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South Dakota State University

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NUTRITIONAL AND HEALTH BENEFITS ENHANCEMENT OF WHEAT-BASED  
FOOD PRODUCTS USING CHICKPEA AND DISTILLER'S DRIED GRAINS

BY  
WALEED ALRAYYES

A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy

Major in Biological Sciences

Specialization in Food Science and Nutrition

South Dakota State University

2018

NUTRITIONAL AND HEALTH BENEFITS ENHANCEMENT OF WHEAT-BASED  
FOOD PRODUCTS USING CHICKPEA AND DISTILLER'S DRIED GRAINS

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of philosophy degree and is acceptable for meeting the dissertation requirement for this degree. Acceptance of this dissertation does not imply that the conclusions reached by the candidate are necessarily the conclusion of the major department.

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I thank Allah (God) who enabled me to successfully complete my work. I would also like to express my gratitude towards Prophet Mohammad Alaih As-Salat-O-Wassalam (prayer and peace upon him) who provided me inspiration to work hard.

I dedicate this dissertation to my family and friends. Without their support and encouragement, I would not be who I am today. I would like to make a special dedication to my mother Raja Al-Rashid and my Father Mohammed Al-Rayyes who led me into the field of healthcare so that I can make a difference in the quality of people's lives.

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## ABBREVIATIONS

|             |   |
|-------------|---|
| <b>AA</b>   | Antioxidant activity                    |
| <b>AACC</b> | American Association of Cereal Chemists |
| <b>ADA</b>  | American Diabetes Association           |
| <b>AOAC</b> | Association of Analytical Communities   |
| <b>APF</b>  | All-purpose flour                       |
| <b>AUC</b>  | Area under curve                        |
| <b>BMI</b>  | Body mass index                         |
| <b>°C</b>   | Celsius                                 |
| <b>Ca</b>   | Calcium                                 |
| <b>CDC</b>  | Center of disease                       |
| <b>CHD</b>  | Coronary heart disease                  |
| <b>CHO</b>  | Carbohydrates                           |
| <b>CP</b>   | Chickpea                                |
| <b>CVD</b>  | Cardiovascular disease                  |
| <b>D</b>    | Distiller's dried grains                |
| <b>d</b>    | Day                                     |
| <b>DDGS</b> | Distiller's dried grains with soluble's |
| <b>DF</b>   | Dietary fiber                           |
| <b>DFC</b>  | Dietary fiber content                   |
| <b>dl</b>   | Deciliter                               |
| <b>EFP</b>  | Emergency food product                  |
| <b>EP</b>   | Energy production                       |

|              |  |
|--------------|--|
| <b>FAO</b>   | Food and Agriculture Organization      |
| <b>FBFs</b>  | Fortified blended foods                |
| <b>FDA</b>   | Food and Drug Administration           |
| <b>FDDG</b>  | Food grade DDGS                        |
| <b>FSANZ</b> | Food standards Australia & New Zealand |
| <b>g</b>     | Gram                                   |
| <b>GI</b>    | Glycemic index                         |
| <b>GLM</b>   | Generalized linear model               |
| <b>GR</b>    | Glycemic response                      |
| <b>HEBs</b>  | High energy biscuits/bars              |
| <b>HPLC</b>  | high performance liquid chromatography |
| <b>IAUA</b>  | Incremental area under curve           |
| <b>IF</b>    | Indigestible fraction                  |
| <b>IMO</b>   | Institute of Medicine                  |
| <b>IRS</b>   | Insulin resistance syndrome            |
| <b>K</b>     | potassium                              |
| <b>Kcal</b>  | Kilocalories                           |
| <b>Kg</b>    | Kilogram                               |
| <b>LDL</b>   | low density lipoprotein                |
| <b>LGR</b>   | Low glycemic response food             |
| <b>LSD</b>   | Least significant differences          |
| <b>MDF</b>   | Mango dietary fiber                    |
| <b>Mg</b>    | Magnesium                              |

|               |   |
|---------------|---|
| <b>ml</b>     | Milliliters                             |
| <b>mmt</b>    | Million metric tons                     |
| <b>P</b>      | Phosphorus                              |
| <b>PCM</b>    | Protein calorie malnutrition            |
| <b>RS</b>     | Resistance starch                       |
| <b>RUFs</b>   | Ready to use foods                      |
| <b>RUTF</b>   | Ready to use therapeutic foods          |
| <b>SDSU</b>   | South Dakota State University           |
| <b>TDF</b>    | Total dietary fiber                     |
| <b>TP</b>     | Total Phenolics                         |
| <b>UNICEF</b> | The United Nation Children's Fund       |
| <b>USDA</b>   | United States Department Of Agriculture |
| <b>W</b>      | Wheat flour                             |
| <b>WFP</b>    | World Food Program                      |
| <b>WHO</b>    | World Health Organization               |

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## ABSTRACT

NUTRITIONAL AND HEALTH BENEFITS ENHANCEMENT OF WHEAT-BASED  
FOOD PRODUCTS USING CHICKPEA AND DISTILLER'S DRIED GRAINS

WALEED ALRAYYES

2018

The first objective of this research was to enhance nutritional, rheological, sensory profiles, and shelf life of wheat-based pita bread using chickpea (CP) and food grade distiller's dried grains (FDDG) as fortification ingredients. CP and FDDG are both high-protein and high-fiber ingredients. Nutritional efficacy was evaluated. Dough rheology and product texture were also analyzed. Chemical, physical, and rheological properties of blends, doughs and finished products were evaluated and the results showed an increase in protein, fat, ash, and total dietary fiber with an increase of FDDG and CP in the wheat-based food formulation. Moisture content was decreased in both flour blends and pita breads with the increase of FDDG and CP substitution levels. Amino acids scores were improved by different fortification levels of either chickpea or FDDG or combinations of the two ingredients in comparison of all treatments to the all-wheat control pita bread. Fortification with 10% FDDG improved amino acid scores by 15%, whereas fortification with 20% FDDG improved AA score by 22% (over control wheat flour pita). Also, fortification with 10% chickpea improved amino acid scores by 20%, whereas fortification with 20% chickpea improved amino acid scores by 28%. Color results indicated decreased L\* values (brightness), and a\*(redness), but increased

b\*(yellowness) levels with increased FDDG levels. With increased chickpea levels in pita formulation, L\* and b\* values decreased, and a\* increased. Rheological evaluation from Mixolab and Farinograph analysis showed that fortification in general, yielded pronounced effects on dough properties. Both FDDG and CP showed increased water absorption, higher dough development time, and lower dough stability time when compared to the wheat-only control. Texture analyzer results showed that the force required to break the dough increased, whereas the dough extensibility declined as the fortification level of either or chickpea and FDDG increased. Texture Analyzer results also showed that fortified pita required a greater force for tearability as determined by the burst rig and the tug fixture tests. Burst distance and tug distance were also reduced with increased fortification level of both chickpea and FDDG. Shelf life evaluation showed that wheat pita bread substituted with 10% chickpea pita bread had the same shelf life time as control pita bread, whereas fortifying with 20CP% increased the shelf life by 6 hours. Also, 10% FDDG fortification increased shelf life by 6 hours whereas fortifying with 20% FDDG increased the shelf life by 12 hours, in contrast to the control pita bread. Fortifying with 20CP-10D% increased the shelf life of the pita bread by 24 hours. The longest shelf life was found in 20 % FDDG-10% chickpea treatment which was 30 hours longer than the control all-wheat pita bread. Sensory analysis was done for all pita breads and showed that all products tested were deemed to be acceptable relative to the control all-wheat flour pita bread. Our findings show that pita breads containing up to by 30% chickpea and FDDG were determined to be acceptable to the sensory panelists.

The second objective of the study was to test the efficacy of high levels of dietary fiber, protein, fat, and antioxidants (phenolic compounds and carotenoids) by employing

ingredients such as chickpeas and food grade distiller's grains in the development of low glycemic response foods. Pita bread containing 10% CP yielded an IAUC of 85.46 mmol.min/L while the 20% CP showed IAUC of 56.32mmol.min/L. FDDG pita breads with 10% FDDG showed IAUC of 81.21 mmol.min/L while the 20% FDDG pita bread resulted in an IAUC of 46.23 mmol.min/L. Moreover, IAUC for the 70W-20CP-10D pita was 40.06 mmol.min/L, and 36.53 mmol.min/L for 70W-20D-10CP pita. Inclusion of CP and FDDG in wheat flour, separately and in combinations (70:20:10 & 70:10:20), brought about improvements in the GR when compared to control wheat pita.

The third objective of this study was to develop formulations for a nutrient-dense energy bar containing wheat flour, chickpea flour, and FDDG and to determine proximate composition and sensory characteristics. It is hypothesized that cereal based foods can be effectively fortified with chickpea and FDDG to produce products of higher nutrient content that can be used in emergency food programs. Results showed significantly higher values for protein, fiber, carbohydrates, and fats content in HEB containing CP and FDDG in contrast to unfortified all wheat HEB. Sensory scores of fortified HEB were acceptable as judged by panelists. HEB, particularly those containing 25% FDDG, 25% CP, and 50% CP, were highly enriched with nutrients and exceeded nutrient content in HEB currently employed by food aid programs. HEB containing 50% FDDG had particularly high protein content (16.6g/100g). Overall sensory results showed that 50% CP fortified HEB received a moderate score (3.86), whereas 25% FDDG, 25% CP, and 50% FDDG HEBs received scores of 4.0, 4.18, and 4.12, respectively. These results show good potential for the use of CP & FDDG in High Energy Bars for emergency food programs

## CHAPTER 1

### 1.1 Introduction

Wheat flour is used as the major ingredient in most of the breads and has been implicated in various health problems in small segments of the population. Highly refined wheat flour is usually used for bread production. Although, wheat is naturally a good source of proteins (8-12%), vitamins such as Vitamin E, minerals such as Iron, Zinc, and dietary fibers, substantial proportions of these nutrients are lost during milling and refining of the wheat grains for flour production. Excessive processing and milling leads to significant loss of fibers due to removal of the outer layer of the wheat (Anjum et al., 2006). Also, wheat, like many cereals, lacks essential amino acids such as Lysine (Khetarpaul and Goyal, 2009). To overcome this problem, one solution is to fortify flour with substances that can supplement the essential nutrients, compensate for the lost nutrients during processing and milling, and reduce the risk of serious nutrition-related diseases such as cancer, diabetes, and cardiovascular diseases. Therefore, to provide nutritious food to consumers, one solution is to use less refined wheat flour and to fortify bread with substances that can compensate for the nutrient loss of wheat flour during processing. Bread is consumed in all parts of the world and is one of the oldest foods known throughout history. Wheat is a major ingredient in bread. To meet the requirements of modern lifestyle, bread is often fortified with various substances to improve its nutrient content and taste. The United States of America is one of the leading countries in the fortification of food. For example, Folic acid has been added to flour, and niacin has been added to bread in the United States since 1938. Other countries have also done many recent studies on fortification. Folic acid is used for fortification of bread in Australia to

prevent folic acid deficiency in the population, especially young girls and women (FSANZ, 2012). Similarly, flax seeds are added to bread formulation to increase its dietary fiber content and for supplementing it with omega-3 fatty acids (Rakcejeva et al., 2007). Furthermore, fruit bits and milk solids are added to bread to improve taste.

Adding nutritional value to bread is one of the ways to provide healthy food to consumers. Nutrients are also added to get desired texture, physical and chemical properties, and to increase the shelf-life of the bread. Since, bread is consumed on a regular basis throughout the world, enrichment of bread with fiber and protein can potentially benefit people of all age groups in having healthy diet and in overcoming various nutritional problems. Moreover, the American Dietetic Association (2002) has reported lower than recommended intake of dietary fiber among US children and adults and has expounded the beneficial role of fibers in controlling diabetes. These facts again support the fortification of bread with fibers and other nutrients so as to ensure daily provision of healthy food to children and diabetics (Lafrance et al., 1998). Cereals being a relatively cheap source of protein and fibers, are an economical choice for fortification of bread. This makes them affordable and nutritious food for use with low income families and food relief programs.

Consumption of low-glycemic index (GI) foods, have been shown to improve glucose tolerance in human subjects. The estimated cost of diabetes in the US is \$245 Billion (ADA, 2013), and it is expected to rise by 53% to more than \$622 billion dollars between the years 2015-2030 (Rowley, Bezold, Arikan, Byrne, and Krohe, 2017). While the consumption of low glycemic response foods (LGR) has increased in recent years (Riccardi, G., Rivellese, A. A., & Giacco, 2008), there is a need for a more diverse range

of such foods in the market that are also affordable. A potential solution to manage diabetes cost is to consume foods that have a low GI. Low GI diets are more expensive than the higher GI equivalents, which affect the consumer buying behavior and food choice (Cleary, J., Casey, S., Hofsteede, C., Moses, R. G., Milosavljevic, M., & Brand Miller, J. 2012).

Malnutrition is a complex problem, and has become a major problem in different countries around the world. Different programs and potential solutions have been suggested as answers to the problem of malnutrition, but most of these are long-term solutions. One of the solutions is the development of cereal and cereal-based products for food supplementations. These products are used throughout the world as inexpensive energy and protein sources (Bulusu et al., 2007, Kent, 1994)

Garbanzo (*Cicer arietinum* L.) flour or Chick pea flour and food grade dried distillers grain (FDDG) are excellent sources for fortification of wheat flour in order to enhance its nutritional content. Chickpea is known to be of excellent nutritional quality. It is rich in carbohydrates, proteins, vitamins and minerals. It is low in fat and sodium content, and as such, it is beneficial for diabetics and hypertensive individuals. It is cholesterol-free and a significant source of both soluble and insoluble fiber. In the scientific literature, it has been reported that chickpea confers various health benefits such as lowering of glycemic index (GI) of diabetic patients, increase in satiety, cancer prevention, and protection against cardiovascular disorders due to its high dietary fiber content (Jukanti et al., 2012). Chickpea seeds are eaten as fresh, boiled, canned, roasted, or fried products. It is ground into powder and used for making various fried snacks. In the Middle Eastern and Mediterranean countries, it is also used as an ingredient in bread

making.

DDGS is the dried fermented grain residues that are rich in protein (27-35%), fiber (5-11%), and (8-12 %) fat, containing up to 10-12% moisture and having almost an indefinite shelf life (Belyea et al, 2004). It is primarily obtained from ethanol plants. Because of low starch, high protein and high fiber content of DDGS and its source, it is thought to be beneficial in the diet of diabetics and individuals suffering from celiac disease. The essential amino acids present in DDGS make it useful for human consumption and one of the functional ingredients to be added in food products. Several studies in past have reported successful incorporation of DDGS in food products (particularly baked products) resulting in nutritionally enhanced products. Some of the baked products in which DDGS have been incorporated are breads, rolls, muffins, and cookies. The composition of DDGS varies from one ethanol plant to other.

The present research is aimed at developing two types of food products. The first product is wheat flour pita bread fortified with fractions of chickpea and a food grade DDGS that is acceptable to consumers, and is capable of lowering glycemic index in human subjects. The second food product is a high energy biscuit (HEB) that is suitable for use in food aid programs during emergencies.

This study is divided into three parts Figure 1.1. The objective of the first part was to optimize the fractions of chickpea flour, food grade FDDG, and wheat flour in the bread dough mixture so as to develop high fiber high protein pita bread. An all-wheat control pita and 6 different blends of chickpea, FDDG and wheat flour combinations were studied. The control and 6 treatments were tested for dough rheology, product texture, chemical and physical properties of blends, and finished product quality. A second

objective was to compare the glycemic response of wheat flour pita bread, chickpea fortified wheat flour pita bread, and chickpea-FDDG fortified wheat flour pita bread to evaluate the efficacy of chickpea and FDDG in diabetic diets. The glycemic response of pita breads developed in first part of the study were measured by administering it into the target subjects. The third objective of the study was to determine the nutritional properties of chickpea-FDDG fortified pita bread as well as to determine its consumer acceptability among trained and untrained panelist of faculty and students. The fourth objective of the study was to evaluate a food product developed for use in food relief programs (High Energy Bars, HEB) to compare it to existing products used in international feeding programs.

In the first part of the study, six different flour blends were developed. The combinations of wheat flour (W), chickpea (CP) and FDDG (D) in wheat-based pita breads. Pita breads were prepared employing flour blends prepared in the following ratios: Control W (100 %), W:CP (90:10 & 80:20), W:D (90:10 & 80:20), and W:CP:D (70:20:10 & 70:10:20). These six flour blends were used for developing pita bread as an alternative to the exclusive use of only wheat flour in the production of traditional Mediterranean pita bread. Seven different types of pita bread-wheat flour pita bread (control), chickpea-wheat flour pita bread, and chickpea-FDDG-wheat flour pita bread, and two FDDG-wheat flour pita bread with two different substitutions levels was baked using a traditional pita bread recipe. Rheological, physical, and chemical properties, as well as shelf life of all the dough and final products were determined and compared. Finally, the consumer acceptability of developed pita breads was determined by means of sensory analysis. Sensory evaluation was carried out by trained and untrained panelists using a



seven-point hedonic scale. Panel members were comprised of undergraduate and graduate students and staff members of South Dakota State University.

In the second part of the study, the glycemic response of all the seven products-wheat pita bread, chickpea fortified pita bread, and chickpea-FDDG fortified pita bread, and the FDDG fortified pita breads (developed in first part of the study), were measured and compared to evaluate the extent of usefulness of chickpea and food grade FDDG in incorporating into the diabetic diet. The test was carried out on healthy subjects selected from the university. Eligible volunteers were given seven different types of pita breads to ingest. After ingestion, blood samples were collected from each subject to determine blood glucose level. Glycemic response of the subjects to the pita bread treatments were evaluated.

The third part of this study was to develop high protein and energy biscuits (HEB) using chickpea and FDDG with 25% and 50% fortification levels. The HEB were then evaluated for their nutritional properties and compared to HEB that's were available and used by food aid agencies.

## **1.2 Purpose of the Study**

Bread is a widely consumed food, made usually from highly refined wheat flour. Excessive processing and milling involved in wheat flour production causes loss of nutrients such as vitamins, minerals, and dietary fibers. Also, wheat lacks essential amino acids such as Lysine. Fortifying wheat flour with alternative flours would be a solution to improve the nutrition of this ingredient which not only will add nutrients to the bread, but it also can help reduce risk of certain nutritional related diseases and health conditions. According to published health reports (2002), the estimated total direct and indirect cost

of diabetes was \$132 billion in the United States alone (WHO, 2006; American Diabetes Association, 2002). This calls for a cost-effective solution to tackle diabetes or in other way to reduce the diabetes cost. Food is one of the best resources for extending health benefits to the population. Fortification of food with potential new ingredients is one way to introduce food with optimal nutritional profile. Therefore, developing a food item rich in protein and fibers will be beneficial for children and adults who are malnourished or who have low intake of protein and fiber, and for diabetic patients. This product will be significant as well for feeding people in emergencies and disasters. Also, it is of considerable importance to develop a low cost food that can be consumed by all income groups throughout the world, especially in disaster refugees. Keeping the above factors in mind, the following objectives are laid down for the present study.

### **1.3 Objectives**

- To investigate nutritional benefits of chickpea flour and chickpea-food grade DDGS blend and to evaluate their potential use as food ingredient, and the possibility of using it as food supplement for malnutrition, low income families, and disasters.
- To determine rheological, physical, and chemical properties of the dough prepared by blend of fractions of chickpea flour, food grade DDGS, and wheat flour.
- To prepare protein and fiber rich pita bread from chickpea-wheat flour dough blend and chickpea-food grade DDGS-wheat flour dough blend.
- To determine physical and chemical properties of the final product (chickpea-wheat flour and chickpea-DDGS-wheat flour pita bread).
- To determine nutrient composition of the final product and compare it with

control (wheat flour pita bread) to evaluate nutritional improvements.

- ⤴ To investigate shelf life of the final product.
- ⤴ To measure and compare the glycemic response of final product and compare it with control to determine efficiency of using chickpea and DDGS within diabetic diet.
- ⤴ To determine consumer acceptability of final product by performing sensory analysis among graduate and undergraduate students, and staff members.

#### **1.4 Hypothesis**

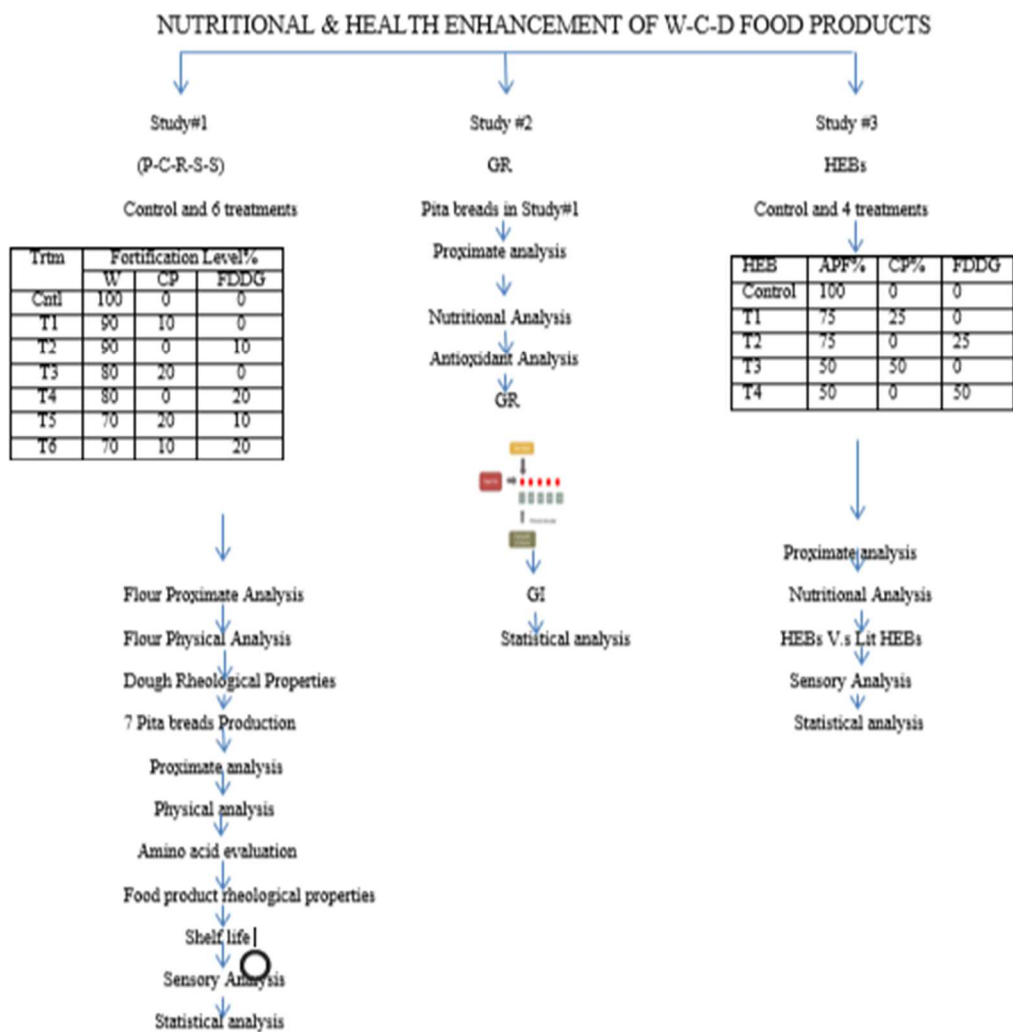
1. Fiber content of the pita bread will be increased by blending wheat flour with other alternative flours.
  - a. Increasing the amount of DDGS in dough mixture will increase fiber content of bread.
  - b. Increasing the amount of chickpea flour in dough mixture will increase fiber content of bread.
2. Protein content of the pita bread will be increased by blending wheat flour with other alternative flours.
  - a. Increasing the amount of DDGS in dough mixture will increase protein content of bread.
  - b. Increasing the amount of chickpea flour in dough mixture will increase protein content of bread.
3. Decreasing the amount of whole wheat flour in the dough mixture of pita bread will reduce firmness and extensibility of the bread.
4. There will be no significant change in the color of the pita bread compared to control, by substitution of a part of wheat flour with chickpea flour.
5. There will be no significant change in the color of the pita bread compared to control, by substitution of a part of wheat flour with chickpea-DDGS blend in the dough mixture.

6. There will be no significant difference in the aroma of the final product pita bread (chickpea-DDGS fortified) and the control.
7. Incorporation of chickpea-DDGS flour in pita bread dough mixture results in significant increase in nutritional properties of the bread.
8. There will be no significant difference in the rheological properties of chickpea-DDGS fortified and control dough.
9. The high-protein high-fiber enriched chickpea-DDGS fortified pita bread will produce a lower glycemic response compared to control.
10. The overall quality of the chickpea-food grade DDGS fortified pita bread will be similar to the control pita bread.

### **1.5 Statistical analysis**

All data collected for physical, chemical, and sensory analysis were analyzed by SAS (Statistical Analysis System, version 9.2) and Microsoft Excel (version 2014) software. T-test and LSD (Least Significant Difference) test were used to determine differences between the means. General Linear Model (GLM) was used to check difference between experimental treatments. A P-value less than 0.05 will be considered for determining the significance of the results.

**Figure 1.1 Flow diagram of research experiment design**



## CHAPTER 2

### Literature Review

#### 2.1. Traditional wheat flour

Wheat is a major cereal crop in many parts of the world. It belongs to the Triticum family, of which there are many species; *T. aestivum* and *T. durum* are the most important commercially (Mckeivith, 2004). Wheat is divided into six classes based on different genetic characteristics. Some of these classes are Hard Red Winter, Hard Red Spring, Soft White, Soft Red, Durum and Hard White (Taylor et al, 2005). Wheat is well adapted to various environmental and soil conditions. It is easy to cultivate and is high yielding. Over the past 10 years, the world has produced nearly 576.3 million metric tons of wheat annually from approximately 218.2 million hectares of land. Wheat is used to produce different kind of foods, such as bread, pasta, noodles, pastry, breakfast cereals and baby foods. In order to produce these products, wheat must first be processed into flour.

Flour is produced from grinding and milling wheat kernels. There are different kinds of flours which are produced for specific purposes. For example, soft wheat flour is used for baking cake and pastry, hard wheat flour is used for bread, and all-purpose flour is a blend of these two which is used to produce many types of the bakery goods (Hiu et al, 2006). Hard wheat flour is generally used to bake bread because of its high gluten levels. Soft wheat flour is a good choice for baked goods that do not need to rely upon high gluten content like pastries (Hillman, 2003). All-purpose flour, on the other hand, can be used to bake a variety of products and it can be found in both bleached and unbleached forms. Bleached flour is better for making cookies, cakes and pastry, while

unbleached flour is desirable in making yeast dough (Daley, 2001). Since the nutritional content of wheat grain is affected by the climate and seasonal changes, brands of all-purpose flours can vary in their nutrition contents over time as well.

Hard wheat flours are used in bread making because they form a strong gluten network in the dough which is necessary in production of bread. The gluten content of hard wheat flour or bread flour which is made from hard red wheat can be between 12.5% and 14%. (Daley, 2001).

### **2.1.1 Nutritional Problems and Challenges**

Although wheat flour comes in different forms, with various nutrient components and attributes, there are some deficiencies and challenges for which they must be fortified or enriched, or even replaced with other cereal grain flours. These problems can be deficiencies of some micronutrient, such as vitamins and minerals, or problems resulting from the gluten of the wheat, which can cause various allergies and diseases in some people.

Naturally, wheat is a good source of vitamins such as vitamin E, as well as iron and zinc (Anonymous, 2010b). But due to milling and refining, many of these nutrient components can be lost. Therefore, the final flour product will not be as nutritious. Wheat flour contains about 8-12% protein and has limited amounts of essential amino acids such as lysine, which is an important nutrient for humans (Khetarpaul and Goyal, 2009).

Another problem which is also caused by the milling process is the loss of dietary fiber owing to the removal of the outer layer of the wheat grain (Anjum et al, 2006). So, highly refined wheat flour is not a good source of dietary fiber. Supplementing wheat flour with alternative flours would be one way to improve the nutrition of this ingredient.

