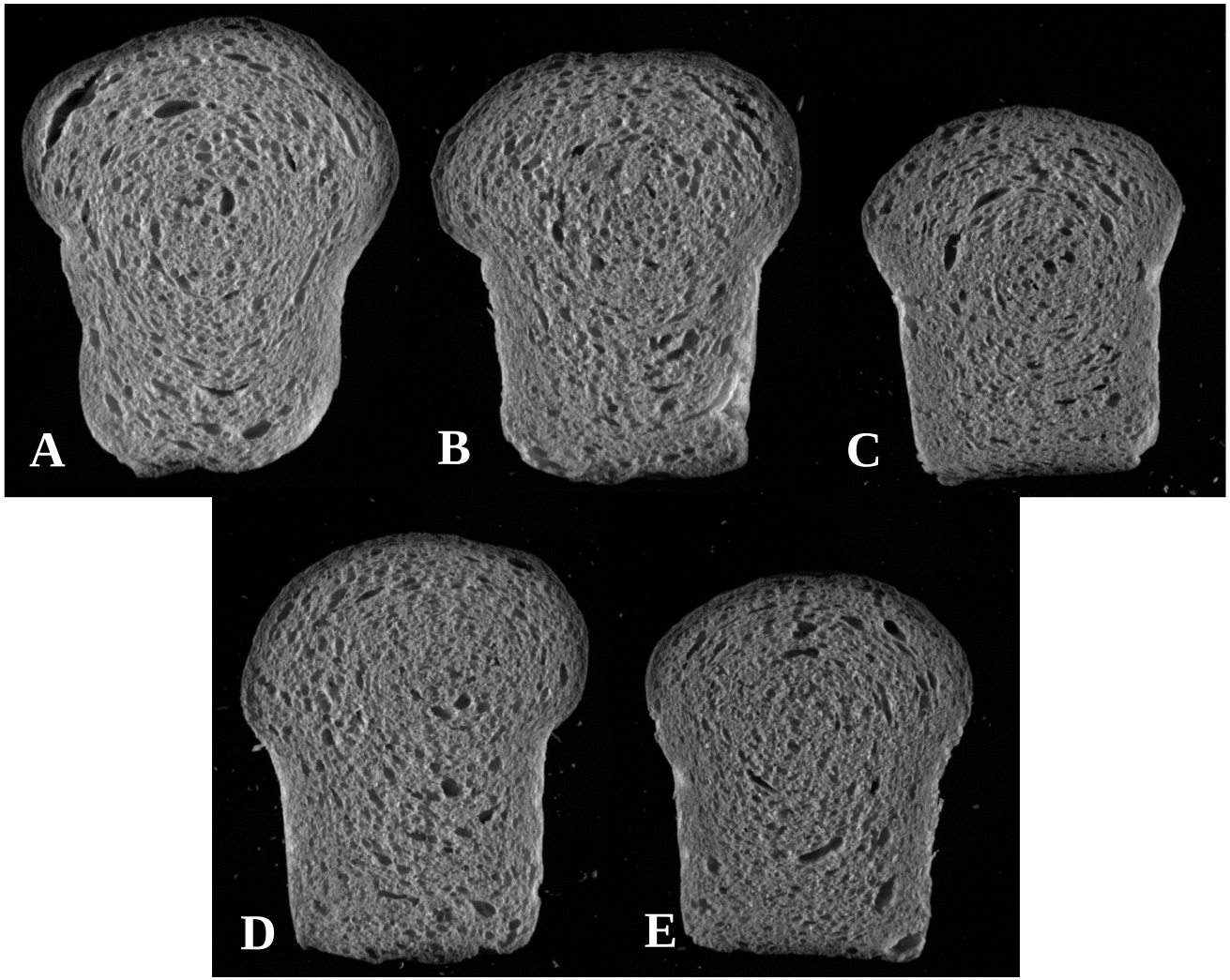

Figure 3.5: Images of bread slices using C-cell Analysis.
A: APF; B: 5% DDGS*; C: 10% DDGS*; D: 5% HP-DDG; and E: 10% HP-DDG.
*DDGS = DDGS with solubles removed as part of washing process.

