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Effects of Particle Size of Distillers Dried Grains and Increased Levels of Flour Enrichment - A Study of Nutritional Composition, Rheology, and Quality of Fiber and Protein Enriched Bread

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EFFECTS OF PARTICLE SIZE OF DISTILLERS DRIED GRAINS AND INCREASED LEVELS OF FLOUR ENRICHMENT - A STUDY OF NUTRITIONAL COMPOSITION, RHEOLOGY, AND QUALITY OF FIBER AND PROTEIN ENRICHED BREAD

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THESIS ACCEPTANCE PAGE

Patra Akaya

This thesis is approved as a creditable and independent investigation by a candidate for the master's 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 major department.

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This work is dedicated to

God Almighty, my creator for his source of inspiration, strength, infinite mercy, and grace upon my life and the strength to go through this program.

In loving memory of my parents Hon. Mr. Henry Chukwuemeka Akaya, I wish to thank you for providing an example of excellence, and for the values you impacted on me early in life and Mrs. Mercy Ijeoma Akaya my beloved mother who could not live to see this day, words cannot express my gratitude for the invaluable advice, humility, perseverance, having a sense of purpose and your seed of hard work and dedication you impacted on me. You have always loved and believed in my ability to make strides and attain greater heights. You may have gone but your belief in me has made this journey possible.

To my sister, Fortune Ogechi Akaya, whom I miss every day of my life, we shared so many memories together which I will continue to hold dear to my heart, you were a beautiful soul. You will always be loved, never forgotten, and forever missed.

Finally, to my siblings for their inspiration, love and encouragement, you all mean the world to me!

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

a: Redness Vs Greenness

a*: Redness Vs Greenness

˚C: Degree Centigrade

µm: Micrometers

%: Percentage

AACC: American Associations of Cereal Chemist

AOAC: Association of Official Analytical Chemist

APF: All-Purpose Flour

AW: Water activity

b: Yellowness Vs Blueness

b*: Yellowness Vs Blueness

BF: Bread Flour

CCT: Corn Cereal and Technology

Coarse: FDDG ground using 0.5 mm mesh

DB: Dry basis

DDG: Distillers Dried Grain

DDGS: Distillers Dried Grain with Soluble

DON- Deoxynivalenol

FDDG: Food Grade Distillers Dried Grain

FDT: Farinograph dough development time (min)

Fine: FDDG ground using 0.2mm mesh

FPWA: Farinograph percent water absorption (%)

FS: Farinograph stability (min)

FU: Farinograph

g: Grams

GI: Gluten index

GMP: Good Manufacturing Practice

GRA: Generally Regarded as Safe

HP: High Pressure

KG: Kilograms

L: Brightness

L*: Brightness

L: Liters

Min: Minutes

MTI: Mixing tolerance index (BU)

NCGA: National Corn Growers Association

PM: Percent moisture

PSD: Particle Size Distribution

ppb: parts per million

ppm: parts per million

PT: Peak time (min)

Sec: seconds

TDF: Total Dietary Fiber

TPC: Total Phenolic Content

USDA: United State Department of Agriculture

USDA: United State Food and Drug Administrations

wb: wet basis

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

EFFECTS OF PARTICLE SIZE OF DISTILLERS DRIED GRAINS AND INCREASED LEVELS OF FLOUR ENRICHMENT - A STUDY OF NUTRITIONAL COMPOSITION, RHEOLOGY, AND QUALITY OF FIBER AND PROTEIN ENRICHED BREAD

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High protein and high fiber residues from ethanol processing streams may be utilized in food applications. Heat-processed wet cake from the ethanol plant was identified as a starting material for further optimization. Food Grade Distillers Grain (FDDG) was produced exclusively for this study employing successive washing schemes, freeze drying and particle size reduction. FDDG was further fractionated into Fine FDDG and Coarse FDDG using a 0.2mm and 0.5mm screen, respectively, in conjunction with an ultra-centrifugal mill. The nutritional compositional and functional traits of FDDG were determined. Wheat flour was fortified using FDDG substitutions at the 0, 5, 10, and 20% levels using fine FDDG and coarse FDDG. The particle size distribution of the various flours were determined. The effects of both particle size and fortification at 5 to 20% levels were compared against Control samples (0% fortified Bread flour). Significant increases in protein content and TDF content were achieved in fortified blends at each level of fortification. Differences in effects of particle size (0.2mm or 0.5mm) were less discernible. Bread produced using the flour blends were evaluated for loaf weight, loaf

volume crumb structure and bread Texture Profile Analysis (TPA). The TPA yielded data on bread hardness, cohesiveness, resilience, springiness and chewiness. The wheat flour used in the study had a significant proportion (77.7%) that ranged from 75 to 150 microns in particle size. A large proportion of the Fine FDDG (88.72%) and Coarse FDDG ranged in particle size from 150 to 400 microns.

FDDG ranged in protein content from 36.01% (Fine FDDG) to 37.59 % (Coarse FDDG). Fine-grinding appeared to result in a slight but statistically significant higher protein content. Fine and coarse FDDG showed no differences in dietary fiber content (TDF). Dietary fiber was the predominant constituent in FDDG at 51.3%TDF in Fine FDDG and 49.5% TDF in Coarse FDDG. Bread wheat flour that had 2.67% TDF, was significantly improved in fiber content with the addition of up to 20% DDGS. TDF content in the blends went up to 13.74% with the inclusion of 20% Coarse FDDG. Enrichment of wheat flour at 5, 10 and 20% levels of substitution brought about a steady and significant increase in fiber content. This was observed with both the fine FDDG and the coarse FDDG.

CHAPTER 1. INTRODUCTION

1.2 Background

The texture of food is an important attribute for food quality and consumer acceptance. Texture traits are used to determine the quality of food (Sloan, 2013). There are substantial, physical, and subjective versatile attributes that differentiate the texture of food, customer perception and acceptability (Christensen, 1984). Food texture is dependent on ingredients that provide the mouthfeel. In food processing, rheology has been observed to have a significant impact in shelf stability and sensory attributes like texture and mouth feel according to (Stokes et al., 2013). Fineness or coarseness of food adjuncts will influence physical characteristics and sensory characteristics of food. Saunders and co workers (2013) showed that DDG refinement which included solvent treatments for color improvement, sterilizing and milling had an enhanced potential on consumer perception and general acceptance. Particle size also had an impact on the outcome of DDG was shown that fine particles generally increased the brightness, reduced the red pigments, and increased the yellowness of DDG (Saunders et al., 2013). The particle size distribution of a powdery component is a set of values which explains the relative quantity of particles present, sorted, and arranged systematically by size. Particle size distribution has been widely used to describe so many other powder components as it imparts functional changes in foods (Barbosa-Canovas et al., 2005). Particle size (PS) is an important consideration for many applications in the food manufacturing industry. Particle size distribution (PSD) is also viewed as a critical parameter in some applications where either the fines or the coarse material may impede the results that the customers are trying to achieve.

Particle size distribution of Distillers Dried Grain (DDG) will directly affect the final product based on its functionality, taste, appearance, color, processability, texture and eating quality. When considering the average particle size, it is important to realize that many measurement instruments give values as if all particles are spherical in shape. All particles are not spherical in nature but have different shapes and sizes, it aids flowability and mixing profile in food industry.

DDG in varied particle sizes, incorporated in other products have been demonstrated to influence the size and general acceptability of baked products (Abbott et al., 1991). Some studies have shown that medium particle size flours separated by sieving have better baking quality, whereas fine particle size flours separated by air classification have better baking quality because of higher protein content in these fractions. (Graeza and Norris (1961) determined that that flour with the same level of protein, but different particle sizes showed different baking results. DDG particle size is essential in other aspects such as optimization in the unit of operation, food formulation, assessment of future enrichment and final product. For food to be acceptable to consumers, it must conform to a set of quality standards. These qualities depend on the nature of the food.

Distillers Dried Grain is considered as waste and partly used to feed livestock's despite their inherent high nutritional value. Distillers Dried Grain (DDG) is underutilized but can be used to enrich flour material for a more nutritional balanced food. This may be beneficial to human health especially for people suffering from chronic diseases like Type II diabetics. There are several steps in converting Distillers Dried Grain (DDG) to Food Distillers Dried Grain (FDDG). Throughout this study, several challenges will be addressed. These challenges involve obtaining correct color, ideal particle size, optimal rheology, and the correct mixing profile while incorporating greater proportions DDG into wheat flour. An additional issue with using corn DDG in food products is the ability to change the characteristics of the final baked goods, for example, its effect on color by (Tsen, Eyestone, & Weber, 1982) and loaf volume (Dresse & Hoseney, 1982) Morad, Doherty, & Rooney, 1984), (Prentice & D'Appolonia, 1977) and the effect of the particle size on food products for example pasta.

Bread quality generally is difficult to define, but some of its attributes include freshness, physical texture, aroma, and appearance according to Scanlon and Zghal, (2001), and Heenan et al, (2008). Bread is a staple food which is consumed in all parts of the world as food. In some countries, it is used mainly for breakfast while some use it as a main course. In production of bread, there are many complicated processes which involve chemistry in metamorphosing flour into a final acceptable product. There are basic steps in bread production which consist of scaling, milling, mixing, fermentation, sheeting, molding, proving, and baking the bread.

1.3 Research Objectives

- To evaluate the effects of particle size (coarse or fine) of Food Grade Distillers Dried Grains ingredient on food functionality traits (physical, proximate, texture, moisture, absorption) in bread production
- To determine the effects of increasing levels of FDDG substitution (0, 5, 10 and 20%) in bread flour on the nutritional composition of bread
- To evaluate the effects of *fine and coarse* FDDG on dough rheology
- To evaluate the effects of *increasing levels* of FDDG substitution on dough rheology
- To determine *bread* quality, nutritional composition, and sensory characteristics of FDDG-fortified bread in order to determine consumer acceptability.

1.4 Hypothesis

H_0: Particle size reduction of Food Grade Distillers Dried Grains will not have a significant effect on food functionality traits (physical, proximate, texture, moisture absorption).

H_1: Particle size reduction of Food Grade Distillers Dried Grains will have a significant effect on food functionality traits (physical, proximate, texture, moisture absorption).

H_0: Increasing levels of FDDG substitution (0, 5, 10 and 20%) in bread flour will not improve nutritional composition of bread.

H_1: Increasing levels of FDDG substitution (0, 5, 10 and 20%) in bread flour will improve nutritional composition of bread.

H_0: Fine FDDG will not improve dough rheology traits compared to Coarse FDDG.

H_1: Fine FDDG will improve dough rheology traits compared to Coarse FDDG.

H_0: There will not be significant differences between the sensory quality of conventional bread and sensory quality of FDDG fortified bread.

H_1: There will be significant differences between the sensory quality of conventional bread and the sensory quality of FDDG fortified bread.

1.5 Justification of Work

This research is aimed at instrumental methods to determine the quality of flour enriched with dietary fiber. Physical and rheological testing methods was used to determine the effect of enrichment of staple foods for the purpose of health benefits and balanced diet. The importance of dietary fiber in food cannot be overemphasizes as research has shown that most Americans consume less than recommended dietary fiber daily intake.

According to AACC report March 2001, “Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine”. “Dietary fiber includes polysaccharides, oligosaccharides, lignin’s, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and or blood cholesterol, and or blood glucose attenuation”. In other words, dietary fiber is very important in food. There is a positive adjustment of levels of serum cholesterol as a result of dietary fiber and increased fiber foods like Distillers Dried Grain (DDG).

The maximum nutritional potential of Distillers Dried Grain (DDG) is not utilized. More than 80% of Distillers Dried Grain has been underutilized but research is ongoing to incorporate Food Grade Distillers Dried Grain (FDDG) into food products. This will solve the needs of the consumers in search of balanced food. Incorporation of Distillers Dried Grain in staple foods will lead to increased nutritional quality of the flour and food enrichment. Shukla, (2003) reported that Distillers Dried Grain (DDG) is a cheap food material that can be easily obtained because of the increase in ethanol production; it is also a good source of cysteine, linoleic acid, vitamin E, methionine and a good source of minerals like phosphorous and potassium.

1.6 Purpose of Research

The purpose of this study was to determine the effects distillers dried grain would have on mixing profile, texture quality, and rheology while utilizing wheat flour supplemented with treated food grade DDG's.

CHAPTER 2. LITERATURE REVIEW

2.1 Corn

Corn is the main cereal grain as measured by production but ranks third as a staple food, after wheat and rice which makes corn a subject of great interest (Gwirtz et al., 2013). Nutritionally, corn contains phytochemicals such as phenolic acids, total phenolic content, which are currently associated with reducing certain chronic disease like cardiovascular heart diseases, certain cancers, type 2 diabetes, and obesity. The type of phytochemicals (carotenoids, anthocyanins, phytosterols, and flavonoids) that are found in corn flour depends on the processing of corn which could be sold as whole or processed.

Generally, all classes of corn contain vitamins (A, B, E & K), plant sterols, dietary fiber, minerals such as phosphorous, magnesium and potassium, flavonoids, and phenolic acids (Siyuan Sheng et al., 2018). However, different classes of corn contain greater proportions of individual phytochemicals in relation to carotenoids and flavonoids. The red and blue colors of corn are due to high amount of anthocyanins which goes up to (up to 325 mg/100 g DW corn), pelargonidin derivatives (5%–10%), cyanidin derivatives (75%–90%) and peonidin derivatives (15%–20%) (Siyuan Sheng et al., 2018).

Yellow dent corn has a composition of 3.8% corn oil, 62% starch, 15% moisture, 19.2% protein and fiber (Corn Chemistry and Technology, 1999). Yellow corn contains substantial amounts of carotenoids (up to 823 g/100 g DW corn) which includes, zeaxanthin (40%), β-carotene (2%), carotene (4%), lutein (50%), and β-cryptoxanthin (3%). High amylose corn contains a minimum of 70% carbohydrates. (Siyuan Sheng et al., 2018).

2.2 Corn Cultivation

The grain is a cultivated cereal crop, and it is utilized both as animal and human feed in many parts of the world with the leading grains being barley, oat, sorghum. Corn ranks as the highest grown crop especially in United States where it constitutes 95% production and usage.

In the United States, 90 million acres of land is used for corn production with a greater percentage of cultivated in the Heartland area which consists of South Dakota and Nebraska, northern Missouri, Indiana, Ohio, western Kentucky with Iowa and Illinois. Ten to twenty percent of corn produced in the United State is transported to other countries thereby contributing to the world corn retail industry ("USDA ERS - Amber Waves," n.d. 2018).

The United State is the highest corn producing country in the world. Much of the yield is used for both human and non-human consumption which includes fuel ethanol production, corn oil, industrial and beverage production, sweeteners, starch, and corn oil. A considerable proportion (40%) of corn produced in the United State is used for ethanol production thereby resulting in a large quantity of ethanol by-products which can be further processed into value-added products.

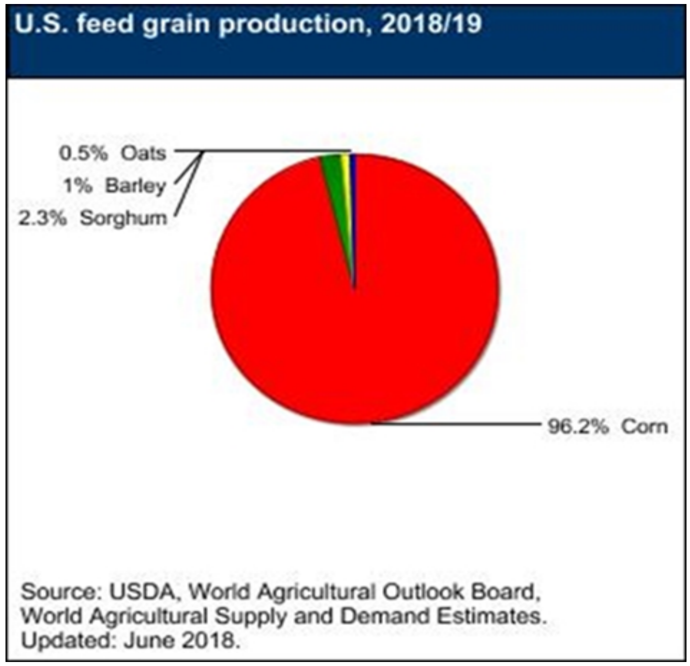


Figure 1. Sources of information: US gain feed production USDA 2018/2019

2.2.2 Effects of corn processing on Corn Composition

Various food technologies are currently used for processing industrially produced maize flours and corn meals in different parts of the world to obtain precooked refined corn flour, and other corn products. These products have different intrinsic vitamin and mineral contents, and their processing follows different pathways from raw grain to the consumer final product, which entail changes in nutrient composition. Dry corn mechanical processing creates whole or fractionated products, separated by anatomical features such as bran, germ, and endosperm. Wet corn processing separates by chemical compound classification such as starch and protein. Various industrial processes, including whole grain, dry milling fractionation, and nixtamalization have been described in the literature. Vitamin and mineral losses during processing are identified and the nutritional impact is outlined.

2.3 Corn Anatomy

2.3.1 Nutritional content of corn

The corn kernel is composed of four primary structures from a processing perspective. They are endosperm, germ, pericarp and tip cap making up 83%, 11%, 5% and 1%, respectively, of the corn kernel. The endosperm is primarily starch surrounded by a protein matrix. Two main types of starch include hard or vitreous and soft and opaque. The vitreous endosperm is negatively related to starch degradability in ruminants. The germ or embryo of the corn kernel is high in fat (33.3%) in addition to enzymes and nutrients for new corn plant growth and development. The germ also contains B complex vitamins and antioxidants such as vitamin E. Corn germ oil is particularly high in polyunsaturated fatty acids (54.7%), which are subject to oxidative and other forms of rancidity resulting in off or objectionable flavors from full-fat corn products. The pericarp is a high-fiber (8.8% crude) semi-permeable barrier surrounding the endosperm and germ, covering all but the tip cap. The tip cap is the structure through which all moisture and nutrients pass through during development and kernel dry down. The black or hilar layer on the tip cap acts as a seal (Eckhoff, S. 2010). The term bran is also used to refer to the fiber-rich outer layer (Pericarp) that contains B vitamins and minerals and tip cap. Corn may be classified according to kernel type as follows: dent, flint, waxy, flour, sweet, pop, Indian and pod corn. Except for pod corn, these divisions are based on the quality, quantity, and pattern of endosperm composition, which defines the size of the kernel. Endosperm composition may be changed by a single gene difference, as in the case of flour (FL) versus (FI), sugary (SU) versus starchy (SU), waxy (WX) versus non waxy (WX) and other single recessive gene modifiers that have been used in breeding special-purpose type of corn (Brown, W & L. Darrah, (1985), and Doebley, J.(2004).

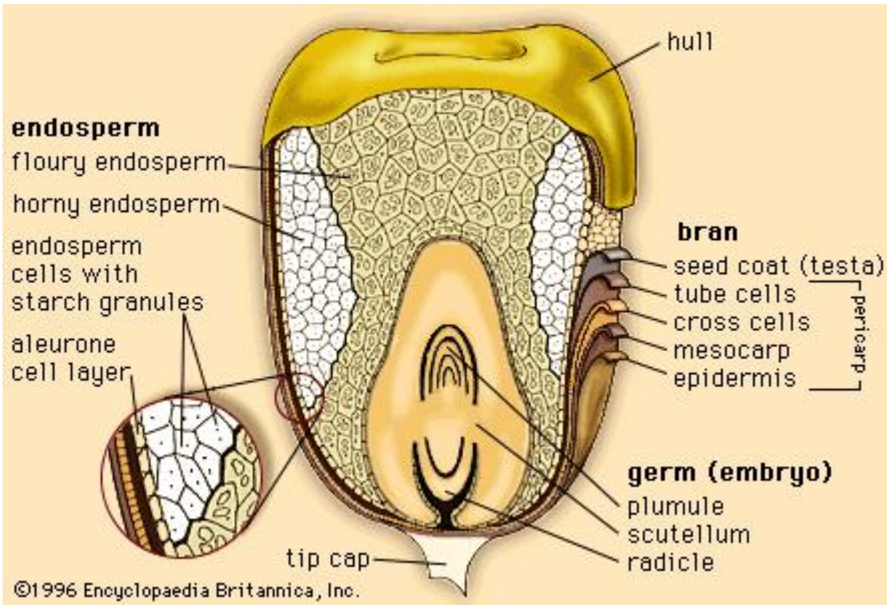


Figure 2. The outer layer & external structure of Maize Corn Kernel Composition (Nutritional, Chemical, and Physical Composition)

Source of Information: Encyclopedia Britannica, Inc 1996 by R. Paul Singh Douglas W. Kent-Jones.

Table 1 and 2 provide the vitamin and mineral analysis of corn, crude bran, and corn starch as available from the U.S Department of Agriculture nutritional database (U.S Department of Agriculture, Agricultural Research Service, 2012).

Table 1. Vitamin content of Whole kernel, crude bran, and corn starch of yellow corn

Nutrient	Units	Corn	Corn	Corn
Vitamin	**100g**	**Whole**	**Bran**	**Starch**
Thiamin	mg	0.39	0.01	0
Riboflavin	mg	0.20	0.10	0
Niacin	mg	3.63	2.74	0
Pantothenic acid	mg	0.42	0.64	0
Vitamin B6	mg	0.62	0.15	0
Folate	μg	19.00	4.00	0
Chlorine	mg	-	18.00	0.40

Source of Information: USDA. United States Department of Agriculture (2013)

As noted in table 1, the corn bran is a significant contributor to corn vitamin and mineral content. The wet milling of corn separates much of its nutrient content away from the starch component. In addition to the chemical composition, physical characteristics of corn in the commercial marketplace influence the value of the grain or the final product. Often, countries will have grading standards for corn entering the supply chain to assist buyers and sellers assessing corn value. Test weight, moisture content, foreign material, and damage are among typical measures of corn quality and value (USDA. United States Department of Agriculture, 2013)

Table 2. Mineral Content of Whole Kernel, Crude bran, and Corn starch of yellow corn

Nutrients	Units	Corn	Corn	Corn
Vitamin	**100g**	**Whole**	**Bran**	**Starch**
Calcium, Ca	mg	7.00	42.00	2.00
Iron, Fe	mg	2.71	2.79	0.47
Magnesium, Mg	mg	127.00	64.00	3.00
Phosphorus, P	mg	210.00	72.00	13.00
Potassium, K	mg	287.00	44.00	3.00
Sodium, Na	mg	35.00	7.00	9.00
Zinc, Zn	mg	2.21	1.56	0.06
Copper, Cu	mg	0.31	0.25	0.05
Manganese, Mn	mg	0.49	0.14	0.05
Selenium, Se	μg	15.50	16.5	2.80

Source of Information: USDA. United States Department of Agriculture (2013)

2.4 Ethanol Production process

In the United States, there was an increase in the manufacturing of ethanol from corn which has increased from 1.6 billion gallons in 2000 to above 14 billion gallons in 2014. In another overview by RFS (2019), the U. S. manufacturing and utilization of ethanol increased in 2018 to a high peak of 16.2 billion gallons of sustainable ethanol production, three millions gallons in a year or more with the a large export of more than over 1.6 billion gallons.

The zeal for sustainable fossil fuel from corn has steadily increased as well as the byproducts as stated by Colleen C et al., (2012). With the increasing demand for ethanol production, there is also a massive increase in production of its by-product, known as Distillers' Dried Grains (USDA Sept 2015). By definition, Distillers' Dried Grains are primary products resulting from the fermentation of cereal grains by yeast into alcohol (J. Zentek, .A. Mader, 2014).