As table 1.1 shows, there are many types of flours produced from different grains. Each of these flours has its own physical, chemical and nutritional properties. Some of these grains and flours will be discussed below.

### **2.1.2 Fortification: a solution to nutrition problems**

To overcome the loss of crucial ingredients during milling and grinding process, one solution is fortification. Food fortification or enhancement is the process of addition of micronutrients (essential trace elements vitamins, and dietary fibers) to food. Flour with substances that can supplement the essential nutrients, substitute for the lost nutrients during processing and milling, and reduce the risk of serious nutrition related diseases such as cancer, diabetes, and cardiovascular diseases etc. Therefore, to provide nutritious food to consumers, one solution is to use less refined wheat flour and to fortify bread with such substances that can compensate for the nutrient loss of wheat flour during processing.

Bread is one of the most widely consumed cereal products and fortification can help prevent certain nutrition-related diseases and problems. One way to fortify bread products is to use alternative flours (Pourafshar et al, 2010a). Different flours have varied nutritional characteristics. For example, oat and barley can enhance the  $\beta$ -glucan content of bread, which can have a significant impact on human health (Marriotti et al, 2006). Barley and oat can contain 3-11% and 3-7% of  $\beta$ -glucan, respectively (Sidhu and Kabir, 2007). Consumption of barley has increased during the past few years because of its association with lowering cholesterol and moderating blood glucose levels (Skendi et al, 2010). The  $\beta$ -glucan in barley flour can increase the quality of bread by modifying the glycemic and insulin response (Gujral and Gaur, 2005). Studies also show that bread



made with a blend of wheat and barley flour has acceptable sensory properties (Skendi et al, 2010). Amaranth has twice the lysine content of wheat protein. It also has cholesterol-lowering properties attributable to its nutrient components; its fiber content is three times higher than that of wheat (Ayo, 2001). In Europe, rye is the most common cereal grown after wheat. Production of this grain is about 15.7 million tons per year (Horszwald et al, 2009). Rye is a health-promoting cereal with high amounts of dietary fiber. Whole grain rye contains 13% to 17% of fiber (Rakha et al, 2010). Another positive nutritional effect of rye flour is the existence of lignin, phytosterols, and phenolic compounds, which are biologically active components that have antioxidant properties (Horszwald et al, 2009). Oat offers health benefits as well because it is high in dietary fiber and protein content. Besides the dietary fiber, oat is rich in essential amino acids, unsaturated fatty acids, minerals and antioxidants (Huttner and Arendt, 2010).

## **2.2 Bread**

Flour and its baked products like breads are relatively cheap sources of energy which are consumed by almost everyone around the world (Kent, 1994). Wheat is the most important consumed cereal grain, which is mostly used in production of different kinds of breads. In more than half the world's countries, bread supplies over half of the total caloric intake. Human beings have become masters of bread-making thousands of years ago. The oldest bakers' oven in the world shows that bread was known in Babylon in 4000 B.C. Production of bread then spread throughout the world to Egypt, Greece and all other countries (Pomeranz and Shellenberger, 1971).

The Middle East is one of the regions of the world in which bread is the main food staple consumed by people. There are different types of breads in Middle Eastern Countries presented in table 1.2, and some of these breads will be discussed below.

### **2.2.1 Pita bread**

More than 60 types of flat breads are made world-wide, and they have been staple foods for many centuries. One kind of flat bread is known as pita (Arabic bread). Similar types of bread are known by different names, e.g., baladi in Egypt, bouri in Saudi Arabia, or souri in Libya and North Africa. Pita bread has a round shape, forms a pocket during baking, and has a golden brown crust color. During baking at high temperatures the dry exterior skin of the proofed flat dough sets, and carbon dioxide and steam expand until the pressure is sufficient to allow separation of the lower and upper layers. This is referred to as pocket formation. Pita bread has a large crust-to-crumbs ratio, which gives the bread the strength and flexibility to be used as a carrier for food, to scoop moist foods, or to hold a filling rolled in the bread to form a convenience food. Flat bread formulations differ from region to region, but the basic ingredients are flour, water, salt, and naturally fermented starter dough with either baking soda or baker's yeast. In addition, sugar, butter, vegetable shortening or non-fat dry milk may be added to enhance taste and aroma (Farvili, Walker & Qarooni, 1995).

### **2.2.2 Fortification of Bread Studies**

In human nutrition, the bread and bakery products play a vital role. Generally, wheat bread is used as an excellent source of energy and irreplaceable nutrients for humans. Bread made from refined flour is nutritionally much poorer and does not fulfill

the demands for a number macro or micro nutrients. It has been reported that bread prepared from refined flour has little micronutrient content (Al-kanhal et al., 1999). The proteins of wheat also have less essential amino acids lysine, threonine and valine. All breads are nutritious, but some are more than others. For example, an average slice of whole wheat bread has 69% of its calories from carbohydrate and 15% from fat (Dalgethy et al., 2006) and one slice of supplemented white bread has 76% of its calories from carbohydrates, 13% from protein and 11% from fat. However, cereals are the major source of calories for many people, and because they are low-cost as well, fortifying cereals, especially breads, is a very important topic among food scientists. Enhancing different kind of breads with components such as Vitamin B1, riboflavin B2, and folic acid is of great help in increasing healthier, nutrient-rich bread, especially for consumption of people of those countries which have high malnutrition. Adding value to breads could be a great step in providing nutrient components to consumers. By adding certain nutrients, we can also change physical and chemical properties, the shelf life, the texture, and the production time of breads (Cauvain, 2003). Summarized in table 1.3, several studies have been done for the fortification of bread in order to enhanced its nutritional, physical, and rheological properties.

The nutritional value of wheat flour can be also nutritionally, physically, and rheologically enhanced using a variety of alternative flours. A number of studies have demonstrated the nutritional value as well as physical and rheological properties of chickpea supplemented wheat flour and its baked product has been improved. Thus one of our objectives is to supplement wheat flour with legume flours, especially chickpea

flour in order to potentially improve the nutritional, physical, and rheological values wheat flour and its products, particularly baked products

## **2.3 Chickpea**

Chickpeas, also called garbanzo beans, are divided into two classes: Kabuli and Desi (figure 1.1). Kabuli is generally contained in salad bars or in soups and is a high-grade bean. The lower-grade desi variety is generally crushed into flour, and used in traditional foods. Worldwide, the desi type of chickpeas is produced in India which accounted for nearly 68 % of production in 2000. In recent years other leading producers of chickpeas are Canada, Turkey, Pakistan and Mexico. Figure 6 shows that in the 1990's the chickpea production in the world varied from 6.5 mmt to 9.25 mmt. In the past two years in the U.S., the production of chickpea has increased dramatically. Chickpeas are grown in Washington, California, North Dakota, Oregon, Montana, and South Dakota. The Alberta and Saskatchewan (provinces of Canadian) are also main production regions (Kevin Mc New, 2011). Chickpea (*Cicer arietinum* L.), also known as Bengal gram or garbanzo bean, is an Old-World pulse and one of the seven Neolithic founder crops in the Fertile Crescent of the Near East (Lev-Yadun et al., 2000). Currently, chickpea is produced in more than fifty countries across the Indian subcontinent, southern Europe, the Middle East, North Africa, the Americas and Australia. Globally, next to field peas and dry beans, the chickpea production is the third most essential pulse.

### **2.3.1 Nutritional content of chickpea**

Chickpea (*Cicer arietinum* L.) is a main pulse crop and is known for its nutritional quality, particularly in the Afro-Asian countries. It is a good source of carbohydrates

containing monosaccharides (fructose, ribose, galactose and glucose), disaccharides (maltose and sucrose) and oligosaccharides (raffinose, ciceritol and verbascose stachyose). The two important galactosides of chickpea stachyose and ciceritol constitute 25 % and 36–43% of total sugars respectively in chickpea seeds (Sanchez-Mata et al. , 1998; Aguilera et al. ,2009).

Chickpea contains of all the essential amino acids except sulphur-containing amino acids. Starch is the main storage carbohydrate along with dietary fiber, oligosaccharides and simple sugars such as sucrose and glucose. Although the lipids are found in small quantity, they are high in unsaturated fatty acids such as oleic and linoleic acids. The chickpea seeds also contain P, Mg and Ca, especially K. Nutritionally important vitamins such as niacin, riboflavin, folate, thiamin and the vitamin A precursor  $\beta$ -carotene are also present in chickpea. It is cholesterol-free and an important source of both soluble and insoluble fiber. Chickpea seeds have a number of phenolic compounds (Wood JA & Grusak MA, 2007). The isoflavones biochanin and formononetin are two significant phenolic compounds of chickpea (Wood JA & Grusak MA, 2007). Matairesinol, genistein, diadzein and secoisolariciresinol are other phenolic compounds found in chickpea oil.

The protein content of seeds of eight annual wild species of the genus *Cicer* ranged from 168 g/kg in *Cicer cuneatum* to 268 g/kg in *Cicer pinnatifidum*, with an average of 207 g/kg over the eight wild species (Ocampo et al., 1998). The protein quality of chickpea is better than some pulse crops such as green gram (*Vigna radiata* L.), black gram (*Vigna mungo* L.), and red gram (*Cajanus cajan* L.) (Kaur et al., 2005).

In chickpea, the total dietary fiber content (DFC) is 18–22 g/100 g of raw chickpea seed (Aguilera, et al., 2009), while pulse contains higher amount of DF. Soluble DFC is about 4–8 g/100 g and insoluble DFC of raw chickpea seed is 10–18 g/100 g (Dalgetty & Baik, 2003). The total DFC and insoluble DFC of desi types are higher compared with the Kabuli types, due to thicker hulls and seed coat in the Desi types (11.5% of total seed weight) compared with the Kabuli types (only 4.3–4.4% of total seed weight) (Rincon et al., 1998).

### **2.3.2 Chickpea fortifications**

Adding nutritional value to bread is one of the ways to provide healthy food to consumers. Nutrients are also added to get desired texture, physical and chemical properties, and to increase shelf-life of the bread. Since, bread is consumed on a regular basis throughout the world. Fortification of bread with fiber and protein can potentially be beneficial for people of all age groups in having healthy diet and in overcoming various nutritional problems. Moreover, the American Dietetic Association (2002) has reported lower than recommended intake of dietary fiber among US children and adults and have advocated the beneficial role of fibers in controlling diabetes. These facts again support the fortification of bread with fibers to ensure providing daily healthy food to children and diabetics (Lafrance et al., 1998). Chickpeas being a relatively cheap source of protein and fibers, are an economical choice for fortification of bread. This makes them affordable for use in feeding low income families and for use in food relief. Table 1.4, presents some studies that have been summarized which used chickpeas for the fortification purpose.

### **2.3.3 Chickpeas and Health**

Pulses have been used for their nutritional qualities for thousands of years (Kerem et al 2007). The interest in pulses as food and their potential impact on human health have been revived, during the past two to three decades. It is also reported that many pulses overcome the risk of chronic diseases and optimize health. Therefore, chickpea is considered as a 'functional food' along with its role in providing protein and fiber. Chickpea contains different vitamins, minerals (Duke, 1981) and several bioactive constituents (phenolic, phytates, enzyme inhibitors, and oligosaccharides, etc.) that could help to reduce the risk of chronic diseases.

#### **2.3.3.1 Diabetes and Blood Pressure**

Chickpea have a higher quantity of resistant starch and amylose. The starch of chickpea is more resistant to digestion in the small intestine, which lowers availability of glucose (Pittaway, et al. 2007). There are several other studies which relate to the use of Chickpea in treatment of diabetes and blood pressure (summarized in the table 1.5).

#### **2.3.3.2 Weight loss/obesity**

Dietary fiber may influence body-weight regulation by physiologic mechanisms involving intrinsic, hormonal, and colonic effects. Ultimately, these mechanisms act to decrease food intake by promoting satiation (lower meal energy content) or satiety (longer duration between meals) or by influencing metabolic fuel partitioning (increased fat oxidation and decreased fat storage). Therefore, it is concluded that fiber-rich diets, contain non-starch fruits, vegetables, whole grains, nuts and legumes, and may be

effective in the prevention and treatment of obesity in children (Pereira & Ludwig, 2001). The use of low-GI foods resulted in an increase in cholecystokinin (hunger suppressant) and increased satiety (Swinburn et al., 2004). Chickpea is considered to be a low-GI food, and therefore may be helpful in the reduction of obesity as well as in weight-loss. Presented Table 1.6, summarizes the reports related to chickpea diet and weight loss/obesity.

### **2.3.3.3 CVD, CHD and cholesterol control**

Foods that contain high amounts of soluble fiber reduced the total cholesterol of serum and LDL-cholesterol (LDL-C) and have an inverse correlation with CHD mortality (Kushi LH, et al. 1999). Chickpea seeds are a comparatively high source of DF and bioactive compounds (e.g. saponins, phytosterols, and oligosaccharides) as well as low glycemic index (GI), therefore chickpea reduced the risk of CVD (Duranti M, 2006). Table 1.7 presents some research that supports the health benefits of chickpea and heart disease.

### **2.3.3.4 Other health benefits**

Chickpea seeds contain sterols, tocopherols and tocotrienols exhibit anti-ulcerative, anti-bacterial, anti-fungal, antitumour and anti-inflammatory properties (Murty, et al. 2010). Carotenoids such as lutein and zeaxanthin, present in chickpea seeds, play a role in senile or age-related macular degeneration (Mozaffarieh, et al. 2003). Vitamin A, is significant in numerous developmental processes in humans such as cell division, bone growth and most importantly, vision (Reifen, 2002). In traditional medicine, the chickpea seeds have been used as tonics, stimulants, and aphrodisiacs (Pandey & Enumeratio, 1993). They are also used as appetizers, for thirst quenching and reducing burning



sensation in the stomach (Aguilera, et al. 2009). In addition to these applications, chickpea seeds are also used for treating skin ailments, ear infections, blood enrichment, and liver and spleen disorders (Warner et al. 1995). For over 2500 years, the Uygur people of China have used chickpea in herbal medicine for treating diabetes and hypertension (Zhang et al., 2007).

### **DDGS (Dried Distillers Grains with Solubles (DDGS))**

DDGS (Dried Distillers Grains with Solubles) is the dried fermented grain residues that are rich in protein (27 - 35%), fiber, and fat, containing up to 10-12% moisture and having almost an indefinite shelf life (Belyea et al, 2004). It is a rich in protein and dietary fiber content and therefore could be used as a high dietary fiber and protein food ingredient for human foods.

#### **2.4.1 Composition of DDGS**

The nutrient composition of distiller's grains is a function of the starting material and the methods used in making ethanol (Weiss, 2007). Distiller's grains have very low concentrations of starch because of the conversion of most of the starch into ethanol. However, concentrations of protein, fiber, fat, and minerals are increased depending on the concentration of starch in the grain. Corn grain comprises about two-thirds starch and when most of the starch is removed, concentrations of the other nutrients are increased about three-fold (Martinez-Amezcuca et al., 2007). Martinez-Amezcuca et al., conduct experiments to evaluate the nutritional value of corn distillers dried grains with solubles (DDGS) and its components of grains and solubles, their results are summarized in Table 1.8 (Martinez-Amezcuca et al., 2007).

Amino acid profiles for wheat and wheat DDGS are given in Table 1.9, which shows that wheat DDGS has higher amino acid concentrations compared to wheat. Therefore, distillers' grains are a valuable source of protein for food (Bonnardeaux, 2007).

Several studies in the past have shown successful incorporation of DDGS in food products, particularly baked products and thus enhancing nutritional value of the products. Breads and cookies have been fortified by Distiller's grains with varying degrees of acceptability (Bookwalter et al., 1984). The composition of DDGS varies from one ethanol plant to other. Some food products were developed by using DDGS are summarized in Table 1.10 (Rosentrater and Krishnan, 2006).

#### **2.4.2 Nutritional value of DDGS**

Distiller's dried grains with solubles (DDGS) are predominately used to provide nutritional value to the diets of animals. DDGS, due to its high nutritional value, is an exceptional feed for animals.

Currently, livestock feed is the ethanol industry's only outlet for the non-fermentable residues, DDGS. Due to the high quantity of residues (approximately 1/3 of the original corn mass) produced from dry-grind processing, it may be ideal to use these co-products as ingredients in human food products (Rosentrater and Krishnan, 2006). Distillers dried grains (DDG) are a good source of fiber (13%) and protein (27%-30%), while remaining relatively low in total carbohydrate (46%) (Miron et al., 2001; Al-Suwaiegh et al., 2002; Davis et al., 1980). The nutritional composition of DDGS can differ, often containing 5-11% fiber, 27-34% protein, 5-6% starch, and 39-62% carbohydrates (UMN, 2007; Belyea et al., 2004; Spiels et al., 2002; NRC, 1998; NRC,

1982). The removal of fermentable carbohydrates, principally starch, to produce ethanol leaves non-fermentable nutrients concentrated three to nine folds in the co product streams (Rosentrater and Krishnan, 2006).

### **2.4.3 DDGS fortifications**

Distillers dried grains with solubles (DDGS) may also be a good source for fortification of cereal-based products. DDGS is a product resulting from the fermentation of cereal grains, mostly corn, for the production of ethanol. DDGS is a source of protein, fiber, minerals and vitamins. Different methods can be used in production of DDGS, and the method chosen then affects the physiochemical properties of the final product (Cromwell et al, 1993); the process used can affect the appearance and the nutritional content of final product. Variation in the composition of corn can affect the composition of the final DDGS (Belyea et al, 2004). The protein content of DDGS can range from 27% to 35%. Research was conducted at South Dakota State University on a traditional Asian flatbread called chapatti. This bread (chapatti) contains more protein and fiber, when fortified with food-grade distiller's grains. The Asian whole wheat unleavened bread eaten in South Asia and East Africa, boosted the fiber from 2.9 to 7.8 %. Using 20 % DDGS in the dough increased the fiber to 10.3 %. Similarly, protein increased from 10.5 to 12.9 %, when used 10% DDGS in chapatti. Using 20 % DDGS increased the protein content to 15.3 %. It was reported by Pourafshar (2011), that DDGS when added to wheat tortillas, it made this flat bread a healthier product. Three levels of DDGS substitution were used (0%, 10% and 20%), and the physical and chemical properties of final tortillas were measured. The objective of this study was to know the impact of substitution of DDGS on the physical and chemical attributes of tortillas. The use of

DDGS in food products can help produce a healthier baked product with a higher amount of fiber and protein. Many studies have reported on the incorporation of DDGS into food products and some of them are presented in table 1.11.

#### **2.4.4 DDGS and Health**

In terms of composition, distiller's grains are low in starch, but high in protein and fiber content. This nutritional content of DDGS appears to match the needs of therapeutic diets for medical conditions such as diabetes and celiac diseases. Short-term studies completed by Arora and McFarlane (2005) established that a low carbohydrate diet resulted in lower HbA1c levels (7.6% +/- 0.3), greater glycemic control, lower postprandial glucose levels, and improved insulin sensitivity when processed into viable food products for diabetic populations. Fiber was not a main concern of this study. Foods higher in starch increase postprandial glucose levels, thus increasing insulin dosage needs. To compensate, insulin dependent (Type I) diabetics would increase insulin injected, while non-insulin dependent (Type II) diabetics would merely restrict the quantity of high starch foods consumed. Perhaps the introduction of distiller's grains into the food market will open up additional food choices for individuals with these medical conditions. This particular application for distiller's grains research is new and has many unanswered questions.

#### **2.5 Diabetes**

Diabetes mellitus, or simply diabetes, is a group of metabolic diseases in which a person has high blood sugar, either due to the lack of production of insulin by pancreas, or cells do not respond to the insulin. Diabetes is mainly a collection of heterogeneous

disorders that have the familiar factor of hyperglycemia and intolerance of glucose". This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). Diabetes is a complicated disease; there are three different types. Type 1 Diabetes is non-preventable and happens when the body does not produce insulin. It accounts for 5 to 10% of all diagnosed cases of diabetes (CDC, 2005b; American Diabetes Association, 2006). Type 2 Diabetes accounts for the other 90-95%, and happens when the body does not use insulin properly. It is associated with obesity, impaired glucose metabolism, physical inactivity, family history and race/ethnicity (CDC 2005b, American Diabetes Association, 2006). Type 2 Diabetes can generally be controlled by maintaining proper blood glucose levels and consuming a healthy diet while exercising and trying to limit excessive weight (CDC, 2005b). Cholesterol and blood pressure should also be controlled in order to prevent further complications from Type 2 Diabetes (CDC, 2005b). The third type, gestational diabetes developed, when pregnant women have high blood glucose level without a previous diagnosis. It may precede development of type 2 DM.

The diabetes epidemic continues to grow, due to a number of factors, including the younger population contracting the health problem, increased obesity in our society, lack of exercise, and an increase in insulin resistance syndrome (IRS) (Bloomgarden, 2004). The road to diabetes can start with low birth weight, along with poor diet, and when this continues to be combined with lack of physical activity, insulin resistance may arise. This can lead to a lifetime battle against cardiovascular disease, renal disease, micro vascular/macro vascular disease, and potentially death (Bloomgarden, 2004). Several treatments are available, and have been met with mixed success.

According to the Center for Disease Control (CDC), in 1980 5.8 million people were living with diagnosed Diabetes in the United States, and in 2004 this number had grown to 14.7 million (CDC, 2005a). Although 14.7 million diabetics are living with the diagnosis, epidemiologists' estimate that 6.2 million are undiagnosed.

Overall, there are 20.9 million diabetics in the United States (CDC, 2005b). Studies have also found that 151,000 children below the age of 20 have diabetes (CDC, 2005c). The 2003 International Diabetes Federation Consensus conference's topic revolved around Type 2 Diabetes in the youth population. They found this to be a major financial concern in our country as this epidemic continues to grow (Bloomgarden, 2004). Globally, Diabetes affects 180 million people and it is likely to double by 2030 (WHO, 2006).

### **2.5.1. Glycemic index (GI)**

The Glycemic Index measures the blood glucose response of a food after consuming the equivalent of 50 g of carbohydrate of the test food, and then comparing this food to a standard of 50 g of glucose solution (or a slice of white bread). The glucose levels of each of these foods are then plotted, and the areas which overlap are then placed in an equation which is used to calculate the glycemic index of that particular food. The equation for glycemic index is as follows (Grete beck et al., 2002):

$$\text{Glycemic Index} = (\text{Blood Glucose Area After Test Food} / \text{Blood Glucose Area After Reference Food}) \times 100.$$

High GI foods tends to release glucose quickly into the bloodstream after carbohydrate is rapidly hydrolyzed during digestion. On other hand, slowly hydrolyzed carbohydrates, release glucose more gradually into the bloodstream, and tend to have a

low GI. In 1980–1981, at the University of Toronto, Dr. David J. Jenkins and colleagues developed concepts (Jenkins et al., 1980) and determined the best foods for diabetic people. The foods contain carbohydrate with lower glycemic index, and which are slowly digested and absorbed. A lower glycemic food may control blood glucose and lipids as it may have response of lowering insulin demand (Jenkins et al., 2008).

Foods with lower glycemic indexes are considered to be more beneficial for diabetics since their glucose is released at a slower rate over a longer period of time. This type of diet is typically hard to follow, and is often not utilized well when trying to control diabetes over a long period of time, or with a combination of foods. (American Dietetic Association, 2006)

### **2.5.2 Glycemic Index Test Protocols**

As illustrated by the literature, Glycemic Index has been a long-debated practice with concern to its methodology and validity. It is a procedure which ranks foods on a glycemic index scale by how fast they enter the blood stream and elevate blood glucose (Miller-Jones, 2002). The faster food is able to increase and elevate blood glucose levels, the higher the glycemic index of the food (Miller-Jones, 2002). Glycemic Index is defined as the incremental area under the curve after an individual has consumed a standard amount of test food. This test food is compared to the glycemic effect of a reference food; often the reference food is 50 g of glucose or a slice of white bread consisting of 50 g available carbohydrate. The difference in glycemic effect between this reference and the test food can then be translated into a glycemic index (Mayod, 2005). Wolever, a researcher of the glycemic index, believes that the knowledge gained by the glycemic index may help to explain the physiological effects of the human diet (Wolever

et al., 1991). It has been suggested that foods with a high glycemic index can increase insulin levels and cause an increase in hunger, which in turn promotes higher caloric intake and storage as adipose tissue (Miller-Jones, 2002). Intake of foods with high glycemic index have been strongly related to a greater risk of Type 2 Diabetes (Schulze et al., 2004). In a study conducted by Miller et al. (2006), consumption of lower glycemic index foods was associated with decreased incidence of diabetes and better glucose control within diabetics, reduced serum lipids, improved insulin levels, and lower risk of colon cancer. Long term compliance with low glycemic foods has also been associated with increased satiety and body weight control (Bloomgarden, 2004; Schultze et al. 2004; Ostman, 2006).

Overall, it has been established that the reference food must contain 50 g of available carbohydrate (typically in the form of white bread or glucose solution). The test food also needs to provide 50 g of available carbohydrate. Subjects generally consume the reference food three different times. Glucose levels are often collected at 0, 30, 60,90, 120 minutes (Granfeldt, Wu and Bjorck, 2006; Hatonen et al., 2006; Wolever et al., 2003). Several inconsistencies exist across the studies. These include the duration of fasting time before the test, whether physical activity needs to be limited in the hours prior to testing, how many subjects to use, and how to collect blood samples, the most debated being a venous versus capillary blood collection site. Many tests have been conducted using glucose meters. This provides a fast, easy, inexpensive, and less invasive way to collect blood samples (Velangi et al., 2005).



### 2.5.3 Fiber and glycemic index

The American Diabetes Association has published their position statement on how best to prevent and treat diabetes (American Diabetes Association, 2002). In their statement, they express the need to optimize metabolic outcomes by keeping blood glucose within its normal range (70-100 mg/dL) and maintain a lipoprotein profile that reduces the risk of macro vascular disease. This can be done through routine glucose monitoring and routine visits to a physician for lab workups. They also show improved health through healthy food selections and physical activity and advice those at risk for diabetes to increase activity to prevent weight gain or maintain a healthy weight (American Diabetes Association, 2002). These healthy food selections will prevent blood glucose and blood lipids from elevating and may also help to produce a feeling of satiety compared to foods containing little or no fiber. Foods that contain fiber may decrease the amount eaten and may help to maintain current weight or even decrease weight.

The American Dietetic Association recognized the significance foods containing carbohydrate, especially those made with whole grains versus those with high starch. Individuals with diabetes should choose foods containing fiber such as fruits, whole grains, and vegetables. High intakes of fiber have been shown to present metabolic benefits for hyperinsulinemia, glycemic control, and plasma lipids (American Dietetic Association, 2002). A dietary fiber intake of 50 g/d has been shown to lower gastric emptying, digestion and the absorption of glucose. This can help to regulate immediate postprandial glucose metabolism and long term glucose control in individuals with diabetes (Lafrance et al., 1998; American Dietetic Association, 2002). The American Dietetic Association has also expressed dietary recommendations and has discussed the

implications of dietary fiber. The recommended intake is 20 to 35 g/d for healthy adults. Dietary fiber has been proven to lower cholesterol and normalize blood glucose levels within the body, which in turn normalizes insulin levels. These processes therefore contribute to the battle against heart disease and Type 2 Diabetes (American Dietetic Association, 2002). Fiber has also been shown to help maintain colon health and decrease the incidence of colon cancer. Diets which are rich in fiber are typically processed /digested slower and thus increase our feeling of “fullness,” leading to lower caloric intake, in turn lowering the incidence of obesity within our population (American Dietetic Association, 2002). Stool weight increases as fiber intake increases, and the fiber tends to normalize defecation frequency to one bowel movement per day, with a gastrointestinal transit time of 2 to 4 d (American Dietetic Association, 2002). The American Association of Cereal Chemists defines dietary fiber as “the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine which compete for partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plants substances. Dietary fibers promote beneficial physiological effects including laxation, and/or cholesterol attenuation, and/or blood glucose attenuation” (American Association of Cereal Chemists, 2000). On the other hand, studies have found that in glucose-controlled diabetics, protein intakes did not increase plasma concentrations (Gannon et al., 2001). Scientists have found that although amino acids are sometimes changed by gluconeogenesis, when glucose levels are not under control, the glucose produced by amino acids is typically not found in circulation after the consumption of protein (Franz, 2000). It is thus important to monitor both carbohydrate and protein levels in order to

maintain adequate glucose levels, and to avoid protein catabolism within the body (Gougen et al., 2000).