3.4.4 Texture Profile Analysis

While texture is important in all foods, it is of particular importance in bread. This is because it is one of its defining characteristics that separates one type of bread from another. For instance, while chapathi and other Asian flat breads are expected to offer a relatively high resistance to chewing (Arra, 2011), sandwich bread is expected to have a soft spongy interior. The texture profile analysis (TPA) test allows us to compare the textures of multiple bread types. Results of mechanical texture tests have been found to parallel texture assessments by touch and sensory measurement (Axford et al., 1968, Bashford and Hartung, 1976, Brady and Mayer, 1985). While there are a multitude of parameters which can be expressed through a TPA, firmness, stickiness, and resilience were the three which were the focus in this experiment. In the scope of this experiment firmness can be defined as the force taken to compress the samples 10.000 mm and is measured in grams. Stickiness is defined as the resistance placed on the probe by the sample as it retracted due to a temporary bond between the probe and the sample. Resilience was defined as the ability of the sample to hold its shape and resist deformation. Since resilience is calculated as the ratio of

The results showed that while both 10% DDGS and 10% HP-DDG samples were statistically firmer than the APF control. The 5% DDGS and 5% HP-DDG were not significantly different in firmness than the control (Table 3.14). Interestingly both 5% DDGS and 5% HP-DDG samples were slightly less firm than the control. Saunders et al. (2014) found that bread stiffness increased as DDG was added. In comparison, a study on

the addition of DDGS to corn bread showed that incorporation of DDGS decreased the force needed to compress loaves (Liu et al., 2011).

No significant differences between the stickiness or resilience of samples was seen. While there were some numerical differences in degree of stickiness, no trends were seen across the samples. The scores of resilience of bread samples were all very close together, showing that all bread samples responded to compression in the same manner.

Vertical segments of bread were taken from the loaves for the TPA test. Previous studies comparing the firmness of breads showed higher resistance to compression in bread samples cut parallel to the long (x) axis of the loaf than in bread samples cut along the y or z axes Fig 2.4 (Hibberd and Parker, 1985, Piazza and Masi, 1995, Keetels et al., 1996). Since bread samples were only cut from one direction of the loaves comparisons cannot be made between compression directions for the loaves in this study. However, it would be interesting in future research to compare other directions of compression.

Table 3.14: Firmness, Stickiness, and Resilience of bread samples made with APF and blends of 5% and 10% DDGS* and 5% and 10% HP-DDG as measured through Texture Profile Analysis**.

	Firmness (g) [a]	Stickiness (g.sec) [b]	Resilience [c]
APF	252ab	0.052a	0.344a
	(± 72.6)	(± 0.089)	(± 0.022)
5% DDGS	179a	4.63a	0.368a
	(± 70.4)	(± 5.80)	(± 0.023)
10% DDGS	397c	1.66a	0.354a
	(± 216)	(± 3.01)	(± 0.014)
5% HP-DDG	213ab	1.76a	0.344a
	(± 92.8)	(± 4.98)	(± 0.016)
10% HP-DDG	323.673c	3.36a	0.346a
	(± 132)	(± 6.62)	(± 0.020)

*DDGS = DDGS with solubles removed as part of washing process.
**Mean values for dependent variables with differing letters within rows are significantly different across treatments ($p<0.05$).
[a] firmness = force to compress sample the first time
[b] stickiness = resistance of sample on probe as it retracted
[c] resilience = ability for sample to hold its shape and resist deformation (ratio of force of first compression to second compression)

3.4.5 Colorimetry

The color of a food can have a large impact on its sensory perception. A Minolta colorimeter was used to evaluate the color of crust and crumb color for each sample on the L*a*b* scale (Tables 3.15 and 3.16). The 5% DDGS (L*= 51.89) and 10% DDGS (L*= 57.20) samples were seen to be significantly lighter than the APF samples. While the 5% HP-DDG and 10% HP-DDG samples were seen to be slightly lighter than the control, the difference was not significant. A similar trend was seen for the degree of yellowness (b*) in sample crusts with the 10% DDGS sample being the only one found to be significantly more yellow than the control. Finally, no significant differences in the degree of redness (a*) were seen between samples. An increase in darkness, redness and yellowness was expected as DDG inclusion increased. Our hypothesis was supported by the results of 10% DDGS samples. However the lack of significant difference in color between the crust of control breads and bread supplemented with HP-DDG was unexpected. The increase in color provided by the DDG was likely overcome by the decrease in the maillard reaction during baking due to lower levels of carbohydrates in samples with DDG inclusions.