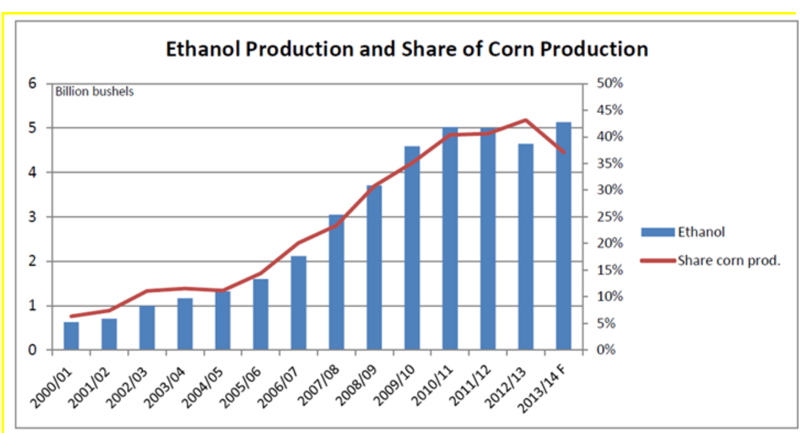


Figure 3. Corn for use in ethanol and ethanol production from Jan 2000 - 2013/2014
Source of Information: USDA (2015)

Ethanol is produced from a wide variety of plant-derived materials which includes corn, rice, sorghum, wheat, barley. Distillers Dried Grain (DDG) can be generated from cereals that have been used for ethanol production, but corn is the principle substrate used in ethanol production. There is ongoing research by food scientists to incorporate distillers dried grain (DDG) as a food additive because of its high nutritional content. Substantial research has been carried out on DDG and most of them have proved DDG to be a rich source of fiber, minerals, vitamins, and protein for consumers and as such has the potential to lower the risk of heart diseases, stroke, diabetes, weight gain and improves skin health. The spent grain (DDG) was underutilized by discarding it or given to animals as feed but recent studies have proved that it contains high amounts of nutritional value and can be incorporated into food to make an edible and nutritional food additive (Rasco and Rubentheraler, 1990).

Ethanol can be produced from corn grains through two primary ways, wet milling, and dry milling. Dry milling is less capital intensive compared to wet milling. There are two co-products generated from the dry milling method, distillers dried grains with soluble (DDGS) and carbon dioxide. Marketing of DDGS is critical to the sustainability of a dry grinding plant. At present, wet milling is an extensive way of ethanol production which requires massive equipment and has a higher output of ethanol production turnover (Singh et al., 2001) to defray production costs (Bothast and Schlider, 2005). According to Singh et al., 2001, dry milling is a less complicated process with a lesser yield of ethanol production. It has gained a high demand as compared to wet milling in the industry (Bothast and Schlider, 2005)

In the beginning stages of ethanol production for biofuel, a high amount of starch is removed which is used in the ethanol production which contributes to high protein 45%, fiber 35%, and 8.2% of fat in the by-product, namely, Distillers Dried Grain (DDG). The spent grain is highly nutritious and can be used to incorporate into food for manufacturing nutritionally balanced food products (Kelzer et al., 2011, and Hoffman et al., 2010).

The starting materials and processes used in ethanol production will determine the overall quality of the main product and by-products of the process. These factors lead to varied differences in the nutritional composition of the Distillers' Dried Grain. Despite the lack of uniformity in the nutritional composition of DDG, it is acceptable in feed applications for livestock while this wide variation in DDG quality and nutrition content is not acceptable in the food market for humans and companion animals (Belyea R.L[1], Rausch K. D, Tumbleson, M.E 2004).

The chemical and nutritional composition of DDG influences food functionality, such as how the ingredient assists in improving food quality traits. These traits include, but are not limited to texture, mouthfeel, particle size, sensory, color, aroma, loaf volume, pasting properties, flow-ability, and rheological properties. DDG can be further processed to food-grade DDG while recovering DDG that is higher in protein content due to the removal of residual corn oil which was not removed in the ethanol production processes. These can be achieved by carefully employing methods to minimize DDG variability, and to ensure uniform food functionality traits. Distillers dried grains with solubles (DDGS) are plant-derived materials which are a by-product of ethanol production, containing DDG and liquid solubles. DDG is the particulate material in the ethanol production process that was sifted out with a mesh. DDG and the soluble are retained separately and the soluble is then concentrated and added back to the DDG. The DDG/solubles mixture is then dried down to yield DDGS (Belyea, 2004).

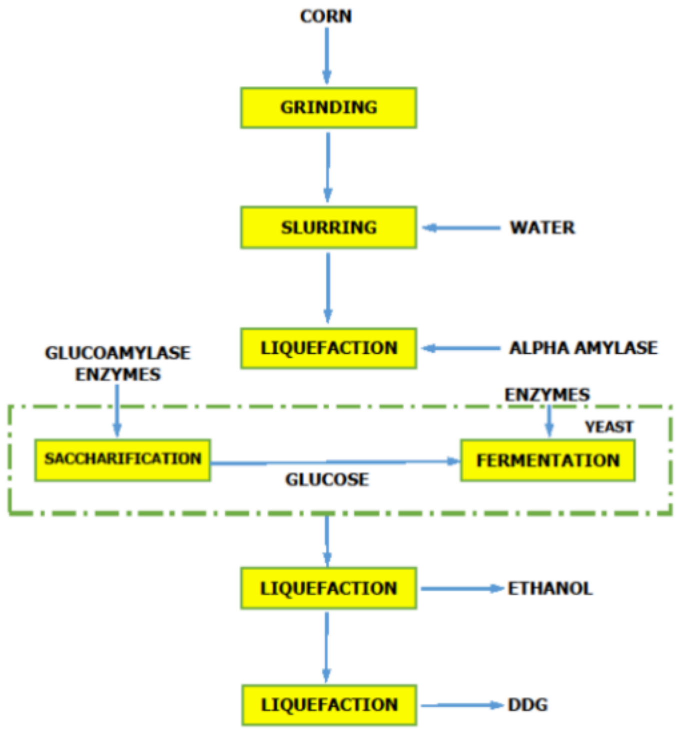


Figure 4. Process flow chart of a typical dry - ground corn to ethanol

2.4.1 Wet Milling corn production

There are two basic categories of industrial processing employed for transforming corn into products for human consumption. They are known as dry and wet milling. In the wet milling process, corn is separated into relatively pure chemical compound classes of starch, protein, oil, and fiber. The products and co-products obtained from wet maize milling are not typically directly used by the consumer and often require further industrial processing before consumption. The products of wet corn milling are not typically produced on a small scale commercially or at home. The primary product, starch, can be processed into a variety of starch products or further refined into a variety of sweeteners sold in liquid and dry forms. Wet milling of corn will not be further addressed here as our research focus is limited to finding new uses for fractions from the dry milling corn ethanol industry. Industrial dry milling includes particle size reduction of clean whole corn with or without screening separation, retaining all or some of the original corn germ and fiber (Brubacher, T; 2002). Because of the high-fat content, these whole or partially degeminated corn products are not particularly shelf-stable. Much of the particle size reduction and separation is accomplished with equipment similar to that employed in wheat flour milling, including hammer mills, stone mills, roller mills, screeners, sifters, specific gravity separators, and aspirators. Specialized hullers or peelers may be employed in maize processing.

Recently, increase in biofuel production, particularly the dry-grind corn-to-ethanol process, creates a sizeable stockpile of its co-product in the form of Distillers' Dried Grain with Soluble (DDGS), which is made by blending distillers' wet grains (DWG) and syrup and drying the mix. The DDGS contains a higher percentage of protein, fiber, lipid contents than those in corn.

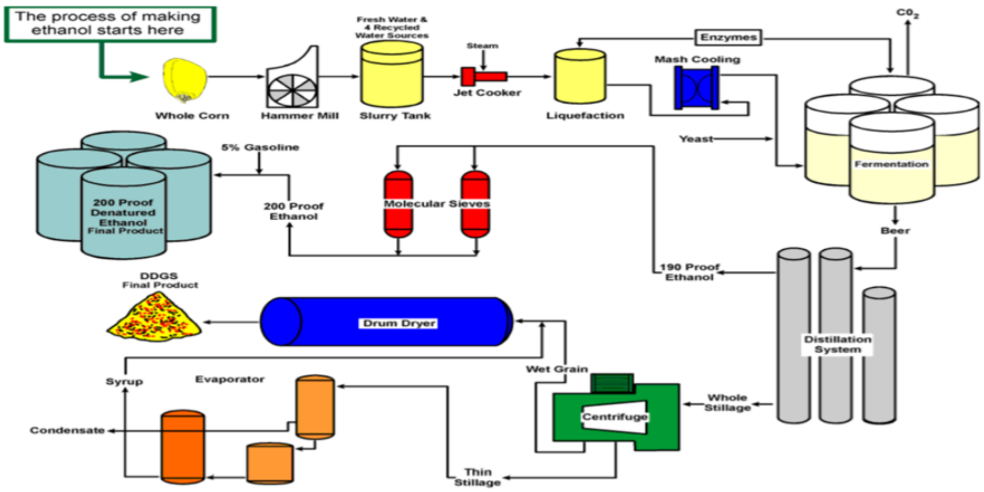


Figure 5. Wet milling corn production process flow chart

Source of Information: G. Shurson* M Spiehs & M. Whitney 2004

Department of Animal Science, University of Minnesota, St Paul USA

2.4.2 Dry milling corn production

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 sugar can be fermented into ethanol by yeast. After the fermentation is completed the ethanol is distilled off leaving behind 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 distillers' dried grains (DDG) which will be used in this study. Often the solubles are condensed after centrifugation then added back to the DDG before drying. This result is distillers' dried grain with soluble (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 and so on which are water-soluble and were removed during centrifugation of the DDG to remove excess moisture before drying (Weiss, 2007). The soluble can be condensed and added back to the DDG to reduce product losses.

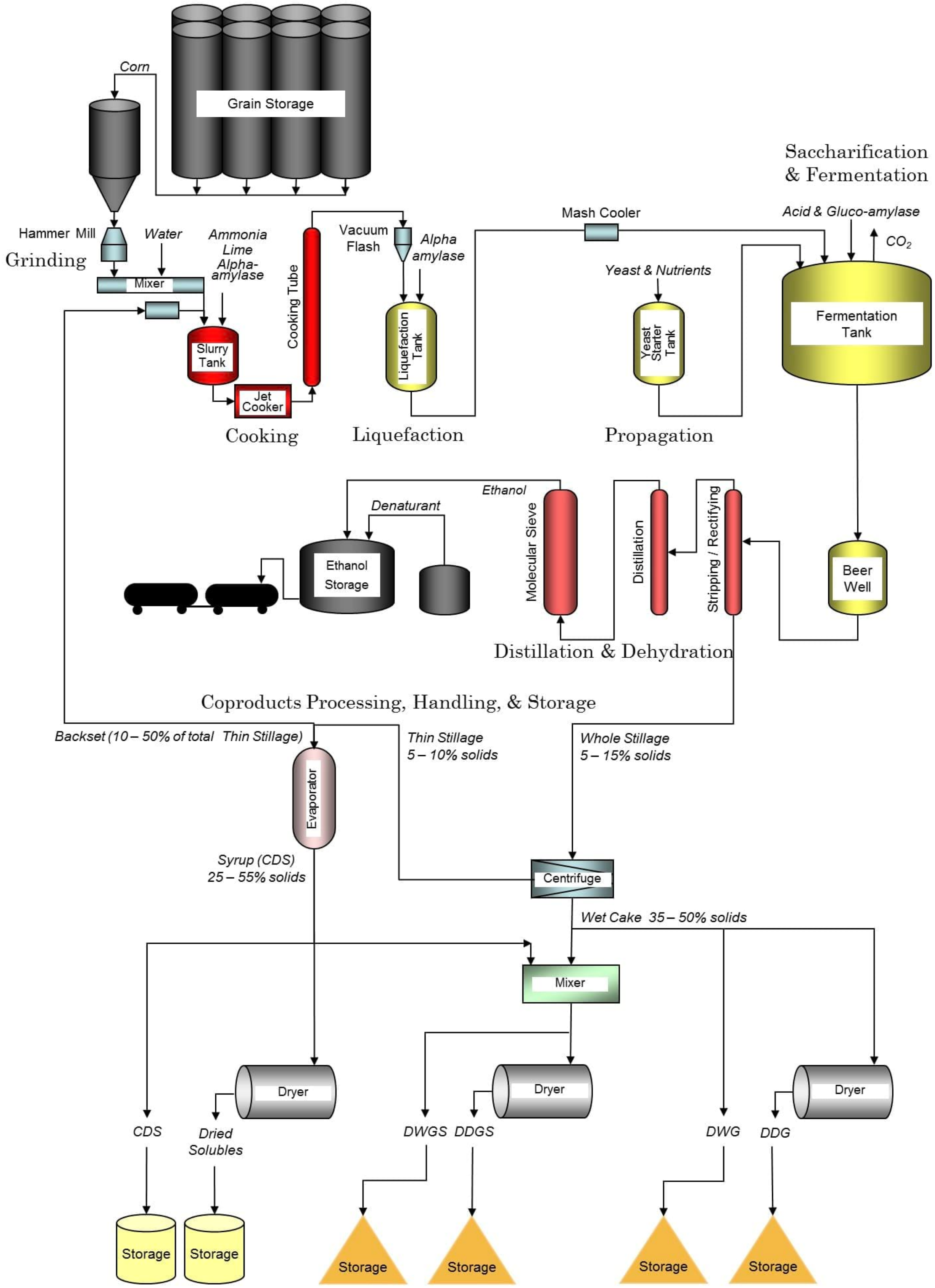


Figure 6. Dry milling corn production process flow chart into fuel and coproducts
Source of Information: Liu and Rosentrater (2011)

2.5 Utilization of DDG and DDGS in animal food

The output of DDGs is very high each year. In 2014 approximately 39 million metric tons of DDGs were produced in the U.S. Most of the DDGs is used as animal feed and some are discarded as waste. When DDGs is discarded, significant resources are wasted as reported by Cromwell, 1993; Lumpkins, 2004; and Stein, 2009. It is known that DDG is an ideal source of dietary fiber, comprising of cellulose and hemicelluloses and a small amount of lignin. Rose, (2010) reported that these fibrous materials are not used in foods for humans. DDGS has many potential applications ranging from animal feed to charcoal production as stated by (Mussatto et al., 2006). Currently, only animal feed garners the significant use of this biomaterial. The main by-product of the distillation of alcohol from maize grain is corn distillers' grain. The processes involved in each production by distillation define the product. For instance, wet distillers' grain (WDG), wet distillers' grain with soluble (WDGS), Dried distillers' grain (DDG), Dried distillers' grain with soluble (DDGs), Condensed distillers soluble (CDS) and dried distillers soluble (DDS). Distillers' by-products have a long and rich history in animal feeding. They are initially considered offal's and were dumped in sewers and rivers. Corn distillers' grains are valuable feed ingredients, rich in protein, moderately rich in fat and relatively poor in fiber and can be fed to all classes of livestock (Hayes, 2008). It is worth to note that as of 2012, corn ethanol by-products are not only relatively recent but are still evolving due to changing technologies.

2.6 Challenges of incorporating DDG into food products

In the United States, Corn is the most common biomass for commercial ethanol production and also an important feedstock for the production of beverages and used in health care and pharmaceutical industries. Distillers Dried Grain with soluble (DDGs) and Distillers Dried Grain are co-products of bio-fuel ethanol and potable ethanol production using mostly corn as the biomass for fermentation. In a typical dry-grind corn-based ethanol production, corn is thermally processed at 90 °C with enzymes to break down corn starch into sugar. The sugar is fermented into ethanol solution at 60^{o}C that is later distilled into 95% pure ethanol solution, dehydrated and denatured to 100% ethanol as an automotive fuel. The underflow from the distillation column, called whole stillage, is centrifuged to obtain distillers wet grain (DWG) that contains 30-35% (w/w) solids. The DWG is dried to obtain Distillers Dried Grain (DDG). Indeed, residues remaining after fermentation the grains are high in Phenolic acids as stated by (Mussatto, Dragone, & Roberto, 2006). DDGS and DDG have many potential applications ranging from animal feed to charcoal production (Mussatto et al., 2006); currently, only animal feed garners the significant use of this biomaterial. However, DDGS has the unique potential for commercial food uses, particularly in baked goods (Bookwalter, Warner, Wall & WU, 1984). The DDGS consists of mainly resistant starch, fiber, protein, and unsaturated lipids (WU, 1994; WU & String fellows, 1982). Inclusion of DDGS could expand markets of consumers in terms of favorable nutritional profile and lower glycemic effect. There is an issue related to incorporating DDGS into food products. It pertains to food safety issue related to potential exposure to mycotoxins (Murphy, Hendrich, & Landgren, & Bryant, 2006) due to fungal infection during the storage of grains

Ewa Peckakielb, (2012) reported that the by-product of the ethanol production by distillation depends on the processes involved, different types of decoctions may be obtained from distilling ethanol from yeast product, dried distiller grains with soluble (DDGS)- the most widely used obtained from wet corn residues (DG) mixed with condensed liquid phase in the form of syrup (CDS) and dried; and high protein dried distillers grain (HPDDG)-bran and germ (rich in fiber and fat) are removed before distillation allowing for the production of dried decoction with high protein content. Foodstuffs in the hydrated form containing dry mass between 5% and 8% (WDG, WDGS, and HPWDG) are cheaper but difficult to transport and to store. Research conducted in recent years has demonstrated the possibilities of corn DDG as feed for livestock due to its high content valuable proteins, high calorific value ad bio elements. Distillers grain has been used as feed for beef and dairy cattle, sheep, swine, and poultry. Recent studies have shown that Distillers dried grain (DDG) can be incorporated into food products for human consumption as it is highly nutritional with high protein and fiber content but there are challenges as DDG do not contain the same functionality as the constituents it replaced. Incorporation of Distillers dried grains impact the sensory qualities of food products, more especially if the inclusion rate is increased. In terms of color, most food products become darker in appearance when distillers' residues are included. In addition, Distillers dried grain do not contribute the same functionality as the components they replace, including resulting volumes and expansion during baking, moisture absorption, texture, and mouthfeel (Rosentrater et al., 2006). Incorporation of distiller by-products at a relatively high level in products also results in a definite negative impact on product flavor; such product has been rated marginally acceptable to not acceptable. Flavor can also be improved, however. Bleaching, and deodorizing distillers grain products can be done before inclusion so the fatty acids and pigments that influence off-flavor development can be neutralized. Due to these challenges, there is currently no food product that incorporates ethanol processing residues in commercial foods.

2.7 Brief History of Wheat

Wheat is the 6th most valuable crop in the United States. Wheat (Triticum aestivum) is one of the major grains in the world with an estimated production of 733MT in 2017/18. (Food and Agriculture Organization of the United Nations) There are six economic classes of wheat which are grown in the United State which includes the following: Hard Red Spring Wheat, Hard Red Winter Wheat, Soft Red Winter Wheat, Soft White Wheat, Hard White Winter Wheat and Durum Wheat. These classes of wheat have different purposes, different functionality and distinctive attributes for the end user or consumer, strength of gluten and the amount of protein present and bran.

Wheat grains contain three parts: the endosperm consisting mostly of lipids and proteins, the bran consisting mostly dietary fiber and the germ consist half percent of the kernel which includes essential amount of protein, B complex vitamins and trace minerals (Marquant, L; Jacobs JRD, Mcintosh, G.H; Poutanen, Reeks, M: 2008). Commercially, wheat flour is produced according to its end use/application. For example, bread making; white flour (WF) differs considerably from the whole wheat flour (WWF) and the difference arises in the selection of wheat cultivar, milling, and chemical composition. The major difference between the two is that the white bread flour is produced only from the endosperm, whereas the WWF includes the germ and bran.

2.8 Utilization and importance of Wheat

The three most important crops that are planted in the U.S consist of corn, soybean, and wheat in 2018/2019. Wheat is considered as the third most important crop that is heavily planted and utilized in the U.S. Wheat is the primary food grain grown in the U.S which consist of three main heterogenetic groups namely, durum, spring and winter wheat. Seventy - 80% of the wheat planted in the U.S are winter wheat which constituent up to 1,100 million to 1,800, this makes winter wheat the highest planted wheat in the USA. Spring wheat is one third of wheat planted in the US which constituents of 400 million to more than 600 million bushels while Durum wheat is the least produced wheat which constitutes 3 to 5% of wheat planted in the U.S with about 75 million bushels (USDA 2018).

In food production, wheat is the most widely used and most cultivated in the world because it has come to be a firm favorite grain and provides diversity in culinary applications. Many different types of wheat grain exist, with two main types being eaten namely bread wheat (Triticum aestivum vulgare) and durum wheat (Triticum turgidum durum). The durum variety is used in the manufacture of pasta while the other wheat-based foods. Bread wheat is typically white and does not have the red color, which typifies most bread wheat grown in the Northern hemisphere. Bread wheat is described as 'hard' or 'soft' according to its protein content. Hard wheat has more protein, including more gluten which makes it purposeful to bake bread, while soft wheat has a much lower protein content, which when milled produces 'cake flour' for sweet biscuits and cakes. Aside from bread wheat and durum, other types of wheat include Spelt, emmer, einkorn, and Kamut. These wheat varieties are commonly referred to as 'ancient' grains and are increasingly being used in the manufacture of niche wheat-based food product. (Grains and legumes nutrition council, 2017).

2.8.1 Wheat Milling Process

The process of roller flour milling of wheat involves breaking open of the grain, scraping the endosperm from bran and germ by break rolls and then gradually reducing the chunks of the endosperm into flour by a series of grindings by reduction rolls, with intermediate separation of products by sifters and purifiers (Bass 1988). The quality of the flour produced depends on a number of factors including particle size. The particle size of the flour produced depends on the wheat quality, sifter sieves opening, type and the adjustment of rolls.

2.8.2 Nutritional Value of Wheat Grain

Wheat grain has numerous nutritional attributes which include:

- Relatively high in protein content (11%-13%) compared with other major grains and contains a protein complex which forms gluten.
- High levels of potassium and low in sodium.
- The endosperm contains glycofraction (similar in structure to insulin) which functions as a prebiotic agent and has similar properties to dietary fiber.
- Contains B-group vitamins such as thiamin, riboflavin, niacin, vitamin B6 (Pyridoxine), folate and pantothenic acid.
- Contains Iron, Zinc, Magnesium, Phosphorus, and Selenium (depending on the soil content of selenium).

- Contains small amounts of copper, manganese, and calcium.
- Contains phytochemicals including lignans, phenolic acids, Phytic acid, Plant sterols and saponins (Grains and legumes nutrition council, 2017).

2.9 DDG as functional ingredients in food

Over the years, various research studies have been conducted to examine the use of ethanol-manufacturing residue as functional ingredients in human foods. Table 3 provides a summary of such articles reviewed by (Rosentrater and Krishnan, 2006) to examine the effect of inclusion of distillers' grains in food product

Table 3. Review of literature relating to food products developed using ethanol co-products as ingredients or functional additives.