Since the postprandial glucose response of DDGS has never been tested before, similar studies in the literature using other food products were examined some of these studies are described in table 1.12.

#### **2.5.4 The effect of distiller's dried grains on glycemic response**

There is a paucity of information on glycemic response to DDG in food products. Bechen (2008) studied the effects of three types of porridge, including all-purpose flour, wheat flour and DDGS (20 g each, in order to achieve 15 g of available carbohydrate) on glycemic response of 10 healthy subjects. The results of this study shows in figure 1.2 revealed an inhibitive property of DDGS which yielded the lowest glucose response while all-purpose flour) demonstrated the highest glucose response (Bechen, 2008).

As shown in Figure 2, the DDGS produced the lowest effect on blood glucose over time. In comparison to all-purpose flour, the whole wheat flour had a delayed blood glucose response, which is consistent with the literature (because fiber and protein can help to delay blood glucose response). The all-purpose flour, with the lowest fiber and protein content, caused blood glucose to rise and then fall. It therefore appears that if treated DDGS were used in various food products as a replacement for either all-purpose or whole wheat flour, or at least a partial replacement, not only would the consumer feel satisfied earlier in their meal, but they could remain satisfied for a longer period of time. This may in turn help to decrease the amount of food consumed, thereby helping to control the overall blood glucose level of the consumer. Thus, DDGS may be beneficial in today's food market.

## 2.6 Antioxidant and health

Numerous studies have shown the potential health benefit of antioxidants against various diseases such as diabetes, cancer, cardiovascular and neurodegenerative diseases such as Alzheimer's disease (Menichini et.al, 2009). Antioxidant and starch hydrolase inhibitory activities are two of the most important mechanisms which are responsible for the prevention of non-communicable diseases such as cardiovascular disease, diabetes, and cancer. While antioxidants provide protection from cellular damage due to free radicals, starch hydrolase inhibitory activity is known to prevent the sudden release of glucose into the physiological system, thereby preventing the biochemical pathways which trigger the production of free radicals inside the mitochondria (Jayawardena et.al, 2015).

### 2.6.1 Antioxidant and diabetes

Diabetes is a major risk factor for premature atherosclerosis and oxidative stress plays an important role in its pathogenesis. One therapeutic approach for treating diabetes is to decrease the post-prandial hyperglycemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes,  $\alpha$ -amylase and  $\alpha$ -glucosidase, in the digestive tract. Inhibitors of these enzymes which is associated with antioxidant intake delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise (Menichini et.al, 2009). Numerous epidemiological studies have demonstrated antioxidant effect on developing diabetes, and accumulating evidences suggested that certain antioxidants such as carotenoids (beta-

carotene and lycopene), phenolic compounds (polyphenols, flavonoids, and tannins) through a variety of mechanisms will result in delays in glucose absorption which leads to suppression of postprandial blood glucose.

Phenolic compounds are diverse secondary metabolites abundant in plant tissues. These compounds play an important role in growth and reproduction, providing protection against pathogens and predators (Bravo, 1998), besides contributing towards the color and sensory characteristics of fruits and vegetables (Alasalvar, Grigor, Zhang, Quantick, & Shahidi, 2001). Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects (Benavente-Garcia, Castillo, Marin, Ortuno, & Del Rio, 1997; Manach, Mazur, & Scalbert, 2005; Middleton, Kandaswami, & Theoharides, 2000; Puupponen-Pimia et al., 2001). Phenolic compounds have been associated with the health benefits derived from consuming high levels of fruits and vegetables (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Parr & Bolwell, 2000). The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity (Heim, Tagliaferro, & Bobilya, 2002).

Structurally, phenolic compounds comprise an aromatic ring, bearing one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymerized compounds (Bravo, 1998). Despite this structural diversity, the group of compounds are often referred to as polyphenols.

Polyphenols possess ideal structural chemistry for free radical scavenging activity, and they have been shown to be more effective antioxidants in vitro than tocopherols and ascorbates. Anti-oxidative properties of polyphenols arise from their

high reactivity as hydrogen or electron donors, and from the ability of the polyphenol-derived radical to stabilize and delocalize the unpaired electron (chain-breaking function), and from their ability to chelate transition metal ions (termination of the Fenton reaction) (Rice-Evans et al., 1997). These changes could sterically hinder diffusion of free radicals and restrict peroxidative reactions. Moreover, it has been seen that phenolic compounds can be involved in the hydrogen peroxide scavenging cascade in plant cells (Takahama and Oniki, 1997).

Flavonoids constitute the largest group of plant phenolics, accounting for over half of the eight thousand naturally occurring phenolic compounds (Harborne et al., 1999). Other than antioxidant activity, certain flavonoids are known to possess the ability to modulate cellular enzyme activities, a trait which is responsible for the inhibition of starch hydrolases such as  $\alpha$ -amylase and  $\alpha$ -glucosidase (Jayawardena et al., 2015). Epidemiological studies suggest that the consumption of flavonoid-rich foods protects against human diseases associated with oxidative stress.

Carotenoids have also been shown to have a number of beneficial physiological actions other than Vitamin A activity, including antioxidant activity, enhanced immune response, and chemoprotective activity against several types of cancer. Lutein and zeaxanthin are both associated with reduced risk of cataracts and macular degeneration. Beta-carotene and carotenoids have both antioxidant and prooxidant activity in vitro, and have also been shown to synergistically enhance the antioxidant activity of tocopherols and tocotrienols in bulk oils and liposomes (Liu & Rosentrater, 2016)

Other studies in the literature have reported an association between intake of carotenoids and glucose metabolism. Dietary carotenoid intake in men is inversely

associated with fasting plasma glucose concentrations, whilst plasma beta-carotene concentrations are inversely associated with insulin resistance, assessed by homeostasis model assessment. An inverse association between serum carotenoids (particularly beta-carotene and lycopene) and fasting serum insulin concentrations has also been noted in the third National Health and Nutrition Examination Survey, and inverse correlations between steady-state plasma glucose and plasma concentrations of alpha-carotene, beta-carotene and lutein have been found (Spence et.al, 2010).

Studies have shown that the postprandial rise in glucose is consistent with depression of serum antioxidants, including carotenoids (lycopene). Presumably, the higher the glycemia, the greater the postprandial depression of serum antioxidants. Finally, supplementing diets with lycopene has been shown to improve glycemic control. Studies such as these suggest a possible beneficial role for low glycemic-index diets by reducing oxidative damage. (Jenkins et.al, 2002).

Carotenoids and vitamins C and E (tocopherols) are important components of the body's defense system against oxidative stress. Oxidative stress may impair insulin action by changing the physical state of the plasma membranes of target cells for insulin action (Ylönen et.al, 2003).

Evidence is mounting about the potential protective role carotenoids potential as antioxidants in the development and course of chronic diseases, especially diabetes. Glucose-intolerant states are now thought to be characterized by increased oxidative stress, as demonstrated by increased reactive oxygen species and lipid peroxidation, and increased free radical activity. Oxidative stress can result in the lowering of antioxidant concentrations in people with glucose intolerance Thus, it is conceivable that both

endogenous and exogenous antioxidants could play a role in the pathogenesis of glucose intolerance (Ford et.al, 1999).

Hyperglycemia has been linked to the onset of the vascular diabetic complications and triggers the generation of free radicals and oxidation-related damage to various organs by stimulating oxidative stress. Oxidative stress has been repetitively shown to be a hallmark of many diseases linked with metabolic or vascular disorders including diabetes and hypertension. Therefore, it is important to control both blood glucose level and cellular redox status for managing these diabetic complications.  $\alpha$ -Amylase and  $\alpha$ -glucosidase are key enzymes involved in starch breakdown and intestinal glucose absorption. Phenolics are also potent inhibitors of  $\alpha$ -amylase and  $\alpha$ -glucosidase, the two important enzymes involved in the regulation of glucose homeostasis (Sreerama et.al, 2012).

### **2.6.2 Antioxidant in chickpea**

The consumption of legumes has been associated with decreasing incidence of diseases, a feature that relates to their high content of antioxidant phenolics, low lipid content, and low glycemic index. Legumes such as chickpeas is seen as staple food and it's nutritious and improve health, known as the meat of the poor people because of its high protein and fiber content. Chickpea is now presented as a staple food for vegetarians and for people affected by nutrition related health problems, such as diabetes, obesity, and over-weight. Such a trend is caused by a general feature of pulses, namely, their appreciable content in slowly digestible carbohydrates (Silva et.al, 2010).

Phenolic compounds are abundant in legumes and their flours. Phenolics from legume flours are potentially safer, and therefore may be preferred alternatives for



inhibition of carbohydrate breakdown and control of glycemic index of food products. Therefore, utilization of legume flours in the development of functional foods with increased therapeutic value would be a significant step toward disease prevention and management through diet. Chickpea is an excellent source of protein, dietary fiber, a variety of micronutrients and phytochemicals with potential health benefits. Chickpea with lower  $\alpha$ -amylase and higher  $\alpha$ -glucosidase inhibitory activities could be used as food ingredients and in composite flours for the delay absorption of dietary carbohydrates in the meal, leading to suppression of an increase in postprandial blood glucose level without adverse effects. Due to favorable flour functionality and phytochemical-associated health benefits, chickpea offers enormous potential for the production of legume composite flours (Sreerama et.al, 2012).

Legumes contain other bioactive compounds beside phenolics such as vitamins and carotenoids that might also behave as antioxidant. (Ghiassi et.al 2012). Carotenoids are fat-soluble pigments (Jayawardena et.al, 2015). Epidemiological studies have shown a positive correlation between ingestion of vegetables and fruits containing carotenoids and prevention of several chronic diseases. The health-promoting properties of carotenoids are due to their free radical scavenging activity through the stabilization of single oxygen by its conjugate double bonds (Quesada et.al, 2011)

### **2.6.3 Antioxidants in DDGS**

The major phenolic compounds present in corn and other cereal grains are cinnamic acid derivatives, mainly consisting of p-coumaric, caffeic, ferulic, and sinapic acids, with ferulic being the most abundant. There is a lack of information on the phenolic composition and the antioxidant capacity of DDGS derived from commercial

dry-grind processing plants (Luthria et.al, 2012). Interestingly, DDGS which is an ethanol industry co-product from corn contains almost three times more quantity of phenolic content than corn (Luthria et.al, 2012). Phenolic acids were concentrated in DDGS as compared to the corn due to starch depletion during fermentation. The results from these workers indicates that phenolic acids were not significantly degraded during dry-grind commercial processing. Antioxidant activity of DDGS showed an approximately three-fold increase. Thus, DDGS is a rich source of phenolic antioxidants. This may be of great interest to corn processors, ethanol manufacturers, and DDGS users since phenolic acids have potential health benefits to diabetic individuals.

A study by Winkler-Moser et.al, (2009) showed that DDG oil is a good source for carotenoids, especially of lutein and zeaxanthin. DDG oil also had a higher carotenoids content than most commercial oils. The results of this study indicate that components such as tocopherols, tocotrienols, carotenoids, and steryl ferulates extracted from DDG oil have contributed to antioxidant activity.

## **2.7 Malnutrition**

Malnutrition is defined as under nutrition that is caused by a deficit. Malnutrition can have many different root causes, such as limited purchasing power, insufficient food supplies, poor health conditions, and incomplete knowledge about nutrition (Berg, 1987). Similarly, Malnutrition happens because of food deficiency, poverty and deprivation. The circumstance, where people cannot get enough food to meet the requirements of their family members, is called food poverty. Food deprivation happens when an individual does not get enough food for his/her daily needs of energy (Marchione, 1999). The other causes of malnutrition include the practices of poor feeding practices, such as insufficient

breastfeeding, consuming the incorrect foods, and not ensuring that the child gets a sufficient amount of nutritious food. According to researchers, the costs of inadequate diet effects physical development, learning ability, capacity to work, behavior and well-being of large segments of populations.

Malnutrition occurs not only in developing countries, but it can also occur worldwide owing to a variety of circumstances. Crises associated with man-made and natural disasters are a major cause of malnutrition and food insecurity, resulting in thousands of deaths each year. Natural disasters may occur suddenly or may develop over a period of time, and relief and rehabilitation responses may vary accordingly. Where resources and socio-economic conditions are favorable, rehabilitation may be short-lived because households can quickly regain food security. If an emergency occurs in conditions of chronic food insecurity, long-term assistance and a variety of interventions will be needed to support the affected people (Thompson et al., 2012).

In the 1990s, war and disaster affected 2 billion people and those individuals requiring food and humanitarian assistance tripled since the mid-1980s. In 2001, aid recipients stood at nearly 34 million, of which 13.7 million were refugees and 20.3 million were displaced persons (Brisske et al., 2006; Grobler-Tanner, 2001). In response to the increasing number of disasters (including natural and man-made disasters) and complex humanitarian emergencies requiring food relief operations, the United States Agency for International Development Bureau for Humanitarian Response sought to create specifications for an Emergency Ration Bar, also called an Emergency Food Product. A committee appointed by the Institutes of Medicine (IOM) of the National

Academies of Science released a report outlining the specifications for an emergency relief bar (Brisske et al., 2006; IOM, 2002).

Increasingly, humanitarian emergencies that are associated with natural disasters and war, have boosted their calls for global action, including reform of food aid. The international community needs an effective mechanism for governing food aid that minimizes disputes, enables rapid response to emergencies, and ensures appropriate resourcing for humanitarian and development objectives. The immediate solution to help people in emergencies is to provide nutritious foods which are also inexpensive (Barrett and Maxwell, 2006).

Malnutrition is generally divided into protein malnutrition and protein-energy malnutrition. The protein malnutrition can result in a disease called Kwashiorkor. In this disease, both hair and skin lose their pigments; also the skin becomes scaly, anemia and edema happen as well. Other forms of malnutrition such as protein-energy malnutrition or protein-calorie malnutrition (PCM) are more prominent in developing countries. Due to insufficient intake of food or as a result of other illnesses, children between 1-3 years old are generally at risk since they are the most prone to infection, and PCM (Alleyne et al, 1977). Malnutrition is a growing crisis. Poverty, natural disasters, war, as well as political problems all contribute to this condition. The other major factor contributing to malnutrition is the sharp increase in population. Malnutrition occurs due to the lack of access to highly nutritious foods and poor distribution of foods (Swinnen, 2007).

Beside protein malnutrition problems, micronutrient malnutrition also can have adverse effects. The studies show that deficiency of micronutrients such as zinc, vitamin A and iron has led to deaths of 3.6 million children under five years old (UNICEF. 1998).

Vitamin A is known as a major factor in reducing mortality from infectious diseases in developing countries (Faisel and Pittrof, 2000).

### **2.7.1 Potential Solutions**

Different programs and potential solutions have been proposed as answers to the problem of malnutrition but most of these are long term solutions such as agricultural development. During the last four decades the nutritional situation for many developing countries has changed significantly. Although cereals provide some nutrition, processing grains by milling and refining, leads to loss of iron, zinc and other micronutrients. On the other hand, bran and husk can be used in food staples as well. Furthermore, Fortification is one source of combating these losses, for both macro and micro nutrient deficiencies. Fortification of cereals can occur by the use of different sources which are rich in vitamins and minerals. These sources can be alternative flours, such as nontraditional flours or even co-products from the production of other materials in industry (Pourafshar, 2010)

Food aid agencies like WFP, USDA, and UNICEF, have developed a wide range of specialized therapeutic foods to improve the nutritional intake in malnourished people who have been affected by emergency and crisis.

#### **2.7.1.1. Therapeutic food**

Currently, the world is combating different forms of malnutrition and the lack of availability of healthy foods. The principal purpose of therapeutic foods is for use for emergency feeding of malnourished children or for use as a supplement for elderly people with special nutritional requirement. The ingredients of therapeutic foods contain

macro nutrients such as carbohydrate, protein, and lipid as well as macronutrients which are vitamins and minerals. (Manary et al. 2006). WHO has worked with UNICEF on the development of a field manual on community-based management of severe malnutrition, and the institutes of medicine IMO guidelines have been revised to take account of the new home-based treatment (Manary et al. 2005).

There are 5 main specialized nutritious foods (therapeutic foods) that were developed by food agencies program following IMO guidelines that are going to be defined below.

- 1) Fortified blended foods (FBFs): blends of partially precooked and milled cereals, soya, beans, pulses fortified with micronutrients (vitamins and minerals). Special formulations may contain vegetable oil or milk powder. Corn Soya Blend (CSB) is the main blended food distributed by WFP but Wheat Soya Blend (WSB) is also sometimes used. FBFs are designed to provide protein supplements in food assistance programs to prevent and address nutritional deficiencies. They are generally used in WFP Supplementary Feeding and Mother and Child Health programs. Also, they are used to provide extra micronutrients to complement the general ration. It is usually mixed with water and cooked as a porridge. It's nutritional value per 100g is as follows: Energy 380 Kcal, Protein 18%, fat 6%, and contain vitamins A, C, B12, D, E, K, B6, Thiamine, Riboflavin, Niacin, Pantothenic acid, Folic acid plus Zinc, Iron, Calcium, and Potassium.
- 2) Ready-to-Use Foods (RUFs): better suited to meet the nutritional needs of young and moderate malnourished children than FBFs. It may contain vegetable fat, dry skimmed milk, malt dextrin, sugar and whey. Used in intervention for prevention

or treatment of moderate malnutrition. Used in addition to breast milk and other food for children (6 to 59 months) which are at high risk of developing malnutrition due to severe food insecurity. It comes in two types, in tubs containing a weekly ration or, comes in one-day sachets. Both can be eaten directly from their containers and are designed to be eaten in small quantities, as a supplement to the regular diet. The first type contains peanuts paste, vegetable fat, skimmed milk powder, whey, maltodextrines, sugar. The second type contains peanut paste, vegetable fat, soy protein isolates, whey, maltodextrines, sugar, cocoa. Both of them has almost the same nutritional value (per 100g) Energy 534Kcal / 545Kcal, protein 12.7g /13.6g, and at 34.5g / 35.7g.

- 3) High energy biscuits (HEBs): Wheat-based biscuits which provide (per 100g) 450kcal with a minimum of 10 grams and max of 15 grams of protein per 100 grams, 15g of fat, fortified in vitamin and minerals. It is always distributed in the first days of emergency when cooking facilities are scarce. Easy to distribute and provide a quick solution to improve the level of nutrition. It contains wheat flour, hydrogenate vegetable shortening, sugar, Soy flour, invert syrup, high fructose, corn syrup, skimmed milk powder, sodium and ammonium, bicarbonates, salt, minerals and vitamins, namely, Calcium, Magnesium, Iron, Iodine, Folic Acid, Pantothenic Acid, Vitamin B1, B2, B6, B12, C, D, E, Niacine, & Vitamin A-retinol.
- 4) Micronutrient Powder/Sprinkles: It is a tasteless powder that contains the recommended daily intake of 16 vitamins and minerals that is to be sprinkled onto home-prepared food just before consumption. It is very useful when fortification

cereal flour is not available. Serving size is one sachet per person. It can be used in school feeding programs and emergencies.

- 5) Compressed Food Bars: Composed of wheat flour, vegetable fat, sugars, soya protein concentrate and malt extract. These bars are used in disaster relief operation when local food can't be distributed or prepared. It is not appropriate for children under 6 months. These bars can be consumed straight from the package or crumbled into water and eaten as porridge. The nutritional value per 56 g bar as follows: energy 250kcal, protein 8.1, fat 9.4 g. It also contains vitamins and minerals such as: A, D3, E, C, B1, B2, B6, B12, Niacin, Folic acid, Pantothenic acid, Biotin, Calcium, Phosphorus, Magnesium, Iron, Zinc, Potassium, Sodium, Copper, Selenium, and Iodine.

The purpose and objective of this research was to develop high energy biscuits and this will be discussed in detail in the next sections.

## **2.8 High Energy Biscuit**

High energy biscuits (HEB) fall under the category of energy-dense nutritional foods in the IOM guidelines. Energy dense nutritional foods according to IOM guidelines can be packaged and stored for extended periods of time in any environment and they present a challenge to the processor. In a natural or man-made malnutrition emergency, these products must also meet the nutritional needs of all age groups from infants to adults and be sufficiently palatable to be consumed for up to two weeks as the sole food. Nutrient profiles for an emergency food product (EFP) have been developed, but the required useful life of the product will be met only through careful consideration and selection of ingredients, processing techniques, and packaging materials. Key considerations include



microbiological and chemical safety, and ease of use. A successful EPF considers five components namely, the EPF must be (1) safe, (2) palatable, (3) easy to dispense, (4) easy to use, and (5) nutritionally complete. The anticipated duration of use is 3 to 7 days, but the product may be used for up to 15 days. The EPF should provide the required energy 2100 kcal daily or 233-250 per EPF, 63-80g protein per 2100 kcal (8-9g/EPF), 82-105g fat per 2100kcal (9-12g/EPF). The remaining calories should be coming from carbohydrates. It also should include vitamins, minerals, and other essential nutrients required for survival during this short time span. The EPF should also exhibit sensory appeal, as well as logistic and cultural convenience (IOM, 2002). Microbiological safety, nutritional value maintenance, and oxidative stability are all important features for a product with extended shelf life under adverse conditions. All of these characteristics are influenced by water content and water activity (IOM, 2002). In addition, the sensory quality of the emergency bar must be acceptable in many cultures (Grobler-Tanner, 2001). To minimize microbiological spoilage, nutrient degradation, and oxidation, the moisture content of the bar should be below 9.5% with water activity of no more than 0.6 (IOM, 2002). Ideally, the final EPF should meet a minimum shelf life requirement of 36 months at 21°C. Each bar should contain approximately 233 kcal. Therefore, adults will need to consume between 9 and 10 bars each day (about 2100 kcal/d). Per the IOM (2002), the primary source of protein could be in the form of a soy product (flour, concentrates, isolates, or textured vegetable protein); partially hydrogenated soybean oil and flaxseed oil will supply the lipid content of the EPF; and a cereal base, vitamin/mineral premix, sugars, and possibly baking and leavening agents will also be constituents of the bar.

Fortification of cereal-based foods would be a great help, since cereals are the most highly consumed food products around the world. Cereal based products are a cheap source of energy and are available to almost everyone. Legumes are rich source of protein that can be used to improve the diet of millions of people (Singha and Muthukumarappan, 2018; Singha and Muthukumarappan, 2017). Supplementing of wheat flour with legume flours, especially chickpea flour has good potential for improving the nutritional value of the flour and its products, particularly baked products. A number of studies have demonstrated the nutritional value of chickpea supplemented flour and food products such as breads (pita breads, chapatti, and toast); cookies, cakes, papads, and pasta (Singh et.al, 1991; Shehata et.al, 1970; Dhinda et.al, 2012) (Dodok et al., 1993; Eissa et al., 2007; Garg and Dahiya, 2003; Hallab et al., 1974; Yousseff et al., 2006). The supplementation of chickpea flour at 15 - 20 percent level in wheat flour biscuits has been reported to not only improve protein quality but also to improve dough texture and sensory attributes in the final product (Masur et al., 2009).

The nutritional value of wheat flour can be also enhanced using a variety of alternative flours and co-products of different industries such as distillers dried grains with soluble's (DDGS) and chickpea flour. DDGS is a major byproduct of the ethanol industry. The starch from cereals serves as the yeast energy source during the fermentation process. Due to the loss of starch, the protein and fiber components are concentrated thus making the dried residue a potentially nutritious food for humans (Singh, 2016). Previous studies have reported on the incorporation of DDGS in various cereal-based products, such as breads (chapatti, naan, corn breads, toast, pita breads), cookies, pizza, tortillas ( Arra, 2011; Pourafshar, 2011; Parmar, 2012; Li, Wang,

Krishnan, 2016 unpublished paper; (Tsen et al., 1982) where the results showed increased/enhanced nutritional potential.

From the literature there were few studies that have employed different types of ingredients for emergency aid programs table 1.13, but only 3 of them have used chickpea flour. To our knowledge, this is the first study to use food grade DDG in such formulations.

Another objective of this study is to develop formulations for a nutrient-dense energy bar containing wheat flour, chickpea flour, and FDDG and to determine proximate composition and sensory characteristics. Chickpea and FDDG are highly nutritious ingredients that were used as principal ingredients for development of extruded snacks. Therefore, it is hypothesized that cereal based foods can be effectively fortified with chickpea and FDDG to produce products of higher nutrient content that can be used in emergency food programs.

**Table 2. 1 Nutrient composition of different flours (adopted and modified from Pourafshar, 2010)**

| <b>Type of Flour</b>                 | <b>Protein (%)</b> | <b>Fat (%)</b>    | <b>Fiber (%)</b>  | <b>Carbohydrate (%)</b> |
|--------------------------------------|--------------------|-------------------|-------------------|-------------------------|
| <b>All-purpose flour<sup>3</sup></b> | 10.32              | 2                 | 14                | 32                      |
| <b>Amaranth<sup>4</sup></b>          | 12.5-17.6          | 6.3-8.1           | 3.6-4.2           | 62.17-64                |
| <b>Arrowroot<sup>4</sup></b>         | 0.3                | 0.1               | 3.4               | 88.15                   |
| <b>Almond<sup>4</sup></b>            | 6                  | 14                | 3                 | 6                       |
| <b>Barley<sup>4</sup></b>            | 11.3               | 1.9               | 0.8               | 85.4                    |
| <b>Buckwheat<sup>4</sup></b>         | 4                  | 1                 | 4                 | 21                      |
| <b>Corn<sup>4</sup></b>              | 2                  | 1                 | 4                 | 22                      |
| <b>DDG</b>                           | 27-30 <sup>1</sup> | 15.2 <sup>2</sup> | 13 <sup>1</sup>   | 46 <sup>1</sup>         |
| <b>DDGS</b>                          | 27-34 <sup>1</sup> | -                 | 5-11 <sup>1</sup> | 39-46 <sup>1</sup>      |
| <b>Millet<sup>4</sup></b>            | 3                  | 1                 | 4                 | 22                      |
| <b>Oat<sup>4</sup></b>               | 4                  | 2                 | 3                 | 16                      |
| <b>Pea<sup>4</sup></b>               | 4                  | 1                 | 4                 | 9                       |
| <b>Potato<sup>4</sup></b>            | 6.9                | 0.34              | 5.9               | 83.8                    |
| <b>Quinoa<sup>4</sup></b>            | 4                  | 2                 | 4                 | 21                      |
| <b>Rice<sup>4</sup></b>              | 3                  | 1                 | 4                 | 8                       |
| <b>Rye<sup>4</sup></b>               | 4                  | 1                 | 7                 | 21                      |
| <b>Soy<sup>4</sup></b>               | 7                  | 4.5               | 4                 | 9                       |
| <b>Spelt<sup>4</sup></b>             | 4                  | 1                 | 4                 | 22                      |
| <b>Tapioca<sup>4</sup></b>           | 0                  | 0                 | 0                 | 26                      |
| <b>White Rice<sup>4</sup></b>        | 5.95               | 1.42              | 2.4               | 80.13                   |
| <b>Whole Wheat<sup>3</sup></b>       | 13.7               | 1.87              | 12.27             | 72.57                   |
| <b>Chickpea<sup>5</sup></b>          | 17-22              | 6                 | 18-22             | 60                      |

1. Rosentrater, and Krishnan, (2006)
2. Qi, (2010).
3. Hyvee all-purpose flour and Hyvee whole wheat flour.
4. Bob's Red Mill Natural Foods, Inc.
5. Ukanti, Gaur, Gowda & Chibbar, (2012).