Table 3.15: Comparison of crust color of loaves made with APF, 5% and 10% DDGS[3] blends and 5% and 10% HP-DDG blends[1].

Parameter[2]	APF	5% DDGS in APF	10% DDGS in APF	5% HP-DDG in APF	10% HP-DDG in APF
L*	45.95a	51.89b	57.20c	48.31ab	49.31ab
	(± 6.68)	(± 2.65)	(± 1.74)	(± 2.25)	(± 2.32)
a*	15.44a	16.79a	15.92a	16.63a	16.04a
	(± 0.96)	(± 1.56)	(± 0.52)	(± 0.83)	(± 0.65)
b*	26.81a	29.65ab	32.15b	29.96ab	27.45a
	(± 3.72)	(± 2.32)	1.27)	(± 1.85)	(± 1.29)

[1] Mean values for dependent variables with differing letters within rows are significantly different across treatments ($p<0.05$).
[2] L: (0 = black, 100 = white); a: (positive = red, negative = green); b: (positive = yellow, negative = blue)
[3]DDGS = DDGS with solubles removed as part of washing process.

A larger difference in crumb color was seen across samples (Table 3.16). The crumb color of both 5% and 10% HP-DDG samples were seen to be significantly darker than the control. Numerically the 5% DDGS sample was lighter than the control, and the 10% DDGS sample was darker. However, neither were found to be significantly different from the control. Values for redness (a*) indicated that all samples were significantly more red than the control. Within treatments 5% DDGS was significantly less red than the others, 10% HP-DDG was reddest, and 10% DDGS and 5% HP-DDG were grouped in the middle. Similarly, the yellowness measurements showed the control being significantly lower than all treatments. Again 10% HP-DDG was significantly the most yellow, and both 5% DDGS and 5% HP-DDG had significantly less yellow coloring than the 10% DDGS sample.

It was expected that the samples with DDGS and HP-DDG would have a darker and more colored crumb. This hypothesis was supported by the results. All treatments yielded crumbs that were more red and yellow than the control. Samples with 10% inclusion levels had higher color concentrations than the 5% inclusion levels. Similarly, Saunders et al. (2014) found that incorporation of DDG into sandwich breads significantly increased the redness and yellowness and decreased the brightness of the crumb. Significant differences for crust color were only seen at the 50% substitution level. Guo et al. (2014) also observed a decrease in brightness and an increase in redness with incorporation of BSG in crackers. They hypothesized that this darkening was partially due to a rise in maillard reaction caused by the presence of additional protein. This is also a possible cause of the darkening observed in our bread.

Table 3.16: Comparison of crumb color of loaves made with APF, 5% and 10% DDGS[3] blends and 5% and 10% HP-DDG blends[1].

Parameter[2]	APF	DDGS		HP-DDG	
		5% in APF	10% in APF	5% in APF	10% in APF
L*	76.20bc	77.35c	75.67b	72.91a	73.31a
	(± 1.67)	(± 1.51)	(± 1.76)	(± 1.50)	(± 1.16
a*	0.04a	0.91b	1.96c	1.93c	3.97d
	(± 0.12)	(± 0.24	(± 0.25)	(± 0.31)	(± 0.22
b*	14.25a	21.15b	25.32c	20.32b	26.61d
	(± 1.05)	(± 1.23)	(± 0.58)	(± 1.17)	(± 0.63)

[1] Mean values for dependent variables with differing letters within rows are significantly different across treatments ($p<0.05$).
[2] L: (0 = black, 100 = white); a: (positive = red, negative = green); b: (positive = yellow, negative = blue)
[3]DDGS = DDGS with solubles removed as part of washing process.