Application	Residue	Feedstock	Inclusion Rate (%)	Functionality	Sensory Panel Analysis	Citation
Blended Ingredients	DDG, DDGS	Corn	0.5, 10	Darker in appearance	Quality poor and unacceptable	Bookwalter et al. (1984)
Blended Ingredients	DDG	Corn	5, 7.5	Darker in appearance	Quality poor; solvent extraction improved flavor to acceptable	Bookwalter et al. (1988)
Blended Ingredients	DDGS	Corn, red wheat, white wheat	0, 24, 73, 29, 09, 31, 68	Poor growth during rat feeding trails due to deficient amino acids	-----	Dong et al (2003)
Blended Ingredients	DDG DDG, DDGS	Corn	0, 2.5, 5, 10	Acceptable digestibility during rat feeding trails	-----	Wall et al (1984)
Blended Ingredients (food aid)	DDG	Corn, soy	0, 5, 10	Darker in appearance	Poor flavor quality	Bookwalter et al. (1984)
Bread	DDG	Barley, corn, rye	5, 10, 15, 20	Poor dough development: Loaf volume decreased; Darker in appearance	Bitter but acceptable	Brochetti et al (1991)
Bread	DDG	Sorghum, (Brown, white, white waxy, yellow)	5, 10, 15	Darker in appearance; loaf volume decreased	Acceptable up to 10%	Morad et al (1984)
Bread	DDGS	Sodium Stearoyl-2Lactilate (SSL)	0, 10, 20	Darker in appearance	-----	P Krishnan (2018)

Bread	DDGS	Wheat	12-23	Darker in appearance; reduced loaf volume	-----	Rasco et al (1991)
Bread	DDGS	White wheat	20	High concentrations of soluble minerals	-----	Rasco et al (1987)
Bread	DDG	Corn and other cereals	10, 20	Decreased dough stability; reduced loaf volume and crumb grain; darker appearance	-----	Tsen et al (1983)
Bread: Baguettes	DDGS	White wheat	0, 12.5, 25	Darker in appearance	Less acceptable	Rasco et al (1989)
Bread: Banana	DDGS	White wheat	30	Darker in appearance	Good to excellent	Rasco et al (1987)
Bread: Carrot coconut	DDGS	Barley, corn, rye	0, 40	Darker in appearance	Acceptable to highly acceptable	O'Palka et al (1989)
Bread: Cinnamon rolls	DDGS	White wheat	0, 12.5, 25	Darker in appearance	Acceptable	Rasco et al (1989)
Bread: Muffin rolls	DDG	Barley, corn, rye	0, 10, 20, 30	Greater water and oil absorption; darker in appearance	Acceptable, but 30% much less acceptable	Brochetti et al (1991)
Bread: Dinner rolls	DDGS	Barley, corn, rye	0, 17, 33	Darker in appearance, decreased volume; chewier	Acceptable to highly acceptable	O'Palka et al (1989)
Bread: Doughnuts DDG	DDG	Barley, corn rye	0, 10, 20, 30	Greater water and oil absorption; darker in appearance	Acceptable, but 30% much less acceptable	Brochetti et al (1991)

Bread: Dough	DDG, DDGS	Barley, red wheat, soft white winter wheat	0, 4, 8	Darker in appearance; decreased loaf volume; decreased crumb grain coarseness; increased water absorption	-----	Rasco et al (1990)
Bread: Hush puppies	DDG	Barley, corn, rye	0, 10, 20, 30	Greater water and oil absorption; darker in appearance	-----	Brochetti et al (1991)
Bread: Muffins	DDG	Barley	0, 15	Cooked volume appearance; decreased volume	Poor	Dawsen et al (1985)
Bread: Nut rolls	DDGS	Barley, corn rye	0, 33	Darker in appearance; decreased volume	Acceptable to highly acceptable	O'Palka et al (1989)
Bread: Oatmeal muffins	DDGS	Barley, corn rye	0, 5, 15, 36	Darker in color	Acceptable	Abbot et al (1991)
Bread: Oatmeal muffins	DDGS	Barley, corn rye	0, 33	Darker in appearance; increased volume	Acceptable to highly acceptable	O'Palka et al (1989)
Bread Tortilla	DDGS	Wheat	0, 10, 20	Darker in color for 10 and 20% substitution	----	Shrin Pourafshar (2015)
Bread: Various	WS	Cereal grains	10-50	-----	-----	Rasco et al (1989)
Bread: white muffins	DDGS	Cereals grains	0, 10, 15, 20	Lighter in appearance	Off-flavors detected at 20%	Reddy et al (1986)
Bread: White	DDGS	White wheat	30	Darker in appearance	Acceptable to good	Raco et al (1987)

Bread: Whole wheat	DDGS	Barley, corn rye	30	Darker in appearance	Acceptable	Raco et al (1987)
Bread: Yeast rolls	DDGS	Barley, corn rye	0, 33	Darker in color	Acceptable	Abbot et al (1991)
Brownie	WS	Cereal grains	10-50	-----	-----	Rasco et al (1989)
Canned: Beef Stew	DDGS	Barley, corn rye	0, 1, 2, 3	-----	Acceptable flavor, appearance, and mouthful	Reddy et al (1986)
Canned: Chili	DGGS	Barley, corn	0, 1, 2, 3	-----	Acceptable flavor, appearance, and mouthful	Reddy et al (1986)
Canned: Hot dog sauce	DGGS	Barley, corn rye	0, 1, 2, 3	-----	Acceptable flavor, appearance, and mouthful	Reddy et al (1986)
Cookies: Bar	DDG	Barley	0, 15, 25	Darker in appearance: Decreased width and thickness	Acceptable, but regular still better flavor	Tsen et al (1982)
Cookie: Chocolate chip	DDG	Barley	0, 15, 25	Darker in appearance; decreased width and thickness	No flavor difference	Tsen et al (1982)
Cookie: Chocolate chip	DDG	Sorghum (Brown, white waxy, yellow)	5, 10, 15	Darker in appearance	Acceptable	Morad et al (1984)
Cookie: Chocolate chip	DDGS	White wheat	30	Darker in appearance	Good to excellent	Rasco et al (1987)
Cookie: Chocolate chip	DDGS	White wheat	0, 12.5, 25	Darker in appearance	Acceptable	Rasco et al (1989)

Cookie: Molasses	DDG	Sorghum (Brown, white waxy, yellow)	0, 12.5, 25	Darker in appearance	Acceptable up to 50%	Morad et al (1984)
Cookie: Molasses raisin	DDG	Barley, corn, rye	0, 10, 2, 30	Greater water and oil absorption; darker in appearance	Acceptable	Brochetti et al (1989)
Cookie: Oatmeal	DDG	Barley	15	Lipid composition degraded during ethanol processing: Bleaching worsened lipid damage	Acceptable; defatted DDG better than defatted bleached DDG, or DDG	Dawson et al (1985)
Cookie: spice	DDG	Barley	0, 15, 25	Lipid composition degraded during ethanol processing, bleaching worsened lipid damage	Acceptable, but without skill had better flavor	Tsen et al (1982)
Cookie: Sugar	DDG, DDGS	Barley, red wheat soft white winter wheat	0, 2, 4, 8	Darker in appearance, variable spread	------	Rasco et al (1990)
Cookie: Sugar	DDG,	Barley	0, 15, 25	Darker in appearance, decreased width, and thickness	-----	Jaques et al (2003)
Cookie: Sugar	DDG	Sorghum (brown, white, white waxy yellow	5, 10, 15	Darker in appearance	Acceptable	Morad et al (1984)
Cookies: Various extruded product	WS DDG	Cereal grains wheat	10-15 \`0, 10, 20, 40	Darker in appearance; unit density and longitudinal expansion	-----	Rasco et al (1989)

				increased; radial expansion increased		
Extruded product	DDG	Barley, corn, oat, rye, sorghum, wheat	0, 20, 50, 100	Unit density and longitudinal expansion increased	-----	Kim et al (1989)
Extruded product	DDG	Corn	0, 10, 20, 40	Decreased expansion	Acceptable up to 20%; poor quality at greater than 20%	Kim et al (1989)
Flour	WS	Cereal grains	10-50	-----	-----	Wampler et al (1984)
Flour	DDG	Corn	100	-----	Extraction produced acceptable flavor	Wu, et al (1990)
Granola	DDG	Barley	7.5	-----	Not as good	Dawson et al (1987)
Granola bar	DDG	Barley	2.4	-----	Not as good	
Health foods	WS	Cereal grains	-----	-----	-----	Dawson et al (1987)
Ingredients	WS	Wheat	100	Bleaching produced much lighter appearance	Bleaching eliminated flavors and odors	Tolle et al (2004)
Ingredients	DDGS	White wheat	100	Antioxidant did not improve lipid stability: Drying method affected lipid stability	-----	Abdel et al (1996)
Muesli	WS	Cereal grains	-----	-----	-----	Tibelius et al (1996)
Pasta	DDG	Wheat	0, 25, 50	Darker in appearance: Cooked weight decreased lower water absorption	Appearance, flavor, texture acceptable at 25%; Unacceptable at 50%	Maga et al (1989)
Pasta	WS	Cereal grains	10-50	-----	-----	Rasco et al (1989)
Ready-to-drinks	WS	Cereal grains	-----	-----	-----	Tolle et al (2004)

Spaghetti	DDG	Corn	0, 5, 10, 15	Increased cooking loss: Decreased firmness	Flavor and texture acceptable at 25%; Unacceptable at 50%	Wu, et al (1987)
Spaghetti	DDG	Red wheat, rye, sorghum, white wheat	0, 5, 10, 15, 20, 30	Cooking quality acceptable, but lower darker in appearance	Poor sensory qualities; unacceptable	Tibelius et al (1996)
Whole Desserts	WS	Cereal grain	-----	-----	-----	Tolle et al (2004)
Yogurt	WS	Cereal grain	-----	-----	-----	Tolle et al (2004)

Source of Information: Rosentrater and Krishnan (2006)

From their review, the authors observed that there are challenges to incorporating distillers' grain in food products because of its impact on sensory qualities of food products especially if the inclusion rates increase. Most food products become darker in appearance when distillers' residues are included. According to their review, distillers' grains do not contribute the same functionality as the components they replace, including resulting volume and expansion during baking, moisture absorption, texture, and mouthfeel. Although the increase of ethanol industry has led to the quest of many plants to extend the use of DDG beyond livestock and ethanol use which have activated the ongoing research on premium standard on the production of food grade DDG, as stated by Rosentrater etal., (2006). In another study by J. A. Saunders et al., (2013), statistical evidence proved that the analytical and physiological results stipulates strong improvement in the protein content, reduction of lipids, red and yellow and pigments and generally increased the brightness of DDG.

However, ongoing research employed in South Dakota State University is generally leading to an improved form of DDG sterilization, consumer acceptance and the demonstration of the importance of DDG as an essential food ingredient.

In this research, particle size of Distillers Dried Grains (fine and coarse) was used as a variable in blends of wheat flour (Bread Flour) to determine efficacy in bread production. In addition, the levels of FDDG substitutions were varied as well to determine the upper limits of enrichment (05, 5%, 10% & 20 %).

CHAPTER 3. MATERIALS AND METHODS

The major raw material (Distillers Dried Grain) was collected from the ethanol plant in Watertown. The first step was to carry out the initial moisture content measurement of the raw DDG before processing. Generally, moisture content in DDG from the plant varies from 50 to 60%. This level of water content is drastically reduced using moisture oven for 103 ^{0}C for 3 hours drying to 2 – 3 percent. Food grade distillers dried grain (FDDG) are plant-derived material that has been treated with solvents - ethanol to reduce pigmentation and improve color attribute thereby making it a shelf stable and wholesome food product, as compared to the same plant-derived materials not treated with solvents. Food grade distillers dried grain was milled by reducing the particle size to coarse and fine samples. The latter were blended with wheat flour using substitution levels of 0%, 5%, 10% and 20%. The blended samples were analyzed for physio chemical properties, compared for particle size distribution of different mesh sieve sizes of #40, #60, #80, #100, #200 and bottom pan. The rheological properties were compared between control and sample blends. Bread was baked using the various FDDG-wheat flour blends. The effect on loaf volume was determined and finally, “bread” was analyzed for sensory and textural attributes to determine acceptance and preference of the final products.

3.1 Experimental Design

3.2. Sample Preparation

Corn Distillers Dried Grain materials was collected from an ethanol plant in Watertown, South Dakota and stored frozen at -80 F until securely for further treatment. For the treatment of Distillers Dried Grain, ethanol was procured from South Dakota State University, Department of Chemistry. Hard Red Winter wheat, and other baking ingredients were procured from Walmart.

Experimental Design

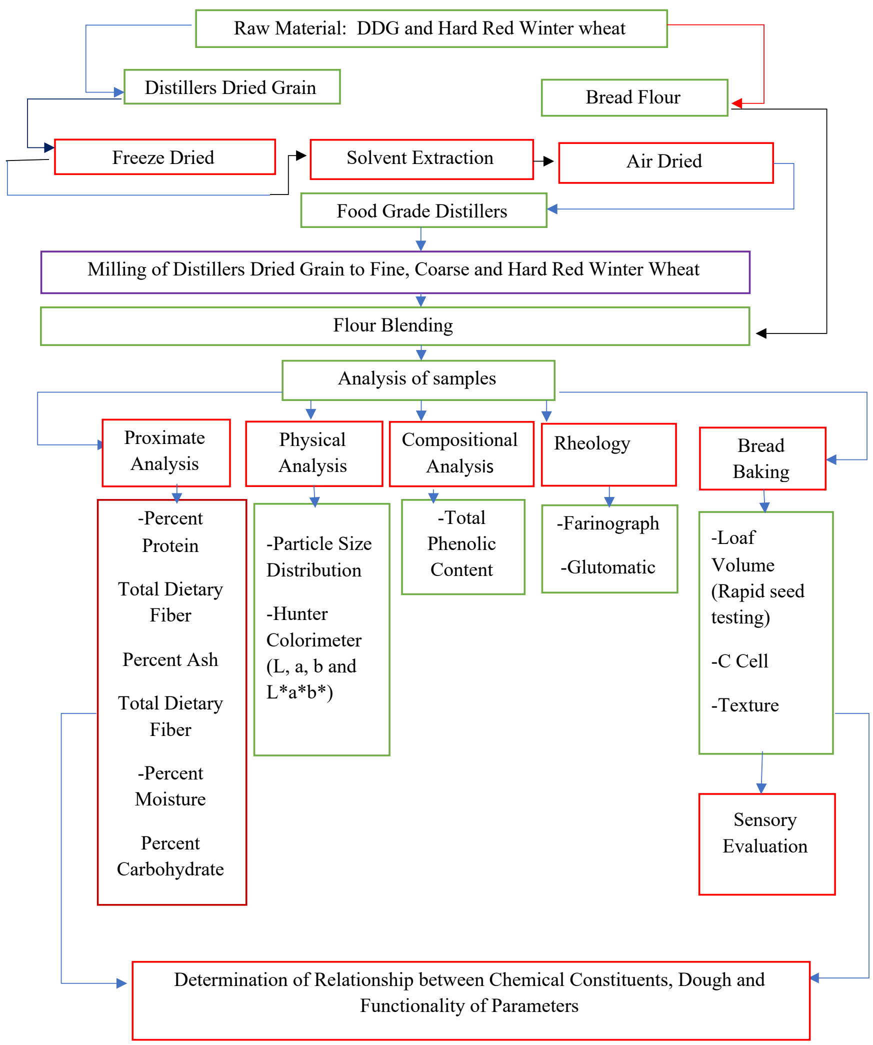


Figure 7. Process flow chart for treatment of DDG and its evaluation in baked bread

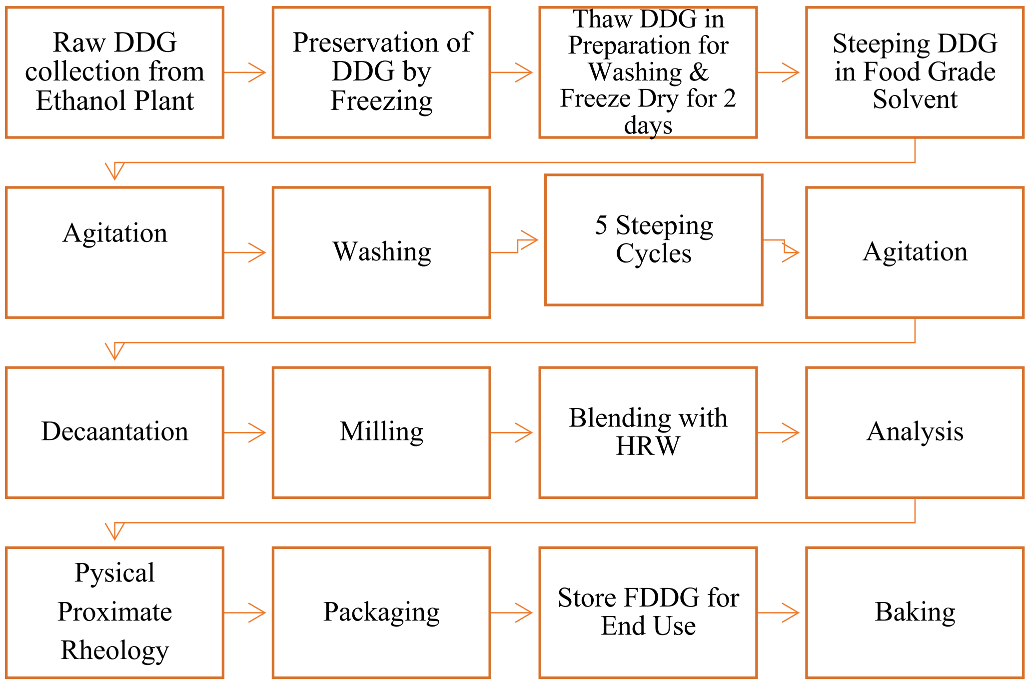


Figure 8. processing steps for Distillers Dried Grain (DDG) products

3.2.1 Flour Formulation

Wheat flours containing various levels of DDG enrichment were created by weight for weight direct replacement of flour using DDG. The blending were made homogenous with the use a crossflow blender. Two sizes (fine and coarse) of FDDG particles were employed. Size distinction of the FDDG particle size was accomplished by the use of 0.2mm and 0.5mm screens used in conjunction with the Retsch Ultracentrifugal Mill.

Blends with the appropriate substitutions in wheat flour were developed as shown below:

- 50g 0f DDG was blended with 950g of Bread flour on 0.2mm sieve mesh
- 50g 0f DDG was blended with 950g of Bread flour on 0.5mm sieve mesh
- 100g of DDG was blended with 900g of Bread flour on 0.2mm sieve mesh.
- 100g of DDG was blended with 900g of Bread flour on 0.5mm unit of mesh
- 200g of DDG was blended with 800g of Bread flour on 0.2mm unit of mesh
- 200g of DDG was blended with 800g of Bread flour on 0.5mm unit of mesh.

3.2.2 Bread Preparation

Ingredients utilized during the preparation of bread included, yeast, salt, sugar, vegetable oil, water, and a blend of bread flour and food distillers dried grain. The bread mix pan was initially removed, and a kneading paddle was installed on the drive shaft. The flat side of the shaft was matched to the corresponding side of the kneading paddle allowing for proper shaft alignment. Next, the ingredients were placed in the mix pan. Liquid ingredients consisting of water and vegetable oil were weighed and placed in the mix pan first. Dry ingredients including flour, sugar, salt, and finally yeast was weighed then added to the mix pan. The mixture was then placed into the baking chamber and the lid secured. Lastly, the bread machine menu was activated to adjust and select the color and size parameters that are desired. For the color, I chose medium on a possible color scale of light, medium, and dark. Weight parameters for this bread machine menu range from 1.5 Ibs to 2.5 Ibs. The weight of the dough was 2 lbs, therefore this was the setting used. This particular model has a temperature that is self-governed based on the entered parameters listed above and type of flour, and the amount of time you want your bread baked for completion. For this project, I used the 3-hour setting.

Table 4. Ingredient formulations used in the production of Control and 5%, 10% and 20% FDDG Bread

Ingredients	(BF) Control	Fine FDDG			Coarse FDDG		
FDDG	0%	5%	10%	20%	5%	10%	20%
Flour Blend	295g	294g	291g	286g	295g	291g	286g
Yeast	9.0g	9.0g	9.0g	9.0g	9.0g	9.0g	9.0g
Salt (Nacl)	2.0g	2.0g	2.0g	2.0g	2.0g	2.0g	2.0g
Sugar	22.0g	22.0g	22.0g	22.0g	22.0g	22.0g	22.0g
Vegetable oil	42.0g	42.0g	42.0g	42.0g	42.0g	42.0g	42.0g
Water	187.5	194	201	230	193	200	272

BF=Bread Flour, FDDG= Food grade Distillers Dried Grains

3.3 Moisture analysis:

3.3.1 Determination of moisture content

The moisture content of Distillers Dried Grain samples was determined according to the American Association of Cereal Chemist (AACCI) oven drying method 44-15.02 (Figure 3.13). Crucibles were washed thoroughly and dried in an oven at 130 ^{0}C for 15 minutes. The hot dried crucibles were cooled in the desiccator for 15 minutes and then weighed.

One gram (1 g) of the sample each were weighed into the crucibles and then dried at 103 ^{0}C for 3 hours in Fisher Isotemp^{TM} oven (Fisher Scientific, PA) forced air convection. Dried samples were removed, cooled in the desiccator, and reweighed. The percentage moisture content was calculated as follows:

Equation 1.1

$$\%\ Moisture\ content = \frac{W_2 - W_3}{W_2 - W_1} * 100$$

Where:

W_1 = Initial weight of empty crucible

W_2 = weight of crucible + weight of sample before drying

W_3 = weight of dish + weight of sample after drying

3.4 Physical analysis

3.4.1 Technique of Sterilization and Washing of DDG

DDG was manually washed in an excess of food-grade solvents employing a protocol developed at South Dakota State University. The raw material was collected from an ethanol plant in Watertown and stored in airtight hermetically sealed containers and stored in a freezer until further use. DDG was thawed at room temperature for 1 hour and freeze-dried for 24 hours. DDG was treated with solvent in five cycles for use as FDDG (Food Grade DDG). Stage 1 began with steeping 1000 gm of freeze-dried material in 2000mL of solvent for 2 hours while agitating. Using a hand-pressing technique to remove the solvent from the DDG, 700mL of steep Ethanol was filtered through #170 sieve. The above step was repeated for an hour while agitating and washing. The entire process was repeated 5 times to achieve a premium and sterilized safe-to-eat product. Total ethanol volume that was used for steeping for 5 cycles was 5000mL while the total used for washing for 5 cycles was 3500mL which is a total of 8500mL, making it 2.2 gallons of ethanol used. DDG was spread out in a freeze dryer tray lined with foil and then air-dried overnight. The air-dried FDDG was milled in a Retsch mill with 0.2 mm (fine) and 0.5-mm (coarse) sieves. Fine and coarse FDDG were used in formulating FDDG-bread flour blends. Bread flour was thus enriched at the 0 (control) 5%, 10%, and 20% and levels. Homogenous distribution of the FDDG within the bread flour was accomplished using the crossflow blender.