**Table 2. 2 Some examples of Middle Eastern breads. (adopted and modified from Pourafshar, 2010)**

| <b>Name of Bread</b>            | <b>Kind of Flour</b> | <b>Country</b>                | <b>Other Characteristics</b>   |
|---------------------------------|----------------------|-------------------------------|--|
| <b>Aish Mehahra<sup>1</sup></b> | Fenugreek & Maize    | Egypt                         | Flat, wide loaves with 50 cm diameter                                      |
| <b>Baladi<sup>2</sup></b>       | Whole Wheat          | Egypt                         | Round shaped, with 15-20 cm diameter                                       |
| <b>Barbari</b>                  | Wheat                | Iran                          | Oval shaped, with length of 67-75 cm                                       |
| <b>Bazlama<sup>3</sup></b>      | Wheat                | Turkey                        | Round shaped, with diameter of 10-25 cm                                    |
| <b>Bolani</b>                   | Wheat                | Afghanistan                   | Flat bread stuffed with different vegetables                               |
| <b>Harsha</b>                   | Semolina             | Morocco                       | Pan fried bread  |
| <b>Injera<sup>6</sup></b>       | Teff, Wheat, Corn    | Eritrea                       | Pancake like bread   |
| <b>Lavash</b>                   | Wheat                | Iran                          | Thin round bread with 50-60 cm diameter                                    |
| <b>Malooga<sup>7</sup></b>      | Wheat                | Yemen                         | Yeasted flat bread, eaten with egg, buttermilk                             |
| <b>Matzo</b>                    | Wheat & Spelt        | Israel                        | Cracker like flat bread, can be made into round shape with a foot diameter |
| <b>Pide<sup>9</sup></b>         | Wheat                | Turkey                        | Soft, chewy texture, it is like Pita                                       |
| <b>Pita<sup>8</sup></b>         | Wheat                | Common in different countries | Flat, round, have a pocket, golden brown crust                             |
| <b>Sangak</b>                   | Whole Wheat          | Iran                          | It is a large bread with the length of 70-80 cm                            |
| <b>Taftoon</b>                  | Wheat                | Iran                          | Round bread with diameter of 40-50 cm                                      |
| <b>Yufka</b>                    | Wheat                | Turkey                        | Thin round bread with diameter of 18 inch                                  |

4 <http://w.about.com>

5 <http://www.cookingwiththebible.com>, <http://members.cox.net>

6 <http://lakenvelderfoodblog.blogspot.com>, <http://www.giverecipe.com>

7 <http://www.ethiopianrestaurant.com>

8 <http://www.blogger.com>

9 <http://www.epicuream.com>

10 <http://w.about.com>

11 Farvili, Walker & Qarooni, (1995).

**Table 2. 3 Some examples of bread fortification studies.**

| Title of the study   | Author/ year             | Brief description of study and results   |
|--|--------------------------|--|
| <b>Nutritional and sensory evaluations of wheat breads supplemented with oleic rich sunflower seed</b>     | Biljana s et al, 2008    | Wholegrain supplemented breads with 8%, 12%, 16% sunflower seed were sensorially acceptable, containing significantly more tocopherols, fat, essential fatty acids, crude fibre, copper and zinc compared to control refined (white) wheat bread.  |
| <b>Utilization of hulless barley in chapati making.</b>  | Sood et al., 1992        | Hulless barley flour added into wheat flour increased protein content. The water absorption capacity of blended samples was on higher side. Color, appearance and texture of chapaties were good up to 30% of hulless barley flours in the blends, but flavor score was slightly decreased. Chew ability of chapati was satisfactory up to 40% of hulless barley flour in the blend. |
| <b>Soy enrichment of chapaties made from wheat and non wheat flours</b>                                    | Lindell and Walker 1984. | improving protein content and nutritive value of wheat flour products where chapaties were enriched with soy flour.  |
| <b>Development of baking procedure for the production of oat-supplemented wheat bread.</b>                 | Marriotti et al, 2006    | Oat improved the protein content of bread and increased the soluble fiber level. Also, both oat and barley enhanced the $\beta$ -glucan content of bread.  |
| <b>Effect of fortification of defatted soy flour on sensory and rheological properties of wheat bread.</b> | Mashayekh et al, 2008    | Adding 3% or 7% defatted soy flour gave as good a loaf of bread as the 100% wheat bread with higher nutritional quality and acceptable consumer attitude with rheological and sensory characteristics  |
| <b>The effect of amaranth grain flour on the quality of bread</b>  | Ayo AJ, 2001.            | The water absorption of the composite flour increase with increased in level of amaranth grain flour. the sensory means scores of the odor taste, color and texture decreased.   |

Table 2. 4 Chickpeas fortification studies

| <b>Title of the study</b>   | <b>Author/ year</b>                   | <b>Brief description of study</b>  |
|---|---------------------------------------|--|
| <b>Supplementation of bread with soybean and chickpea flours</b>  | Yousseff, Sale m, Abdel-Rahman (1976) | Water absorption was reduced by adding raw chickpea flour. Also dough mixing time, and stability increased but the mixing tolerance index decreased. Loaves were slightly smaller in volume than control. Moreover, bread score and panel evaluation showed deterioration of bread characteristics above 15% chickpea level of supplementation. Chemical analysis of the supplemented bread showed a positive trend of increasing protein, fiber, and ash contents by increasing the levels of chickpea. |
| <b>nutritive value and organoleptic properties of white Arabic bread supplemented with soybean and chickpea</b>   | Hallab, Khatchadourian & Jabr, (1974) | Supplementation with 10, 20, 30, 40 and 50% chickpea flour significantly enhances the nutritive value (protein and fiber) of Arabic bread, but 20% supplementation is most acceptable organoleptically. But supplementation with chickpea flour above 30% level in the preparation of bread impaired the quality of bread, while at lower levels it was acceptable.  |
| <b>Rheological properties and quality evaluation on Egyptian balady bread and biscuits supplemented with flours of ungerminated and germinated legume seeds or mushroom</b> | Eissa , Hussein , Mostafa ,(2007)     | Wheat flour fortified with 5,10,15% of chickpea flour showed an increased water absorption, decreased dough extensibility, and increased dough strength. Chickpea fortified Balady Egyptian bread showed an increased protein content.   |
| <b>Impact of adding chickpea (Cicer arietinum L.) flour to wheat flour on the rheological properties of toast bread.</b>  | Hefnawy, El-Shourbagy, Ramad (2012)   | Chickpea flour at 15 and 30% substitution levels increased the stability and the tolerance index of the dough. The volumes of the breads decreased as the level of chickpea flour increased. Substitution at 15 and 30%, gives parameter values at least as good as the control sample and produces an acceptable toast bread, in terms of weight, volume, texture and crumb structure.  |
| <b>The effects of chickpea on the functional properties of white and whole wheat bread.</b>   | Yamsaengsung et al, (2010)            | The addition of 10 and % of chickpea altered amount of water on the functional properties (bread volume, color of crust, crumb texture and crumb porosity) compared to white and whole wheat bread. Addition of chickpea increased crumb firmness and slightly decreased bread volume in both bread types. Chickpea addition increased darkness and yellowness of the bread.   |

|  |   |  |
|--|---|--|
| <b>Nutritional evaluation and shelf life studies of papads prepared from wheat-legume composite flours</b>   | Garg and Dahiya, (2003)                               | Fat and ash content was significantly higher in chickpea flour supplemented papads. Total carbohydrates decreased significantly on supplementation with chickpea flour. Copper content increased significantly on supplementation. Storage studies showed that chickpea flour supplemented papads can be stored safely for 60 days and wheat papads for 30 days both at room and refrigeration temperatures.   |
| <b>Alternative Use of Chickpea Flour in Breadmaking: Chemical Composition and Starch Digestibility of Bread<br/>Chickpea flour ingredient slows glycemic response to pasta in healthy volunteers</b> | Utrilla-Coello, Osorio-Dí'az, and Bello-Pe'rez (2007) | 20, and 40% chickpea fortified bread did not show differences in moisture, lipids and ash content, but had higher protein, RS and DF amount than control bread (all-wheat)   |
| <b>Quality Characteristics of Spaghetti as Affected by Green and Yellow Pea, Lentil, and Chickpea Flours.</b>  | Zhao et al, (2005).                                   | Firmness, pulse flavor, and color intensity of the pasta products increased with the increase in the percentages of legume flour fortification up to 30%, whereas the intensity of the shiny appearance, elasticity, and overall quality decreased. Consumers preferred control spaghetti (without legume additives) more than the spaghetti containing legume flours and they slightly liked the spaghetti with 15% lentil or green pea and the spaghetti with 20% chickpea or yellow pea |
| <b>Effect of durum flour enrichment with chickpea flour on the characteristics of dough and lasagne.</b>   | Sabani et al., (2006).                                | Supplementing lasagne with 5–20% chickpea flour improves the physical characteristics of dough. Sensory analysis improved with a low proportion of chickpea flour. Total protein increased along with the level of fortification.  |
| <b>Nutritional Evaluation and Functional Properties of Chickpea Flour and the Improvement of Spaghetti Produced from its</b>   | Abou Arab et al, (2010)                               | Spaghetti produced from wheat flour by replacement with different chickpea flour at levels 10, 15, 20, 25 and 30 % increased protein content and enhanced amino acid scores.   |
| <b>Effect on protein quality of supplementing wheat flour with chickpea flour.</b>   | Shehata & Fryer, (1970).                              | Chickpea flour 5, 10, 15 or 20% added to hard red winter wheat flour decreased moisture content and had little effect on physical properties of the dough or acceptability of Egyptian bread.  |



**Table 2. 5 Use of Chick pea diet in diabetes and blood pressure**

| <b>Title of the study</b>  | <b>Author/ year</b>   | <b>Brief description of study &amp; results</b>  |
|--|---|--|
| <b>Pasta added with chickpea flour: chemical composition, in vitro starch digestibility and predicted glycemic index.</b>                                    | Osorio-Díaz P, Agama-Acevedo E, Mendoza-Vinalay M, et al., 2008                             | Protein, ash, lipid, and fiber content increased while total starch decreased with the chickpea flour level in the composite pasta. The starch hydrolysis index (HI) decreased as chickpea flour in the pasta increased, reflecting the slow and low digestion of the starch in chickpea. Predicted glycemic index was lower in spaghetti added with chickpea flour than in durum wheat-control pasta. |
| <b>Chickpeas may influence fatty acid and fiber intake in an ad libitum diet, leading to small improvements in serum lipid profile and glycemic control.</b> | Pittaway JK, Robertson IK & Ball MJ, (2008)   | Incorporating chickpeas in the habitual ad libitum intake of 45 healthy participants for 12 weeks resulted in reduced serum total cholesterol, fasting insulin concentration. This may benefits in a more hypercholesterolemic and hyperglycemic population.   |
| <b>The effect of yellow pea protein and fibre on short-term food intake, subjective appetite and glycemic response in healthy young men</b>                  | Christopher E. Smith, Rebecca C. Mollard, Bohdan L. Luhovyy and G. Harvey Anderson, (2012). | Yellow pea consumption suppressed mean pre-meal BG compared to control. In conclusion, protein is the component responsible for the short-term effects of yellow peas in the regulation of glycaemia.  |
| <b>Chickpea flour ingredient slows glycemic response to pasta in healthy volunteers</b>  | Goni et al, (2003).   | Incorporation of 25% of chickpea flour into wheat pasta significantly lowered starch hydrolysis than in white bread. Chickpea flour, evidently provide a food with a low glycemic response and could help in achieving a wider range of low-GI foods.  |

**Table 2. 6 Use of Chick pea diet in Obesity/weight loss**

| <b>Title of the study</b>   | <b>Author/ year</b>            | <b>Brief description of study</b>  |
|---|--------------------------------|--|
| <b>Dietary chickpea reverses visceral adiposity, dyslipidaemia and insulin resistance in rats induced by a chronic high-fat diet.</b> | Yang, et al., (2007)           | Chickpea supplementation in the diet prevented increased body weight and weight of epididymal adipose tissues. Chickpea is reported to decrease fat accumulation in obese subjects. This aids in improving fat metabolism and could be helpful in correcting obesity-related disorders |
| <b>Chickpea supplementation in an Australian diet affects food choice, satiety and bowel function</b>                                 | Murty, Pittaway & Ball (2010). | Chickpea supplementation in the diet resulted in increased satiety and fullness.   |

**Table 2. 7 Use of Chick pea diet in CVD, CHD and cholesterol control**

| <b>Title of the study</b>   | <b>Author/ year</b>                                 | <b>Brief description of study</b>  |
|---|---|--|
| <b>A hypocaloric diet enriched in legumes specifically mitigates lipid peroxidation in obese subjects</b>   | Crujeiras et al. (2007)                             | Fibre-rich chickpea-based pulse diet has been shown to reduce the total plasma cholesterol levels in obese subjects.   |
| <b>A pulse-based diet is effective for reducing total and LDL-cholesterol in older adults</b>   | Abeysekara, Chilibeck, Vatanparast & Zello, (2012). | Pulse-based diet is effective for reducing LDL-C and total cholesterol in older adults and that's why reduces the risk of CVD.   |
| <b>Non-soy legume consumption lowers cholesterol levels: a meta-analysis of randomized controlled trials</b>  | Bazzano et al, (2010)                               | pulse-rich diet decreases total and LDL cholesterol.   |
| <b>Dietary Supplementation with Chickpeas for at Least 5 Weeks Results in Small but Significant Reductions in Serum Total and Low-Density Lipoprotein Cholesterols in Adult Women and Men</b> | Pittaway et.al, (2006)                              | Inclusion of chickpeas in an intervention diet results in lower serum total and low-density lipoprotein cholesterol levels as compared with a wheat-supplemented diet. |

**Table 2. 8 Average composition of corn grain and corn distiller's grains with solubles (Adopted from Martinez-Amezcuca et al., 2007)**

| <b>Nutrients (%)</b> | <b>Corn Grain</b> | <b>Corn Distillers Grains</b> |
|----------------------|-------------------|-------------------------------|
| <b>Dry material</b>  | 87.2              | 87.1                          |
| <b>Crude protein</b> | 22.33             | 27.11                         |
| <b>Crude fat</b>     | 9.75              | 6.98                          |
| <b>Ash</b>           | 4.60              | 2.00                          |
| <b>Phosphorus</b>    | 0.72              | 0.39                          |

**Table 2. 9 Amino acids profile of wheat and wheat DDGS**

| <b>Amino acids (%)</b> | <b>Wheat</b> | <b>Wheat DDGS</b> |
|------------------------|--------------|-------------------|
| <b>Isoleucine</b>      | 0.363        | 1.165             |
| <b>Leucine</b>         | 0.719        | 2.257             |
| <b>Lysine</b>          | 0.321        | 0.679             |
| <b>Methionine</b>      | 0.178        | 0.568             |
| <b>Phenylalanine</b>   | 0.505        | 1.602             |
| <b>Threonine</b>       | 0.540        | 1.783             |
| <b>Tryptophan</b>      | 0.163        | 0.283             |
| <b>Valine</b>          | 0.475        | 1.517             |

**Table 2. 10 Some food products developed by using DDGS (Adopted from Rosentrater and Krishnan, 2006).**

| <b>Application</b>             | <b>Feedstock</b>                           | <b>Functionality</b>   | <b>Taste Panel</b>                       |
|--------------------------------|--|--|--|
| <b>Blended ingredients</b>     | Corn                                       | Darker in appearance   | Flavor quality was poor and unacceptable |
| <b>Blended ingredients</b>     | Corn, red wheat, white wheat               | Poor growth during rat feeding trials, due to deficient amino acids                                    | ---                                      |
| <b>Blended ingredients</b>     | Corn                                       | Acceptable digestibility during rat feeding trials   | ---                                      |
| <b>Bread</b>                   | Wheat                                      | Darker in appearance; reduced loaf volume  | ---                                      |
| <b>Bread</b>                   | White wheat                                | High concentrations of soluble minerals  | ---                                      |
| <b>Bread - baguettes</b>       | White wheat                                | Darker in appearance   | Less acceptable flavor                   |
| <b>Bread – banana</b>          | White wheat                                | Darker in appearance   | Good to excellent                        |
| <b>Bread – carrot coconut</b>  | Barley, corn, rye                          | Darker in appearance; decreased volume   | Acceptable to highly acceptable          |
| <b>Bread – cinnamon rolls</b>  | White wheat                                | Darker in appearance   | Acceptable flavor                        |
| <b>Bread – dinner rolls</b>    | Barley, corn, rye                          | Darker in appearance; decreased volume; more chewy   | Acceptable to highly acceptable          |
| <b>Bread - dough</b>           | Barley, red wheat, soft white winter wheat | Darker appearance; decreased loaf volume; decreased crumb grain coarseness; increased water absorption | ---                                      |
| <b>Bread – nut rolls</b>       | Barley, corn, rye                          | Darker in appearance; decreased volume   | Acceptable to highly acceptable          |
| <b>Bread – oatmeal muffins</b> | Barley, corn, rye                          | Darker in color  | Acceptable                               |

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|                                |  |  |   |
|--------------------------------|--|--|---|
| <b>Bread – oatmeal muffins</b> | Barley, corn, rye                          | Darker in appearance; increased volume   | Acceptable to highly acceptable               |
| <b>Bread – wheat muffins</b>   | Cereal grains                              | Lighter in appearance  | Off flavors detected at 20%                   |
| <b>Bread - white</b>           | White wheat                                | Darker in appearance   | Acceptable to good                            |
| <b>Bread – whole wheat</b>     | White wheat                                | Darker in appearance   | Acceptable to good                            |
| <b>Bread – yeast rolls</b>     | Barley, corn, rye                          | Darker in color  | Acceptable                                    |
| <b>Canned – beef stew</b>      | Barley, corn, rye                          | ---  | Acceptable flavor, appearance, and mouth feel |
| <b>Canned – chili</b>          | Barley, corn, rye                          | ---  | Acceptable flavor, appearance, and mouth feel |
| <b>Canned – hot dog sauce</b>  | Barley, corn, rye                          | ---  | Acceptable flavor, appearance, and mouth feel |
| <b>Cookie – chocolate chip</b> | White wheat                                | Darker in appearance   | Good to excellent                             |
| <b>Cookie – chocolate chip</b> | White wheat                                | Darker in appearance   | Acceptable flavor                             |
| <b>Cookie - sugar</b>          | Barley, red wheat, soft white winter wheat | Darker appearance; variable spread   | ---   |
| <b>Ingredient</b>              | White wheat                                | Antioxidants did not improve lipid stability; drying method affected lipid stability | ---   |

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**Table 2. 11 Studies used DDGS for fortifications**

| Title of the study  | Author/ year         | Brief description of study and results   |
|---|----------------------|--|
| <b>Evaluation of distillers dried grain flour as a bread ingredient.</b>                      | Tsen, et al., (1983) | Bread supplemented with 10%, and 20% DDG contain higher amount of protein, fat, fiber, and ash when compared to whitebreads. Breads supplemented with 10% DDGF-B and DDGF-C were superior to whole wheat breads in loaf volume crumb grain and color.  |
| <b>Evaluation of the quality of cookies supplemented with distiller's dried grains flour.</b> | Tsen, et al., (1982) | DDGS at 15-25% replacement level increased fiber and protein, and decreased the width and thickness and darkens the color of cookies.  |
| <b>Evaluation of spaghetti supplemented with corn distillers dried grains.</b>                | Wuet al., (1987)     | Supplemented spaghetti with 10% DDG resulted in higher protein and dietary fiber than control Spaghetti.   |
| <b>Making quick breads with barley distillers dried grain flour.</b>                          | Eidet et al., (1984) | Incorporation of barley DDG flour into quick breads enhanced fiber and protein content.  |
| <b>Utilization of dried distillers grains from sorghum in baked food systems.</b>             | Morad et al., (1984) | Replacement of wheat flour with 15% sorghum DDGS decreased stability volume and mixing time of the dough. Crumb color was also affected, with the exception of color the quality of DDG sugar cookies was comparable to that of controls   |
| <b>Incorporation of corn distillers dried grains with solubles in Asian wheat flat breads</b> | Arra , (2011)        | Fortified chapathi, naan, and tandoori with different levels of DDGS showed significant changes in color, texture, and water absorption. protein, fat, fiber and ash levels were improved as the DDGS substitution level increased. sensory panelists preferred whole wheat flour chapathi with 20% DDGS among all levels of DDGS substituted chapaties. |
| <b>Utilization of corn distillers' grains in chapathies</b>                                   | Ahmed, (1997)        | Substitution of wheat flour chapathies with DDG at 5, 7, and 10% (w/w) levels, showed significant increase of protein and fiber contents.  |



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|---|----------------------------|--|
| <b>Analysis of physical and chemical properties of Persian barbari bread and Latin American tortilla (you did wheat tortilla, there is also corn tortilla) substituted with distillers dried grains with solubles</b> | Pourafshar, (2011)         | Fortification with 20% DDGS in wheat flour had the highest value of protein 12.55% and fiber 3.57% as compared to control. It was concluded that that the addition of DDGS as an ingredient in the preparation of wheat tortilla and barbari bread not only increase the nutritional value but also improve the textural properties of these two breads.   |
| <b>Protein and Fiber Fortification of White Pan Bread Using Food-Grade Distiller's Dried Grains</b>   | Adamski, (2016)            | Incorporation of DDG into breads led to smaller, denser loaves with fewer air cells. Substantial increases in protein content, where increases in fiber were noted only in the 10% DDGS loaves. Sensory analysis showed that all bread treatments were accepted.   |
| <b>Effects of corn distillers dried grains on dough properties and quality of Chinese steamed bread</b>   | Li, Wang, Krishnan, (2016) | 10%, 15%, 20% and 25% DDG fortified chinese breads resulted in protein and dietary fiber improvements. Dough demonstrated higher water absorption while dough development time and dough stability were decreased. Extensibility of dough decreased significantly at each level of flour replacement. Substitution of DDG reduced the brightness (L*) of flour blends and CSB. Rheological and sensory analysis showed that up to 15% DDG was tolerated. |

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**Table 2. 12 Use of high fiber diet in diabetes.**

| <b>Title of the study</b>  | <b>Author/ year</b>    | <b>Brief description of study</b>   |
|--|------------------------|---|
| <b>Effect of a Viscous Fiber Bar on Postprandial Glycemia in Subjects with Type 2</b>  | Flammang et al. (2006) | Compared postprandial glucose levels of Type 2 Diabetic patients who consumed an experimental guar fiber bar as compared to two other commercial crispy bars. Results showed adding viscous guar fiber to the test foods, caused a reduction in postprandial glycemic response compared to the other two types of bars.   |
| <b>Effect of Fiber Bread on the Management of Diabetes Mellitus.</b>   | Nizami et al. (2004)   | The postprandial glucose levels were found to be significantly lower after incorporating the 8 times higher -fiber bread when compared to control.  |
| <b>Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women</b>         | Schulze et al., 2004   | The objective of the study is to examine the relation among glycemic index, glycemic load and dietary fiber and the risk of type 2 diabetes in a large group of young women. Increasing evidence suggests an important role of carbohydrate quality in the development of type 2 diabetes. A diet high in rapidly absorbed carbohydrates and low in cereal fiber is related with an increased risk of type 2 diabetes. For diabetic subjects, moderate carbohydrate, high fiber diets compared to moderate carbohydrate, low fiber diets are associated with significantly lower values for postprandial plasma glucose. High carbohydrate, high fiber diets compared to moderate carbohydrate, low fiber diets are associated with lower values for: fasting, postprandial and average plasma glucose; hemoglobin A1c. |
| <b>Carbohydrate and Fiber Recommendations for Individuals with Diabetes: A Quantitative Assessment and Meta-Analysis of the Evidence</b> | Anderson et al., 2004  |   |

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|  |                       |   |
|--|-----------------------|---|
| <b>In Vitro Study of Possible Role of Dietary Fiber in Lowering Postprandial Serum Glucose</b> | Shiyi Ou et al., 2001 | The results showed that dietary fibers lowered postprandial serum glucose levels at least by three mechanisms.  |
| <b>Whole-grain and fiber intake and the incidence of type 2 diabetes</b>                       | Montonen et al., 2003 | Cereal fiber intake was associated with a reduced risk of type 2 diabetes. An inverse association between whole-grain intake and the risk of type 2 diabetes was found. The similar result for cereal fiber intake suggests that the whole-grain association is due to cereal fiber or another factor related to cereal fiber intake. |

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**Table 2. 13 Fortified high energy biscuits (HEB) studies from literature.**

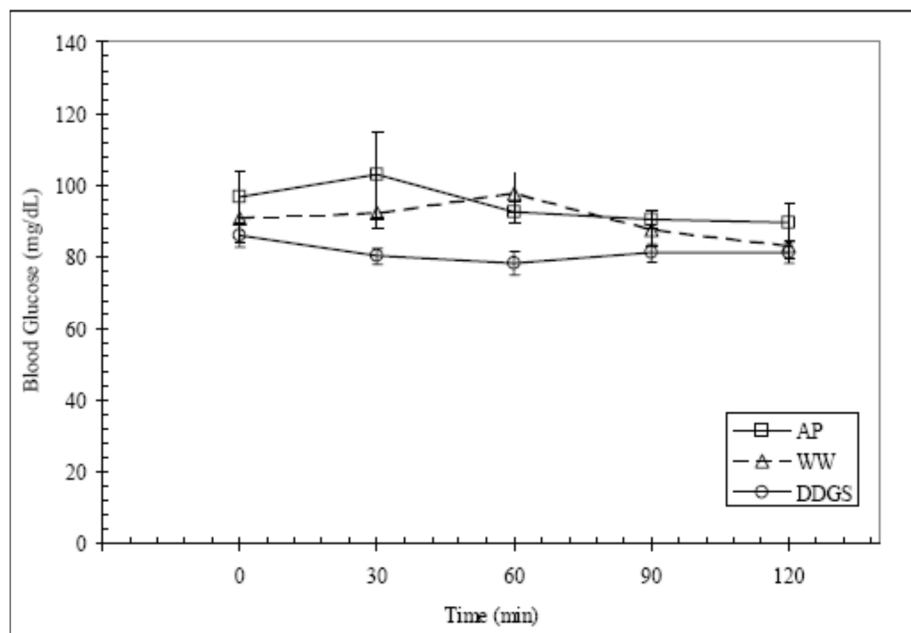
| <b>References</b>            | <b>Product &amp; ingredients</b>  | <b>Results</b>   |
|------------------------------|---|--|
| <b>Naseem et.al, (2013)</b>  | CP (5,10,15,20%) fortified HEB  | HEB was developed for malnourished children in Pakistan. Supplementation increased protein, fat, fiber, iron, and zinc   |
| <b>Sharmal et.al, (2012)</b> | CP (20,40,60%) fortified biscuit  | To develop rich protein and fiber source food. Supplementation increased protein, fiber, and ash.  |
| <b>Masur et.al, (2009)</b>   | CP (10,15,20,25) fortifies biscuit  | Increasing nutritional awareness among consumers. CP fortified high protein biscuit improved the nutritional and textural quality of biscuits                                    |
| <b>Young et al. (2007)</b>   | HEB fortified with eggs, soy oil, and dried milk.   | Developed to be used in feeding programs to prevent malnutrition after disaster. The adopted recipe was satisfactory in achieving nutritional values when compared to literature |
| <b>Briske et al. (2006)</b>  | Prototype nutrient-dense Bar, soy based, corn syrup, granulated sugar, high fructose corn syrup | Was developed as emergency product for refugees and displaced persons. Proximate composition met general specifications of IMO.  |

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CP: chickpea flour, HEB: high energy biscuits, IMO: Institution of medicine



**Figure 2. 1 Kabuli vs desi chickpeas**



**Figure 2. 2 Blood glucose levels over time. Error bars represent +/- 1 standard error of the mean (adopted from Alyssa Bechen, 2008)**

AP= All purpose

WW = Whole Wheat

DDGS = Distiller's Dried Grains

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### Chapter 3

#### **Physico-chemical traits, rheological properties, and shelf-life of chickpea-FDDG fortified pita breads**

##### **Abstract**

Consumers demand healthier food products that are also wholesome, safe and economical. Foods that provide excellent aesthetic and sensory qualities are also desired. New ingredients that impart improved functionality in food products, particularly in bread, may lead to improvements in nutrition, sensory characteristics and food rheology. Bread is a unique vehicle for fortification and nutritional enrichment as bread baking is common to all communities in the world. The blending of wheat flour, corn co-products and compatible legume flour such as chickpea can bring about improvements of wheat-based flat breads such as pita breads. The objective of the first study was to enhance nutritional, rheological, sensory profiles, and shelf life of wheat based pita bread using chickpea (CP) and food grade distiller's dried grains (FDDG). Flour blends with varied proportions of wheat, corn (10% and 20% FDDG) and chickpea (10% and 20% CP) were used in pita bread formulations. Pita bread with Nutritional efficacy was evaluated. Dough rheology and end-product texture were also analyzed. Chemical, physical, and rheological properties of blends, doughs and finished products were evaluated and the results showed an increase in protein, fat, minerals (ash), and total dietary fiber content with an increase of FDDG and CP in all-wheat flour. Moisture content decreased in both flour blends and pita breads with the increase of FDDG and CP substitution levels. Amino acids scores were improved by of either chickpea or FDDG or combinations of the two ingredients in comparison to the all-wheat control pita bread. Fortification with

10% FDDG improved amino acid scores by 15%, where fortification with 20% FDDG improved by 22% (over control wheat flour pita). Also, fortification with 10% chickpea improved amino acid scores by 20%, whereas fortification with 20% CP improved amino acid scores by 28%. Color values showed decreased L\* values (brightness), and a\* value (redness), but increased b\*(yellowness) levels in pita bread containing increased FDDG levels. However, L\* and b\* values decreased, and a\* increased with increased chickpea fortification. Rheological analysis of dough from Mixolab and Farinograph evaluation showed that fortification in general, yielded pronounced effects on dough properties. Flour replacement with FGGD and chickpea yielded dough with higher water absorption, higher dough development time, and lower dough stability time when compared to the wheat-only control. Texture analyzer results showed that the force required to break the dough increased, whereas the dough extensibility declined as the fortification level of either or chickpea and FDDG increased. Texture Analyzer data also showed that fortified pita required a greater force for tearability as determined by the burst rig and the tug fixture tests. Burst distance and tug distance was also reduced with increased fortification level of both chickpea and FDDG. Shelf life study showed that wheat pita bread substituted with 10% chickpea pita bread had the same shelf life time as control pita bread, whereas fortifying with 20CP% increased shelf life by 6 hours. Also, 10% FDDG fortification increased shelf life by 6 hours only when compared to control. However, fortifying with 20% FDDG doubled the shelf life time which increased by 12 hours when compared to control pita bread. Fortifying with 20CP-10D% increased the shelf life of the pita bread by 24 hours. The longest shelf life was encountered in 20 % FDDG-10%CP which is 30 hours more than the control pita bread. Sensory analysis was done for

all pita breads and showed that all products tested on a scale of 1 (poor) to 5 (excellent) were rated to be acceptable relative to the control all-wheat flour pita bread. Our findings show that formulation of pita breads by replacing up to 30% of wheat flour with chickpea and FDDG yielded comparable pita breads that were judged to be acceptable by the panelists.