3.5 Sensory Analysis

A sensory analysis study was conducted to evaluate the sensory acceptance of each type of bread containing food-grade DDG in comparison to a control. The same basic process was used to make the samples for sensory analysis as was used to make all other loaves. However, 1 pound loaves were made rather than 100 gram loaves so some minor modifications had to be made to the process. A mixing time of 6 minutes in a Globe stand mixer was used. The sheeter was set to a 6 in roll width and $^{7}/_{32}$ in roll spacing. The loaf pans used had dimensions of 9 ½ in x 5 ¼ in (top inside) and 8 ½ in x 4 ¼ in (bottom outside). Proofing and oven temperatures as well as proofing and baking times were kept the same.

Bread samples were packaged loosely in a gallon plastic bag after they were cool. The following day samples were sliced for analysis. Slices were ½ in thick, all slices were then cut in half again. Each participant was given a small glass of water and a plate with a half-slice of each sample on it. Samples were given random 3 digit numbers and were organized randomly on the testing sheet to give no particular preference to any one sample. Participants were then given a sheet to chart their rankings on (see appendix).

There were ten participants for the sensory analysis study. The study was not repeated. As seen in Table 3.17, APF samples were in general ranked higher than the other samples. However, appearance was the only trait that showed a significant difference. Here, the APF sample was found to be significantly more liked than the 10% DDGS or 10% HP-DDG sample. No statistical difference was seen between the sensory ranking of samples in taste, texture, aroma or overall liking.

Figure 3.6 shows a comparison of the appearances of all bread samples used for sensory testing. This image shows that the two predominant differences are color and size. Depending on the sample there may also have been visual differences in the air cell distribution or density which could have caused people to dislike one sample more than another. The 5% HP-DDG sample was the most liked overall of the samples containing DDGS or HP-DDG, and scored highest of the 4 treatments in appearance, taste, and texture. The bread treatments faired relatively well scoring between “4” which was labeled as neither like nor dislike and “6” which was labeled as like moderately. Samples which received an average score between 4 and 5 could be described as palatable but preferred less than other options. It is likely that the color imparted by the DDG on bread samples had a negative effect on sensory acceptance. This is supported by a similar trend in crumb color and appearance scores for samples.

Other studies testing the sensory acceptability of baked products including BSG and DDG had positive results. Rasco et al. (1987) evaluated acceptability of white bread, whole wheat bread, banana bread, and chocolate chip cookies containing wheat based DDG on a 5-point hedonic scale and found that the most common ranking for all samples was “4” (ranking of good). A similar study in which bar, spice, and chocolate chip cookies including DDGS were evaluated by elementary school students also found all samples to be acceptable. Finally, in the sensory evaluation of DDGS supplemented chapathies, no significant difference was seen between the 10% DDGS treatments and control chapathies in texture, aroma, taste, and chewability (Arra, 2011). However significant differences were noted in the scores of 20% DDGS samples and among appearance scores for all samples.

Table 3.17: Average sensory ranking of APF, 5% and 10% DDGS, and 5% and 10% HP-DDG bread samples on a 7-point hedonic scale*.

	Appearance	Taste	Texture	Aroma	Overall
APF	6.4b (± 0.92)	5.9a (± 1.51)	5.9a (± 1.14)	6.1a (± 1.04)	6.1a (± 1.04)
5% DDGS in APF	5.3ab (± 1.35)	4.8a (± 1.40)	5.0a (± 1.95)	5.2a (± 0.98)	5.2a (± 1.47)
10% DDGS in APF	4.5a (± 1.63)	5.0a (± 0.77)	5.2a (± 1.40)	5.3a (± 0.90)	5.1a (± 0.94)
5% HP-DDG in APF	5.6ab (± 0.66)	5.2a (± 1.08)	5.3a (± 1.68)	5.0a (± 0.89)	5.5a (± 1.28)
10% HP-DDG in APF	5.2a (± 0.98)	4.6a (± 0.80)	5a (± 1.18)	5.2a (± 1.08)	5.1a (± 1.04)

*Mean values for dependent variables with differing letters within columns are significantly different across treatments ($p<0.05$).