Table 5. General steeping and washing procedure for the production of FDDG from DDG

Number of Washing Steps	Ethanol volume (in mL)	
	Steeping volume	Washing volume
0	Material from first steeping step	700
1	1000	700
2	1000	700
3	1000	700
4	1000	700
5	1000	700
Total volume (in mL)	5000	3500

Figure 9. Technique for Washing, Freeze Drying and Sterilization of DDG

Figure 10. Dry grinding of DDG using Retsch mill employing a 0.2- and a 0.5-mm sieve. Retsch mill (Retsch Brinkmann, GmbH & Co.KG, 5657 HAAN1, and Germany)

3.4.2 Particle Size Distribution Analysis

The Particle Size Distribution of Distillers Dried Grain (both raw and processed) was analyzed with a Ro Tap Sieve Shaker, employing different sieve mesh units. The sieve sizes were 40, 60, 80, 100, 200 and with 40-sieve mesh placed on top according to official AACC Standard 55-60.01 (AACC, 2011). A collection pan recovered the material flowing through the bottom-most 200 mesh sieves. Aliquots of 100 grams of the samples were weighed out and placed on the top sieve (#40 mesh) to see the particles size distribution determined by the various sieves. The shaker was operated for five minutes. At the end of 5 minutes, DDG material from the top of the sieves and the collection pan were collected and weighed. The particle size was measured in 'microns (μ)' and the flour fractions retained on the sieves were expressed as 'percentage (%)' since the initial 100 gram sample weight. Color values (L, a, & b) were determined for each sieved fraction. Statistical associations were made between color values and the particle size fractions.

Figure 11. Stacked Tyler Sieves for determination of particle size distribution

Figure 12. Processes used in determining particle size distribution of flour samples

3.4.3 Colorimetry (L*a*b)

A Minolta colorimeter was used to evaluate the color profile of DDG samples using the L*a*b scale for color. On this scale, 'L' refers to the "brightness" of the sample and it is a score ranging from 0 (black) to 100 (pure white). Parameters 'a' and 'b' are scored on positive and negative scales. The a scale was reflective of (redness (+a) versus greenness (-a). The b scale was reflective of yellowness (+b) versus blueness (-b). Color evaluation was performed before and after DDG washing to determine effectiveness of washing. The same color evaluation system was also used to compare color differences between the control unfortified bread and DDG-fortified bread (Figure 13 & 14).

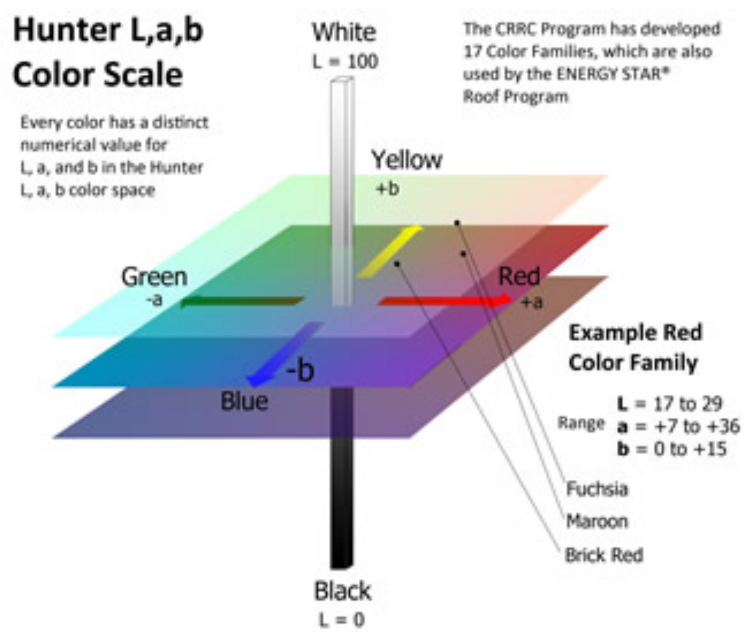


Figure 13. Hunter L, a, & b Color Scale (Image ethanol plant fractions)
https://cindyallen.files.wordpress.com/2015/02/hunter-l-a-b-color-scale.jpg

Figure 14. Calorimeter - Konica Minolta CR-400 chromameter (Image ethanol plant fractions)

3.4.4 Water activity measurement:

Water Activity was determined on the flour formulation by the use of Aqua Lab water activity meter for flour formulation (Figure 16). Before the experiment, the water activity meter was calibrated by the use of LiCl (8.57 molal solution) in water reference standard with an Aw = 0.500. Raw and wet DDG were measured by the use of LiCl (13.41 molal) in water reference standard with an Aw= 0.250.

3.5 Proximate Analysis:

Samples were analyzed according to the officially accepted methods for protein content (AOAC official method 990.03), fat content (AOAC American Oil Chemists' Society (AOCS), Am 5-04 (2005), ash content (AACC 08-03 method), moisture content (AACC1 official method 44-15.02), and Total dietary fiber (TDF) content - (AOAC official method 991.43).   Carbohydrate content was calculated by difference (Kraisid et al; 2003).

3.5.1 Determination of protein content

The protein content of samples was determined according to an official method (AOAC 990.03 Crude Protein in the animal feed) employing the N/protein analyzer rapid MAX N Exceed (Elementar, Germany Lakewood, NJ). % Crude protein was calculate using a %N to % protein conversion factor of 6.25 (Council 2012). This procedure employs a combustion method at 900°C in the presence of oxygen.

Figure 15. Flash EA 1112 N/Protein analyzer

Figure16. Water Activity Meter

3.5.2 Determination of fat content

The percent crude fat content in samples was determined using the modified standard method approved by American Oil Chemists' Society (AOCS), Am 5-04 (Society 2005) with the use of ANKOM Fat extractor (Model XT115) with petroleum ether as the solvent for extraction. The extraction was done in 90ºC and the loss in the weight of the sample was measured after fat extraction. The samples where dried prior to extraction at 103ºC for 3 hours in an oven and expressed in dried weight after extraction, cooling and weighing. The following formula was used to calculate the fat percentage:

Equation 1.2

$$\%\,Crude\ oil = \frac{W_2 - W_3}{W_1} * 100$$

Where W1 = Original weight of sample

W2 = Weight of pre-extraction dried sample and filter bag

W3 = Weight of dried sample and filter bag after extraction

Figure 17. ANKOM Fat extractor (Model: XT115)

Figure 18. Moisture Oven

3.5.3 Determination of Ash content analysis

The ash content of the blends of flour was determined in a muffle furnace (Box Furnace, 51800 series). Samples were incinerated at 525°C for 12 hours. AACC 08-03 method was used to estimate the total inorganic mineral content by dry oxidation method. Ash content was expressed in dry weight basis (Figure 20).

3.5.4 Determination of carbohydrate content (CHO).

Carbohydrate was determined by the difference which was done by subtracting the sum of % ash, % protein, % fat, % moisture, and % crude fiber from 100 %.

% Carbohydrate = [100%- (protein%, + fat% + ash%, + moisture%)].

3.5.5 Total Dietary Fiber:

The Official Standard AOAC method 991.43 was used to analyze soluble and insoluble dietary fiber content by the use of a gravimetric method. The instrument uses Megazyme assay test kits. The sample and Diatomaceous earth was weighed separately. The filter bag was installed in the TDF machine and clipped, diatomaceous earth and samples where added into the bags. The sample was digested, and the PH was checked. Rinsed with acetone, IDF/SDF was weighed. The automated fiber recovery was done by first digesting samples within using enzyme treatments, the instrument collected the IDF and SDF residue using two separate filter, the TDF residue was collected. There was correction of results by determining protein and ash content and the dietary fiber value was calculated and expressed in dry weight basis.

Figure 19. ANKOM TDF fiber analyzer

Figure 20. Muffle furnace by Cole Parmer (Model: Box furnace, 51800 series)

3.6 Dough Rheological Analysis:

3.6.1 Dough Rheological Properties

The Farinograph analysis was done to evaluate the dough rheological properties of blends according to the standard method of AACC (1990) 54-20. This machine is made up of two mixer blades to mix flour samples with water, and measures water absorption, mixing tolerance index (degree of softening), development time, departure time, peak, dough stability and farinograph quality number.

The Farinograph consist of a drive unit with speed control to evaluate the rheological properties of dough. Dough Resistance against the mixing blades, is measured in torque. The instrument is a recording dough mixer. Optimal mixing conditions including optimal water requirements and optimal mixing times provide valuable information to the baker about specific flours.

3.6.2 Farinograph Percent Water Absorption

This is the required for the dough to give the miller or the baker the amount of water required for the baking which helps the miller or baker know the required yield needed for baking. The amount of water added to balance the farinograph curve on the 500-FU line, expressed as a percentage of the flour (14% mb). This parameter is useful in adjusting the water relationships in commercial doughs when flour changes. Farinograph water absorption helps the baker to determine the maximum strength of dough in production of bread.

3.6.3 Farinograph Dough Development Time

Farinograph dough development time is an essential parameter in dough testing as it provides the optimal mixing time for the dough. This is seen as the time in minutes between beginning of the curve and the point where it reaches its maximum peak. Dough development time can be interchangeable with peak time. This is the stage where the dough reaches it maximum consistency before it begins to break down.

3.6.4 Mixing Tolerance Index (MTI)

Mixing Tolerance Index (MTI) is determined by taking the difference in Brabender Units ranging from the peak of the curve and point along the curve measured 5 minutes after the peak arrival. MTI demonstrates the mixing tolerance of sample and can be seen to be good if the value is 30 B.U and excellent if less than 30 B.U. The mixing tolerance of 50 B.U shows less tolerance and will usually be challenging when used. Generally, a low numerical value is desired for MTI.

3.6.5 Farinograph Dough Stability

Farinograph Dough Stability is the intersection of the 500 BU. The point where curve intercepts to where the point drops which are known as the arrival and the departure time. Long stability indicates longer mixing time. Stability is measured in minutes. Long stability times indicate good dough strength.

3.6.6 Farinograph Quality Number

Farinograph quality number show the global description of the attributes measured in a farinograph. This shows that sample with higher farinograph value tend to have a better baking characteristic as compared to the ones with lower farinograph quality number.

3.6.7 Gluten analysis:

The Glutomatic system (Perten Instruments, Waltham, MA) was used and the official AACCI method 38-12.02 was followed. The instrument separates gluten from flour by washing off starch. Ten gram of flour sample was weighed in duplicates and mixed with water to form a dough. The washing sieve was wet with 2% NaCl to prevent losses. The washing chamber with 88 µm polyester sieve was washed with 3.5 – 5 mL of 2% NaCl solution. The flour formed a dough after mixing for 20 seconds, after mixing, washing was done for 5 minutes which removed starch and formed a gluten mass. After washing the gluten mass was transferred to the centrifuge which was done for 6000 rpm. The gluten network that passed through the sieve was weighed. The good wet gluten was added to the balance to get the total wet gluten weight which was finally dried in Glutork for 4 minutes and the weight of the wafer was taken. Dry gluten index was calculated and expressed in 14% moisture basis.

3.7 Bread Loaf Volume

Samples of the bread was allowed to cool completely for 2hours before measurement of weight and volume was taken by the use of AACCI method 10-05.01. Weight of each sample was taken by weighing on a scale with a maximum weight limit of 200.0g. The rapeseed displacement test was used to determine the loaves of bread. Each loaf was placed in a container; rape seeds was added until a volume of 200ml is reached. The volume of rapeseeds was measured in a graduated cylinder to determine loaf volume by difference.

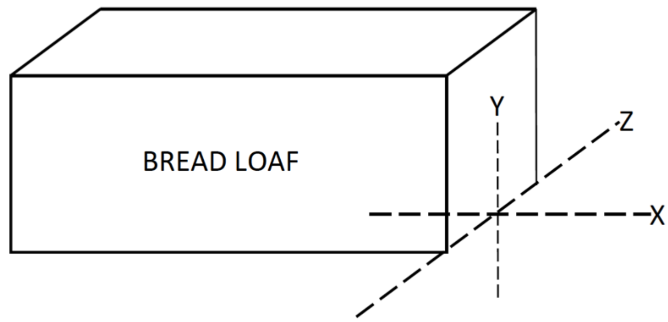


Figure 21. Bread loaf description of Sample cutting for texture analysis

3.8 C-Cell Analysis

Digital image analysis of the bread cell structure was determined with the use of C-cell machine (cc.300.06, caliber control International Ltd, Warrington, UK). Bread was sliced to 0.5 inches thick and the images of slices were compared across all bread formulations. Cell size, number of cells, cell wall thickness, uniformity, and overall shape of the bread slices were obtained. The c-cell machine is a device that is used as a digital machine to evaluate the important features of a new developing products. The c-cell evaluates the functionality of a new product as regards to so many paramerters.

Figure 22. C-Cell

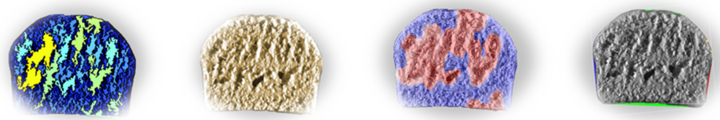


Figure 23. Image analysis of bread slice by C-Cell

Figure 24. The Glutomatic System

3.8.1 Texture Analysis

A TAXT Plus Texture Analyzer was used to test for bread texture through texture profile analysis (TPA). A cylinder 1¼ in tall in length and 7/8 inches was cut out along the Y-axis (Crowley et al; 2002, Minarro et al; 2010). A probe with a trigger force of 5.0g was used to compress to 10.000 mm. 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 (Figure 25).

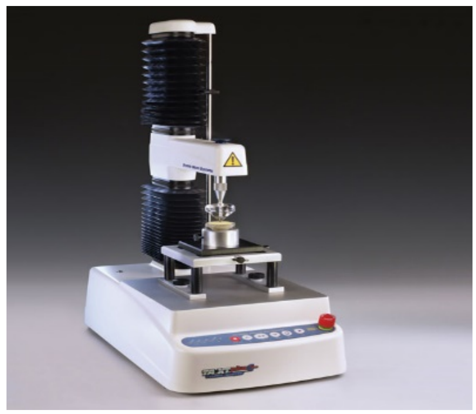


Figure 25. Texture Analyzer

CHAPTER 4. RESULTS AND DISCUSSION

In this study, a product of the ethanol processing plant, namely Distillers Dried Grains (DDG), was further processed into a food grade material that will be referred to as Food Grade Distillers Grains (FDDG). This material was further fractioned into Fine FDDG and Coarse FDDG using mill. Each fraction was used to fortify wheat bread formulations at the 5,10, and 20% levels of substitution. The resulting flour blends, their dough, and bread produced from the fortification were evaluated for nutritional and food functional traits. The outcomes of the various physical and chemical analyses are reported in this chapter. The information will be organized into sections to reveal the nature of the raw materials used as ingredients in bread making for the production of high-fiber and high-protein DDG fortified bread, The effects of using two particle sizes of FDDG (fine and coarse) and increasing levels of each of these (0, 5,10, & 20 % substitution) in wheat flour will be studied. Effects on changes in nutrient content of the resultant blends and the changes in the dough functionality will also be studied. And finally, the effects on the quality of bread loaves produced from the various blends will be reported.

In essence, a factorial experimental design was pursued involving 2 types of FDDG (fine and coarse), four levels of inclusion (0, 5, 10, & 20 %), and resulting in eight treatments. An Analysis of Variance (ANOVA) thus provided for statistical comparison to determine the nutritional and food functional merits of FDDG enrichment of bread using a product obtained from the ethanol production processing streams.

4.1 Comparison of Wet and Freeze Dried DDG Sample

4.1.1 Yield

Percent yield was calculated based on the starting raw materials and recovered dry DDG product. Table 5 shows the percent yield of DDG to be between 36% - 39%. Losses in DDG processing are encountered due to loss of material in the waste stream as well as loss of moisture. Raw DDG form the ethanol plant varies in moisture content up to 50 - 60%. Much of the loss of weight was, therefore, due to drying and moisture removal. Freeze-drying was effective in producing a dry product that was shelf stable due to efficient removal of water content. The yield of DDG was approximately consistent throughout all the batches that was used for the analysis.

Table 6. Yield of batches of wet DDG before and after Freeze Drying

Batch number	Flour Type	Initial weight of wet Sample (g)	Final weight of freeze-dried sample (g)	Percent Yield
1	DDG	6.000	2.2655	37%
2	DDG	4.5565	1.6855	36%
3	DDG	2.5270	0.9585	37%
4	DDG	3.000	1.1755	39%
5	DDG	3.000	1.1230	37%
6	DDG	3.000	1.1750	39%
7	DDG	3.0000	1.1660	38%

Tables 7 and 8 provide the overview ANOVA summary statements on the various analyses done in this study. Statistical analysis was done using R studio software and in replicate to determine the effects of various treatments of physical, chemical composition and rheological behavior of finished products.

Table 7. Analysis of variance for physical & chemical properties of solvent-treated dried products

Parameters	Df	Mean	Mean square	Sum of square	F value	LSD	Significance
% TDF	10	20.38	845.2	8452.3	2378.7	1.328	***
% Crude Protein	10	23.97	197.2	1972.4	2967.5	0.575	***
% Moisture	10	8.168	197.2	1972.4	2967.5	0.142	***
% Fat	10	1.654	11.42	114.2	969.2	0.241	***
%Ash	10	1.686	5.606	6.06	3225.3	0.093	***
mg GAE/100gm TPC	10	0.199	0.046	0.461	138.6	0.041	***
% CHO	10	43.94	1972	1972	4055.6	1.553	***
Particle size distribution	53	17.47	653.6	35296	11.3634	NA	***
Color L* value	51	88.55	166.5	8658	165.8	NA	***
Color a* value	51	-0.20	1.3	65.5	57.4	NA	***
Color b* value	51	21.68	107.4	5583	223.9	NA	***
Water Activity	9	0.335	0.061	0.551	2350.4	0.012	***

Significance codes: '***' 0.001 '**' 0.01 '*' 0.05; Df: Degrees of freedom; N.A.: Not Applicable; N.S.: Not significant, DDG: Distiller's dried grains without solubles, BF: Bread flour; DDG: Distiller dried grain; FDDG: Food grade distillers died grain; HRW: Hard red winter wheat; TDF: Total Dietary Fiber; TPC: Total phenolic content

Table 8. Analyses of variance for rheology, loaf volume, c- cell bread crumb structure and texture of finished product (bread)

Parameters	Constituents	Df	Mean	Mean square	Sum of square	F value	LSD	Significance
Rheology	FPWA	6	68.47	60.77	364.64	815.48	0.668	***
Farinograph	PM	6	11.54	2.414	14.48	375.59	0.196	***
Gluten	FDT	6	8.6	9.258	55.55	27.95	1.408	***
Analyzer	FS	6	12.53	402.39	67.06	3.909	10.134	NS
	MTI	6	61.14	2274.6	13647.7	93.660	12.058	***
	PT	6	8.571	10.74	64.43	21.48	1.730	***
	GI	6	97.69	1.769	10.615	45.65	0.481	***
RSD	SW	12	486.7	542.5	3255	33.88	7.118	***
	WSD	12	1916	4131	2479	70.21	136.4	***
C_Cell	Slice Area	30	14866	4331	2599	3.759	400.2	**
Bread	Height Max	30	128.1	8.777	52.66	3.105	1.982	*
Crumb	Height Ave	30	126.7	7.390	44.34	2.239	2.142	NS
	Breath	30	127.5	12.227	73.36	1.132	3.874	NS
	Height Breath	30	1.005	0.0003	0.002	0.380	0.0356	NS
	Number Cell	30	8896	3579	2147	16.569	547.96	***
	Number Holes	30	0.964	2.781	16.68	3.360	1.072	*
	Area of Cell	30	53.3	19.220	115.3	29.434	0.952	***
	Area of Hole	30	0.404	0.274	1.641	1.597	0.488	NS
	Non-Uniformity	30	1.683	0.632	3.794	1.003	0.936	NS
	Wrapper Length	30	455.7	183.1	1098.7	3.905	8.074	**
	Wall Thickness	30	0.449	0.001	0.007	15.19	0.010	***
	Cell Diameter	30	2.263	0.219	1.317	4.436	0.262	**
	Slice Brightness	30	88.43	500.5	3002.9	168.67	2.031	***
Texture	Hardness	51	823.3	4844	2906	55.39	NA	***
Analysis	Springiness	51	16.08	503.89	3023.3	5.72	NA	***
	Cohesiveness	49	0.875	0.158	0.946	11.11	NA	***
	Gumminess	49	685.7	2846	1707	59.03	NA	***
	Chewiness	49	753	2965	1779	10.80	NA	***
	Resilience	49	0.555	0.015	0.089	5.206	NA	***

Significance codes: '***' 0.001 '**' 0.01 '*' 0.05; Df: Degrees of freedom; LSD: Least significant difference; N.A.: Not Applicable; N.S.: Not significant; BF: Bread flour; Coarse: FDDG ground using 0.5 mm mesh; DDG: Distillers dried grain; FDDG: Food grade distillers dried grain; Fine: FDDG ground using 0.2mm mesh; AC: Area of cells; AH: Area of hole; CD: Cell diameter; SB: Slice brightness; NC: Number of cells; NU: Non-uniformity; NH: Number of holes; SA: Slice area; WL: Wrapper length; WT: Wall thickness

4.2 Proximate composition of raw ingredients

Table 9 provides the proximate composition of raw ingredients.

4.2.1 Moisture Content

Table 9, figure 26 and 27 provides information on the proximate composition of the materials used in product formulation. Moisture content of the various ingredients ranged between 3.39 to 10.99 %. All ingredients exhibited significant differences in moisture content. Ingredient formulation brings materials of significantly different moisture content into a common food system.

Such blends were made homogenous by rigorous mixing in a cross flow blender in order to ensure homogenous distribution within the blends. In general, FDDG-Flour blends containing the same level of substitution FDDG, whether fine or coarse FDDG, showed the same moisture content. This implied that the final equilibrium moisture content within the FDDG-flour blends are not influenced by the particle size of the fiber inclusion.

4.2.2 Fat content:

Table 9, figure 26 and 27 provides the fat content of the various ingredients and blends made from them. The ether extracted fat content of the FDDG samples were below 0.5 %. This was expected as the FDDG fractions were extracted with solvents that were selective for fats and oils. Reduction to the low levels of fat is desirable as this will contribute to shelf stable finished products. Degradation of fats and oils and the development of fat rancidity are major factors that reduce the wholesomeness of foods.

When these blends are incorporated with other bread making ingredients in the food processing schemes, further levels of fat are added. It is therefore important for starting ingredients to be low in fat content. It was noted that Supercritical Extracted DDG was the raw material containing the highest level of fat (8.19%) of all the fractions studied. SCFE employs carbon dioxide as a solvent in contrast to ethanol used for most of the other ingredients

Table 9. Proximate composition of Bread Flour, Fine-Ground Food Grade DDG and Super Critical CO2 Extracted DDG and DDGS and Bread flour blends containing 5 to 20% FDDG

Flour type	Parameter	Substitution	%Protein	%Moisture	%Fat	%Ash	%TDF	%CHO	TPC
			Mean Values (W/W%)						mgGAE/100gm
Bread Flour	**Control**		14.78g	8.46e	0.4d	0.5f	2.67i	73.19a	0.02j
FDDG	**Fine**		37.59a	3.4h	2.11c	2.01c	51.27a	3.27h	0.36c
FDDG	**Fine**	**5%**	15.99f	10.8b	0.35d	0.66e	4.08h	68.092b	0.04ij
FDDG	**Fine**	**10%**	16.89e	10.3c	0.38d	0.71de	4.1h	67.46b	0.16ef
FDDG	**Fine**	**20%**	18.1d	8.46e	0.4d	08d	7.22f	64.16d	0.17e
FDDG	**Coarse**		36.02b	4.32g	1.9c	1.93c	49.45b	5.99g	0.40b
FDDG	**Coarse**	**5%**	15.80f	11.0a	0.32d	0.66e	3.79hi	68.38b	0.07hi
FDDG	**Coarse**	**10%**	17.15e	10.34c	0.44d	0.69e	5.53g	65.73c	0.13fg
FDDG	**Coarse**	**20%**	18.85d	8.46e	0.4d	0.79d	13.74e	57.68e	0.09gh
DDG	**SCE**		37.54a	5.69f	3.33b	5.05a	39.94d	8.01f	0.45a
DDGS	**SCE**		34.31c	8.66d	8.19a	4.77b	42.45c	1.35i	0.28d

Legend:
BF: Bread flour
DDG: Distiller dried grain
DDGS: Distillers dried grain with soluble
FDDG: Food grade distillers died grain
HRW: Hard red winter wheat
SCE: Supercritical extraction
TDF: Total Dietary Fiber
TPC: Total phenolic content
Means with the same letter within a column are not significantly different from each other ($P \leq 0.05$)

4.2.3 Ash content

Table 9, figure 26 and 27 provided the ash content of the ingredients used in this study. Ash content is the expression of the total mineral content. Ash is the remainder of inorganic residue after complete oxidation of organic matter in food. It is the residue encountered in the porcelain crucible after incineration of samples at high temperature of 550 ^{0}C. Ash content is the ash residue weight expressed as a percentage of the weight of starting material.