### **3.1 Introduction**

Wheat is considered as a very important cereal crop and consumed all around the world in form of different foods. While cereals supply 50 % of total proteins humans consume, wheat contributes one third to total cereal protein production (Greg & Dahiya, 2003). Due to the ever-increasing demand of wheat for bread making, the prospects of replacing a part of wheat flour with alternative sources of starch have been deliberated (Hefnawy et.al, 2012). Prospects of fortifying wheat flour with fiber, protein and lysine to improve protein and essential amino acid content of final baked foods like bread have also been explored (Hallen et.al, 2004). An excellent approach to meet the growing demands of wheat and fulfilling protein needs would be to combine cereal grain protein that are low in lysine with high lysine containing legumes.

Legumes inherently are rich in proteins, carbohydrates, fat, vitamin B complex like thiamine and niacin along with minerals like calcium, magnesium, zinc, iron, and phosphorus. The protein quality can be enhanced by consuming cereals and legumes in the same meal. (Greg & Dahiya, 2003). Legumes can add diverse texture and taste to cereal diets. Chickpea rich in complex carbohydrates, vitamins and minerals can be an excellent source to enhance nutritional quality of bread flour and therefore bread itself.

Chickpea has a high lysine and low methionine content that could complement the lysine poor wheat flour proteins (Hefnawy et.al, 2012).

The principle determinant of protein quality is the availability of key amino acids. These nutrients play a vital part in the development, reproduction and support of the human body. Amino acid content in food is used to compute the amino acid score, which gives an idea about how effectively the protein will meet an individual's amino acid needs. The technique depends on comparison of the concentrations of the first limiting amino acid in the test protein with the concentration of the same amino acid in a reference (scoring) pattern. The requirements of amino acid in milligrams/gram of dietary protein as percentages in an “ideal” protein can be expressed by reference amino acid scoring pattern (Caire-Juvera, Vázquez-Ortiz & Grijalva-Haro, 2013). The FAO/WHO/UNU has stipulated that the composition of amino acids in local and regional diets can be taken into consideration to decide the chemical composition of diets and to have the capacity to evaluate the protein quality of the diets.

Most plants do not contain adequate amounts of essential amino acids, vitamins and minerals. A well-balanced diet provides satisfactory amounts of all essential amino acids. Issues associated with under-nutrition emerge when the diet is confined to a solitary plant source. For instance, cereal storage proteins are lacking in lysine and threonine while legumes do not have adequate sulfur-containing amino acids methionine and cysteine. A diet exclusively containing one of these protein sources will likely be lacking in one or more crucial amino acids (Hefnawy et.al, 2012).

Production of wheat has not been adequate to take care of the expanding demand for bread to satisfy human needs. More recently, new endeavors have been undertaken to

replace a portion of the wheat flour by other plant materials sources. Flours from corn, barley, cassava and chickpea are among the most widely studied flours for the production of composite flour breads. Legumes such as beans and chickpea are considered critical crops due to their high nutritional quality. They are excellent sources of complex carbohydrates, vitamins and minerals. Legumes have been viewed as a rich source of protein all through the world and contain approximately three times more protein content than cereals. Chickpea (*Cicer arietinum* L.) is one of the main legumes when the amount of grain produced is taken into account. It has been utilized for the preparation of different conventional foods including bakery products. Chickpea flour can be suitable choice for enhancing the nutritional properties of the bread. The high lysine and low methionine content of chick pea complements the amino acids of wheat flour protein, which are poor in lysine and generally higher in the Sulfur-containing amino acids. (Hefnawy et.al, 2012).

Distillers dried grains with solubles (DDGS) is a co-product, which is produced during ethanol production from corn. It is the dried residue remaining after the starch fraction of corn is fermented, using selected yeasts and enzymes, to produce ethanol and carbon dioxide. It is currently sold at low price as an animal feed (Singh and Muthukumarappan, 2016; Singh and Muthukumarappan, 2017a; Singh and Muthukumarappan, 2017b). DDG has been determined to be a promising human food ingredient, because it is a source of protein and fiber. It is low in starch, high fiber and high protein ingredient and can be used in formulating foods for diabetic and celiac disease patients (Bechen, 2008). DDGS contain 25-30% crude protein, 8-12% of fat. In addition, it contains 42.2 insoluble fiber, and 0.7 soluble fiber (Shukla, 2003; Parmar



2012). DDGS also has the essential amino acid composition which is needed for the human consumption (Wu et al., 1980). In terms of human food, scientists have explored the use of distillers grains (such as DDG and DDGS) in food systems over the years. Distillers grains have been incorporated into breads, cookies, and pasta with varying degrees of acceptability (Rosentrater & Krishnan, 2006).

Since there has been a growing interest in fortifying wheat flour with high lysine materials, to improve the amino acid balance in baked products, our objective was to fortify wheat flour with high protein ingredients (chickpea and FDDG) to improve amino acid composition of pita bread.

The health benefits of dietary fibers were identified and proven in 1980s, and have since then generated an interest in food industry as a source to enhance fiber content in foods (Dhinda et.al 2012). Although the demand of dietary fiber enriched breads are on the rise, the incorporation of dietary fiber in bread poses many challenges. Dietary fiber enrichment not only modifies the dough rheology but also affects the sensory attributes like texture, taste and appearance (Ktenioudaki et.al, 2012).

In the search for alternate sources of dietary fiber to overcome the above mentioned challenges, we could use Distillers grain, the by-product of ethanol production, having high dietary fiber and protein content to fortify foods especially breads. The use of Food Grade Distillers Dried Grain (FDDG) as a bakery ingredient has been researched extensively during the last 20 years and the results indicated a poor texture and flavor to the final products (Roth et.al, 2016). A recent trend in baking industry has been the use of a mixture of grains and legumes to increase dietary fiber and

protein content of baked foods in addition to improved taste, aroma, appearance, nutritional and rheological properties.

Bakery products like bread have a short shelf life. The shelf life of bakery products can be extended by modifying the process of bread making along with the packaging materials and condition of storage. The main defect in bakery products that limit their shelf life causing spoilage and food waste is mold growth. The issue of mold growth can be controlled to some extent by use of preservatives such as sorbates and propionates which need to be declared in the ingredient statement. The use of commercial ingredients like Sonextra Natural Preserve Soft can be added to preserve all kinds of bread and to add extra softness. However, these ingredients do not lead to a clean labeled product. There has been a growing demand for foods to be labeled clean by eliminating any foreign agents and limiting ingredients. To follow up on these consumer demands, researchers have to develop natural preservatives or ingredients that extend the shelf life of bread products. DDG and chickpea with antioxidant properties could be potential natural agents that may inhibit or slow mold growth, in clean labelled breads. Dreese and Hosney (1982) concluded that products high in fiber such as DDGS and chickpea also had increased quantity of water absorption. Fiber plays many roles in food system, such as providing structure and bulk, modification of rheological properties, as well as other functions (Fennema, 1996, Brochetti et al., 1991; Waelti & Ebeling, 1982; Wu et al., 1984; Rasco et al., 1987).

To cater to the growing demands for cleaner, healthier and cost effective food products with enhanced sensory qualities, chickpea-DDGS fortified flour/bread could be feasible alternative. To this end, this study was undertaken to develop chickpea-DDGS

fortified flour/bread with improved nutritional quality, with regards to protein and fiber content, good sensory and rheological attributes with an extended shelf life.

## **3.2 Materials and methods**

### **3.2.1 Materials**

Corn distillers dried grains with solubles (DDGS) was obtained from a commercial ethanol plant and was stored at  $-80\pm 1^{\circ}\text{C}$  until further processing for food applications. Other ingredients for preparation of pita bread, such all-purpose flour, chickpea, salt, sugar, active dry yeast, and olive oil, were purchased from a local grocery.

### **3.2.2 Methods**

#### **3.2.2.1 Sample preparation**

##### **3.2.2.1.1 Preparation of chickpea flour**

Chickpea flour was prepared by milling dry chickpea in a Retsch mill (Company: GmbH & Co. Germany, Model: KG 5657HAAN1) into a fine powder. The powder obtained after milling was sieved using 0.5mm sieve to get fine flour.

##### **3.2.2.1.2. Preparation of FDDG**

FDDG was processed specifically for food applications in this study. The DDGS obtained from commercial ethanol plant was placed in stainless steel trays lined with cheesecloth, and then washed extensively with absolute alcohol i.e. 99.5% pure ethanol to remove pigments and oil. De-fatted samples were then washed multiple times with distilled water to remove traces of ethanol. The samples were then freeze-dried for 3-4

days in a shelf freeze dryer (Company: Virtuis, Model: USM15). Freeze dried DDG powder was milled in Retsch Ultra centrifugal mill (Company: GmbH & Co. Germany, Model: KG 5657HAAN1) at the centrifugal speed of 20,000 rpm. Using a 0.5mm sieve, the powder obtained after milling was sieved and then stored in air-tight glass jars and sterilized in an autoclave at 15 psi (per square inch) pressure for 15 minutes. Sterilized FDDG flour was stored in a freezer to ensure maximum quality.

### **3.2.2.2 Preparation of flour blends**

Control flour containing 100 % wheat (W) and six treatment blends containing wheat, chickpea and FDDG blends containing varied proportions of chickpea and FDDG were prepared as shown in table 3.1 The control consisted of a 100% All Purpose Flour (APF). The flour blends were mixed to ensure homogeneity in a V-shaped twin-shelled dry blender (Company: Peterson Kelly Co. Inc. Stroudsburg, PA) at a constant speed for 45 minutes to ensure uniform mixing of the ingredients.

### **3.2.2.3 Pita bread formulation**

Seven different types of pita bread, corresponding to the flour blends and differing in ingredient composition (W, CP and D) were prepared (table 3.1). These were control all-purpose wheat flour pita bread (W:100), chickpea-only wheat flour pita breads (10% or 20% replacement level, W90:CP10 & W80:CP20), FDDG-only fortified pita bread (10% or 20% replacement level, W90:D10 & W80:D20), and finally, chickpea-FDDG fortified wheat flour pita breads (W70:CP20:D10 & W70:CP10:D20).

The pita recipe and baking procedure were provided by a professional chef from a Mediterranean/Middle Eastern restaurant. This method of pita bread was followed

consistently for the control and all 6 treatments. The basic formula for pita bread for 4-5 servings included 187.5 grams (g) flour, 14.3 g sugar, 59 ml (milliliter) lukewarm water, 1.2 g salt, 14.3 g yeast, and 4.8 g (5 ml) olive oil. In pita production, sugar, yeast and water were mixed and set aside for 10 minutes at room temperature for activation of yeast. Yeast growth was confirmed by liberation of bubbles from the mixture. The dough was prepared in an automatic dough mixer (Kitchen Aid, Model: KSMQO). First, flour was added in the mixer followed by yeast mix. The dough was mixed at a low speed for 1.5 min. Salt was added, followed by olive oil. Mixing was done at faster speed this stage. The dough was then covered and leavened at room temperature for 1.5 h in a proofing cabinet. The flour blends were mixed using a dough hook head using the Hobart mixer.

#### **3.2.2.3.1 Rolling and Shaping of the dough**

Rolling and shaping of the dough was done manually. Before dough handling, it is advisable to rinse the hands with cold water to prevent sticking of dough to hand. From each dough mix, 4-5 dough balls of equal size were made and spread on a table using dough roller. Before rolling, the table was sprinkled with flour to prevent sticking. After rolling, the flattened dough was laid on parchment paper and kept for re proofing for about 5 minutes before baking.

#### **3.2.2.3.2 Baking of pita bread**

The pita breads were baked in an oven at 525°Fahrenheit (274 °C) for 60-90 seconds. After the specified baking time, the bread was removed from oven and allowed to cool for 1-2 hours at room temperature 77° Fahrenheit (25±1°C). Each piece of pita bread was

cut into 8 slices using a bread knife, sealed in plastic bags and refrigerated further analysis.

#### **3.2.2.4 Proximate analysis**

**Moisture:** Moisture content was measured using oven the drying method according to AACCI approved method 44-19.01 (AACC 2000).

**Fat:** Fat content was determined using AOAC method 920.39 (AOAC, 1990) in an automated Soxhlet extractor using petroleum ether as solvent (CH-9230, Buchi laborotechnik AG, Flawil, Switzerland).

**Protein:** Protein content of the pita bread samples was analyzed for using the Dumas combustion analysis method (AOAC 17th ed., method 968.06) using a Rapid N cube (Elementar Analysen Systeme, GmbH, Hanau Germany). Nitrogen content was then multiplied by a conversion factor of 6.25 to calculate Crude Protein % (CP).

**Amino acid:** Amino acid analysis was done by HPLC and post column derivatization method (15-06.1 AOAC).

**Amino acid evaluation:** The amino acid score was calculated using the ratio of a gram of the limiting amino acid in the food to the same amount of the corresponding amino acid in the reference diet multiplied by 100. The scoring patterns suggested by the FAO/WHO/UNU6 was used for this purpose.

**Ash:** Ash content of the pita bread samples was determined using incineration (Method. 08-03, AACC, 2000) in a muffle furnace (Company: Model: Box furnace, 51800 series). The dried pita bread samples were ashed at 525°C for 12 hours in muffle furnace to estimate inorganic content (minerals) in the bread.

Total Dietary Fiber (TDF): Fiber content was analyzed by enzymatic gravimetric method employing AOAC method (Method 30-25) for non-digestible fibers. The Megazyme assay test kit was used.

Carbohydrates: The (CHO) in pita bread samples was calculated by difference [100% - (protein%, + fat% + ash%, + moisture%)].

### **3.2.2.5 Rheological analysis**

Mixolab (Company: Chopin Technologies, France)) was used to study the rheological behavior of all the seven types of dough and evaluate the effect of flour blends on rheology.

Farinograph: analysis was done using method 54-20 (AACC,1990) for dough development dough stability time and water absorption (Model C.W Brabender, Instruments, Inc, South Hackensack, NJ).

Texture: Texture analysis of pita bread was performed using Texture analyzer (Company: Texture Technologies Corp., New York, Model: TX.XT-plus) to determine extensibility, chewability, and shear force required to tear the pita bread. The extensibility of the dough was measured using Kieffer extensibility rig. A 15-gram dough ball was oiled (to prevent sticking to the mold surface) and placed in Kieffer press and molded. The excess dough was removed using knife. The Kieffer press was held in rested position for 45 minutes for gluten network relaxation. After resting period, the press was removed and dough strips of approximately same dimensions (length, breadth, height) will be obtained. The dough strings were clamped between the two plates of Kieffer extensibility rig and force required to break the string was recorded by an automated software installed in the system. It is to be noted that test was performed immediately after obtaining dough strips

to avoid deformation of the strips.

#### **3.2.2.6 Nutrient Profile of test food**

Physico-chemical properties such as moisture, protein, total dietary fibers, fat, ash, and carbohydrates were determined for the control and 6 treatments of pita bread.

All seven types of pita bread were freeze-dried for 3-4 days in a shelf freeze dryer (Company: Virtis, Model: USM15) prior to milling in Retsch mill (Company: GmbH & Co. Germany, Model: KG 5657HAAN1) at the centrifugal speed of 20,000 rpm. The powder obtained after milling was sieved using a 0.5mm sieve to obtain homogenous fine flour.

#### **3.2.2.7 Sensory analysis**

Sensory evaluation was carried out by 45 trained and untrained panelists using a seven-point hedonic scale. Panel members were comprised of undergraduate and graduate students and staff members of South Dakota State University.

#### **3.2.2.8 Shelf life**

Shelf life of control and chickpea and FDDG fortified of wheat breads were studied. Breads were analyzed for apparent spoilage by visual observation for mold growth under ambient temperature. The shelf life of pita breads was observed visibly for 24 hrs to 1 week at room temperature ( $25\pm 1^{\circ}\text{C}$ ), for growth of molds



### **3.3 Results and discussions**

#### **3.3.1 Proximate analysis**

##### **3.3.1.1 Proximate analysis of raw ingredients**

Table 3.2 provides the nutritional composition for the raw starting materials used in the pita bread production, namely all-purpose flour, chickpea flour and food grade distiller's grains. These materials varied considerably in their content of moisture, protein, fats, minerals and carbohydrates as reflected by their composition. Their diversity thus provided for unique properties in the finished products when they were brought into the pita bread formulations in fixed ratios described earlier in table 3.1. Food Grade DDG was composed of protein (31.0%), TDF (30.9%), fat (5.1%), and ash (3.1%) in composition. Chickpea flour in contrast to all-purpose flour, had almost twice the amount of protein (22.3%), about four times higher TDF (21.1%) and ash content (2.6%), and the fat content was almost doubled (3.2%).

Table 3.3 provides the proximate composition of pita bread samples. The results showed that fortification levels of 10 and 20% of chickpea and FDDG individually, or as a combination of the two ingredients, resulted in significant increases in protein, fat, ash, and TDF contents while, moisture content and carbohydrates content were reduced.

##### **3.3.1.2 Proximate analysis of pita bread**

###### **3.3.1.2.1 Moisture content**

Table 3.3 shows that as the fortification levels of chick pea and FDDG increased, moisture content in the pita bread, decreased. Control pita bread with all-wheat flour had the highest level of moisture while the breads containing 70% wheat flour showed the

lowest moisture content (30%). Other workers have reported reductions in moisture content in baked goods such as naan breads, cookies, and pizza fortified with DDG (Staudt and Zeigler, 1973; Ahmed 1997; Arra, 2011; Tsen et.al,1983; Maga and Van Everen,1988; Parmar, 2012; and Saunders et.al, 2014). Differences in the initial moisture levels in the ingredients may explain this phenomenon. Initial ingredient moisture content of FDDG was 7.2% while All Purpose flour had a moisture content of 12%. The reduction of pita bread moisture content could be also due to the high protein and fiber content of FDDG. FDDG fiber content was 30.9% when compared to that of APF (5.24%), and FDDG protein content was 31.0% where as that of APF was 12%.

In the present study, an increase in Chickpea supplementation led to a decrease in pita breads moisture content. This result is consistent with earlier reports (Shehata et.al, 1970, Hefnawy et.al, 2012). The decrease in moisture could be attributed to the inherent low moisture content of chickpea flour (8.6%), compared to the wheat flour (12%). It could be also due to the high fiber content of chickpea flour which was (21.1%) when compared to APF (5.24%), and CP protein content was (22.3%) where APF was (11.95%). Several studies have reported that high fiber content flour would lead to higher absorption of free water, thus decreasing the moisture content of the final baked product (Kurek & Wyrwiesz, 2015; Parmar,2012; Dreese and Hosenev 1982). Incorporation of dietary fibers to food products such as bread imparts functional properties such as increased water holding capacity (Sivam, Sun-Waterhouse, Young Quek, Perera, 2010). This mechanism may lead to reduced pita bread moisture content owing to greater non-gluten ingredients like fiber and protein that tie up moisture in the final product.

### 3.3.1.2.2 Fat content

Table 3 shows that, in general, there were significant differences between the fat content of pita bread. Overall however, fat content was less than 1% in the pita breads and ranged 0.11% to 0.28% on a dry weight basis. This low-fat content shows pita bread to be an inherently low fat food entrée in accordance to FDA labeling regulations.

Results showed that since FDDG had higher fat content than chickpea (table 3.3) pita bread with FDDG generally was higher in fat content in comparison to the pita bread having chickpea as an ingredient. All treatments, with the exception of 10% CP pita breads, were higher in fat content in comparison with the all-wheat control pita bread.

It is thus shown that as DDG fortification level increased, fat content increased correspondingly. These results agreed with findings of previous researchers who fortified different types of food items, breads, and different baked products (cookies, Naan, Lavash, pizza, and steamed bread) with different levels of DDG. (Joseph et.al, 1988; Arra,2011; Pourafshar, 2011; Parmar, 2012; Tsen et.al, 1983). The phenomenon of increased fat content may be due to the initial higher fat content occurring in the DDG (5.10%) compared to all-purpose flour (1.89%). Another reason for this perhaps was the lower level of gluten in the dough network which contributed to reduced interactions of protein and lipid and reduced fat retention in dough compared to that of the control sample (Pourafshar, 2011). The result of our study demonstrated that incorporating chickpea flour into wheat flour increased fat content as well. Similar results were concluded by (Yousseff et.al, 1976; Hallab et.al 1974; Dhinda et.al, 2012). Chickpea flour was endowed with higher fat content (3.2%) than the all-purpose flour (1.89%).

### 3.3.1.2.3 Total Dietary Fiber Content

Table 3.3 demonstrated that all pita bread samples were found to be significantly different from each other in TDF content. With a range of 5.21g-17.44g/100g, it can be concluded that as the fortification level increased, TDF% increased as well. Fortification with 10%D yielded double the amount of TDF (7.21%). And fortification with (20%D) increased amount of TDF by two and half times (13.05%) when compared to control (5.21%). Similar result where found by Li et.al, 2016 in an unpublished paper where they fortified steamed bread with FDDG. Fairly similar results were reported by different researches where they fortified different types of food items, breads, and different baked products with different levels of DDG. (Joseph et.al, 1988; Arra, 2011; Pourafshar, 2011; Parmar, 2012; Tsen et.al, 1983; Wu et.al, 1987). These workers reported increased Neutral detergent and crude fiber levels at the higher substitution levels of DDG. This was because DDG had higher fiber levels compared to the all-purpose flour itself. It was also concluded in our result that as the fortification level of chickpea. Fortification with (10%CP) increased the TDF by 50% ratio (7.21%), where fortification with (20%C) has doubled the TDF content (11.74%) when compared to control (5.21%). Similar results were concluded by different study in the literature when they fortified different types of breads with chickpea flour (Yousseff et.al, 1976; Hallab et.al 1974; Dhinda et.al, 2012). The reason behind increased TDF is that both chickpea and FDDG fiber content were higher (21.10%) and (30.90%) when compared to control (5.24%).

#### **3.3.1.2.4 Protein**

From Table 3.3, it can be observed that there were significant differences in protein content among all treatments when compared to the all-wheat control. It was noted that as the level of FDDG in the pita bread increased, the protein content of the pita bread also increased. These results agreed with results from several studies where they fortified food items, particularly, breads, and different baked products with different levels of DDG (Joseph et.al, 1988; Arra, 2011; Pourafshar, 2011; Parmar, 2012; Liu et.al, 2011; Tsen et.al, 1983; Li, Wang, and Krishnan, 2016 unpublished paper. This increase occurred owing to the fact that DDG has almost three times the protein content (31.0%) when compared to all-purpose flour (12%). It was also found in our current study that as chickpea fortification level increased, protein level increased as well. The results are in agreement with the work of others (Eissa et.al 2007; Yousseff et.al, 1976; Hallab et.al.,1974; and Dhinda et.al, 2012). These workers showed that the increase in protein content was the direct result of the appreciably higher protein content of chickpea flour in foods.

#### **3.3.1.3 Amino Acid Evaluation**

Amino acid analysis was done by HPLC and post column derivatization method AOAC Official Method 982.30 E (a, b, c), chp. 45.3.05, 2006.

The amino acid score was calculated using the ratio of the amount of the limiting amino acid in the food to the same amount of the corresponding amino acid in the reference diet multiplied by 100. The scoring pattern suggested by the FAO/WHO was used for this purpose (FAO/WHO, 1985). The different amino acids recovered were

presented as g/100g protein. The amino acids scores were calculated according to the method of Abou Arab et al., (2010) and Chavan, et al., (2001).

$$\text{Amino acid score (\%)} = \frac{\text{mg of amino acid in 1 gm of test protein}}{\text{mg of amino acid in 1 gm of reference protein}} * 100$$

Table 3.4 shows that both chickpea flour and FDDG had higher amino acid content when compared to all-purpose flour. When comparing chickpea to FDDG it was found that FDDG is higher in all of the amino acids except for lysine where it was higher in chickpea. Lim & Yildirim-Aksoy, (2008) reported that DDG composition is good in amino acids but it is deficient in lysine and methionine. Also, according to the literature, pulses including chickpea are a high value crop, that are rich source of lysine (Tulbek, 2006).

Table 3.5 provides amino acid content and amino acid scores of controls and six treatments. The results showed that lysine was the first limited amino acid in control as well as all other 6 treatments. Fortification with 10% FDDG improved amino acid scores by 15%, where fortification with 20% FDDG improved by 22% (over Control wheat flour pita). Also, fortification with 10% chickpea improved amino acid scores by 20%, where fortification with 20% improved amino acid scores by 28%. This improvement can be due to the fact that chickpea has a higher amount of lysine when compared to FDDG. Previous research findings reported that cereal storage proteins like maize, wheat, and rice are deficient in amino acids such as lysine and methionine while legumes lack the sulphur amino acids such as Methionine and Cysteine.

Our findings are in agreement with Arab et.al, 2010, who fortified spaghetti with chickpea flour (10,15,20,25, & 30%). They concluded that chickpea flour compared to wheat flour were higher in amino acid composition. The authors found out that as

chickpea fortification level increased in spaghetti, the amino acid scores increased correspondingly.

Our findings were also in agreement with early findings reported by Hefnawy et al, 2012. These workers fortified flour with chick pea flour which resulted in increased lysine content (Zhu et al., 2010).

Parmar, (2012) fortified pizza crust with ddg and found that incorporating pizza with ddg increased amino acid content. It can be concluded that fortifying wheat flour with food grade DDGS and chickpea flour will improve amino acid profile.