Figure 3.6: Bread loaves used for sensory evaluation.
(Left to Right: APF, APF breads containing: 5% DDGS*, 10% DDGS, 5% HP-DDG, 10% HP-DDG)
*DDGS = DDGS with solubles removed as part of washing process.

4 CONCLUSIONS

4.1 Food-grade DDG washing procedure and comparison

From the evaluation of food-grade DDG and its manufacturing procedure a variety of things were learned. First, it is necessary to increase the yield of the washing process or generate value added streams from the used ethanol or water. This could be done through the extraction of pigments or oils lost in the process. The washing procedure is also inefficient in its use of ethanol and water. The amount of ethanol used to wash 1 kg of DDG is several times the amount generated when 1 kg of DDG is produced. An alternate process to washing DDG would be to use supercritical CO_2 extraction. Carbon dioxide is also a coproduct of ethanol manufacture and has previously been shown as a viable solvent for extraction of pigments and aromatic compounds from DDG (Gachumi, 2016). Supercritical CO_2 extraction could also increase yield through reduction in loss of fines and increase the amount of pigment removal to result in a brighter, and whiter product.

The first objective of this study was to test the quality and HP-DDG and DDGS for the manufacture of food-grade DDG. Proximate analysis of washed DDGS and HP-DDG samples showed significant differences in fat, ash and protein. The significant loss of fat from DDGS during washing and the insignificant difference between TDF content in washed HP-DDG and DDGS samples suggested that it would be beneficial to fractionate corn prior to fermentation. This not only results in a higher protein content, but would also allow the oils to be harvested and sold rather than lost in the washing process.

Finally, while the particle size distribution of washed DDGS and HP-DDG differed slightly the majority of particles in both were between 150 µm and 400 µm. food-grade DDG particle sizes attained in this study were not seen to have a negative impact on dough quality. Further testing to evaluate whether smaller particle size would increase loaf volume or sensory acceptance would be necessary to verify this.

4.2 Dough and Bread Testing

The second and third objectives of this study were to compare the effects of EDDG and DDGS and the effects of 5% and 10% food-grade DDG inclusion rates on dough and bread quality. Dough development times for the HP-DDG blends were found to be much longer than the APF and DDGS blends. Other research has suggested that this is due to the increase in time necessary for particle hydration (Dreese and Hoseney, 1982, Tsen et al., 1983, Ktenioudaki et al., 2013b). There was not a large difference in development times between 5% and 10% blends.

While there were differences between the average extensibility of the dough types, only the extensibility of dough from the 5% HP-DDG blends was found to be significantly different from the other samples. Small, but insignificant, differences were seen between doughs with 5% and 10% inclusion rates, suggesting that extensibility may be significantly decreased at inclusion rates greater than 10%.

A clear positive correlation was seen with density and inclusion of food-grade DDG. While at the 10% levels there was a small difference between densities of breads

made with HP-DDG and DDGS, no significant difference was seen at the 5% level. APF loaves had the lowest densities, followed by 5% DDGS and HP-DDG, 10% HP-DDG and 10% DDGS.

There was a significant difference in protein and fiber content of breads containing food-grade DDG as compared to the control. Breads with 10% food-grade DDG were significantly higher than 5% and APF breads in protein. HP-DDG contributed significantly more protein than DDGS this is likely due to the higher percentage of protein in the initial sample. The inclusion of food-grade DDG hopefully was seen to increase the protein and TDF to levels above what is present in commercially prepared white bread. If food-grade DDG is approved as a food ingredient we would then be able to claim that breads containing 5% or more DDGS or HP-DDG was fortified with protein and breads containing 10% DDGS were fortified with protein and fiber.

Slices of bread made with APF and 5% DDGS blends had significantly more air cells than the other breads. However, the percent area of cells was significantly higher in slices from APF and 5% HP-DDG loaves. This correlates well with the density data in that slices with more air cells had larger volume and were less dense. The air cells in the 10% HP-DDG samples had the thinnest cell walls. This could possibly be explained by the cell walls being too thin to hold the air inside or by large DDG particles puncturing the thin cell walls and causing coalescence of cells.