Bread flour (control) had the lowest mineral content (0.50%) compared to Fine FDDG (2.01%), Coarse FDDG (1.93%), Supercritical DDG (5.05%) and Supercritical DDGS (4.77%). Pourafshar et al. (2014) and Tsen et al. (1983) observed higher amount of ash in the resultant bread compared to the control after the supplementation of wheat flour with 10 % DDGS flour. DDG samples contribute mineral content when used as supplements or enrichment ingredients to wheat flours.

4.2.4 Protein content

An overarching goal in this research is to bring about modest improvement in protein content to bread flour by inclusion of protein (and fiber) sources from other traditional cereal crops. Corn protein that originated as 8% protein in the corn kernel, is significantly concentrated in the FDDG by the selective and sequential removal of carbohydrates such as sugars and starch which can make up to 80 % of the corn kernel by weight.

The resulting residue after ethanol fermentation is significantly enhanced in protein content (in excess of 36%) and serves as an enrichment agent that has unique nutritional and functional properties. Table 9 provides the protein content of the various materials studied. FDDG ranged in protein content from 36.01% (Fine FDDG) to 37.59 % (Coarse FDDG). Fine-grinding appeared to result in a slight but statistically significant higher protein content, (Keshun Liu 2008) also, concluded in his study that finer DDGS fraction had higher protein content than coarser fractions. Supercritical-CO2 Extracted FDDG showed a high level of protein content at 37.54% owing to oil removal from the DDG matrix.

Bread wheat flour, at 14.78% protein content, was significantly improved by the addition of FDDG, particularly at the 20% level of FDDG enrichment (18.1 to 18.85%). Such increases are possible owing the high protein content of the enrichment medium, which was FDDG in our case. Cronwell et al. (1993), observed high percentage of protein content in DDG.

4.2.5 Total dietary fiber content (TDF%)

Table 9, figure 26 and 27 provide information on the Total Dietary Fiber content of the various ingredients used in this study. While FDDG was high in protein content, it was also significantly high in TDF content as well. Plant constituents that made up a significant proportion of the structural plant material when the plant was alive, are now concentrated by the removal of other moieties.

Dietary fiber was the predominant constituent in FDDG at 51.3%TDF in Fine FDDG and 49.5% TDF in Coarse FDDG. Bread wheat flour that had 2.67% TDF, was significantly improved in fiber content with the addition of up to 20% DDGS. TDF content in the blends went up to 13.74% with the inclusion of 20% Coarse FDDG. Enrichment of wheat flour at 5, 10 and 20% levels of substitution brought about a steady and significant increase in fiber content. This was observed with both the fine FDDG and the coarse FDDG.

4.2.6 Total Phenolic Content:

Total phenolic content was determined by Singleton, Orthofer and Lamuela-Raventos method (1999). The total phenolic acid content was measured using Folin–Ciocalteu reagent in each extract. A calibration curve was used to ($y = 0.02x + 0.0049$, $R^2 = 0.9993$) of gallic acid (0–20 µg/mL) and expressed in gallic acid equivalents (GAE) per gram dry extract weight.

In comparing DDG versus DDGS, Supercritical DDG was significantly higher with 4.53 GAE mg/100g (or mg%) than supercritical DDGS (0.28mg %). Coarse FDDG (0.404 mg%) was significantly higher than fine (0.36mg%) FDDG and control bread flour (0.017

mg%). There was an increased trend in Total phenolic content (TPC) with increased inclusion FDDG (5, 10, & 20%) in the treatment blends (Table 9, figure 26 and 27).

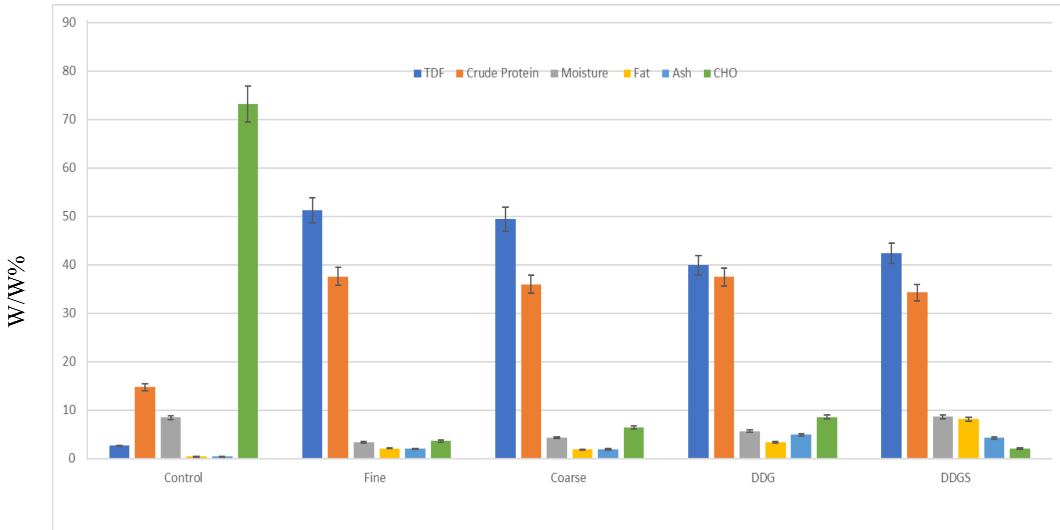
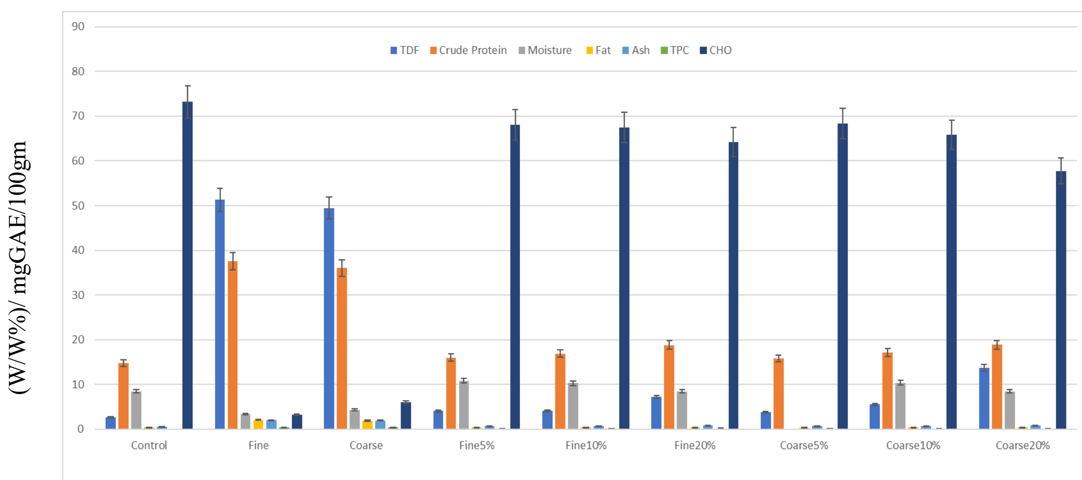


Figure 26. Bar plot of proximate composition comparing Bread Flour with Fine-Ground Food Grade DDG and Super Critical CO2 Extracted DDG and DDGS



Parameter

Figure 27. Bar plot of proximate composition comparing Bread Flour with Bread flour blends containing 5-20% FDDG

4.3. Physical Analysis

4.3.1 Particle Size Analysis

Table 10, figure 28 and 29 shows the particle size distribution (>400 µm, >250 – 400 µm, >180 – 250 µm, >150 – 180 µm, >75 150 µm, ≤ 75 µm) for un-milled FDDG, coarse FDDG, and fine FDDG.

The particle size distribution of food grade DDG, Control wheat flour and wheat flour-fddg blends was done using a stacked set of sieves having different sieve sizes. The 5 sieves and collection pan separated particles ranging from 75 microns to 400 microns. Particle size analysis was measured on air-dried FDDG, Un-milled FDDG, coarse FDDG, and fine FDDG samples.

Control wheat flour: The wheat flour used in the study had a significant proportion (77.7%) that ranged from 75 to 150 microns in particle size.

Un-milled FDDG: A significant proportion of Un-milled FDDG (44.6%) was found to be retained on top sieve (40mesh, <400um) which was significantly different from that seen with the fine and coarse flour blends. Hammer mills employed in ethanol plants are not designed for fine grinding.

Fine FDDG was milled using a 0.2mm sieve attachment in the Retsch mill. A large proportion (88.72%) of this material ranged in particle size from 150 to 400 microns.

Coarse FDDG was milled using a 0.5mm sieve attachment in the Retsch mill. Coarse FDDG had a significant proportion (78.8%) of this material that ranged from 150 to 400 microns in size. In contrast, wheat flour that served as the backbone of the bread formulations had only 61.7% of its material in the comparable particle size range of 150 to 400 microns.

Fine FDDG, therefore, had 10% greater proportion of its material in the 150 to 400 micron size range in comparison to the Coarse FDDG. Differences in particle size in flour blends prepared with wheat flour and either Fine FDDG or Coarse FDDG may be manifested into functionality differences in dough mixing, water absorption, gas retention in dough and texture differences in the ultimate baked products.

Table 10 also provides particle size distribution information on the flour blends prepared with 5, 10, and 20% levels of substitution with each of the Fine FDDG and Coarse FDDG.

For FDDG-Wheat flour blends prepared with fine FDDG, the single largest proportion of the flour blend was retained in the 3^{rd} sifter (150 to 180 microns). This sifter retained 57.9%, 64.2%, 39.4% of the 5%, 10% and 20% respectively, of the Fine FDDG-wheat flour blend.

For Coarse FDDG-wheat flour blends, the 150 to 180micron sifter retained 36.4%, 58.0% and 54.7% of the 5%, 10% and 20%, respectively, of Coarse FDDG-wheat flour blends.

For contrast, it is noted that the Un-milled FDDG retained 44.6% of it material above 400 micron sieve, the top most sieve in the sifter stack. The Control Wheat Flour had 58.7% of its material retained on the 150 to 180 micron sifter.

Fine grinding and coarse grinding did indeed alter the particle size distribution of the FDDG and the flour blends made with them. These contrasts are more obvious in the bar graphs showing particle size distribution of various blends used in this study.

4.3.2 Color Measurement

Table 10 a and b provide color values for various FDDG flour blends prepared with fine FDDG and Coarse FDDG.

Color comparison of FDDG and FDDG-wheat flour blends were determined using a Minolta Spectrophotometer. A three-dimensional L*, a* and b* scale was used to quantify color.

The L scale provides a range for brightness (100) versus blackness (0). The a* scale provides values of redness (+a*) versus greenness (-a*). The b* scale describes yellowness (+b*) versus blueness (-b*). L values provide information on the effectiveness of processing steps in improving the brightness of the FDDG ingredient. Repeated washing with solvents have a cumulative effect on brightness of the finished product. The washing steps remove plant pigments and phenolic substances that originated in the corn and were concentrated in the spent grain. It is necessary therefore to begin with an unpigmented (reduced redness or yellowness) to ensure brightness in the food products.

The control wheat flour, in general, yielded the brightest products with L* values in excess of 90.

FDDG sifted fractions ranged in L* scores from 65.4 to 94.4. FDDG blended fine fractions for (5, 10, 20) ranged from 60.05 to 98.54, 70.54 to 96.75, 77.04 to 97.01 respectively. FDDG sifted fractions ranged in b* scores from 16.32 to 29.79.

b* values for control flour ranged from 10.13 to 11.87. b* values for fine fractions for (5, 10, 20) ranged from 13.2 to 25.26, 15.63 to 25.95, 10.56 to 20.61, respectively.

For coarse group, L* scores ranged from 74.08 to 92.22. Coarse blends for (5, 10, 20) ranged from 73.88 to 97.57, 76.37 to 96.32, 81.99 to 95.08, respectively and un-milled FDDG ranged in value score of 83.28 to 89.51.

b* values for coarse group ranged from 26.02 to 33.13. Substituted coarse FDDG for (5, 10, 20) ranged from 12.94 to 30.58, 12.7 to 28.93, 16.23 to 29.77, respectively and un-milled FDDG ranged from 28.42 to 33.01.

Table 10a. Particle size distribution of bread flour, and bread flour blends containing fine FDDG

Parameter	Particle size um	Moisture	100g	L*	a*	b*
Bread Flour (Control)	>250 - 400	8.46	0.297pq	90.92lmn	-0.16ijkl	11.87uvw
	>180 -250		2.758nopq	90.685mn	-0.405lmn	11.73vw
	>150-180		58.69ab	99.275ab	-0.695nopqrs	11.12wx
	>75 - 150		19.03hijkl	99.09ab	-0.735opqrs	11.1wx
	≤ 75		13.75ijklmnopq	99.31a	-0.76opqrst	10.135x
Fine	>400	3.4	0.224q	88.935nop	0.14ghi	27.845hi
	>250 - 400		35.68def	90.92mn	-0.04ij	28.31ghi
	>180 -250		41.2cde	83.42q	-0.34klm	27.15ij
	>150-180		11.84jklmnopq	94.365hij	-0.2jkl	29.79def
	>75 - 150		7.12klmnopq	65.38v	-1.05tuv	18.82no
	≤ 75		1.63opq	94.975ghij	-0.5lmnop	16.325qr
5%	>400	10.8	0.14q	74.13t	1.33ab	28.13hi
	>250 - 400		0.52pq	77.33s	1.04bcd	25.26k
	>180 -250		0.88opq	98.54abc	-0.84rst	13.2tu
	>150-180		57.94ab	97.265bcde	-0.695nopqrs	14.65s
	>75 - 150		17.74ijklmn	60.05w	-1.3v	16.3qr
	≤ 75		17.18ijklmn	88.03op	-0.1ijk	31.1bcd
10%	>250 - 400	10.3	0.5578pq	82.26q	1.3ab	22.43l
	>180 -250		2.55nopq	96.755cdefg	-0.501lmnop	15.635rs
	>150-180		64.21a	95.54efghi	-0.57mnopqr	16.445qr
	>75 - 150		15.97ijklmno	70.54u	0.32fgh	25.95jk
	≤ 75		10.93jklmnopq	93.82ijk	-0.81qrst	20.44m
20%	>250 - 400	8.46	1.43opq	90.62mn	-0.47lmno	20.615m
	>180 -250		28.14efghi	95.43efghi	-1.29v	18.33op
	>150-180		39.38de	94.76ghij	-0.965stu	20.12mn
	>75 - 150		18.881hijklm	77.04s	-0.52lmnopq	10.56wx
	≤ 75		5.357klmnopq	97.015cdef	-0.485lmno	15.335rs

Legend:
BF: Bread flour
DDG: Distillers dried grain
FDDG: Food grade distillers died grain
Fine: FDDG ground using 0.2mm mesh
Means with the same letter within a column are not significantly different from each other ($P \leq 0.05$)

Table 10b. Particle size distribution of bread flour and flour blends made with coarse FDDG

Parameter	Particle size um	Moisture	100g	L*	a*	b*
Bread Flour (Control)	>250 - 400	8.46	0.297pq	90.92lmn	-0.16ijkl	11.87uvw
	>180 -250		2.758nopq	90.685mn	-0.405lmn	11.73vw
	>150-180		58.69ab	99.275ab	-0.695nopqrs	11.12wx
	>75 - 150		19.03hijkl	99.09ab	-0.735opqrs	11.1wx
	≤ 75		13.75ijklmnopq	99.31a	-0.76opqrst	10.135x
Coarse	>400	4.32	0.90opq	74.08t	0.58ef	26.02jk
	>250 - 400		22.55fghij	87.915op	0.925cd	33.13a
	>180 -250		36.16def	90.09n	0.395fgh	32.39ab
	>150-180		20.21ghijk	92.225klm	-0.475lmno	29.715def
	>75 - 150		14.79ijklmnopq	90.08n	-0.785pqrst	28.265ghi
	≤ 75		2.66nopq	88.78nop	-1.28v	26.06jk
5%	>400	11	0.93opq	73.88t	1.56a	23.08l
	>250 - 400		1.59opq	89.02nop	0.43fg	17.33pq
	>180 -250		34.81defg	97.575abcd	-0.855rst	12.94uv
	>150-180		36.40def	94.96ghij	-0.54mnopq	15.24rs
	>75 - 150		11.48jklmnopq	97.07cdef	-0.59mnopqr	14.52st
	≤ 75		10.93105jklmnopq	93.615ijk	0.17ghi	30.58cde
10%	>250 - 400	10.34	1.642opq	76.37s	1.32ab	28.93fgh
	>180 -250		2.9373nopq	80.07r	1.1bc	28.66fgh
	>150-180		58.04ab	94.43hij	-0.53mnopq	14.825s
	>75 - 150		19.37hijkl	94.525hij	-0.745opqrs	14.46st
	≤ 75		13.13ijklmnopq	96.325defgh	-0.93stu	12.7uv
20%	>250 - 400	8.46	3.72mnopq	81.99qr	0.77de	29.65efg
	>180 -250		4.73lmnopq	83.51q	0.43fg	29.77def
	>150-180		54.70abc	93.165jkl	-0.925stu	18.98no
	>75 - 150		15.46ijklmnop	94.355hij	-1.2uv	16.52qr
	≤ 75		15.19ijklmnopq	95.08fghij	-1.265v	16.23qr
Un-milled FDDG	>400		44.63bcd	87.48p	0.88cd	33.015a
	>250 - 400		33.55defgh	88.66nop	0.12hi	31.955abc
	>180 -250		9.53jklmnopq	NA	NA	NA

>150-180	4.42lmnopq	89.51no	-0.75opqrs	28.42fghi
>75 - 150	3.76mnopq	83.28q	0.96cd	31.91abc
≤ 75	0.27pq	NA	NA	NA

Legend:
BF: Bread flour
Coarse: FDDG ground using 0.5 mm mesh
DDG: Distillers dried grain
FDDG: Food grade distillers died grain
Means with the same letter within a column are not significantly different from each other ($P \leq 0.05$)

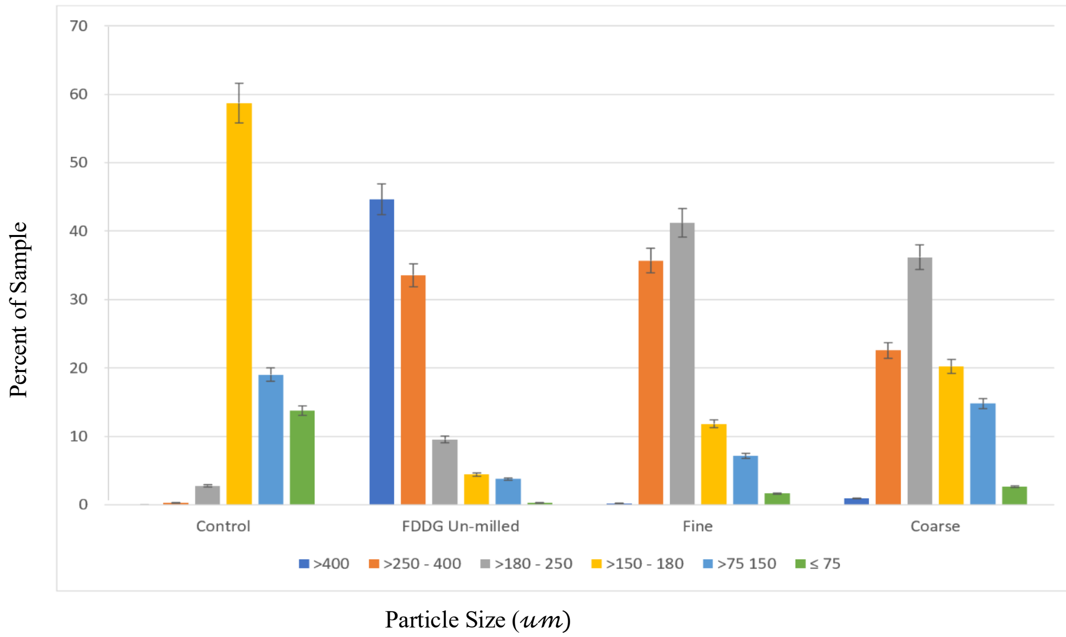


Figure 28: Bar plot comparing particle size of Bread Flour, Un-milled FDDG, Fine FDDG, & Coarse FDDG

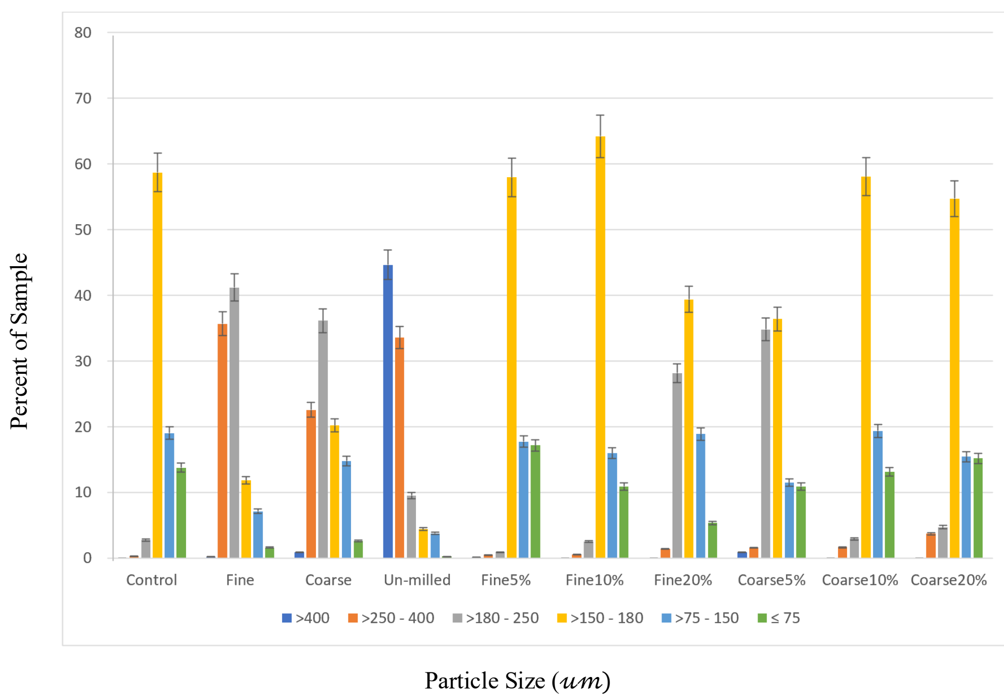


Figure 29. Bar plot comparing particle size with Bread Flour, Un-milled, Fine, Coarse, with Bread flour blends containing 5-20% Food grade distillers dried grain

4.4.0: Water Activity

Table 12 and figure 30, provide the water activity (Aw) of ingredients used in making bread, namely, bread wheat flour, Un-milled FDDG, Fine FDDG, Coarse FDDG and blends containing 0 -20% FDDG. The table shows that control flour and 5% FDDG-Flour blends did not have significantly different Aw. The five % level DDG substitution did not alter the water activity of the resulting blend. Control flour was however, significantly different from that of the 10 and 20% blends. Particle size of fine FDDG or course FDDG did not alter the Aw of 10% blends. Particle size differences in Aw were not discernible at the 20% level of FDDG substitution as well. As the levels of FDDG increased in the flour (5, 10, 15, & 20%) however, Aw of the resulting blends showed a reduction of Aw. The Aw of fine FDDG and Coarse FDDG were 0.08 and 0.1, respectively. This was in contrast to the Aw of Control Flour (Aw=0.51). Changes in the final FDDG-Wheat flour blends reflect new moisture equilibrium reached as a result of blending materials with diverse Aw. The Aw of all ingredients studied however, indicated that Aw of no greater the 0.5 was encountered. Generally, Aw of greater the 0.65 is seen in food that are susceptible to microbial spoilage.