It can be concluded also that amino acids scores were improved by different fortification levels of either chickpea or FDDG or combinations of the two ingredients. The amino acid deficiencies in wheat could be enhanced by combining wheat flour with other ingredients that are rich in the missing amino acids.

The combination of legume with cereal-based products could be an option for expanding the intake of legume consumption. Moreover, legume proteins are rich in lysine and poor in sulfur containing amino acids, while cereal proteins lack lysine, but have sufficient quantities of sulfur amino acids. Thus, the mix of grain with legume proteins would provide amino acid balance and to combat the world protein calorie undernourishment problem (Yousif & Safaa, 2014). It has been demonstrated that it is promising to utilize chickpea flour and food grade DDGS to partially replace wheat flour in the expansion of bread and perhaps other food products. The substitution rate may be experimentally determined for every situation relying upon the sort of bread or food product as well as the pursued goal of the study.

Cereals are one the most consumed foods all around the world. They are inexpensive sources of energy and protein, and because of their moderate prices many people can afford to buy them. But the problem with most baked products, especially those in which wheat flour is used, is that many nutrient components, such as minerals, vitamins, and fiber can be lost due to milling. Also, another problem is that cereals are deficient in some of the essential amino acids such as lysine and threonine. To overcome these problems, fortification is the solution. This solution will help people receive more nutrient components it is important to add nutrients (i.e. fortify) to cereal products.

Fortification of flours and their products is one way to achieve that goal. In order to add value to these products, alternative grains can be used as well. Various cereal grains have many health benefits and nutritional components, so their flours can be used as alternatives in for production of different products. Another source of fortification can be co-products from cereal grain processing, such as DDGS, as well as the legume chickpea which is high in protein and fiber. The combination of these three ingredients will make up a more complete protein meal. Most of these fortification sources are relatively inexpensive, so improved or altered flours may be an effective way for people to consume more nutritious foods.

### **3.3.2 Physical analysis**

#### **3.3.2.1 Water activity**

Water activity is defined as the ratio of the vapor pressure in a food sample to the vapor pressure of pure water (Fennema, 1996). One of the important factors for analyzing water activity is a homogenous distribution of flour blends.



Table 3.6 shows significant differences in water activity levels when comparing control to all treatments. The water activity scores ranged between (0.41-0.57). The highest value for water activity was found for control and the lowest water activity was found in treatment 6 (20%D-10%C). These scores fall within the accepted range of flour moisture which is according the aqualab water activity meter (0.40-0.50).

In our study chickpea and FDDG had significant effects on water activity. Our results showed that the higher the fortification levels of substitutions caused significant decrease in water activity. This may be due to higher protein levels in the flour blends that resulted in a significant decrease in water activity levels, as water binds to the protein (Arra, 2011). It can be also due to higher fiber level in the flour blends. Soluble fibers have water holding capacity which make it hold water and make it less available (Frost, Adhikari & Lewis, 2011). In contrast, a study by Liu et.al, (2011) found that the water activity of corn breads fortified with different levels of DDGS did not change with the addition of DDGS.

### **3.3.2.2 Color profile**

Color values contribute to the appearance of food products that is considered as one of the most important properties in sensory evaluation in addition to consumer acceptability, adaptability, and preference. Color change is one of the quality indicators for protein-based cooked materials in the food and feed industries (Brown et al., 2015). Fortification of flour may affect sensory qualities such as (color, taste, as well as smell) if it is not implemented appropriately. Different raw materials used for fortification of wheat flour can affect flour color, which may have a great impact on the color of the final product.

Chickpea and DDGS are ingredients which may have a positive or negative impact on finished products. Table 3.6 shows that the color values obtained for control and all the flour blends. The comparison showed the effects of varying substitution levels of DDGS and chickpea flour in the wheat flour. All treatments yielded significantly different color values from each other. It can be observed that increased level of DDGS resulted in decreased brightness and increased yellowness of the flour blend. Similar results were obtained by (Saunders, 2008; Arra, 2011; Maga and Van Everen, 1988, Parmar, 2012; Li, Wang, & Krishnan, 2016 unpublished paper). Redness value was found to be lowest with the highest DDGS substitution level. Similar results were found by (Li et.al, 2016 unpublished paper). In contrast, Maga and Van Ever (1988) reported increased redness with the increased level of DDG in pasta flour due to the higher level of pigmentation associated with DDG.

It can be observed that increased level of chickpea resulted in decreased brightness and yellowness, but increased redness value. Similar results were reported by Esmat et. al (2012) when they produced fortified wheat flour spaghetti with different processed chickpea flours (10, 15, 20, 25 and 30 %).

### **3.3.3 Rheological properties**

Rheology is defined as the study of flow and deformation of materials. It uses a well-defined deformation (strain) on a material over period of time to measure behavior of material (stress). Traditionally, dough quality was evaluated manually by bakers using a number of methods (Darly-Kinelspire 2013). A rheological knowledge of wheat flour is essential for a high-quality end product. The Farinograph and the Mixograph are commonly used instruments in the study of dough rheology.

The Brabender Farinograph developed in 1930 is the most widely used instrument for studying dough rheology. A number of parameters can be obtained from the Farinograph curve (Farinogram) such as flour water absorption, dough development time, mixing stability and Mixing Tolerance Index (MTI). The Farinograph has a constant mixing speed and temperature during operation. Water absorption (WA) is important since it quantifies how much water needs to be added to the flour to form dough with optimum consistency. It is expressed as a percentage of the flour weight. The optimum consistency of the dough is defined by the moment the middle of the mixing curve reaches the 500 Farinograph Units (FU) line. The arrival time is the moment the mixing curve first crosses the 500 FU line and the departure time corresponds to the moment when the mixing curve drops below the 500 FU line. The time that elapses during the arrival and the departure time is called dough mixing stability. Mixing Stability (Stab) is measured in minutes. The peak time or development time corresponds to the time at which the mixing curve reaches its maximum. The MTI is another parameter that is obtained from the farinogram. It is measured as the difference between the dough consistency at peak time and the dough consistency five minutes after peak time. It is an indicator of dough strength. The lower the value of MTI, the stronger is the dough. Flours with good bread making characteristics usually have a higher water absorption, long dough development time and good resistance to mixing. The Farinograph is often used to assess the extent to which new ingredients affect the rheological properties of dough (Ozcan, 2009; Ozturk et al., 2009; Komlenic et al., 2010).

As compared to Farinograph, the Mixolab is a newer instrument developed by Chopin Technologies. The latter can work at variable temperatures enabling the study of

mixing and pasting of the dough. (Le Burn and Dubat, 2006; Koksel et.al, 2009; and Darly-Kinelspire, 2013). A typical Mixolab output consists of 5 stages: Development C1, protein reduction C2, starch gelatinization C3, amylase activity C4, and starch gelling C5.

The first stage corresponds the dough formation and development, and ends when the curve reaches peak, which corresponds to the optimum dough consistency. This peak is called C1 and corresponds to a torque of  $1.10(\pm 0.07)$  Newton meter (Nm). The second stage corresponds to the protein weakening which occurs because of the dual action of mixing and heating. The breakdown stage ends with C2, the lowest point of the Mixolab curve. This stage is used to evaluate protein quality. The rate of breakdown of the protein network is quantified by alpha, the slope of the curve. The increase in consistency observed during the 3<sup>rd</sup> stage is due to the swelling of the starch granules. The 3<sup>rd</sup> stage which ends with C3; beta is the gradient of the curve between C2 and C3. The 4<sup>th</sup> stage characterized by a decrease in the dough consistency; gamma, the slope curve estimates the gel stability and the alpha amylase activity in the dough system. This stage ends with C4. Finally, the 5<sup>th</sup> stage measures starch retardation. The final torque of the test is C5. Because the Mixolab is a fairly new instrument, there are a limited number of studies available in the literature that have employed the instrument. Several studies showed that Mixolab was useful in measuring the effects of different additives in dough rheology (Pourafshar, 2011; Arra, 2011; Darly-Kindelspire, 2013).

A Mixolab gives additional information on flour performance during the entire bread making process including phases of heating and cooling. The Mixolab can provide information on the baking performance differences based on starch- protein interaction, enzyme activity, environmental factors and gelatinization (Saunders et.al, 2007:2014).

The Kieffer rig, burst rig, and Tug fixture are all attachments that can be mounted on the Texture analyzer TA.XT. Plus and can perform different rheological test for both dough and final product. The SMS/Kieffer rig is a test for dough and gluten extensibility. It offers an effective simple test than the traditional extensibility test done by Extensigraph (Darly-Kinelspire,2013).

Burst Rig is an attachment to the texture analyzer that allows the evaluation of the extensibility and strength of the baked product. The final product should have a balanced burst force and extensibility, but still needs to break easily during chewing.

The Tug Fixture is an attachment of the Texture Analyzer. With the use of the Tug fixture, the bread tug tests for extensibility uses TA-226 Tug Fixture to conduct tests on four different varieties of bread to measure each product's extensibility and resistance to tearing.

### **3.3.3.1 Farinograph results**

#### **3.3.3.1.1 Water absorption**

Water absorption in baking industry gives the baker an idea about the water requirement for optimal dough production. Dough is made by adding water to the flour and subsequent mixing. It is a very important parameter for the bakers. Water absorption gives an idea about estimated yield to the bakers.

Table 3.7 provides Farinograph data on wheat doughs prepared with varying levels of DDG and chickpea. It can be observed from Table 3.7 that water absorption was found to be significantly different for the control in contrast to all FDDG or chickpea treatments. Figures 3.1 and 3.2 from our results shows that water absorption by the dough has a strong positive direct relationship with protein ( $R^2 = 0.88$ ) and fiber ( $R^2 = 0.98$ )

contents in the flour. As the fiber and protein levels increased, the water absorption increased as well. Hence, as the amount of DDGS increased, the water absorption increased. This phenomenon is in agreement with other published studies (Tsen, et.al 1983; abbot, 1986; Krishnan and Darly-Kindelspire, 2013; and Roth et.al, 2016). These findings suggest that proteins and fibers exert high water holding capacity. Therefore, more water is required to hydrate the blend (Krishnan and Darly-Kindelspire, 2013). The addition of protein ingredients to baked products also impart additional functionality such as dispersibility, swelling, water holding, gelation, and viscosity (Saunders et.al, 2013). Also, since DDGS is a fibrous material, many studies have concluded that adding different fiber sources to wheat flour increased water absorption (Roth, Döring, Jekle, & Becker, 2016; Saunders et.al, 2013; Sivam, Sun-Waterhouse, and Quek, & Perera, 2010)

Table 3.7, in our study also showed that water absorption increased with increasing levels of chickpea flour in the dough. Similar findings were noted by other researchers (Hefnawy, et. al 2012; Abou Arab, et.al 2010; Mohammed, 2012; Sabanis et.al, 2006; Eissa et.al, 2007; Dhinda et.al, 2012). Eissa et.al (2007) who fortified Balady Egyptian bread with chickpea flour found that addition of raw chickpea flour mainly increased the flour water absorption. The differences in water absorption are mainly caused by the greater number of hydroxyl groups which exist in the fibrous structure allowing more water interaction through hydrogen bonding. Another reason for water retention is that raw legumes flour contains more fiber, sugars and higher protein content (Eissa et.al 2007) compared to all-wheat flour. Hefnawy et.al, (2012) tested the impact of adding chickpea flour to wheat flour on the rheological properties of toasted bread. Their results showed that water absorption increased with increasing levels of chickpea flour

ratio in the dough. Dhinda et.al, (2012), who tested the effects of several ingredients on the rheological, nutritional and quality characteristics of high protein, high fiber and low carbohydrate bread also reported similar findings. These workers fortified wheat flour with SPOBCP blend (soy protein, oat bran, and chickpea) in different fortifications levels. They demonstrated that increasing the amount of SPOBCP in the blend significantly increased water absorption. The increase in the water absorption of the dough can be attributed to the increase in the protein and fiber contents in the blends. It was noted that the higher the number of hydroxyl groups existing in the fiber structure, the greater is the interaction by hydrogen bounds (Dhinda et.al, 2012). Hence, the higher flour moisture absorption. Similar results were reported by Sabanis et.al (2006) when they fortified durum wheat flour with chickpea flour and tested the characteristic of lasagna dough. The components of chickpea flour are hydrophilic, so they allowed the water content of the product to decrease and minimize the losses in cooking, thus improving the yield in the product (Sabanis et.al, 2006). Dodok et.al, (1993) investigated the importance and utilization of chickpea in cereal technology. They found that water absorption, in their study, increased as the amount of chickpea flour increased. In this study, pasta was fortified with chickpea flour and the functional properties of dough were evaluated. According to Kaur and Singh (2005), and Amon et.al, (2014) flours with more hydrophilic groups such as polysaccharides absorb more water. Therefore, the higher water absorption capacity of chickpea fortified flour could be attributed to the presence of greater amounts of hydrophilic constituents in them. The inherent proteins in chickpea flour may also have played some role in the higher water absorption capacity (Abou arab et.al, 2010). Hallen et.al, (2004) found a correlation between the flour water absorption

and increasing level of cowpea flour. According to their study, the water absorption capacity increased at lower moisture content, higher bran content, higher protein content, higher pentosan levels, higher damaged starches, and higher enzymatic activity. Their results also showed that at higher protein content due to increased fortification level, water absorption also showed an increase. A plausible reason for this phenomenon is that legumes generally contain more proteins than cereals. Approximately 70-90% of dry bean protein are water soluble, whereas gluten, the major fraction constituting approximately 80-90% of total wheat flour protein, are water insoluble. The higher water absorption of the composites could therefore, be explained by the higher water absorption of the legume (Hallen et.al, 2004).

Yousseff et.al, (1976), however, showed that substitution of wheat flour with different chickpea flour levels reduced water absorption. According to them, water absorption is generally related to the hydration capacity of protein. Gluten had the strongest imbibition power compared to protein from other sources. Replacement of wheat flour with chickpea flour, which is gluten free, resulted in decreased water absorption despite the elevated protein content (Yousseff et.al, 1976). Rawar and Darappa, (2015) investigated the effect of ingredients on rheological, nutritional and quality characteristics of fiber and protein enriched baked energy bars. Their results showed that substitution of 0 to 75% of brown flour with protein rich flour decreased the water absorption. This indicated lower water binding capacity of protein rich ingredients when compared to gluten protein. Luz Fernandez and Berry (1989) studied the rheological properties of flour and sensory characteristics of bread made with germinated chickpea. They found that addition of chickpea flour to wheat flour led to decreased



water absorption. The results suggested that water absorption maybe related to the type rather than the quantity of protein, and will vary depending on the legume used for substituting wheat flour (Luz Fernandez and Berry,1989).

Our results are similar to the studies that report on increased water absorption with increase in the fortification levels of fiber and proteins. Many studies have demonstrated that water absorption increased with the addition of fiber although the data were usually obtained using a Farinograph or Mixograph. Such results could be due to the hydroxyl groups in the fiber structure, which allowed more water interactions through hydrogen bonding (Gmomez et.al, 2003). Almeria et al (2010) showed that increased fiber in the wheat flour brought about increased water absorption. They observed that the addition of different fiber sources in wheat flour increased the water absorption in the dough. This is due to the high water-holding capacity of most fibers.

Many studies have also concluded that the increased water absorptions could be attributed to increased total protein and pentosan content, as well as ribose and deoxyribose as it refers to RNA sugars (non-starch polysaccharides) (Sathe et al., 1981; Fernandez and Berry, 1989; Narpinder et al., 1991; Shahzadi et al.,2005; Collar et al., 2007; and Anton et al., 2008). An increase in water absorption, following incorporation of various vegetable protein concentrates or isolates to wheat flour, has also been reported by other researchers (Mohammad et.al, 2012) who attributed the water absorbing capacity of these protein preparations to their ability to compete with other constituents in the dough system for water. The ability of these proteins to absorb high quantities of water resulted in doughs that exhibited increased farinograph water absorption values. Hence, the quantity of added water is considered to be very important

for the distribution of the dough materials, their hydration and the gluten protein network development.

### **3.3.3.1.2 Dough Development Time**

Dough formation occurs when the flour protein (glutenins and gliadins) are hydrated and form a cohesive mass, which is a protein composite commonly referred to as gluten.

Dough development time (DDT) or peak time in minutes indicates the stage where the dough reaches maximum viscosity before the gluten start to break down, which is the highest point of the curve. It can be observed from Table 3.7 that dough development time of the control and treatments were significantly different. It can be observed that DDT has a direct positive relation with proteins and fiber content in the flour. As the fiber and protein level increased the DDT increased as well. Similar results were also reported by Krishnan and Darly-Kindelspire (2013) and Roth and coworkers (2016).

In our study, the time required for the control dough to reach 500BU consistency was also modified by the addition of chickpea. During this phase of mixing, the water hydrated the flour components and the dough was developed. DDT was significantly ( $P < 0.05$ ) higher when the ratio of chickpea to wheat flour was greater than the control. Similar results were reported by Sabanis et al. (2006) when they fortified durum flour with chickpea flour and tested the characteristic of lasagna dough. They demonstrated that the inclusion of chickpea flour delayed Farinograph development time.

Strong flours are characterized by long DDT, high stability with a small degree of softening, and high F.q.n, while poor flour weaken quickly, resulting in low quality number of F.qn. It is known that the proteins of leguminous flour are made up of albumin and globulin. In chickpea flour, legumins are the main storage proteins. So a flexible

network begins to form, but it's quality is not as good as that of gluten protein (Sabanis et.al, 2006). The deterioration in farinograph characteristics with the higher levels of chickpea flour supplementation was due to the fact that chickpea flour is gluten-free. The amount of gluten decreased as the concentration of chickpea flour in wheat flour increased (Sabanis et.al, 2006).

Eissa et.al (2007) fortified balady Egyptian bread with chickpea flour and found that chickpea addition increased the DDT dough development time. Rawar and Darappa, (2015) observed similar results of increased DDT for energy bars when substituting 50% BF with PRIM. This indicated that there was a delay in the development of gluten in the presence of PRIM.

Dhinda et.al, (2012) tested the effects of ingredients on rheological, nutritional and quality characteristics of high protein, high fiber and low carbohydrate bread. They fortified wheat flour with SPOBCP blend (soy protein, oat bran, and chickpea) in different fortifications levels. They demonstrated that increasing amount of SPOBCP in the blend significantly increased DDT. The increased DDT could be explained due to the interaction between non-wheat protein, fibers and gluten leading to a delay in hydration and development of gluten in the presence of these ingredients (Dhinda et.al, 2012). In contrast, Luz Fernandez and Berry (1989), Dodok et.al, (1993), Hefnawy et.al, (2012) found that DDT decreased as the amount of chickpea flour increased by increasing the chickpea proportion.

The increase in DDT resulting from chickpea addition could have been due to the differences in the physicochemical properties between the constituents of the chickpea and those of the wheat flour. Higher chickpea substitution levels weakened the gluten

network during the kneading. This is attributed to an intense incompatibility between the protein of chickpea and wheat gluten protein. It was assumed that increasing chickpea flour in the blends, increased the energy requirements for the optimal development of dough consistency which in turn, led to increased requirement for mechanical agitation of non-gluten proteins in the dough system through the chickpea proportion. One other reason for the weakening of dough strength was explained by addition of vegetable protein addition. The substitution of gluten proteins by the non-gluten-forming vegetable proteins caused a dilution effect and consequently weakened the dough. This conclusion is consistent with the results of studies by Roccia et al. (2009) who found that the substitution of wheat protein by soy protein decreased mixture elasticity, indicating dough network weakening. One other reason for the weakening of dough strength resulting from vegetable protein addition could stem from the fact that the substitution of gluten proteins by the non-gluten-forming vegetable proteins causes a dilution effect and consequently weakening of the dough. (Mohammed et.al, 2012).

#### **3.3.3.1.3 Dough stability**

The points between the arrival and the departure time on the 500 Brabender Units line on the farinogram is defined as dough stability in the farinograph. Figure 3.3 shows a typical farinogram profile. Dough stability is measured in minutes. In general, dough stability value is an index of the dough strength. Higher values indicate stronger dough. Dough stability can be affected by the amount of substitution of different types of ingredients into the dough flour.

From table 3.7, it was observed that all treatments compared to the control had significant ( $P < 0.05$ ) differences between them, and had significantly lower dough

stability than the control. Stability of treatment blends ranged from 4.7 to 7.2 min, where the control had a stability of 8.8 min. Roth et.al, (2016) studied the mechanism behind DDG grains and its impact on wheat dough and bread quality. They reported that an increased fraction of DDG into wheat flour decreased dough stability due to the competition of fiber for free water leading to incomplete hydration of starch and gluten and thus causing weakness during processed dough development.

Parmar (2012) found no significant differences in dough stability when wheat flour was fortified with 15% of soy protein-DDG blend while a 5%-10% of substitution levels increased stability, and more than 15% decreased dough stability. The probable reason could be that protein present in DDGS and/or soy protein may have interrupted the native structure of wheat protein (gluten) which may have led to increased dough stability.

Hefnawy and coworkers (2012) had reported a decrease in dough stability with increase in the chickpea proportion to 15 and 30%. This weakening was a result of the breakdown of gluten network after elapsing of appropriate time. The latter is consistent with our findings. Protein in the wheat flour-chickpea mixture was of a low functional quality because of its deficiency in gluten and therefore the dough weakening potential was increased (Hefnawy et.al, 2012). Dodok et.al, (1993) found that dough stability decreased as the amount of chickpea flour increased with increase in the chickpea ratio. Mohammad et.al (2012) found that dough samples containing 10% chickpea exhibited higher stability and resistance to mechanical mixing value than the control, while it decreased as the substitution level increased from 20-30%. In general, the stability value is an index of the dough strength, with higher value indicating stronger dough. The increase in stability time was related to the amount of the substitution. The reduction in dough stability with

the higher chickpea substitution level demonstrated to weaken of the gluten network during the kneading (Mohammad et.al, 2012). Rawar and Darappa, (2015) studied the effect of ingredients on rheological, nutritional and quality characteristics of fiber and protein enriched baked energy bars. These workers fortified the control brown flour (BF) (blend of refine wheat flour and whole wheat flour in the ratio of 50:50) with a blend of PRIM flour (chickpea, sesame flour, soya protein isolate, and whey protein concentrate). Their results showed that substitution of 0 to 75 % BF with PRIM decreased the dough stability. The decrease in the stability value could be due to dilution of gluten. Luz Fernandez and Berry (1989) studied the rheological properties of wheat flour and sensory characteristics of bread made with germinated chickpea. They concluded that the addition of chickpea flour to the wheat flour resulted in reduced dough stability.

Some published results are in agreement with our findings. Shehata et.al, (1970) fortified wheat flour with chickpea and showed that there was a slight decrease in dough stability as the percentage of chickpea increased. Dhinda et. al, (2012) tested the effect of ingredients on rheological, nutritional and quality characteristics of high protein, high fiber and low carbohydrate bread. They fortified wheat flour with SPOBCP blend (soy protein, oat bran, and chickpea) in different fortifications levels. They demonstrated that increasing amount of SPOBCP in the blend significantly decreased dough stability time. The decreased dough stability time could be explained due to the interaction between non-wheat protein, fibers and gluten leading to a delay in hydration and development of gluten in the presence of these ingredients.

In contrast, Eissa et.al (2007), who fortified Balady Egyptian bread with chickpea flour, found that addition of raw chickpea flour mainly increased dough stability.

Hefnawy et.al, (2012) showed that dough stability increased with increasing the level of chickpea flour in the formula. Yousseff et.al, (1976) supplemented bread with parboiled and raw chickpea. They found that stability increased as the amount of parboiled chickpea increased. In the case of raw chickpea, dough stability did not change significantly when comparing to control bread (wheat flour) with different chickpea supplementation levels. It was concluded that as the chickpea flour increased, dough stability decreased.

#### **3.3.3.1.4 Mixing Tolerance Index**

The mixing tolerance index (MTI) is determined by taking the difference in Barbender unit (BU) between the peak time and 5 minutes after the peak time is reached. It gives an idea to the bakers about dough breakdown over a period of mixing. MTI is inversely proportional to the strength of the dough. Higher values of MTI indicate lower strength, lower dough stability and poor tolerance to mixing.

From table 3.7, it can be seen that supplementation of wheat flour with lower percentages of 10% FDDG and 10% chickpea did not impact mixing intolerance indices. Similar results were found by Krishnan and Darly-Kindelspire, (2013) and Parmar (2012) who concluded that there were no significant ( $P < 0.05$ ) differences that were noted in MTI when Alice flour, a strong bread flour, was fortified with different levels of DDG.

Fortification with higher percentages (20% and 30%) increased mixing tolerance index (MTI). The reason for increased mixing tolerance index (MTI) may be due to the dilution of gluten protein with the fiber content. This maybe also due to the interaction between fibrous materials and gluten, which in turn affects the dough mixing properties (Sudha et.al, 2007).

Rawat and Darappa, (2015) showed that replacement of BF (brown flour) with PRIM (chickpea flour, sesame, soya protein isolate, whey protein concentrate) mix resulted in increased mixing tolerance index (MTI) which indicated poor tolerance of the dough to mixing in the presence of PRIM.

In contrast to our results, Eissa et.al (2007), demonstrated that when they fortified balady Egyptian bread with chickpea flour, mixing tolerance index MTI decreased

Yousseff et.al, (1976) fortified wheat flour bread with parboiled and raw chickpea flour and found that mixing tolerance decreased as the amount of parboiled chickpea increased. In the case of raw chickpea, dough mixing tolerance was only slightly affected. Comparing control bread (wheat flour) with different chickpea supplementation levels, increased chickpea fortification level decreased mixing tolerance.

### **3.3.3.2 Mixolab results**

#### **3.3.3.2.1 Water absorption**

It was observed from table 3.7 that as the amount of FDDG increased in the mixture, there was an increase in water absorption of flour mixtures. This was due to the increased water binding capacity owing to the presence of DDGS, which requires additional water in order to soften and to be incorporated into a dough ball (Arra, 2011; Ahmed, 1997; Saunders et.al, 2014, Parmar 2012, Arra, 2011, Ahmed 1997, & Li et.al, 2016). In contrary to findings, Pourafshar (2011) demonstrated that water absorption was highest when there was no fortification of DDGS.

In the case of chickpea, it was also demonstrated in table 3.7 that as the amount of chickpea increased, the water absorption also increased. These results are in agreement with the work of Tulbek (2006), who demonstrated that water absorption value increased



with increased fortification with fermented chickpea flour in bread. In another study by (Dalgetty and Baik (2006), the fortification of bread with hulls and cotyledon fibers isolated from peas, lentils, and chickpeas led to increased water absorption even at 1 % level of chickpea fiber fortification. The increase could be due to the high amount of fiber material in chickpea and the high protein fraction of the dough. Another possible explanation could be due to increased hydrophilic groups in dietary fiber and their greater association with water molecules as described by Rosel and coworkers (2007). Similarly, wheat flour-bran blends with higher content of dietary fiber showed increased water absorption (Sudha et al., 2007). An increase in water absorption was observed with the addition of pea fiber in flour blends (Jia et al. 2011, Bojňanská et al. 2014, and Wang Initials, 2002). Higher water absorption capability of with dietary fibers can improve the water holding capacity of bread, which may contribute towards the freshness of the product (Li et.al, 2016).

#### **3.3.3.2.2 Stability**

Table 3.7 provides data on the stability of the dough as measured using the Mixolab, a second rheology instrument. It was observed that as the presence of FDDG increased, the stability of the dough decreased. Similar results were found by (Saunders et.al, 2014; Li et.al, 2016; Pourafshar, 2011). This could be due to the fact that DDGS contained no gluten proteins, to aide the wheat gluten network (protein) thus resulting in the dough system having reduced stability (Saunders et.al, 2014). In contrast to our results Krishnan and Darly-Kindelspire, (2013) demonstrated that there was no significant difference in dough stability between control (100% wheat) and ddg fortified (5, 10, &15%) wheat flour.

Dalgetty and Baik, (2006) fortified bread with hulls and cotyledon fibers isolated from peas, lentils, and chickpeas. Dough fortified with chickpea hulls had lower stability than the control dough. Tulbek et al (2006) noted that an increase in the amount of fermented chickpea resulted in increased dough stability. The increase in stability could be attributed to the high protein content. (Chevan et.al, 1986; Tulbek, 2006).