While the texture of 5% DDGS and 5% HP-DDG were not significantly different than the control, both 10% DDGS and 10% HP-DDG samples were statistically firmer than the APF samples. No significant differences between the stickiness or resilience of

samples was seen. From this we can concluded that the addition of DDG increased the density and the firmness of bread samples, but that there were no differences in breads supplemented with different types of food-grade DDG.

Both types of food-grade DDG caused significant increases in color of the bread crumb over the control. However, differences in their effect was seen primarily at the 10% level where bread made with HP-DDG had a statistically darker, more yellow, and more red crumb than bread made with DDGS.

Based on these results our first hypothesis was proven wrong. There was a significant difference in dough and bread quality between the control and treatments. However, our second hypothesis was proven to be correct. Significant differences were seen between the 5% and 10% incorporation levels in both dough and bread quality.

4.3 Sensory Analysis and Future Research

The fourth objective of this study was to evaluate the sensory acceptability of breads fortified with 5% and 10% food-grade DDG in comparison to the APF control. The 5% HP-DDG sample was the most liked overall of the samples containing DDGS or HP-DDG, and scored highest of the 4 treatments in appearance, taste, and texture. The results showed that breads containing DDGS and HP-DDG at the 10% level received significantly lower scores for appearance than the APF control. It is likely that the color imparted by the food-grade DDG on bread samples was the source of this negative effect on sensory acceptance. With the exception of the appearance scores, the results matched my hypothesis that there would be no significant difference in the sensory acceptability of samples.

The results of this study show that there is a potential for bread fortified with food-grade DDG in the consumer grocery market. While samples including 10% food-grade DDG did not fare particularly well in all analysis 5% food-grade DDG incorporation did much better. It is possible that with future research 10% food-grade DDG bread could be improved to be acceptable as an alternative for conventional sandwich bread. For example, Dreese and Hoseney (1982) found that the inclusion of BSG in bread decreased loaf volume, but that the addition of SSL increases the volume of loaves containing BSG. Further research on the incorporation of dough conditioners such as SSL should be evaluated in DDG containing breads to determine whether an acceptable product can be generated.

In addition, this study did not compare food-grade DDG fortified breads to whole wheat breads. Future research should also evaluate the sensory acceptability and nutritional composition of these two breads to determine how they compare as products. Finally, given that staling is an important sensory factor in bread quality (Heenan et al., 2008), it would also be valuable to evaluate the shelf life of bread containing food-grade DDG in comparison to white and whole wheat bread.

While the corn-based ethanol industry continues to thrive there will not be an absence of DDGS. Developing methods to use this coproduct will benefit the industry through increasing the economy and efficiency of the process. Furthermore, the development of the food-grade DDG manufacture process and the inclusion of food-grade DDG into products such as sandwich bread will significantly impact consumers through increased fiber and protein consumption. While the final product may not be

quite ready to be sold on store shelves the results of this experiment have shown the viability of this product as a substitute for conventional white bread.

APPENDIX

Bread Sensory Evaluation Sheet

Rank each sample attribute by circling the number corresponding to your perceived level of like or dislike for the sample. Please rinse your mouth with water between samples.

Sample 135

Appearance						
dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7
Taste						
dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7
Texture						
dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7
Aroma						
dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7
Overall						
dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7

Sample 267

Appearance						
dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7
Taste						
dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7
Texture						
dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7
Aroma						
dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7
Overall						
dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7

Sample 479

Appearance

dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7

Taste

dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7

Texture

dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7

Aroma

dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7

Overall

dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7

Sample 803

Appearance

dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7

Taste

dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7

Texture

dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7

Aroma

dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7

Overall

dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7

Sample 549

Appearance

dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7

Taste

dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7

Texture

dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7

Aroma

dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7

Overall

dislike very much	dislike moderately	dislike slightly	Neither Like nor dislike	like slightly	like moderately	like very much
1	2	3	4	5	6	7

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