Table 1 2. Water activity of ingredients used in bread baking

		Food Grade Distillers Dried Grains								
Parameters	**Control**	**Fine FDDG**	**Coarse FDDG**	**Fine FDDG in Bread flour**			**Coarse FDDG in Bread Flour**			**Un-milled FDDG**
DDG Substitution				**5%**	**10%**	**20%**	**5%**	**10%**	**20%**	**Un-milled**
Water Activity (Aw)	0.51±0.004a	0.078±0.002e	0.10±0.00d	0.51±0.001a	0.42±0.0025b	0.35±0.0005c	0.51±0.00 a	0.42±0.005b	0.34±0.008c	0.11±0.003d

Legend:
BF: Bread flour
DDG: Distiller dried grain
FDDG: Food grade distillers died grain
Means with the same letter within a column are not significantly different from each other ($P \leq 0.05$)

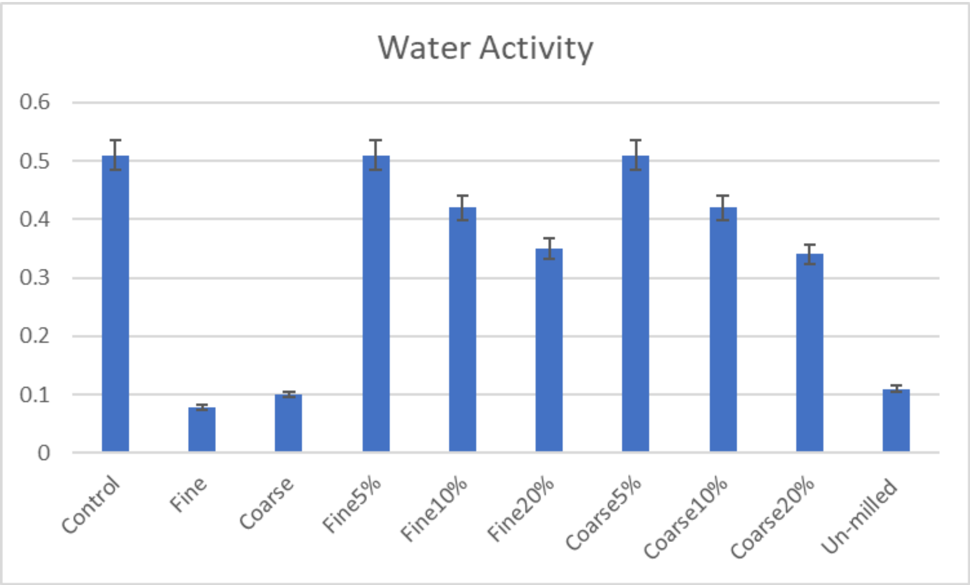


Figure 30. Bar plot comparing water activity with Bread Flour, Un-milled, Fine, Coarse and Bread flour blends containing 5-20% Food grade distillers dried grain

4.5 Rheology

4.5.1: Farinograph

The Farinograph measures the dough property of flour, namely, shear and consistency (viscosity) of a mixture of flour and water and its resistance to deformation. Additional parameters are obtained such as farinograph development time (min), Farinograph percent water absorption (%), Farinograph stability (min), Mixing Tolerance Index (BU), and Peak Time (min). This instrument yields a curve that shows the strength or weakness of the dough. All of this information is necessary to develop an optimal dough that is mixed to perfection. Table 12 and Figure 4.6 provide a comparison of flours substituted at various levels (5 to 20%) DDG. Changes in water absorption, peak mixing time, dough stability and tolerance to mixing are noted.

4.5.2: Farinograph Water Absorption

Table 13 and figure 31 shows that there were significant differences in water absorption between the control samples and the blends for water absorption.

Increasing levels of FDDG in the flour blends from 5 to 20%, significantly and predictably increased flour water absorption (WA).

Flour water requirement increased with addition of FDDG, from 65.2 and 64.6% in the 5% DDG blends to 76.8 and 75.8% in the 20% substitutions. FDDG inclusion resulted in a competition for water owing to the water holding capacity for the DDG fibers. Control flour, however, had low WA at 63% WA as water requirements arose from the wheat flour alone without any other inclusion.

Differences in the WA of the blends between fine and coarse DDG were noted only at the 20% level of substitution.

4.5.3: Farinograph Dough Development Time

In general, Dough Development Time was reduced by addition of DDG to the flour blends.

The highest peak time was observed in the control flour with mean value of 13.5 minutes. In looking at the table, PT shows that all of the FDDG-wheat flour blends showed a significant lower PT than the control, unfortified flour. Addition of extraneous material in a gluten dough system brings about a dilution of gluten and weakening of the cohesive nature of the dough. peak mixing is thus affected. While reduced mixing time is not a detriment in itself, complete development of the dough’s ability to trap moisture and retain gasses down stream may be impacted.

No particles size effects were discernible (fine versus coarse FDDG)

Peak dough mixing time was reduced from 13. 5minutes in the control to 6 to 7 minutes in the 20% FDDG-wheat flour blends. (Table 13 and figure 31).

4.5.4: Mixing Tolerance Index (MTI)

Table 13 and figure 31 provide the mixing tolerance index (MTI) of bread flour and FDDG blends. All the FDDG treatment blends showed high MTI with the highest MTI observed in 20% FDDG –flour blends both (fine and coarse). It was observed that the increasing level of fiber increased the mixing tolerance index. It should be noted that most of the particles for fine and coarse FDDG blends were retained in <150 – 180 mesh size sieves. Control (Bread Flour) was significantly different than the FDDG blends. At each level of FDDG substitution (5,10, and 20%), there were no significant differences noted in MTI between fine and coarse blends. Particle size within a particular level of flour replacement of the fibers did not, therefore, have any effect on Mixing Tolerance Index.

MTI increased substantially, however, at the higher levels of flour substitution with FDDG. Control flour MTI at 16.5 BU increased to 106 BU at the 20% level of FDDG substitution. This pattern of increases in MTI with higher levels of FDDG substitutions in the flour was consistent regardless of the particle size (fine FDDG or Coarse FDDG). FDDG substitutions levels (0, 5, 10, 20%) was more detrimental to dough mixing tolerance than the nature of the FDDG particles size (fine versus coarse).

4.5.5: Dough Stability

Table 13 and figure 31 show mixing the Stability (Stab)) of bread flour and FDDG blends. Fine and Coarse FDDG were observed to decrease farinograph dough stability as compared to the control with the mean value of 21.4 minutes (control sample). This means that addition of fiber decreases the dough stability. It is observed that the stability for fine FDDG is slightly better than coarse FDDG. Control (Bread Flour) was significantly different than 20% fine and coarse FDDG blends. All FDDG blends were not significantly different from each other. This is consistent with the finding of by Hyma Gajula (2017), who said that addition of bran for fine and coarse decreased dough stability of flour.

4.5.6: Gluten Index

Table 13 and figure 31 provide the Gluten Index measured using the Glutomatic System. High GI are indicative of the desired gluten strength. GI is a ration reflecting the proportion of the "Good gluten" expressed as a percentage of total gluten content. The higher the gluten index, therefore, the better the baking characteristics of the bread. Control flour had slightly higher GI than the rest of the FDDG-Flour samples. Increase in fiber in the flour decreases the gluten content of the blends. This is referred to as gluten dilution. Fine FDDG or Coarse FDDG did not impact GI at any of the levels of DDG substitution showing the particle size differences were not a factor for undermining gluten strength. No statistical differences were noted when substitutions were done at the same level. Particle size did not undermine gluten strength in comparing 0.2mm and 0.5mm size DDG.

Table 1 3. Rheological Properties and Gluten Characteristics Bread Flour and Blends Containing (5-20%)

Ingredients			Farinograph						Glutomatic
Flour	Constituents	Substitution	FPWA	PM	FDT	FS	MTI	PT	GI
			(%)	(%)	(min)	(min)	(BU)	(min)	(%)
BF	Control	0%	63±0.5e	12.6±0.00a	13.35±0.65a	21.45±7.45a	16.5±1.5d	13.5±0.5a	99.40±0.27a
BF-FDDG	Fine	5%	65.2±0.00d	12.45±0.15a	7.8±0.00bc	16±0.00a	38.5±4.5c	8±0.00bc	98.19±0.13b
BF-FDDG	Fine	10%	67.1±0.00c	11.4±0.00b	8.1±0.4bc	13.15±0.15ab	58.5±0.5b	8±1.00bc	97.34±0.06c
BF FDDG	Fine	20%	76.8±0.00a	10.1±0.00c	6.9±0.1c	5.75±0.55b	106.5±3.5a	6.5±0.5c	96.95±0.00cd
BF FDDG	Coarse	5%	64.6±0.1d	12.6±0.00a	8.35±0.45b	13.9±0.1ab	43±1.00c	8±0.00bc	98.05±0.16b
BF FDDG	Coarse	10%	66.9±0.3c	11.5±0.00b	8.2±0.2bc	12.75±0.75ab	60±6.00b	9±0.00b	97.31±0.02c
BF FDDG	Coarse	20%	75.75±0.05b	10.1±0.00c	7.5±0.5bc	4.7±0.2b	105±1.00a	7±0.00c	96.61±0.00d

Legend:
BF: Bread flour
Coarse: FDDG ground using 0.5 mm mesh
DDG: Distillers dried grain
FDDG: Food grade distillers dried grain
Fine: FDDG ground using 0.2mm mesh
FDT: Farinograph dough development time (min)
FPWA: Farinograph percent water absorption (%)
FS: Farinograph stability (min)
GI: Gluten index
MTI: Mixing Tolerance Index (BU)
PM: Percent moisture
PT: Peak time (min)
Means with the same letter within a column are not significantly different from each other ($P \leq 0.05$)

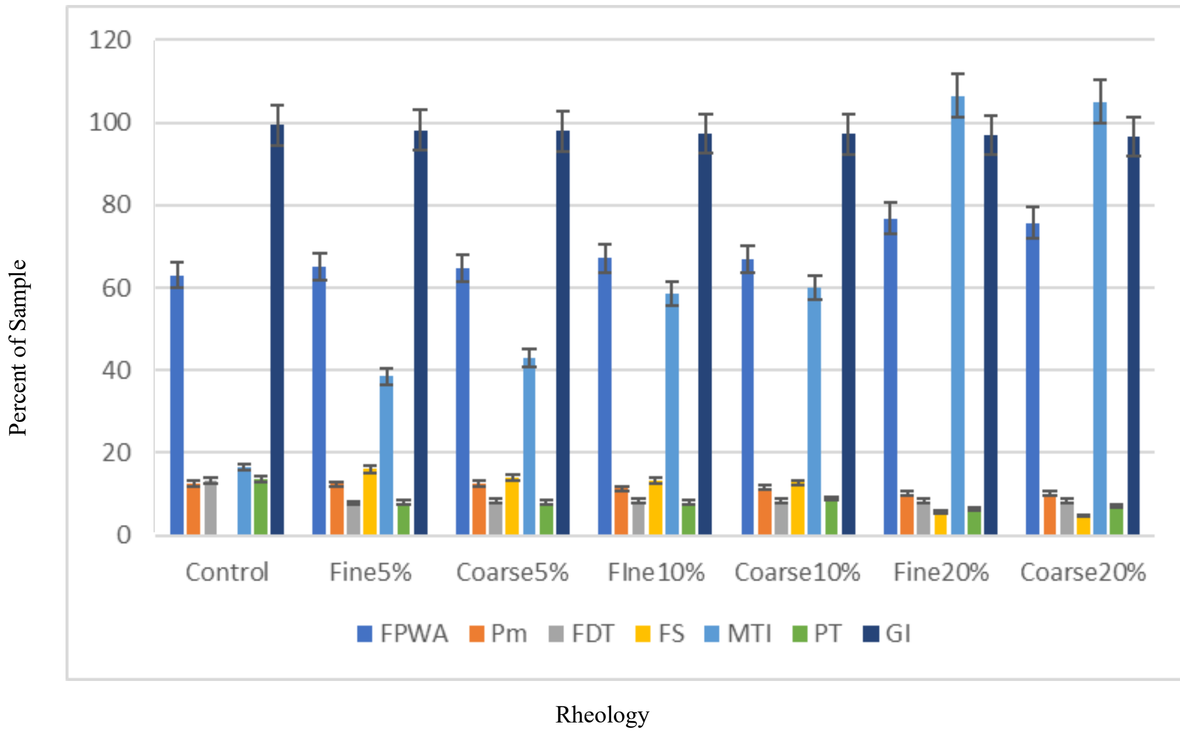


Figure 31. Bar plot comparing Farinograph percent water absorption (%), Percent moisture, Farinograph development time (min), Farinograph stability (min), Mixing Tolerance Index (BU), Peak time (min)and Gluten index with Bread Flour, Un-milled, Fine, Coarse and Bread flour blends containing 5-20% Food grade distillers dried grain

4.6.0: Bread Making

4.6.1: Bread Making with Wheat Flour, Fine FDDG-Wheat flour blend and Coarse FDDG-Wheat Flour Blends

In general, bread weight increased as the level of fortification increased. This was the case with fine FDDG fortification as well as coarse FDDG fortification. The specific volume calculated based on weight and volume measurements revealed that the 20% FDDG breads had the lowest specific volume regardless of the particle size of the FDDG ingredient (fine or coarse). This finding suggests that the high level of FDDG fortification undermined gas cell production and the ability of the gas cells to retain gases. (Table 14, figure 32 and 33)

Figure 32. Control Bread loaves without FDDG fortification

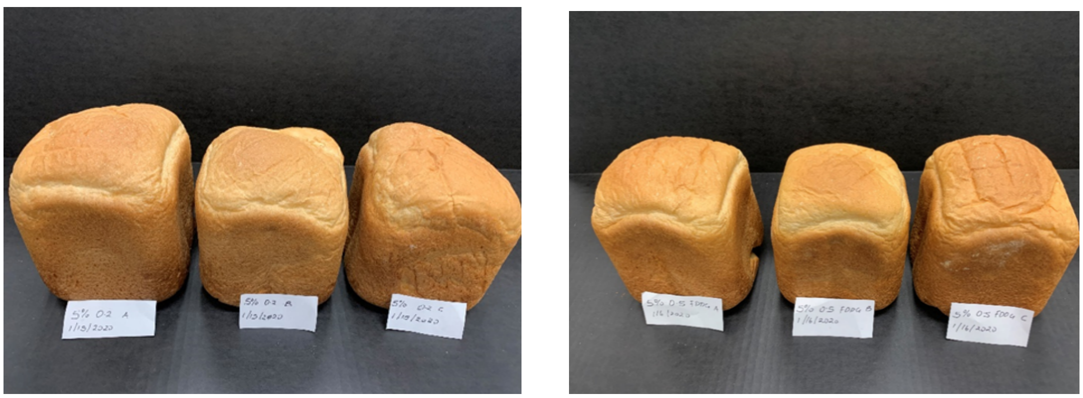


Figure 33. Control Bread loaves and Bread treated with fine and coarse FDDG fortified at various levels (5%)

Figure 34. Control Bread loaves and Bread treated with fine and coarse FDDG fortified at various levels (10%)

Figure 35. Control Bread loaves and Bread treated with fine and coarse FDDG fortified at various levels (20%)

Figure 36. Control Bread loaves and Bread treated with fine and coarse FDDG fortified at various levels (0 and 20%)

Table 1 4. Weight and volume of Breads made with Control Wheat Flour, fine FDDG-Wheat flour blend and Coarse FDDG-Wheat Flour Blends

Constituents	Parameter	Substitution	Mass (g)	Loaf Volume (cm3)	Specific Volume (cm3/g)	Density (g/cm3)
BF	**Control**	**0%**	465.3d	2183a	4.69	0.21
FDDG	**Fine**	**5%**	488.3b	2226a	4.56	0.22
FDDG	**Fine**	**10%**	488.7b	2031b	4.16	0.24
FDDG	**Fine**	**20%**	503.0a	1446d	2.87	0.35
FDDG	**Coarse**	**5%**	483.3b	2283a	4.72	0.21
FDDG	**Coarse**	**10%**	476.0c	1863c	3.91	0.26
FDDG	**Coarse**	**20%**	501.8a	1380d	2.75	0.36

Legend:
BF: Bread flour
Coarse: FDDG ground using 0.5 mm mesh
DDG: Distillers dried grain
FDDG: Food grade distillers dried grain
Fine: FDDG ground using 0.2mm mesh
Means with the same letter within a column are not significantly different from each other ($P \leq 0.05$)

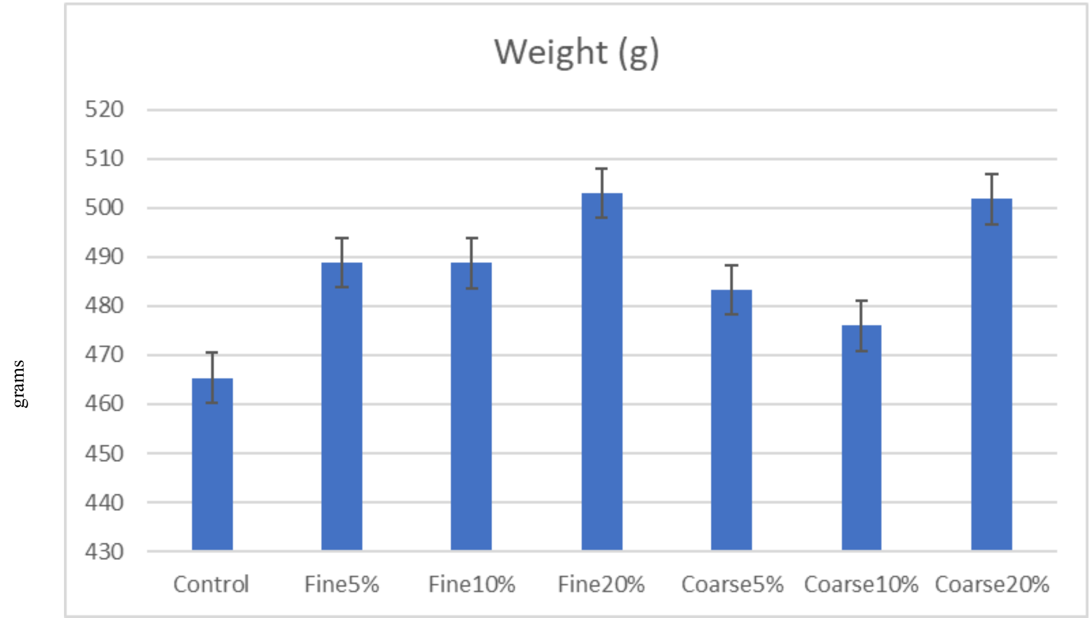


Figure 37: Bar plot of weight for Bread

4.6.2 Bread Crumb Internal texture

Table 14 provides the image analysis of slices of bread from the various fractions of FDDG, both fine FDDG and Coarse FDDG. The number of air cells increased as you progressed from the Control loaf to loaves containing 20% FDDG. Large increase of FDDG yielded greater cell number. This pattern was seen both the fine grind FDDG as well as the coarse FDDG.

Table 1 5. Bread crumb internal structure

Constituents	Parameter	Substitution	SA	Height Max	Height Avg	Breadth	HeightB	Number Cell	Number Holes	Area Cells	Area Holes	NonUni	WL	Wall Thickness	CellDia	Slice Brightness
			(mm)	(mm)	(mm)	(mm)	(mm)			(%)	(%)	(mm)	(mm)	(mm)	(mm)	
Bread Flour	Control	0%	1466c	127.7bc	126.5abc	126.4ab	1.012a	9282b	1.592ab	54.15a	0.592ab	1.343a	455.6bc	0.452bc	2.391ab	91.27b
FDDG	Fine	5%	1481bc	127.9abc	126.6abc	127.8ab	1a	8541c	0.668abc	54.25a	0.308ab	1.725a	453bc	0.463a	2.561a	92.35b
FDDG	Fine	10%	1528a	129.6ab	127.8ab	130.2a	0.997a	8499cd	0.172c	54.03a	0.218ab	1.904a	448.3c	0.446c	2.067c	77.13d
FDDG	Fine	20%	1521ab	129.8a	128.6a	127.6ab	1.018a	9901a	1.738a	50.88b	0.678a	1.765a	454.7bc	0.447c	2.253bc	98.52a
FDDG	Coarse	5%	1481bc	127.7bc	126.3bc	127.9ab	1a	8256cd	0.292c	54.78a	0.115b	1.291a	454.1bc	0.421d	2.029c	98.63a
FDDG	Coarse	10%	14632c	126.6c	125.2c	125.9b	1.007a	7968d	0.618bc	54.62a	0.333ab	2.217a	466.3a	0.460ab	2.374ab	83.78c
FDDG	Coarse	20%	14661c	127.1c	126.2bc	126.6ab	1.003a	9827ab	1.672ab	50.6b	0.58ab	1.541a	457.7b	0.453bc	2.1703bc	77.38d

Legend:
BF: Bread flour
Coarse: FDDG ground using 0.5 mm mesh
DDG: Distillers Dried Grain
FDDG: Food Grade Distillers Dried Grain
Fine: FDDG ground using 0.2mm mesh
Area Cells: Area of cells
Area Holes: Area of hole
CellDia : Cell diameter
Slice Brightness: Slice brightness
Number Cell : Number of cells
Non Uni: Non-uniformity
Number Holes: Number of holes
SA: Slice area
WL: Wrapper length
Wall Thickness: Wall thickness
Means with the same letter within a column are not significantly different from each other ($P \leq 0.05$)

4.6.3 Texture Profile Analysis of Control and FDDG Fortified Bread

Observations: Control Bread without fortification with FDDG hard a hardness of 303.8g. With the exception of the 5% Fine FDDG Bread, control bread was the softest in texture. The 20% Coarse FDDG Bread had the hardest texture at 2283 g. This was the force necessary to compress the bread slice.

Springiness declined significantly from 18.9 in the control bread to 1.11 in the 20% Coarse FDDG Bread. The 5% Coarse FDDG Bread had the same Springiness as the Control bread. Even at 20% substitution with fine FDDG, springiness was not significantly changed.