#### **3.3.3.2.3 Dough Development Time (DDT)**

Results from our study (table 3.7) showed that as FDDG level increased in the formula, DDT increased as well. Similar result were reported by Parmar (2012) and Pourfshar (2011).

The explanation for increased DDT may be due to the increased amounts of fiber and protein that were added to the flour from FDDG and chickpea. Dough Development Time has a direct relation to the amount of fiber and protein present in the dough (Almedia et.al, 2010; Parmar 2012).

In contrast to the results obtained in our study, Tsen and coworkers in 1983, observed that with replacement of flour with 10 to 20% DDG, there was a reduction in dough development time.

Also in contrast, Li et.al (2016) concluded that DDVT decreased with increased DDGS fortification level in dough developed for steamed bread. The development time and stability of the dough reflect the strength of the protein network structure in the process of dough mixing (Rosell et al. 2010; Bojňanská et al. 2014). The downward trend in dough development time and stability indicated that the addition of DDG weakened the gluten strength, decreased endurance to mixing, and contributed to difficulty in forming a continuous gluten network. Incorporation of legumes and soluble

fibers showed inconsistent changes in mixing and developing time of the dough. Fortification required longer mixing and development time than the control (Dalgetty and Baik, 2006).

In our study, as the amount of chickpea fortification increased, the dough development time also increased. These results are in agreement with Tulbek, (2006), who demonstrated that DDVT value increased with increased fortified bread with fermented chickpea flour due to the interference in gluten development. Development time has direct relation to the amount of fiber and protein presented in the dough (Almedia et.al, 2010; Parmar 2012).

### **3.3.3.3 Texture analysis**

#### **3.3.3.3.1 Dough texture**

This rheological information provided by the Texture Analyzer are mainly dough extensibility (Ermax), and dough strength (Rmax). When the dough extensibility increased, dough strength decreased. Force (strength) and extensibility (distance) are inversely proportional to each other.

Significant changes in dough properties were observed for the bread flour blends in Table 3.8

Table 3.8 provides information about dough extensibility and strength required to break dough strand containing various ingredients (wheat, chickpea and FDDG). The force required to break the dough increased, whereas the dough extensibility declined as the fortification level of either or chickpea and FDDG increased. Similar results were found by (Arra, 2011; Parmar, 2012; Krishnan and Darly-Kindelspire, 2013). Substitution with

higher fiber and protein flour resulted in decreased extensibility and increased need for force to stretch the dough (Parmar, 2012).

In contrast to our results, Parmar (2012) fortified pizza with DDGS and concluded that as DDGS levels increased, dough strength decreased owing to an increase in fibrous material.

In bread making studies, researchers have reported that the fiber absorbs water to a greater degree than other particles and can prevent them from being fully integrated into the starch/gluten matrix which can in turn, affect dough texture. Parmar (2012) showed that as the extensibility decreased, the force required to stretch the dough was increased. This occurred primarily because of the high amount of fibrous material present in dough. Fiber addition was thus, not conducive for the formation of a gluten network.

The extensibility of dough is an indicator of the dough processing characteristics. Table 3.8 shows that increased chickpea flour supplementation decreased the dough extensibility. Fiber content was strongly inversely correlated to extensibility ( $R^2=0.93$ ). Protein content was also inversely correlated to extensibility ( $R^2=0.95$ ) (Figures 3.4 & 3.5). Eissa et.al, (2007) reported that extensibility values were greatly reduced by the addition of raw legumes flour. This reduction of extensibility can be due to the deficiency of gluten in chickpea flour protein. This indicated that the fortified dough was softer and weaker than the unfortified control wheat flour (Eissa et.al, 2007). In the absence of the strengthening effect, there was thus a gluten dilution effect that weakened chickpea fortified wheat doughs.

Buresova et.at, (2014) tested the relationship between rheological characteristics of gluten-free dough and quality of leavened bread. When they compared the wheat flour

control sample to chickpea fortified sample, they found that extensibility was significantly lower in the chickpea fortified sample. The authors attributed this to the absence of gluten in chickpea to provide support to the dough matrix. Mohammed and coworkers (2012) evaluated dough rheology and bread quality of wheat fortified chickpea flour blends. Decreased extensibility of dough was noted with increased chickpea fortification level.

Similar results were concluded by Sabanis et.al (2006) when they fortified durum flour with chickpea flour with the objective of evaluating characteristics of lasagne dough. Dough extensibility decreased with increasing chickpea flour levels. This weakening effect is the result of dilution of the durum wheat by the added chickpea protein. The speculated that the affects may also be accentuated by the presence in the chickpea flour of undesirable enzymes or constitutes that interact strongly with gluten proteins and thereby inhibit development of desirable rheological properties.

Dodok et.al, (1993) fortified wheat flour with chickpea flour and found that as the amount of chickpea flour increased, the extensibility of wheat dough decreased. Tulbek (2006) fortified bread with fermented chickpea flour and reported that increased chickpea flour incorporation into wheat flour decreased the extensibility of the dough. These researchers attributed this to weakening of gluten network by fermented chickpea protein, starch and lipids,

Results shown in Table 3.8 showed that with increase in chickpea flour supplementation the dough strength also increased. The ( $R^2$ ) values in figures 3.6 & 3.7 shows that there is a strong positive correlation between dough strength ( $R_{max}$ ), and fiber content ( $R^2 = 0.97$ ) and also between  $R_{max}$  and protein content ( $R^2 = 0.93$ ) content.

Similar correlation was also reported by Eissa et.al (2007) who fortified Egyptian balady bread with chickpea flour and found that incorporation of raw chickpea flour increased dough strength. The reason behind increased strength may likely be due to the interaction between polysaccharides and proteins present in flour blend. This explains why dough becomes harder in the presence of legume flour (Eissa et.al, 2007).

Buresova et.at, (2014) tested the relationship between rheological characteristics of gluten-free dough and quality of leavened bread. Tests were performed using a Texture Analyser TA.XT to compare the wheat flour control sample to chickpea fortified samples. These workerd determined that dough strength was significantly higher in the chickpea fortified samples.

Results reported by Mohammed et.al, (2012) are in agreement with our results when they tested dough rheology and bread quality of wheat-chickpea flour blends. They concluded that the dough strength increased with increased chickpea fortification levels. Sabanis et.al (2006) had similar results to ours where they fortified durum flour with chickpea flour and tested the characteristic of lasagne dough. Dough strength increased with increasing chickpea flour ratio. In the milling and baking industry, the extensograph is an essential tool in the production of flour of reproducible quality (Sabanis et al., 2006). Extensibility indicates the ability of the dough to extend during fermentation and gas production by the yeast. High extensibility values result in weak and slack dough which collapses during the proofing stage or while baking in the oven. In contrast to extensibility and strength results in our study, Shehata et.al, (1970) found that on fortifying wheat flour with chickpea flour, the extensibility and strength of dough were not affected to any extent.

### 3.3.4 Pita bread properties

#### 3.3.4.1 Physical properties of pita bread

##### 3.3.4.1.1 Color profile

Table 3.9 shows the main effects of the varied flour composition on the color properties of pita bread. The results indicated decreased L\* values (brightness), and increased a\*(redness) and b\*(yellowness) levels. Maga and Everen, (1989) reported results that were identical to our findings where they fortified whole wheat pasta with DDG. These workers demonstrated a decreased L\* values (brightness), and increased a\*(redness) and b\*(yellowness) levels in their pasta.

Hunter value L\* decreased as the quantity of FDDG increased, which means the product became browner and decreased in brightness. Similar results were found by (Saunders et.al, 2014; Rasco et.al, 1990; Brochetti et.al, 1991). As the level of FDDG increases, it caused Maillard reaction and caramelization during baking which contributed to browning (Saundres et.al, 2014).

Liu et.al, (2011) fortified cornbread with DDGS and found that as the DDGS supplementation level increased L\* level decreased. Also, a\* values significantly increased as the FDDG supplementation level in steamed bread increased, which indicated more redness in the product. The redness was attributable to initial red pigments present in FDDG rather than those from AP flour (Saundres et.al, 2014). Liu et.al, (2011), yielded results that are in agreement with our results when they fortified cornbread with DDGS, and they concluded that as the DDGS supplementation level increased a\* level increased. In contrast to our results, Rasco et.al, (1990), demonstrated that breads made with various types of DDG decreased hunter a\* value as DDG level increased.

Table 3.9 provides the yellowness –blueness value determined in colorimetric analysis. The b values indicate a range in yellowness and blueness. The b\* value significantly increased with increased FDDG supplementation level. Similar results were reported by Saunders et.al, (2014). Liu et.al, (2011), fortified cornbread with DDGS, however and they found that as the DDGS supplementation level increased, the b\* level decreased. Pita bread color was also affected by fortifying wheat flour with chickpea flour. As the supplementation level of chickpea increased, the bread become darker with decreased brightness (L\*). Similar results were concluded by Mohammed et.al, (2012), and Eissa et.al, (2007) when they fortified pita bread with chickpea flour.

The a\* and b\* values increased as chickpea flour increased, indicating a greater redness and greater yellowness of the pita bread. These findings are are also in agreement with work of Mohammed et.al, (2012)

The work of Eissa et.al, (2012) yielded results that are in agreement with our results. These workers fortified balady breads and biscuit with chickpea flour. They found that redness and yellowness of biscuit was increased with increased chickpea flour fortification. Redness of balady bread increased with 5% and 10%, but decreased with 15%. Redness of balady bread increased with 5% supplementation, but slightly decreased with 10% and 15%.

The darker color of bread may be due to Maillard reactions occurring during baking. In the Maillard reaction, reducing carbohydrates react with free amino acid side chains of proteins, mainly lysine that are present in chickpea flour, and lead to amino acid sugar reaction products (polymerized protein and brown pigments). (Hallen et.al, 2004; Mohammed et.al, 2012)



### **3.3.4.2 Rheological properties of pita breads**

#### **3.3.4.2.1 Pita bread texture analysis**

The texture of cooked pita bread was evaluated on the Texture Analyzer using a Burst Rig. The Burst Rig is an attachment to the texture analyzer that allows the evaluation of the extensibility and strength of the baked product. The final product should have a balanced burst force and extensibility, but still needs to break easily during chewing.

Table 3.9 provides Burst Rig data on the texture of pita breads. Pita bread with DDGS had less extensibility than the control. It was observed that higher DDGS substitution in pita bread had lower extensibility, and higher force was required to tear the pita bread. Similar results were reported by Arra, (2011) in relation to DDGS fortified chapatti and naan. It was also observed that higher chickpea substitution in pita bread had lower extensibility and higher force was required to tear the pita bread. Greater levels of the incorporation of chickpea and/or DDGS resulted in the greater fibrous material, which affected the dough rheology. This was visible in the final baked products. Pitas with chickpea and/or DDGS had lower extensibility. The latter were harder to break in comparison to the all-wheat control.

The Tug Fixture (TA-226) and the bread tug tests for extensibility were used in conjunction with the TA.XT Plus Texture Analyzer to evaluate the different varieties of bread for product extensibility and resistance to tearing. From table 3.9 and figure 3.8, it can be seen that there were significant differences in tear resistance between treatments. As the chickpea and FDDG fortification level increased, the tear resistance of bread increased as well. Bread extensibility decreased effect of most fiber has been previously

reported and connected to the diluting gluten content and crumb structure disruption encompassing an impairment in gas retention (Collar et.al, 2007).

Advanced instruments such as Farinograph, Mixolab, and Texture Analyzer remove the guesswork in estimation of optimal water content, mixing requirements while providing explanations for starch and protein interaction and other changes in the functional nature of the food constitutes (Krishnan and Darly-Kindelspire, 2013)

Protein and fiber constitutes in food adjuncts change the water holding abilities of dough owing to the competition for water in the food system. Such trade-offs are manifested as reduced dough volume, decreased dough stability, changes in machinability and also reduced eating quality. There is a need to balance the formulation to retain the desirable traits of sensory and rheology.

The difference in water absorption are believed to be attributed to the protein content of the flour. Both the quantity and quality are evaluated. Proteins which are naturally present in flour, including gluten forming proteins are able to absorb one to two times their weight in water. Therefore, slight changes in the protein content of flour can contribute to large differences in the water absorption of samples (Goldstein et.al, 2010)

The increase in water absorption is believed to be related to the presence of cellulose fibers. Cellulose fibers are able to hold many times their initial weight in water, and the hydroxyl groups present in cellulose fiber allows for more interactions with water through hydrogen bonding (Goldstein et.al, 2010)

Each type of fiber acts in the mixture differently and unexpectedly (Kučerová et.al, 2013). The changes in dough characteristics upon addition of chickpea flour are attributed to dilution of gluten forming proteins causing changes in dough. Competition

between chickpea and wheat flour proteins for water of hydration and variation in their hydration behavior due to differences in the nature of protein may be another reason for the changes in dough characteristics (Singh et.al, 1991). The rheological characteristics of wheat dough were mainly affected by the properties of the gluten protein network (Buresova et.al, 2014). A combination of good strength and good extensibility results in desirable dough properties. (Buresova et.al, 2014). A study by Sudha et.al, (2007) concluded that an increase in the dough development time indicates that an increase in fiber content in the blends slowed the rate of hydration and development of gluten.

The decrease of the dough extensibility and the increase of dough strength of extension for the pure wheat flour dough can be due to the increase of thiol groups or a sulfhydryl groups (SH), that oxidize the dough with oxygen through the mechanical action. The transformation of SH-bonds in disulfide bond (SS-bond) and this newly formed SS-bond contribute to the increased elasticity of the gluten and the dough (Mohammed, 2012). These effects may be accentuated by the presence of undesirable enzymes in the chickpea flour or constitutes that interact strongly with gluten proteins and thereby inhibit development of desirable rheological properties (Mohammed, 2012).

The addition of both chickpea and FDDG to wheat flour modified physical, chemical, and rheological properties of the dough and the final food products. Chickpea flour and FDDG had similar influence on the brightness of the end product. Chickpea addition increased the redness and at the same time decreased yellowness and DDGS addition decreased the redness but increased the yellowness. Fortifying wheat flour with DDGS decreased the brightness and redness, but at the same time it increased the yellowness of food products. Chickpea decreased brightness and yellowness, but

increased the redness. Combination of chickpea and FDDG function differently than when any one of them is added alone. Decreasing levels of water activity occurred as the FDDG and chickpea flour substitutions level increased. This increases the possibilities of slowing down microbial growth in the product thereby increasing the shelf life.

Fortification with chickpea and FDDG had different impacts of dough rheology. It increased water absorptions, dough development time, MTI, and decreased dough stability. It also increased dough strength and decreased dough stability. Fortified pita required a greater force for tearability as determined by the burst rig and the tug fixture tests. Burst distance and tug distance was also reduced by FDDG addition. These changes were due to the increased fiber and protein contents that increased with increased fortification level of both chickpea and FDDG. It is also believed that the difference in these parameters not only effected by the quantity but also the type of fibers and proteins.

This study demonstrated that bread of high fiber, high protein content can be prepared by partial substitution of wheat flour with chickpea flour, and or FDDG, or as combination.

Adding value to breads could be an excellent step in providing nutritional components to consumers. By adding certain nutrients, we can also change physical and chemical properties, the shelf life, the texture, and the production time of breads.

### **3.4 Sensory analysis**

Wheat-based pita breads were prepared employing ingredients incorporated in the following ratios: Control W (10 %), W:CP(90:10 & 80:20), W:D (90:10 & 80:20), and W:CP:D (70:20:10 & 70:10:20), and evaluated by a panel of 45 trained and untrained judges. Control all-wheat pita bread and 6 treatments blends having varied ratios of CP

and FDDG were scored using a 5-point hedonic scale. Blends containing a combination of the three ingredients were also used as treatment variables for pita bread production.

The purpose of this study was to study the changes in sensory attributes of wheat flour (W) pita breads that were enriched with varied proportions of chickpea (CP), and food grade distiller's dried grains FDDG (D).

Addition of new ingredients to the basic formulation of a food product may significantly enhance the nutritional value and sensory attributes of a product. The substitution of wheat flour with alternative flours is very common in baking products such as bread, cakes, etc. Flour can be fortified with many different macro and micro nutrients such as protein, dietary fibers, vitamins and minerals to enhance sensory qualities of a product (Jambrec et al., 2011).

Rawat and coworkers (2015) reported on the incorporation of a combination of grains and legumes that are high in protein and dietary fiber in order to derive multiple benefits in baked goods such as improved color, taste, aroma, texture, and overall acceptability as well as nutritional quality. Bread is a frequently used food item in the human diet and it is consumed on a daily basis. Therefore, this food staple can be enhanced employing a variety of bioactive ingredients that are beneficial for health. The researcher attempted to make a new nutrition-rich bread recipe without compromising the inherent physical and functional properties of bread. All-purpose flour (APF) was fortified with different amounts of FDDG and chickpea flour in the pita bread formulation. The effects of the formulations on bread quality and sensory properties were studied. A panel of forty five trained and untrained trained judges consisting of undergraduate and graduate students, faculty, and staff members of South Dakota State

University evaluated the Control Pita Bread and Pita bread made with 6 treatments blends enriched with 10 to 20% FDDG or Chickpea, or combinations of chickpea and FDDG.

The pita bread was evaluated for overall acceptability (color, aroma, texture and taste) and was carried out using 5-point hedonic scale rating 1-5 (1=dislike extremely, 2=dislike moderately, 3= neither like or dislike, 4=like moderately, 5=like extremely), where scores are defined as poor (1), fair (2), acceptable (3), good (4), and excellent (5) as far as indicating consumer acceptability. All samples were identified with three-digit random numbers and all samples were presented in a complete randomized order to panelist. All of the panelists were given a printed response sheets with the evaluation procedure instructed prior to the test. Freshly prepared pita bread samples were presented on paper plates and were cooled to room temperature (28.0 C) degrees C for about 20-30 minutes prior to sensory analysis.

The data from the sensory analysis of samples were subjected to Analysis of Variance (ANOVA) using Fisher's least significant difference (LSD) test. The results were calculated using the statistical tools of Microsoft Excel and listed in the table below:

Scores that are reported in the table 3.10 below are presented on a scale of 1 to 5. The lowest score awarded by panelists was 3.42, whereas the highest score was 4.30. Scores of 3, 4 and 5 were designated acceptable, good and excellent, respectively. Table 3.10 provides the sensory analysis data from the evaluation of control wheat pita and 6 treatments containing different levels of flour substitutions using chickpea and FDDG.

### 3.4.1 Color scores

Color is produced through a process of visual perception in the eyes resulting from the stimulation of the retina by light (wavelengths between 380 and 760 nm). Color is the foremost and most important sensory attribute that influences consumer preference and acceptance for any product especially in food products (Lori Walker, 2012). The sensory panel results showed that among all control and 6 treatments, treatments 1 (10%CP), treatment 3 (20%CP), treatment 5 (20CP-10D%), and treatment 6 (20D-10CP%) had pita bread receiving the highest color scores. These were the treatments containing chickpea. The presence of chickpea in the formula yield consistently higher color score when compared to control. This could be due to the fact that fortification with chickpea flour yielded a desirable salmon-white color. Hefnawy et al. (2012) reported that adding chickpea flour to wheat flour in toasted bread improved color acceptance as judged by their panelist. Similar results were reported by Fernandez and Beery (1989) who fortified bread with 10% chickpea flour. The authors found that chickpea fortified breads had higher color scores than the control bread. Similar results were reported by Yousseff et.al (1976), when they supplemented wheat flour bread with different ratios of chickpea flour. They found that as the chickpea fortification levels increased to 5%, 10%, and 15 % substitution levels, sensory scores for color also increased, where fortifying with a 20% ratio decreased in color score .This result indicated that wheat flour probably should not be replaced with higher than 20% of chickpea flour for acceptable quality as judged by the color of the product.

Others have found the opposite results. Hallab et.al (1974) studied the nutritional value and organoleptic properties of white Arabic bread supplemented with (10%, 20%, 30%, 40% &50%) of chickpea flour. They demonstrated that color scores decreased with increased chickpea flour fortification level in the final product even with the lower levels (10%, 20%) This is in contrast to our results where low level of chick pea fortification (10%, 20%) improved color score for acceptability.

Our results showed that the lowest color scores were observed in treatments 2 (10%D), and 4 (20%D). The color of bread reduced statistically significantly with the addition or increasing amounts of FDDG in the product. Treatment 2 with 10 % FDDG, and treatment 4 with 20 % FDDG gave a darker brown color to the bread which was not liked by the panelist. Similar results were reported by Rosentrater and Krishnan (2006) and Arra et.al (2009) where food grade DDGS was incorporated in many different food products such as white pan breads, flat breads and cookies.

It can be concluded that addition of chickpea flour to bread up to the level of 20% substitution improved the color of pita bread. Chickpea flour can be used alone or in conjunction with other ingredients such as FDDG to increase color/appearance acceptance of the bread.

### **3.4.2 Aroma**

Aroma is an intricate physiochemical process which requires aroma molecules to reach the olfactory bulb in the nose. Breathing air transfers the aroma molecules which interacts with the olfactory cells in the olfactory mucosa and stimulate a chemical sense which is perceived as aroma. Aroma has the ability for adaptation where one odor usually has little effect on perception however it can interfere with the perception of similar



odors. Various factors also affect aroma which includes age, gender, smoking and olfactory disorders (anosmia, hyposmia, hypersomnia and dysomia). (Lori Walker, 2012).

Data from Aroma scores in table 3.10 showed that treatment 1 (90W-10CP%) and Treatment 3 (80W-20CP%) had the lowest aroma scores of all treatments. Treatments 2 (90W-10-D), 5 (70W-20CP-10D%), and 6 (70W-20D-10CP%), with no significant difference, ranked intermediate. Treatment 4 and the control, with no significant difference, ranked highest in aroma score.

It can be concluded that breads fortified with FDDG alone did not influence the aroma relative to the control, whereas the blends made with FDDG and chickpea or with chickpea alone scored lower for aroma in contrast to the control. Pita breads made with FDDG alone had score above 4.0 while those made with chickpea alone received scores below 4.0. Similar results were demonstrated by Mohammed et.al, (2012) who tested dough rheology and bread quality of wheat-chickpea flour blends. Their results showed that as the level of chickpea flour increased in wheat flour, the aroma scores decreased. Dodok et.al, (1993) demonstrated the same results when they fortified wheat flour bread rolls with 10%, 20% chickpea flour. The breads fortified at either level (10 or 20%) had lower aroma scores than the control. In contrast, Fernandez and Beery (1989) who fortified bread with 10% chickpea flour, found that chickpea fortified breads had higher aroma scores than the control bread.

From our study, it can be concluded that the reduction in aroma can be attributed to the beany odor that chickpea flour imparted to the bread. Beany odor of chickpea is considered as one of the important factors that may influence the quality as well as

acceptability of any food product that is fortified with chickpea or chickpea flour (Gonzales et.al., 2014).

### 3.4.3 Taste

Taste is a chemical sense stimulated by the taste receptors upon interaction with taste stimuli on the tongue. In general, humans can distinguish between five to six basic tastes –sweet, sour, bitter, umami, fatty, and salty. Each taste can be distinguished up to intensity levels of 20 to 30. Factors affecting taste sensitivity include age, smoking, product viscosity, taste disorders (hypogeusia, ageusia, dysgeusia) and temperature (Lori Walker, 2012).

Control pita bread received the highest score for taste (4.12). However, this score was not sig different from taste scores for most of the other treatments with the exception of Treatments 1 and Treatment 3, which were the 10% and 20% Chickpea pita breads, respectively. The presence of chickpea by itself yielded lower scores in the pita bread. The presence of FDDG in combination with chickpea, however, appeared to improve acceptability in taste scores.

Sensory evaluation results of pita bread indicated that no significant differences in taste of the bread was found. Control and Treatments 2 (10%D), 4 (20D), 5 (20CP-10D%), and 6 (20D-10CP%). Treatment 1(10CP%) and Treatment 3 (20CP%) were not significant different from each other for taste scores. The two treatments received significantly lower scores that the other treatments. Incorporation of chickpea flour into the pita bread imparted a distinct bitter beany flavor, which could be the reason for low taste scores. Similar results were observed by Finney et.al (1982); Kefalas et.al (2009) in their studies. The chickpea flour may have exerted a negative influence on sensory taste

scores. Also, similar results were demonstrated by Fernandez and Beery (1989) who fortified bread with 10% chickpea flour. They found that chickpea fortified breads received lower taste scores than control bread. Dodok et.al, (1993) demonstrated the same results when they fortified wheat flour bread rolls with 10% and 20% chickpea flour. The breads fortified with both levels had lower taste scores than the control. They recommended the use of some additives to mask the flavor of chickpea flour, for a more desirable food product.

Mohammad et.al (2012) made the same conclusion. They tested dough rheology and bread quality of wheat-chickpea flour blends. Their results showed that as the ratio of chickpea flour increased in wheat flour, the taste scores decreased. Another similar conclusion by Yousseff et.al, (1976) where they supplemented wheat flour bread with different ratios of chickpea flour. They found that as the fortification level increased, taste scores decreased.

In an additional study, Hallab et.al (1974) studied the nutritive value and organoleptic properties of white Arabic bread supplemented with soybean and chickpea. They demonstrated that taste scores decreased with increased chickpea flour fortification level in the final product.

Based on the results, it can be concluded that fortification with FDDG alone did not affect the taste of the bread, whereas fortification with chickpea flour reduced likability of the pita bread taste. The formulation with combination of both FDDG and chickpea have not changed the taste of bread compared to control bread that can be justified from the taste scores. Combining chickpea flour with FDDG may be a good solution to reduce the distinct bitter beany flavor caused by the chickpea.

### 3.4.3 Texture

The sum total of kinesthetic (muscle sense) and cutaneous sensations derived from manual and oral manipulation is known as texture. It involves mouth feel, masticatory properties, and residual properties, visual and auditory properties of food. The initial phase of texture includes mechanical characteristics of hardness, fracturability, and viscosity and any geometrical characteristics which are observed in the first bite. The second or masticatory phase encompasses mechanical characteristics of chewiness, gumminess, and adhesiveness and any geometrical characteristics observed during chewing. Changes engendered in the mechanical and geometrical characteristics through mastication occur in the third phase (residual phase). The feel of food is interlinked with other sensations which transpire concurrently during “normal” eating (Lori Walker, 2012).

The texture scores from our study showed that there were no statistically significant differences between the treatments. The average scores of treatments for control, Treatment 1, Treatment 2, Treatment 3, Treatment 4, treatment 5 (3.88), and treatment 6 (3.98) were 4.0, 4.08, 4.06, 4.12, 4.18, 3.88 and 3.98, respectively. While the treatments were not significantly different from each other, a range of 3.88 to 4.18 indicated an overall high sensory value for all pita bread treatments on a scale of 1 through 5. Similar results by Fernandez and Beery (1989) who fortified bread at 10 and 20% levels with chickpea flour concluded that there were no significant differences between the two treatments. It shows that panelist were unable to observe any differences in the texture pita breads. In contrast, Yousseff et.al, (1976) had a different conclusion, when they supplemented wheat flour bread with different ratios of chickpea flour (10%,

15%). They found that as the fortification level increased, texture scores decreased. The findings of Youseff are also in agreement with the results by Hallab et.al (1974) who studied the nutritive value and organoleptic properties of white Arabic bread supplemented with soybean and chickpea. They demonstrated that texture scores decreased with increased chickpea flour fortification level in the final product.

Maga and Van Everen (1989) fortified pasta with two levels of DDGS (25%, 50%), and found that as DDGS levels in formula increased, the texture score decreased. These results are in contrast to our result.

Saunders et.al (2014) used a different method to quantify a quality by using texture analyses machine. They fortified breads with different levels of DDGS and concluded that softness and tenderness of bread decreased with increased DDGS fortification level.