Cohesiveness declined significantly from the control at the 10 and 20% Fine FDDG fortification.

Gumminess was noted only in the highest level of fine FDDG fortification (at 20% substitution). With Coarse FDDG, gumminess in bread slices relative to the control bread was noted at the 10% and 20% levels of DDG fortification.

Chewiness was measured in grams of force. Control Bread had a chewiness of 5763.3g. No predictable pattern was noticed in the Chewiness of the bread slices with fine and coarse FDDG at any level of fortification.

Resilience of the Control Bread Slice was 0.559. The most distinct sample that showed the most significant difference from the control bread was the bread made with 20% Fine FDDG (Table15, figure 38 and figure 39).

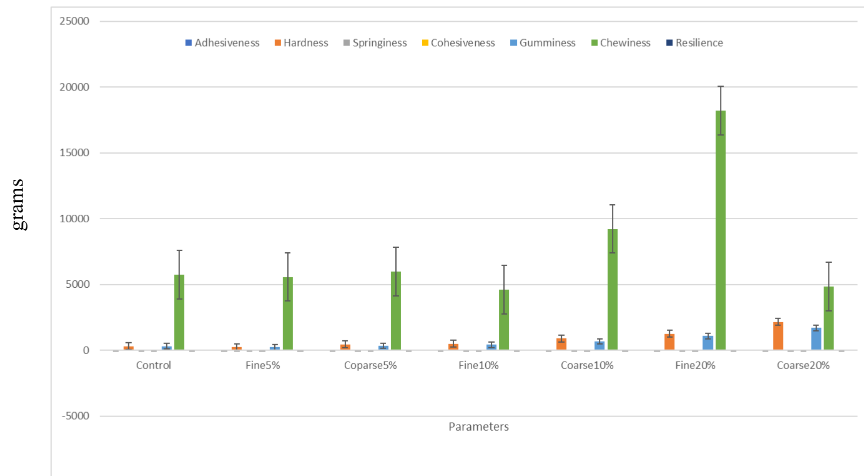


Figure 38. Bar plot of fresh bread for adhesiveness, hardness, springiness, cohesiveness, gumminess, chewiness, resilience containing Bread Flour, Processed FDDG (Milled), Bread flour blends containing 5-20% FDDG

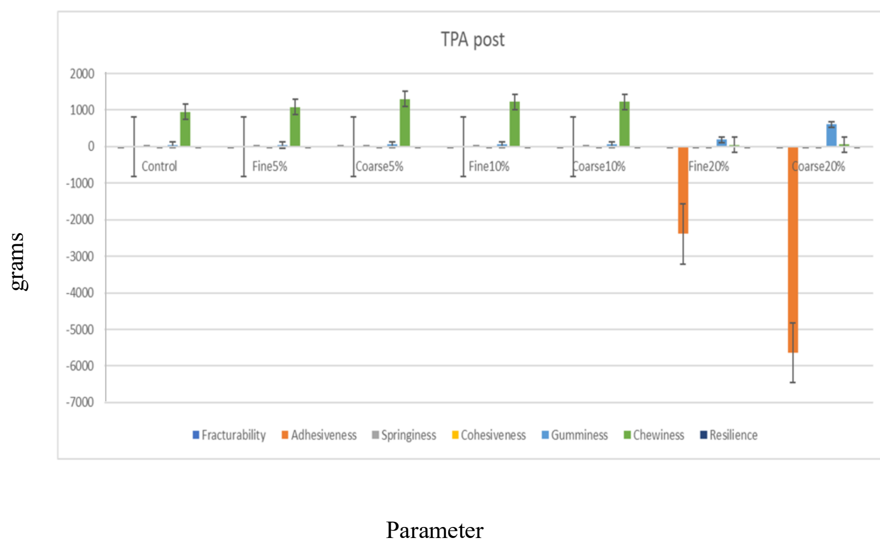


Figure 39. Bar plot of bread after 7 days evaluated for adhesiveness, hardness, springiness, cohesiveness, gumminess, chewiness, resilience containing Bread Flour, Processed FDDG (Milled), Bread flour blends containing 5-20% FDDG

Table 1 6. Texture evaluation of bread made with bread flour and FDDG-flour blends containing 5-20% FDDG – Fresh

Constituents	Parameter	Substitution	Hardness (g)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)	Resilience
Bread Flour	Control	0%	303.76d	18.93ab	1.053a	315.04d	5763.31bc	0.559b
FDDG	Fine	5%	246.27d	24.71a	1.029a	238.79d	5584.19bc	0.572b
FDDG	Fine	10%	510.40d	12.65b	0.846b	429.32d	4610.72bc	0.527bc
FDDG	Fine	20%%	1255.86b	20.63ab	0.874b	1086.15b	19549.36a	0.624a
FDDG	Coarse	5%%	457.81d	18.77ab	0.784bc	359.219d	5989.49bc	0.495c
FDDG	Coarse	10%	901.08c	15.11b	0.7015c	676.454c	9234.89b	0.564b
FDDG	Coarse	20%	2282.94a	1.11c	0.791bc	1789.007a	1909.17c	0.541bc

Legend:
BF: Bread Flour
Coarse: FDDG ground using 0.5 mm mesh
DDG: Distillers Dried Grain
FDDG: Food Grade Distillers Dried Grain
Fine: FDDG ground using 0.2mm mesh
Means with the same letter within a column are not significantly different from each other ($P \leq 0.05$)

4.7 Sensory Evaluation

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 acceptability. Descriptors which corresponded to the points ranged from “Dislike extremely-1 Dislike Moderately -2 Dislike slightly-3 Neither like nor dislike-4 Like slightly-5 Like Moderately-6 Like extremely-7. Twelve participants were used for this study, the study was conducted once. Ten participants where used for this study. Each participant tested half slice of the sample for each treatment.

Table 17 and figure 36 describe bar plots of bread for sensory evaluation of appearance, taste, texture, aroma, and overall acceptability containing Bread Flour, Processed DDG (Milled), Bread flour blends containing 5-20% FDDG for Bread.

Observations:

There were no statistical analysis done on the sensory data except for means and standard deviation. A seven-point hedonic scale was used.

Control bread without fortification and the 5% Fine DDG-Wheat flour Bread received the highest scores.

5% Fine FDDG Bread scored 4.0 points for most traits with the exception of aroma.

The higher levels of FDDG substitution with both fine FDDG and coarse FDDG resulted in bread with lower scores. Scores as low as 2.2 were records for overall acceptability.

On a scale of 1 through 7, a score of 4 would be deemed to be acceptable. Based on these criteria, of the treatment groups, the 5% FDDG breads made with fine FDDG will be acceptable to consumers. Scores of 3.5 and less reflect neutrality and diminished acceptance.

In some respects, the 5% Fine FDDG Bread received consistent and high scores of 4 0 for Appearance, Taste, Texture and Overall acceptability. Numerically, it performed better than the Control Bread which contained no FDDG.

Table 1 7. Mean comparison of sensory evaluation of appearance, taste, texture, aroma and overall acceptability of bread

Parameters	Control	Food Grade Distillers Dried Grains					
		Fine FDDG in BF			Coarse FDDG in BF		
DDG Substitution		**5%**	**10%**	**20%**	**5%**	**10%**	**20%**
Appearance	3.66 ± 2.14	4.0 ± 2.16	2.75 ± 0.95	2.60 ± 2.07	3.66 ± 1.52	2.75 ± 2.87	3.75 ± 2.62
Taste	6.0 ± 2.0	4.0 ± 2.64	4.0 ± 2.0	2.66 ± 2.12	2.75 ± 2.21	3.66 ± 1.52	4.0 ± 2.87
Texture	3.25 ± 2.06	4.0 ± 3.36	2.75 ± 0.5	2.5 ± 1.64	3.66 + 2.08	3.0 ± 1.15	2.4 ± 1.51
Aroma	4.66 ± 1.15	3.0 ± 2.36	3.0 ± 1.83	1.75 ± 1.34	2.2 ± 1.78	2.75 ± 1.15	2.6 ± 1.82
Overall Acceptability	4.0 ± 3.60	4.0 ± 2.60	2.75 ± 1.25	2.2 ± 1.09	3.66 ± 3.78	3.66 ± 2.70	3.0 ± 2.16

Legend:
BF: Bread flour
Coarse = FDDG ground using 0.5 mm mesh
DDG: Distillers dried grain
FDDG: Food grade distillers dried grain
Fine = FDDG ground using 0.2mm mesh
HRW: Hard red winter

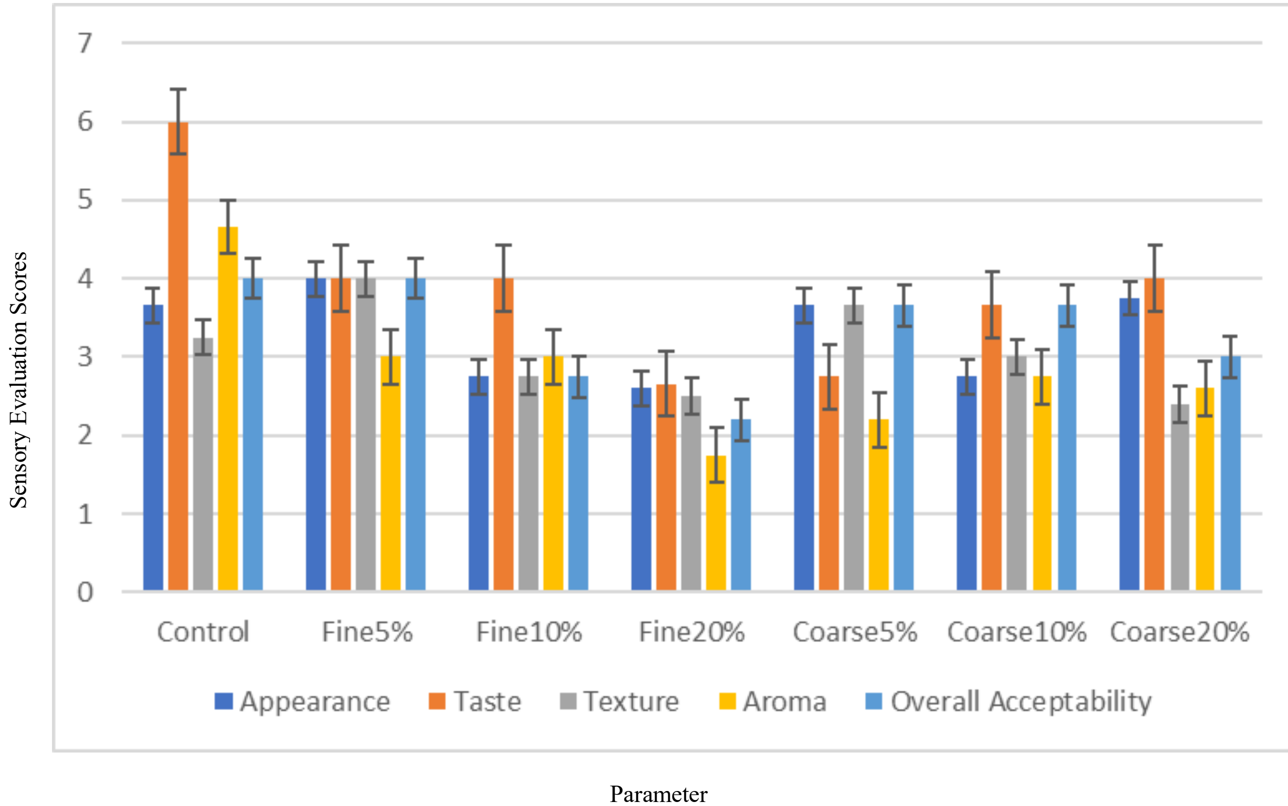


Figure 40. Standardized (0 – 10) bar plot of bread for sensory evaluation of appearance, taste, texture, aroma and overall acceptability containing Bread Flour, Processed DDG (Milled), Bread flour blends containing 5-20% FDDG for Bread

Figure 41. Picture of Bread for Sensory Analysis

5. CONCLUSION

Ethanol production has become a very important industry in the US in view of its contribution to energy production. Efficient utilization of co products of this industry is critical to sustainability of the industry. The yield of FDDG from the raw material received from the ethanol plant ranged from 36% - 39%. This was due to removal of water to make it a shelf stable product. While this is an energy intense process, a dry DDG matrix is conducive to further processing for effective use of solvents and recovery of useful moieties that can be used in food processing.

There is need for optimized efficient techniques in washing of DDG to minimized losses and improve productivity and efficiency as manual DDG washing takes time and use of solvents. The protein content of bread is influenced by the type of flour which is used in baking and the degree of extraction in the flour. Further nutritional improvements of bread can be brought about by addition of FDDG, which has significant amounts of both protein (36-40%) and Dietary fiber (40%).

The research attempted to identify and develop a nutritive food ingredient that has a high content of TDF and protein and that can be used at high levels of inclusion in foods. We explored high substitution levels (up to 20%) in bread formula. We also investigated the potential for particle size reduction of the key ingredient to match the particle size of the base wheat flour. The nutritional and sensory attributes of the FDDG fortified bread were evaluated to determine the upper limits of fortification without undermining acceptability of the enriched bread. Modest levels of substitution at 5 and

10% were found to be acceptable. Significant improvements in protein content and fiber content were achieved in fortified bread.

The effects of particle size reduction were not as significant as the effects of levels of supplementation in terms of rheology of the dough. Fine grinding of FDDG yielded 10% additional material in the 150 to 400 micron particle size range in comparison to coarse grinding. This did not bring about drastic changes in the ingredient properties of FDDG.

6. RECOMMENDATIONS

Ethanol as a costly reagent was mostly used for processing technique and this incured some cost during processing. In this research manual processing technique for DDG was carrieed out which can be improved with the use of a machine so that DDG can be processed in a larger scale, better processing techniques can be developed to reduce cost (ethanol) and limit waste during processing.

There is no difference in 0.2 and 0.5 mm sieve, future study should include the use of 0.08 mm seive in place of 0.2 mm sieve to grind the particles finer to verify further changes in the particle size distribution, loaf volume, and chemical properties of DDG. Further investigation can also be conducted in the inclusion rate of DDG as DDG incorporation can be elevated in bread if vital gluten can be added during the mixing processs to help develop the structure and volume of the bread compared to that of white bread. FDDG can be used as an ingredient in the world feeding programs as this research showed that it adds major nutritional value.

APPENDIX

Farinograph

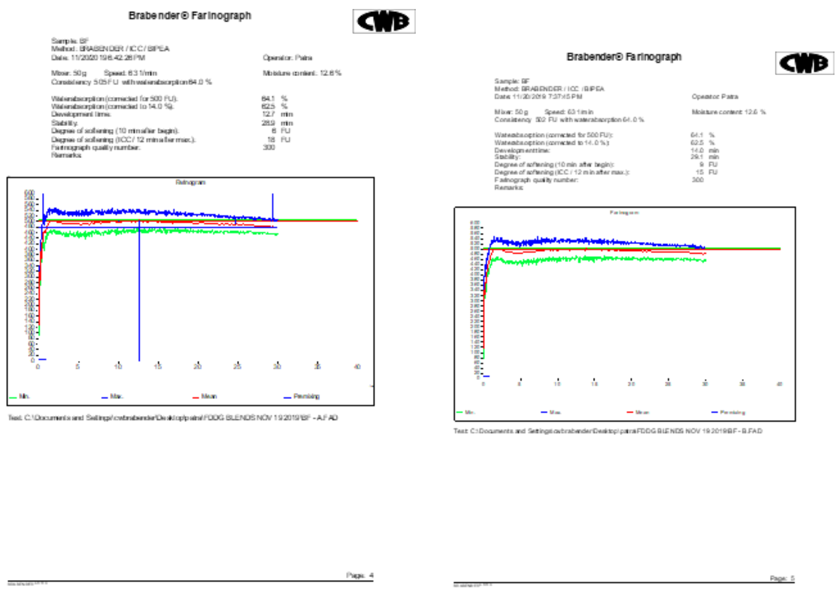


Figure 42. FFDDG and 100% BF

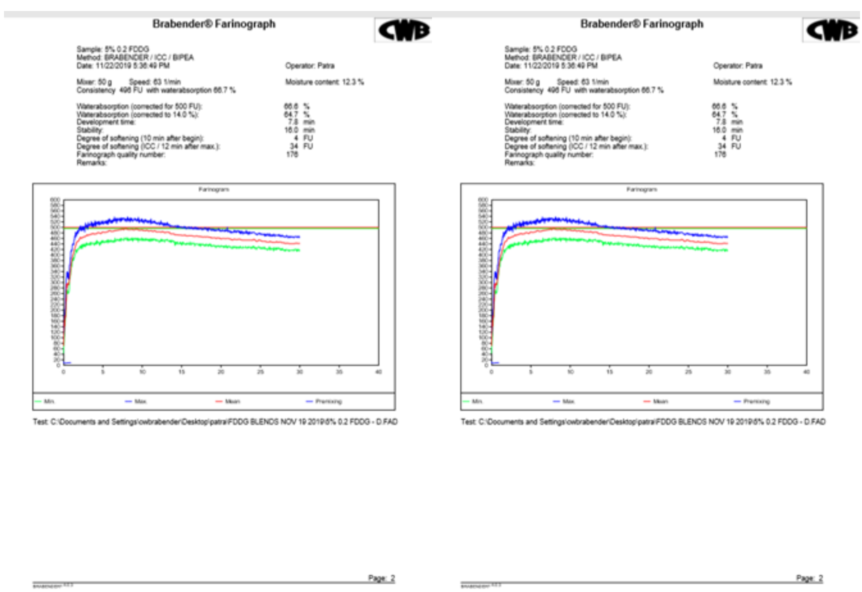
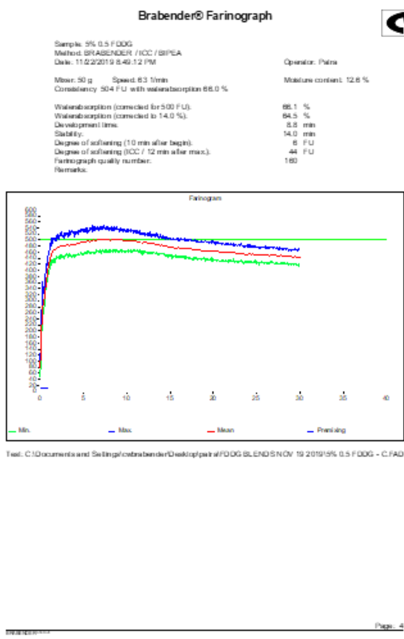


Figure 43. 5% 0.2 FDDG and 95% BF



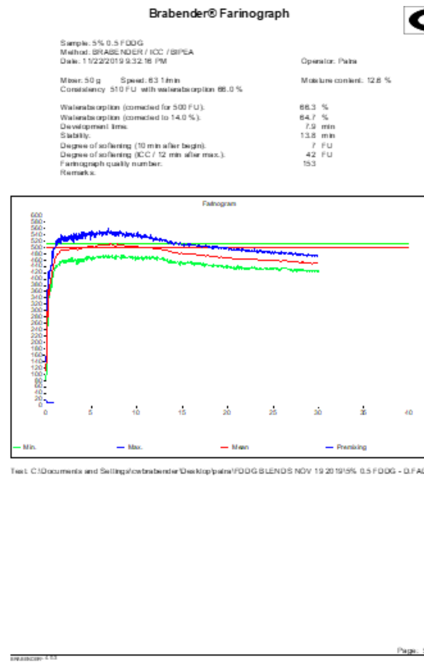
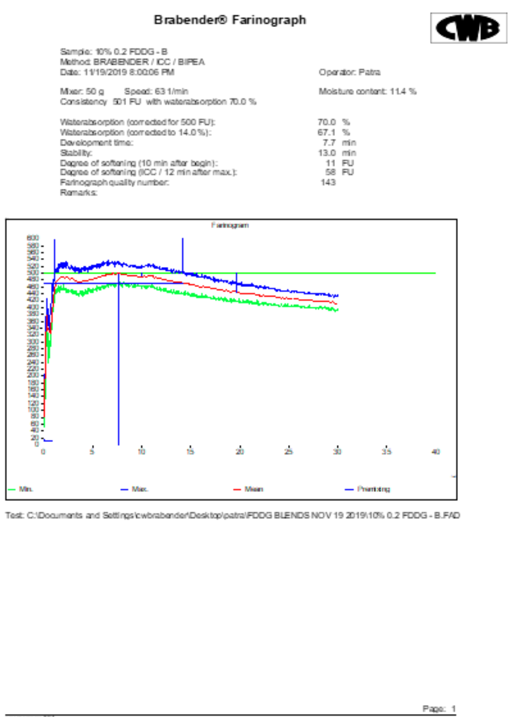


Figure 44. 5% 0.5 FDDG and 95% BF



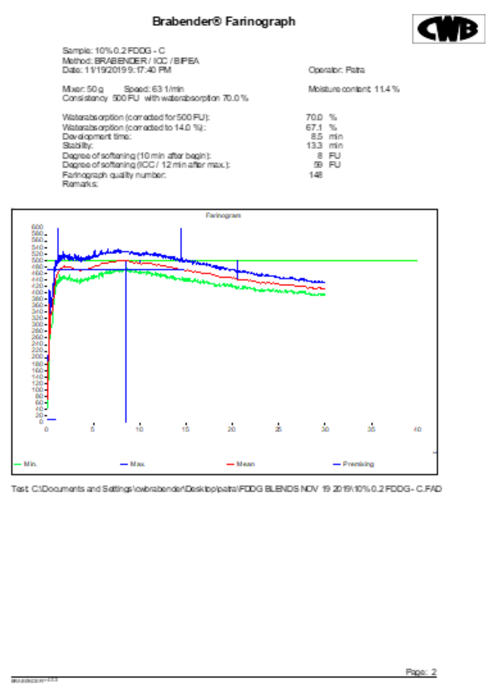
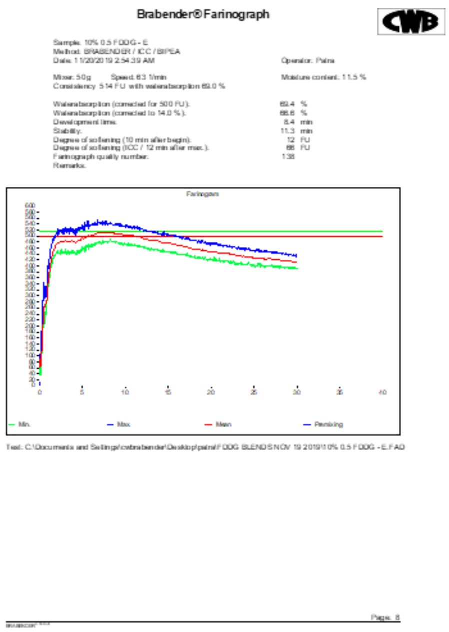


Figure 45. 10% 0.2 FDDG and 90% BF



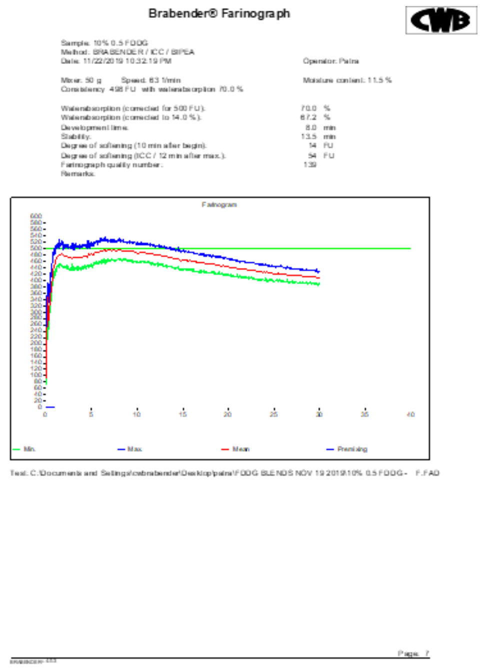
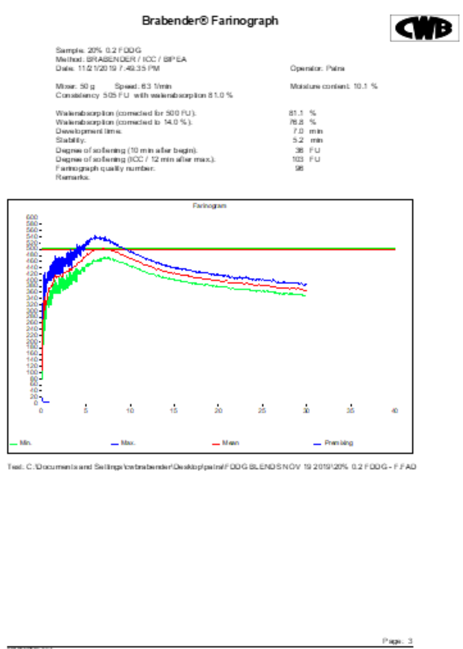


Figure 46. 10% 0.5 FDDG and 90% BF



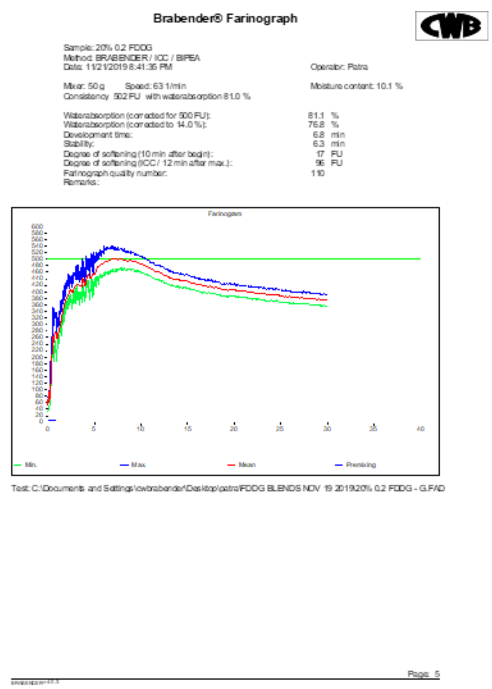


Figure 47. 20% 0.2 FDDG and 80% BF

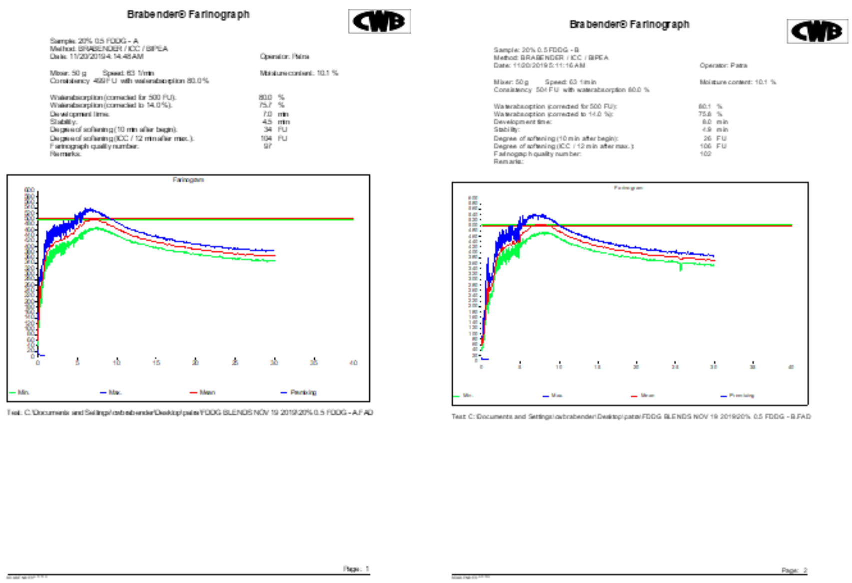


Figure 48. 20% 0.5 FDDG and 80% BF

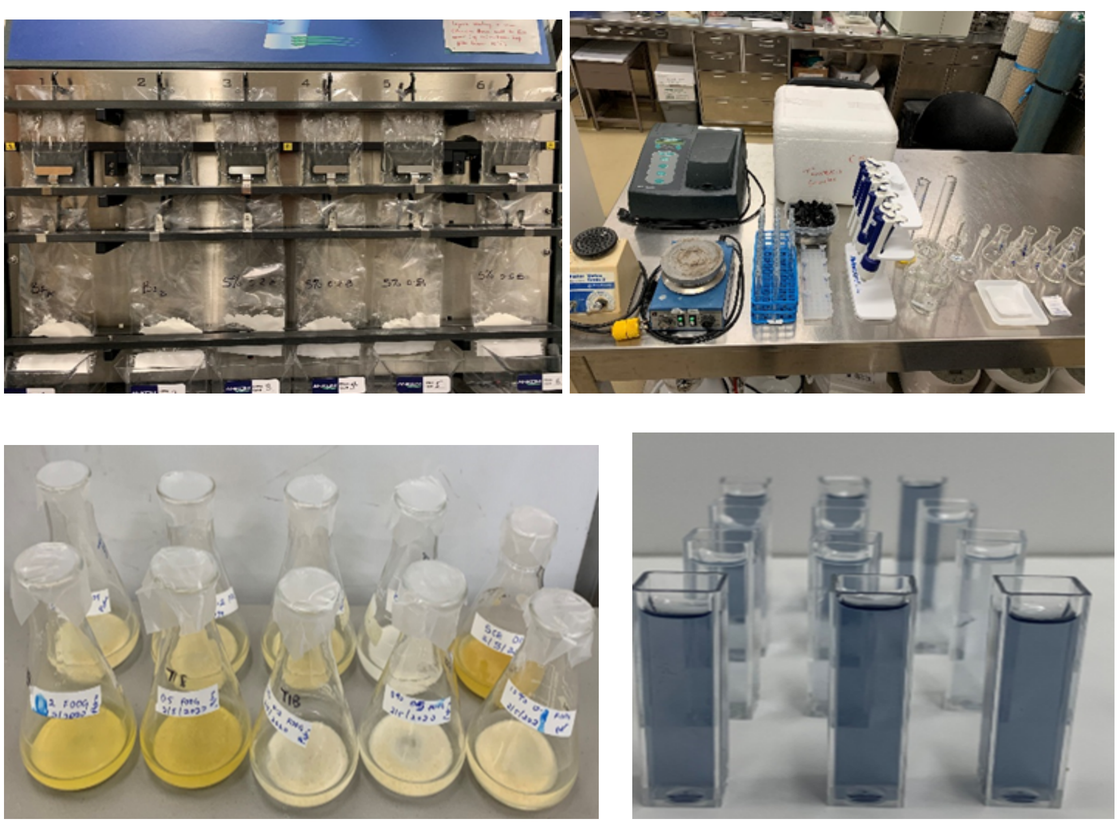


Figure 49. TDF, and total phenolic content analysis

Table 17. Zero-order correlation coefficients (r) between variables of proximate analysis of TDF, Protein, Moisture, Fat, Ash, CHO for Composition of Bread Flour with Bread flour blends containing 5-20% FDDG

	TDF	Protein	Moisture	Fat	Ash
TDF					
Protein	0.97				
Moisture	**-0.68**	**-0.64**			
Fat	0.39	0.42	0.40		
Ash	0.49	0.66	0.07	0.74	
CHO	**-0.97**	**-0.98**	0.52	**-0.56**	**-0.67**

Note: Bold coefficients are significant (P<.05)

Legend:
BF: Bread flour
DDG: Distiller dried grain
DDGS: Distillers dried grain with soluble
FDDG: Food grade distillers died grain
HRW: Hard red winter wheat
TDF: Total Dietary Fiber
TPC: Total phenolic content

Table 18: Zero-order correlation coefficients (r) between variables of particle size distribution of Bread Flour, Processed DDG (Milled and Un-milled) for >400, >250 – 400, >180 – 250, >150 – 180, >75 150, ≤ 75

	>400	*>250 - 400*	*>180 - 250*	*>150 - 180*	*>75 - 150*
>400					
>250 - 400	0.439048				
>180 - 250	-0.44042	0.584129			
>150 - 180	-0.54289	-0.97691	-0.51336		
>75 150	-0.70896	-0.91214	-0.21296	0.896144	
≤ 75	-0.47332	-0.96396	-0.58204	0.993497	0.840172

Table 19. Zero-order correlation coefficients (r) between variables of particle size distribution of bread flour, processed DDG (Milled and un-milled) and bread flour blends containing 5-20% FDDG for >400, >250 – 400, >180 – 250, >150 – 180, >75 150, ≤75

	>400	*>250 - 400*	*>180 - 250*	*>150 - 180*	*>75 - 150*
>400					
>250 - 400	0.573061				
>180 - 250	-0.12768	0.491648			
>150 - 180	-0.59659	-0.9137	-0.67926		
>75 - 150	-0.70728	-0.8408	-0.39871	0.836594	
≤ 75	-0.5164	-0.84488	-0.66076	0.907686	0.687215

Table 19. Relationship between variables >150 – 180 for Particle Size comparison of L*, a*, b* of Bread Flour, Processed DDG (Milled and Un-milled), Bread flour blends containing 5-20% FDDG

Variables	Multiple R	R Square	Adjusted R Square	Standard Error	*t Stat*	*Coefficients*	*P-value*	*Lower 95.0%*	*Upper 95.0%*
L*	0.0185	0.0003	-0.1246	5.554	-0.052	-0.019	0.959	-0.868	-0.825
a*	0.849	0.722	0.687	2.827	-4.559	-6.993	0.001	-10.530	-3.456
b*	0.307	0.094	-0.018	0.620	0.912	0.0279	0.389	-0.042	0.099

Table 20. Relationship between estimated L with color a of Bread Flour, Processed DDG (Milled and Un-milled), Bread flour blends containing coarse group FDDG for >150 – 180

Variables	Multiple R	R Square	Adjusted R Square	Standard Error	*t Stat*	*Coefficients*	*P-value*	*Lower 95.0%*	*Upper 95.0%*
L*	0.809	0.654	0.611	3.571	-3.897	-1.570	0.004	-2.500	-0.641
a*	0.392	0.154	0.049	2.880	-1.207	-3.600	0.261	-10.481	3.279
b*	0.657	0.430	0.359	0.258	2.460	0.029	0.039	0.001	0.056

Table 21. Summary of Regression Statistics for Particle Size color comparison L*, a*, b* of Bread Flour, Processed DDG (Milled and Un-milled), for >75 – 150

Variables	Multiple R	R Square	Adjusted R Square	Standard Error	*t Stat*	*Coefficients*	*P-value*	*Lower 95.0%*	*Upper 95.0%*
L*	0.877	0.768	0.739	2.756	-5.146	-1.579	0.0009	-2.286	-0.870
a*	0.1220	0.0149	-0.109	3.152	0.348	1.403	0.737	-7.907	-7.907
b*	0.301	0.091	-0.022	0.263	0.894	0.011	0.397	-0.019	0.040

Table 22. Summary of Regression Statistics for Particle Size color comparison of L*, a*, b* of Bread Flour, Processed DDG (Milled and Un-milled), for >75

Variables	Multiple R	R Square	Adjusted R Square	Standard Error	*t Stat*	*Coefficients*	*P-value*	*Lower 95.0%*	*Upper 95.0%*
L*	0.878	0.770	0.659	8.709	5.493	0.1684	0.0004	0.099	0.24
a*	0.758	0.573	0.462	61.839	-3.479	-75.292	0.007	-124.24	-26.35
b*	0.790	0.626	0.514	0.583	-3.874	-0.042	0.004	-0.067	-0.02

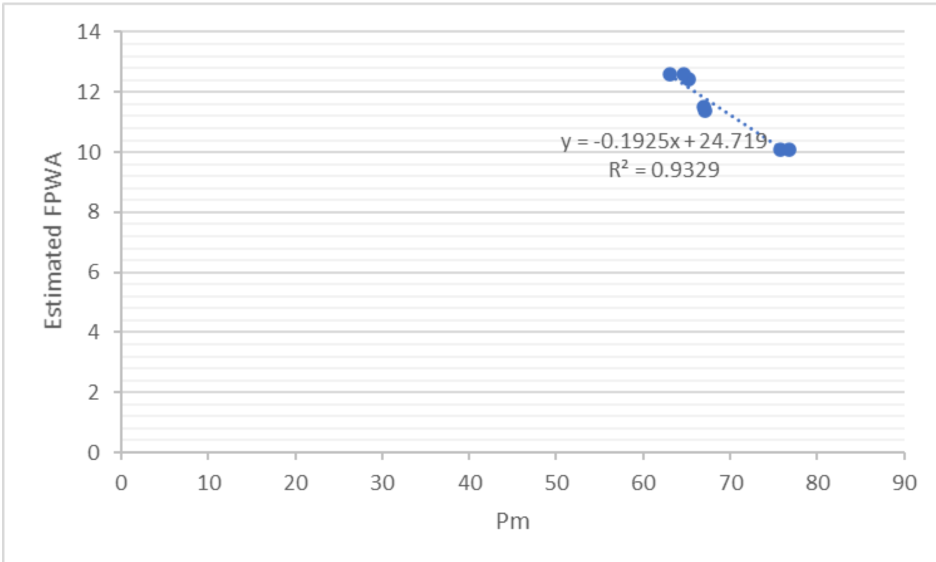


Figure 50. Relationship between estimated Farinograph percent water absorption (%), with Percent moisture for Bread Flour, Processed DDG (Milled and Un-milled), Bread flour blends containing coarse group FDDG

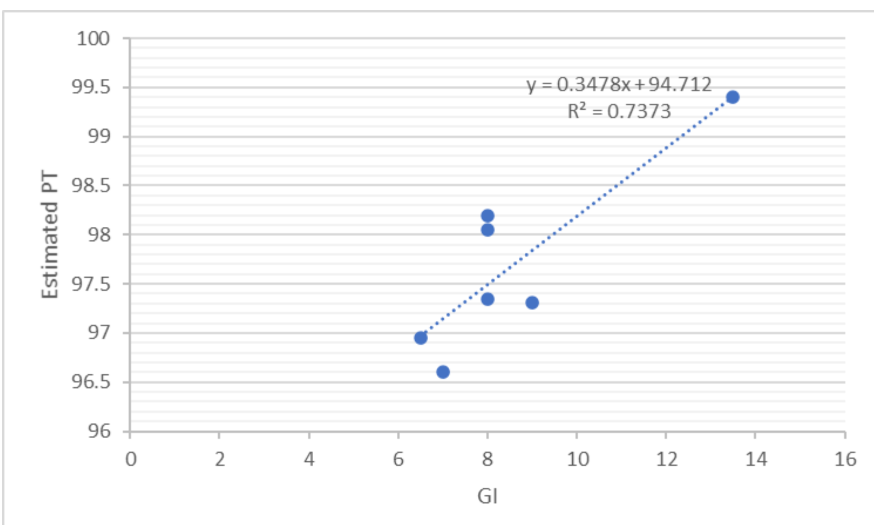


Figure 51. Relationship between peak time with gluten index for Bread Flour, Processed DDG (Milled and Un-milled), Bread flour blends containing coarse group FDDG

Table 23. Rheological Properties and gluten characteristics (5-20%)

Variables	Multiple R	R Square	Adjusted R Square	Standard Error	*t Stat*	*Coefficients*	*P-value*	*Lower 95.0%*	*Upper 95.0%*
PT	0.779	0.608	0.440	46.449	3.049	6.057	0.022	1.194	10.919
MTI	0.642	0.412	0.247	11.279	2.053	0.128	0.086	-0.024	0.028
FS	0.950	0.903	0.737	3.077	7.490	0.639	0.0002	0.8479	0.4302
FDT	0.983	0.967	0.800	2.270	13.271	1.242	1.13E-05	1.013	1.472
PM	0.987	0.974	0.808	11.887	15.087	5.853	5.35E-06	4.903	6.802
G1	0.971	0.944	0.778	2.247	10.112	0.087	5.43E-05	0.067	0.109

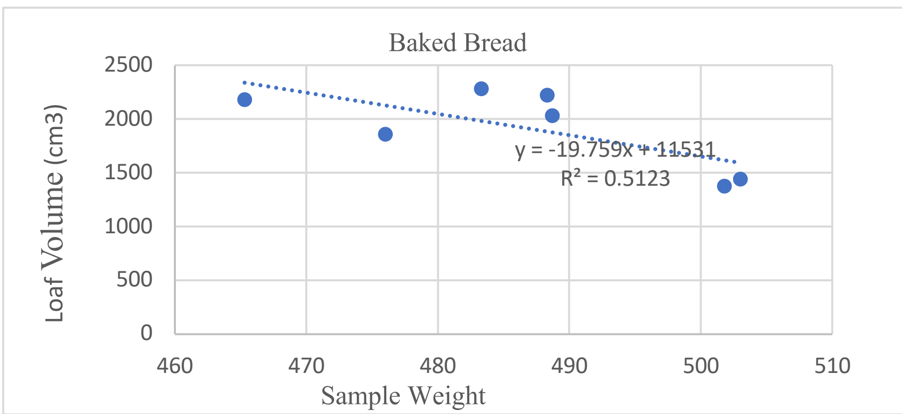


Figure 52. Relationship between sample weight, with Loaf Volume (cm^3) for baked bread

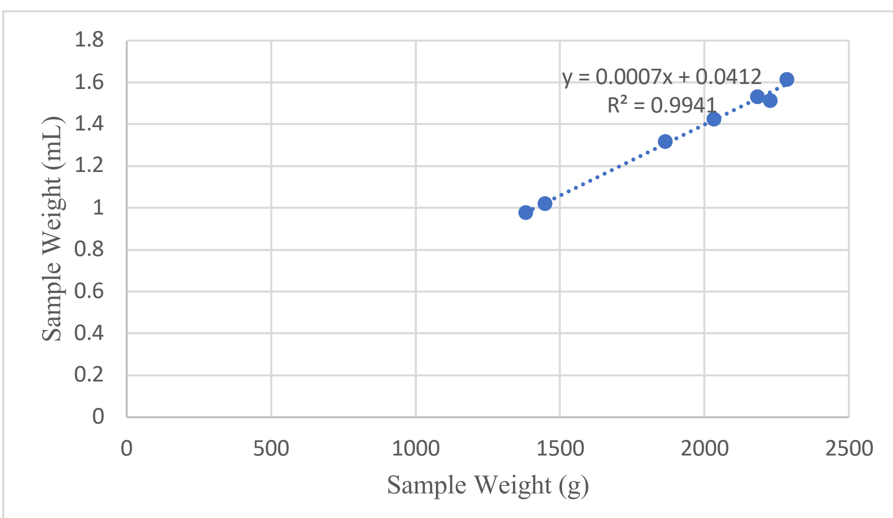


Figure 53. Relationship between sample weight (mL), and grams for baked bread

Table 24. Summary of Regression Statistics variables for bread crumb for Bread Flour, Processed DDG (Milled and Un-milled), Bread flour blends containing 5-20% FDDG

Variables	Multiple R	R Square	Adjusted R Square	Standard Error	*t Stat*	*Coefficients*	*P-value*	*Lower 95.0%*	*Upper 95.0%*
Area Cells	0.760	0.579	0.494	0.110	-2.622	-.0289	0.047	-0.573	- .0.005
Volume Holes	0.873	0.762	0.714	0.024	-4.002	-0.098	0.010	-0.161	-0.035
Wall Thickness	0.426	0.181	0.018	466.8	-1.054	-492.2	0.340	-1692.4	707.9
Cell Diameter	0.798	0.638	0.565	0.019	2.970	0.057	0.031	0.007	0.107
Non-Uniformity	0.625	0.391	0.269	0.205	1.793	0.368	0.132	-0.159	0.897
WL	0.250	0.062	-0.124	0.025	0.578	0.014	0.587	-0.058	0.080
SliceB	0.084	0.007	-0.191	0.269	-0.190	-0.051	0.856	-0.744	0.641

Legend:
WL: Wrapper Length
SliceB: Slice Brightness

Table 25. Summary of Regression Statistics variables for texture analysis of post for adhesiveness, hardness, springiness, cohesiveness, gumminess, chewiness, resilience for Bread Flour, Processed DDG (Milled and Un-milled), Bread flour blends containing 5-20% FDDG

Variables	Multiple R	R Square	Adjusted R Square	Standard Error	*t Stat*	*Coefficients*	*P-value*	*Lower 95.0%*	*Upper 95.0%*
Hardness	0.443	0.197	0.036	0.000	*1.105*	*0.000*	*0.319*	*0.000*	*0.000*
Springiness	0.721	0.520	0.424	20.089	- 2.328	-46.767	0.067	98.407	2459.0
Cohesiveness	0.399	0.159	-0.008	23.489	0.973	22.862	0.375	37.518	83.243
Chewiness	0.291	0.085	-0.097	0.029	0.681	0.020	0.526	-0.056	0.096
Resilience	0.536	0.287	0.144	47008.6	1.419	66711.9	0.215	- 54127.7	187551.7

Table 26. Summary of Regression Statistics variables for texture analysis of post for adhesiveness, hardness, springiness, cohesiveness, gumminess, chewiness, resilience for Bread Flour, Processed DDG (Milled and Un-milled), Bread flour blends containing 5-20% FDDG

Variables	Multiple R	R Square	Adjusted R Square	Standard Error	*t Stat*	*Coefficients*	*P-value*	*Lower 95.0%*	*Upper 95.0%*
Adhesiveness	0.233	0.054	-0.134	0.001	*0.536*	*0.000*	*0.6149*	*-0.003*	*0.005*
Springiness	0.896	0.803	0.763	85.660	4.517	387.01	0.006	166.81	607.21
Cohesiveness	0.986	0.972	0.967	0.847	13.323	11.295	4.26E-05	9.116	13.474
Gumminess	0.786	0.616	0.539	0.000	-2.834	-0.001	0.036	-0.003	-0.000
Chewiness	0.787	0.619	0.543	0.104	-2.852	-0.297	0.036	-0.564	-0.029
Resilience	0.969	0.939	0.928	77.245	-8.827	-681.88	0.000	-880.4	-483.3

Table 27. Summary of Regression Statistics variables for comparison of sensory evaluation of appearance, taste, texture, aroma and overall acceptability of bread

Variables	Multiple R	R Square	Adjusted R Square	Standard Error	*t Stat*	*Coefficients*	*P-value*	*Lower 95.0%*	*Upper 95.0%*
Taste	0.969	0.939	0.772	0.896	9.599	0.812	7.310	0.606	1.019
Texture	0.957	0.914	0.748	1.263	8.013	1.222	0.0002	0.849	1.596
Aroma	0.957	0.914	0.748	0.987	8.021	1.006	0.0002	0.699	1.312
Overall Acceptability	0.974	0.949	0.782	0.726	10.563	0.857	4.233	0.658	1.054

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