Two components, namely fiber and gluten, play an important role in the bread texture profile; fiber can absorb greater amounts of water than other particles and can prevent them from being fully integrated into the starch/gluten matrix and will also lead to a harder texture (Gould et al, 1989). Golmoohammadi and co-workers showed that gluten plays a more important role in the texture of the bread. Even though fiber had increased and gluten content had decreased in all treatment which negatively influenced the texture of the bread. Subjects were unable to tell the differences in texture.

### **3.4.5 Overall acceptability**

The sensory evaluation scores for overall acceptability indicated that the Control and Treatments 5(70W-20CP-10D), and Treatment 6 (70W-20D-10CP) were the most acceptable pita breads overall. Treatment 5 and Treatment 6 received Overall

Acceptability scores of (4.29) and (4.26) comparable to that of Control (4.3) indicating that breads made with T5 and T6 were as acceptable as control. They were the highest flour replacement treatment groups with 30% wheat flour removed from the formula. Varying either the FDDG or CP at 10 to 20 % in the formula with the other ingredient present at 10 to 20% did not bear out any differences to the panelists and they considered the 70% wheat flour pita breads as high as the control 100% wheat pita breads. Panelists were unable to differentiate control T5, and T6 in terms of aroma, texture and taste. Moreover, the color score of T5 and T6 was more favorable than color score of control indicating.

No differences were discerned among all the other treatments. Treatments 1 (90W-10CP) and 4 (80W-20D) follows after that with no significant differences. The lowest scores were found in treatments 2 (90W-10D) and 3 (80W-20CP) with no significant difference that can be attributed to the distinct beany flavor and odor caused by the chickpea flour. Similar results were found by Mohammad et.al (2012). They tested dough rheology and bread quality of wheat-chickpea flour blends. Their results showed that low fortification level with chickpea flour did not influence overall acceptability of chickpea fortified bread. The opposite results were concluded by Fernandez and Beery (1989). They fortified bread with 10% chickpea flour and found higher overall scores in the fortified bread in comparison to control bread. This can be explained by the lower amount of chickpea flour used in their study compared to 20% in our study.

Hallab et.al (1974) studied the nutritive value and organoleptic properties of white Arabic bread supplemented with soybean and chickpea. They demonstrated that over all acceptability scores decreased with increased chickpea flour fortification level in the final

product. This was in contrast to findings from our study. This can be due to both beany flavor and odor of chickpea which are considered important factors that may influence the quality as well as acceptability of any food products that is fortified with chickpea or chickpea flour (Gonzales et.al., 2014).

Another reason behind the lower overall scores may be due to the darker color that was caused by the FDDG fortification. Similar results were concluded by Rosentrater and Krishnan, (2006); Li et.al, (2016) unpublished paper; Maga and Van Everen (1989) ; Rasco et al. (1987); Tsen et al. (1983), Liu et al. (2011), Singh et al. (2012) and Pourafshar (2011); Arra, (2011).

Another reason for the darker color of bread may have been due to increased Millard reaction during baking due to the lysine in chickpea flour. In the Millard reaction reducing carbohydrates react with free amino acid side chains of proteins, mainly lysine that are present in chickpea flour, and lead to amino acid sugar reaction products (polymerized protein and brown pigments). (Hallen et.al, 2004; Mohammed et.al,2012).

Even though there were differences in scores, the sensory panel found pita breads from all treatment combinations to be acceptable. Our results indicated that combination of FDDG and chickpea flour had greater overall acceptability than mere addition of either of the ingredients alone with wheat flour.

Recently, new efforts have been systematically undertaken to replace part of the wheat flour by other types of flours in order to improve its nutritional and sensory properties. In this study, we used chickpea flour and FDDG as substitutes to determine the effect of adding different levels of chickpea and FDDG on the sensory properties of pita bread.

Darkness in color of bread increased as FDDG increased which also negatively influenced the color scores. Our results showed that addition of chickpea in conjunction with FDDG showed substantial improvement in the color of the bread. Beany odor and flavor was increased as the percentage of chickpea increased which showed adverse effect on the taste and aroma scores. Quality and acceptability of legume products is influenced by beany odor and flavor which can be reduced by the addition of FDDG. The color of the baked product is of paramount importance in the initial acceptability by consumers (See et.al., 2007).

Table 3.10 provides Texture analysis results showed statistically no differences between treatments. For all pita breads. Panelist was unable to discern differences in the texture attributes in the various treatments of pita breads when compared to control. Therefore, it can be concluded that it is feasible to produce bread with acceptable texture by using chickpea flour and FDDG substituted in wheat flour. Also, the overall acceptability of bread was found greater with combination of FDDG and chickpea flour. The findings in this research can be useful for both researchers and industry to understand the impact of FDDG and chickpea flour on the nutritional and sensorial qualities of bread. It should be noted that addition of excessive amounts of FDDG and chickpea can adversely affect the color and aroma & taste of bread. Therefore, the substitution percentage should be experimentally determined depending on the kind of bread, and the goals of the research (Hefnawy et.al 2012).

### **3.5 Shelf life**

The industrialization of the food industry, including baked goods, is the result of the consumer's demand for products with high quality, convenience, longer shelf life, easier



storage condition, and high appeal to sight, touch, taste, and smell. To meet the above demands the baking industries are required to use functional food additives. There is an increasing demand for the use of natural antioxidants in foods, especially in bakery products. Natural antioxidants such as  $\beta$ -carotene has already been used in bakery products. Natural antioxidants have antimicrobial activities in addition to their antioxidative properties and have been found to be effective in enhancing the shelf life of bakery products (Nanditha & Prabhasankar, 2008).

Incorporating bioactive compounds such as antioxidants, namely, polyphenolic compounds can improve the safety and shelf life of food products (Yang, Lee, Won, & Song, 2016). Because DDG is a value source for phenolic compounds with potential antioxidant activity (Inglett, Rose, Stevenson, & Biswas 2009; Luthria & Memon, 2012), it may be beneficial to use fractions of DDG in improving shelf life and stability of bakery products. DDG also contains phytochemicals which are valued for their antioxidant activity, namely, carotenoids (Winkler-Moser & Vaughn, 2009), thus it can be a good agent to inhibit lipid peroxidation in food products and improve food quality.

It has already been shown that legumes contain antifungal compounds which are responsible for extension of shelf life of baked foods (Rizzello, Lavecchia, Gramaglia & Gobbetti, 2015). This can be due to the antioxidant activity. Such bioactives may present in chickpea, which can chelate metal ion responsible for lipid oxidation (Arcan, & Yemenicioğlu, 2010; Han & Baik, 2008). Chickpea is a rich source of phenolics and carotenoids (Han & Baik, 2008; Thavarajah, 2012; Jukanti, Gaur, Gowda, & Chibbar, 2012), which are associated with antioxidant activity that is acting as antimicrobial compounds which may help in increased shelf life of baked products.

There has always been interest among researchers to improve quality and shelf life of baked products such as bread. Some stabilizers have been used to extend the shelf life of baked bread by two days while retaining the sensory attributes and to enhance water retention capacity, improve texture, volume and cell structure of the products. Currently, the average shelf life of breads is short, sometimes as few as three days. Considering the significant shelf life issue of bread industry, the present study was designed to compare the effect of different food ingredients on the overall quality of bread, and to assess the suitability among the tested combinations to prolong the shelf life of bread (Latif et al., 2005).

Since there was no research done on the fortification of wheat flour with a combination of FDDG and chickpea flour, we have attempted to determine the combined effectiveness of the blend in the shelf life of the pita bread. Though several researchers have explored FDDG and chickpea flour individually to enhance the shelf life of baked products, their combined effects have not been tested.

The objective of this section of the study was to evaluate the effectiveness of various combinations of FDDG, chickpea and wheat flour in pita bread formulation and to determine its effectiveness in limiting bread spoilage at various storage intervals. Table 3.11 provides information on the inspection of baked pita bread to determine visual changes that degrade the bread quality, namely fungal growth and mold. Baked breads were allowed to cool for 2 hours and were stored at room temperature (18.7-22.9°C). No spoilage was noted up to the morning of the 4th day for control bread (100%W), and bread from Treatment 1(90W-10CP%). Bread from Treatment 2 (90W-10D%) showed fungal growth on the evening of the 4<sup>th</sup> day. Bread from Treatment 3 (80W-20CP)

showed fungal growth on the morning of the 5<sup>th</sup> day and on the evening of the 5<sup>th</sup> day of storage. Bread from Treatment 4 (80W-20D%) showed mold growth. Treatment 5 (70W-20CP-10D%) showed mold growth on the morning of day six. Treatment 6 (70W-20D-10CP) showed mold grow in the evening of the 6<sup>th</sup> day.

Figures (3.9, 3.10, 3.11, 3.12, 3.13, and 3.14) track the mold growth in hours in relation to moisture, TDF, protein total phenolics, AA, and carotenoids. Figure 3.9 shows significant high coefficient of determination ( $R^2$ ) and a strong negative correlation between molds growth in hours and moisture content (-0.84) of pita breads. The high  $R^2$  also demonstrated that there is a strong positive correlation between TDF ( $R^2=0.99$ ), protein (0.98), total phenolics content ( $R^2=0.85$ ), AA ( $R^2=0.94$ ) and carotenoids ( $R^2=0.97$ ) values when related to molds growth per hour.

To our knowledge this is the first study that tests the correlations between visuals molds growths in hours (dependent variable) which was converted from subjective to objective variables in relations to all other independence variable. Based on the results of this study, we can draw a conclusion pertaining to extending the shelf-life of chickpea-FDDG fortified pita breads. There is a paucity of information on the shelf life of pita breads in the literature. Estimated shelf life of a pita bread in room temperature is 72 hours. Fortification could impart positive attributes to the quality of bread while offering better protection against microbial spoilage to the bread. Our results showed that wheat pita bread substituted with 10% chickpea has increased shelf life by 12 hours, whereas fortifying with 20CP% increased shelf life by 36 hours, when compared to the control pita bread. Also 10% FDDG fortification increased shelf life by 24 hours. However, fortifying with 20% FDDG doubled the shelf life, which increased by 48 hours, when

compared to control pita bread. Fortifying with 20CP-10D% increased the shelf life of the pita bread by 60 hours over control bread. The longest shelf life was exhibited in pita bread with 20 % FDDG- 10% chickpea which had 72-hour longer shelf life than the control pita bread. The difference in shelf life of pita breads fortified with FDDG and chickpea flour can be due to the higher protein and fiber content and lower moisture and water activity values observed from the chemical analysis.

Proteins of legume origin have been reported to possess antioxidant activity. The proteins owe their antioxidant activity to their constituent amino acids such as aromatic, sulfur containing and basic amino acids which have the ability to donate protons to free radicals. The basic and acidic amino acids also have chelating properties that are responsible for initiation of lipid oxidation in foods. The cationic proteins help electronic repulsion metal ions away from lipid droplets, whereas surface active characteristics enable binding unsaturated lipids (Arcan and Yemenicioglu, 2010).

Addition of 10% DDGS flour in the bread formulation was found to increase the loaf volume, color, and shelf life compared to whole wheat bread (Tsen et al 1983). Incorporation of DDGS in the formulation results in migration of water between the flour and DDGS particles. The shelf life of bread is highly dependent on the moisture content of flour i.e. flour with low moisture content offers longer shelf life (Staudt and Zeigler, 1973; Butt et al., 2004). The substitution of FDDG resulted in substantial reduction in the moisture content of the flour mixtures with increase in FDDG content. The bread with 10% and 20% FDDG content in the flour mixtures had considerably less moisture when compared to 100% bread flour (control flour). Ahmed (1997) obtained similar results in his study on Chapathis, a whole wheat Indian flatbread. He reported that moisture

content of chapathi was inversely proportional to the amount of DDGS in the product. Therefore, low moisture content leads to low water activity in the product. This signifies that shelf life of bread can be extended significantly by incorporation of FDDG. Similar reduction in moisture content was in bread with increase in levels of dough additive from 2% to 5% (Arra, 2011). Bread made from durum wheat flour substituted with chickpea sourdough was found to have distinct flavor, better taste and a prolonged shelf life (Kefalas et al., 2009). White pan bread fortified with chickpea flour was found to show enhanced nutritional quality and shelf life by several days (R.D. Report, 2004). Garg and Dahiya (2003) found that papads prepared with wheat flour fortified with chickpea flour ranging from 10%-30% showed higher acceptability, nutritional quality and better keeping quality (Garg and Dahiya, 2003).

A study by Yust and coworkers (2012) concluded that the use of chickpea protein can be used as a preservative to prevent rancidity, owing to its antioxidant activity to its antioxidant activity and carotenoids content. Proteins from legumes have been reported to possess antioxidant activities, which are capable to donate protons to free radicals. Proteins also have the ability to chelate metal ions that are responsible for initiation of lipid oxidation in foods (Arcan and Yemenicioglu, 2010). In our pita bread treatments, it is plausible that the antioxidant activity is the result of phenolic compounds and carotenoids originating in both the chickpea as well as the FDDG. More recently, several workers have advocated the extraction of antioxidants such as carotenoids and their use in reducing oxidative damage to prevent deterioration of commercial food products (Wahyuono, Hesse, Hipler, Elsn, & Böhm, 2016).

Table 3.14 provides data on the total phenolics, antioxidants activity, and total carotenoids content of wheat flour control and wheat pita bread flour blends containing chickpea and FDDG. As the fortification levels of the two enrichment ingredients (chickpea and FDDG) increased in the pita breads, total phenolics (TPC), antioxidant activity (AA), and total carotenoids content increased significantly at each level of fortification. Each treatment was statistically different and higher than the wheat-only control in relation to TPC, AA, and Carotenoids content. Chickpea and FDDG treatments also resulted in significant difference between treatments for the same constituents. FDDG fortification, more dramatically increased all of the three above constituents when compared to chickpea. For example, when compared to the control all-wheat bread, TPC increased by 78% when bread was fortified with 20% FDDG, whereas it increased by 63% when bread was fortified with 20% chickpea.

Vergara-Valencia, Granados-Pérez, Agama-Acevedo, Tovar, Ruales, & Bello-Pérez, (2007) fortified bread and cookies with rich carotenoids and polyphenols mango dietary fibers (MDF). They concluded that bakery products fortified with MDF showed higher TDF than respective controls, and the products maintained significant antioxidant capacity associated to longer shelf life. Hidalgo, & Brandolini (2008) fortified wheat flour with carotenoids, and reported that carotenoids contribute to improved freshness and shelf life of bakery products due to the stability of carotenoids in flour. The bioactive compounds such as carotenoids and phenolics in legumes, behave as antioxidants and effectively prevent oxidation of the food products (Ghiassi, Gharachorloo, Baharinia, & Mortazavi, 2012). Rababah, Feng, Yang, & Yücel (2012) conducted a study to fortify potato chips with natural plant extracts to enhance their sensory properties and storage

stability. They found that potato chips with the highest total phenolics and antioxidant activity minimized lipid oxidation and increased shelf life.

A plausible reason for increased shelf life noted in food products in our study maybe due to the increased minerals content which act as antioxidants. Our results showed that mineral content in FDDG and chickpea were significantly initially higher than the all-wheat flour.

Antioxidants are found in certain foods and may prevent some of the damage caused by free radicals. The best known antioxidants are vitamin A, beta-carotene, vitamins C and E, the minerals Selenium, Zinc, Manganese, Copper, and Iron (Evans & Halliwell, 2001). Antioxidants can function in different ways. Some vitamins donate their electrons to free radicals to stabilize them. Some minerals act to destroy free radicals (superoxide dismutase, catalase and glutathione peroxidase).

Many studies from the literature have related the use of minerals in bread fortifications and its antioxidants activity to increase shelf life (Katina, Hartikainen & Poutanen, 2017; Duodu & Taylor, 2012; De Valdez, Rollán, Gerez & Torino, 2011; Clarke & Arendt, 2005; Guerzoni, Gianotti & Serrazanetti, 2011; Hartikainen & Katina, 2012).

Sourdough has been used to improve bread quality parameters such as volume, texture, flavor, nutritional value, increase bread shelf life by retarding staling and protect bread from mold and bacterial spoilage. This is because it increases the availability of minerals such as magnesium, iron and zinc which increases the function of antioxidants, which retard mold growth and longer shelf life (Bryszewska et al., 2007).

Islam & Ho-Min, (2018) studied the effect of iron, iodine and selenium on quality, shelf life and microbial activity of cherry tomatoes. They found that fungal incidence and

microbial activities were lower in selenium-treated cherry tomatoes compared with iron and iodine treatments. However, all had significant higher shelf life and lower microbial activities when compared to control not treated tomatoes.

Baking has been reported to increase the antioxidant activity of whole wheat bread compared with refined flour and that the crust of white bread contained slightly more phenolic compounds than the crumb, owing to Maillard reactions (Yu & Nanguet, 2013). Bread products which have browning reactions, especially caramelization intermediates, show increased antioxidant potential (Sivam, Sun-Waterhouse, Quek, & Perera, 2010). A study by Capuano, Garofalo, Napolitano, Zielinski & Fogliano, (2010) concluded that antioxidant activity increased during toasting as a consequence of Maillard reaction product formation. Their explanation is that the rate of Maillard reaction is higher in whole rye flours compared to brown and white rye flours because of their higher free amino acid and protein content.

Figures (3.9, 3.10, 3.11, 3.12, 3.13, and 3.14) tracks the shelf life time of various pita breads in relation to initial moisture, total dietary fiber content, protein content, total phenolics content, AA, and carotenoids content. The graphics show strong coefficients of determination ( $R^2= 0.84$ ) between shelf life and moisture content. The high  $R^2$  also show that there is a strong positive correlation between shelf-life and protein content ( $R^2=0.98$ ); shelf life and TDF% ( $R^2 =1.0$ ); shelf life and TPC ( $R^2=1.0$ ); shelf life and AA ( $R^2=0.93$ ); shelf life and total carotenoids ( $R^2=0.97$ ).

The increase in shelf life of pita breads may be attributed to a number of reasons. Increased fiber and protein content which may have resulted in increased water binding capacity that caused decreased available water in the pita breads, increased antioxidants



such as carotenoids and phenolics which act as antimicrobials. Adding value to breads through FDDG and chickpea fortification could be a significant step in providing nutrient components to consumers. By adding certain nutrients, we can also change physical and chemical properties, the shelf life, the texture, and the production time of breads. Both chickpea and FDDG fortification significantly increased the shelf life of bread. It was observed that the same level of fortification with FDDG increased the shelf life of bread by 12 hours when compared to its chickpea counterpart. Thus, enrichment of breads with chickpea and FDDG that contain valuable components such as fiber protein, phenolic, and carotenoids, can be a significant step in increasing its shelf life.

**Table 3. 1 Experimental design showing proportions of All Purpose wheat Flour (W), Chickpea (CP) and Distillers Dried Grains in control and treatment blends.**

| Treatment (T)<br>APF:CP:FDDGS | Fortification Level      |                        |                           |
|-------------------------------|--------------------------|------------------------|---------------------------|
|                               | All-purpose flour<br>(W) | Chickpea flour<br>(CP) | Food grade DDGS<br>(FDDG) |
| <b>Control</b>                | 100                      | 0                      | 0                         |
| <b>T1(90:10:0)</b>            | 90                       | 10                     | 0                         |
| <b>T2 (90:0:10)</b>           | 90                       | 0                      | 10                        |
| <b>T3(80:20:0)</b>            | 80                       | 20                     | 0                         |
| <b>T4(80:0:20)</b>            | 80                       | 0                      | 20                        |
| <b>T5 (70:20:10)</b>          | 70                       | 20                     | 10                        |
| <b>T6 (70:10:20)</b>          | 70                       | 10                     | 20                        |

FDDG: Food grade Dried Distillers Grains W= wheat, (APF) All Purpose Flour

CP=chickpea

D= DDG

**Table 3. 2 Proximate composition of raw ingredients used in pita breads employed in the glycemic response study**

| Nutrient        | %                        |                        |                           |
|-----------------|--------------------------|------------------------|---------------------------|
|                 | All-Purpose flour<br>(W) | Chickpea flour<br>(CP) | Food grade DDGS<br>(FDDG) |
| <b>Moisture</b> | 12.0a                    | 8.60b                  | 5.80c                     |
| <b>Protein</b>  | 12.0c                    | 22.30b                 | 31.0a                     |
| <b>Fat</b>      | 1.89c                    | 3.20b                  | 5.10a                     |
| <b>Ash</b>      | 0.61c                    | 2.60b                  | 3.10a                     |
| <b>TDF</b>      | 5.24c                    | 21.1b                  | 30.9a                     |
| <b>CHO</b>      | 68.3a                    | 42.2b                  | 24.1c                     |
| <b>Total</b>    | 100%                     | 100%                   | 100%                      |

FDDG: Food grade Dried Distillers Grains TDF: Total dietary fibers, CHO: Carbohydrates.

Means with the same letter within rows are not significantly different ( $P < 0.05$ ).

**Table 3. 3 Chemical properties of pita breads enriched with 10 to 20% chickpea or Distillers grains and 30% flour replacement with combinations of FDDG and chickpea (dry basis)**

| <b>Nutrient<br/>s</b>                         | <b>Control<br/>100W%</b> | <b>T1<br/>90W-<br/>10CP%</b> | <b>T2<br/>90W-<br/>10D%</b> | <b>T3<br/>80W-<br/>20CP%</b> | <b>T4<br/>80W-<br/>20D%</b> | <b>T5<br/>70W-<br/>20CP-<br/>10D%</b> | <b>T6<br/>70W-<br/>20D-<br/>10CP%</b> |
|---|--------------------------|------------------------------|-----------------------------|------------------------------|-----------------------------|---------------------------------------|---------------------------------------|
| <b>Protein</b>                                | 14.8g<br>(0.05)          | 16.7f<br>(0.06)              | 17.3e<br>(0.09)             | 18.1d<br>(0.11)              | 18.6c<br>(0.10)             | 18.9b<br>(0.02)                       | 19.6a<br>(0.13)                       |
| <b>Fat</b>                                    | 0.11f<br>(0.00)          | 0.11f<br>(0.00)              | 0.12e<br>(0.00)             | 0.13d<br>(0.00)              | 0.16c<br>(0.00)             | 0.21b<br>(0.00)                       | 0.28a<br>(0.00)                       |
| <b>Ash</b>                                    | 0.59g<br>(0.00)          | 0.6f<br>(0.00)               | 0.61e<br>(0.00)             | 0.62d<br>(0.00)              | 0.72c<br>(0.00)             | 0.97b<br>(0.00)                       | 1.06a<br>(0.00)                       |
| <b>Moisture</b>                               | 40.3a<br>(0.25)          | 38.6b<br>(0.50)              | 34.2c<br>(0.28)             | 32.0d<br>(0.05)              | 31.e<br>(0.09)              | 30.1f<br>(0.16)                       | 30.0f<br>(0.20)                       |
| <b>TDF</b>                                    | 5.21g<br>(0.31)          | 7.21f<br>(0.31)              | 10.04e<br>(0.28)            | 11.74d<br>(0.31)             | 13.05c<br>(0.22)            | 15.64b<br>(0.54)                      | 17.44a<br>(0.81)                      |
| <b>Kcal<br/>/100 g</b>                        | 267.50                   | 263.0                        | 254.1                       | 247.00                       | 234.00                      | 212.5                                 | 201.0                                 |
| <b>Av<br/>(CHO)<br/>in 100 g</b>              | 49.2                     | 45.3                         | 41.0                        | 37.5                         | 33.0                        | 25.9                                  | 21.4                                  |
| <b>Amt. ser.<br/>TA/50 g<br/>Av<br/>(CHO)</b> | 101.5                    | 110.4                        | 122.1                       | 133.4                        | 151.4                       | 192.8                                 | 234.0                                 |

TDF: Total Dietary Fibers, Kcal: Kilocalories, g: grams, Amt.: Amount, ser.: served, TA: to achieve, Av: available, CHO: Carbohydrates W=wheat flour, D=food grade DDGS, G=garbanzo/chickpea flour

Means across rows with the same letter are not significantly different (P<0.05)

**Table 3. 4 Amino acid profile for raw ingredients Font size is still inconsistent. Heading has smaller print than table content**

| <b>(g/100g protein)</b> | <b>APF (W)</b> | <b>AA Score</b> | <b>CP</b> | <b>AA score</b> | <b>FDDG</b> | <b>AA Score</b> | <b>FAO/WHO Ref.Pat</b> |
|-------------------------|----------------|-----------------|-----------|-----------------|-------------|-----------------|------------------------|
| <b>Leucine</b>          | 0.76           | 11              | 1.69      | 24              | 4.07        | 58              | 7.00                   |
| <b>Isoleucine</b>       | 0.42           | 11.5            | 1.07      | 27              | 1.36        | 34              | 4.00                   |
| <b>Lysine</b>           | 0.28           | 5               | 1.63      | 30              | 1.15        | 21              | 5.50                   |
| <b>Methionine+</b>      | 0.4            | 11.5            | 0.65      | 18.6            | 1.35        | 38.5            | 3.50                   |
| <b>Cystine</b>          |                |                 |           |                 |             |                 |                        |
| <b>Phenylalanine+</b>   | 0.54           | 8               | 1.30      | 19              | 1.75        | 26              | 6.80                   |
| <b>Tyrosine</b>         |                |                 |           |                 |             |                 |                        |
| <b>Threonine</b>        | 0.29           | 7               | 0.82      | 20.5            | 1.22        | 30.5            | 4.00                   |
| <b>Valine</b>           | 0.46           | 9               | 1.07      | 21.5            | 1.71        | 34              | 5.00                   |

APF= all purpose-flour, AA= amino acid, CP= chickpea, FDDG= food grade ddg  
Essential amino acid (EAA) (g amino acid/16 g N) pattern of the FAO/WHO standard protein:

Amino acid scores (AA) calculated by the formula:  
(FAO/WHO, 1985) reference pattern (Ref.Pat)

**Table 3. 5 Amino acid profile and amino acid scores (within parenthesis) for pita breads made with wheat flour (W), chickpea(CP) and Food Grade distillers dried grains (D)**

| EAA<br>(g/100g<br>protein) | Contro<br>1<br>100W | T1<br>90W- 10CP | T2<br>90W-10D | T3<br>80W-20CP | T4<br>80W-20D | T5<br>70W-<br>20CP-<br>10D | T6<br>70W-<br>20D-<br>10CP | Ref.Pat |
|----------------------------|---------------------|-----------------|---------------|----------------|---------------|----------------------------|----------------------------|---------|
| Leucine                    | 3.8<br>(55)         | 5.77<br>(82)    | 7.51<br>(107) | 7.55<br>(107)  | 8.92<br>(127) | 9.11<br>(130)              | 9.92<br>(141)              | 7.00    |
| Isolucine                  | 2.4<br>(60)         | 3.76<br>(94)    | 4.03<br>(101) | 3.95<br>(99)   | 4.17<br>(104) | 4.24<br>(106)              | 4.48<br>(112)              | 4.00    |
| Lysine                     | 2.2<br>(47)         | 3.24<br>(59)    | 3.1<br>(55)   | 3.6<br>(65)    | 3.34<br>(60)  | 4.27<br>(77)               | 3.96<br>(72)               | 5.50    |
| Meth+<br>Cystine           | 1.9<br>(54)         | 2.4<br>(68)     | 3.1<br>(89)   | 3.6<br>(102)   | 4.2<br>(120)  | 4.7<br>(128)               | 4.6<br>(130)               | 3.50    |
| Phenyl+<br>Tyrosine        | 3.3<br>(49)         | 4.5<br>(66)     | 5.2<br>(76)   | 5.3<br>(78)    | 5.9<br>(89)   | 6.4<br>(94)                | 6.9<br>(101)               | 6.80    |
| Therionine                 | 2.1<br>(52)         | 2.7<br>(66)     | 3.06<br>(76)  | 3.25<br>(81)   | 3.59<br>(90)  | 3.5<br>(88)                | 3.7<br>(91)                | 4.00    |
| Valine                     | 2.5<br>(50)         | 3.4<br>(73)     | 4.1<br>(85)   | 5.5<br>(90)    | 6.1<br>(95)   | 6.5<br>(98)                | 6.9<br>(103)               | 5.00    |

APF= all purpose-flour, AA= amino acid, CP= chickpea, FDDG= food grade ddg  
Essential amino acid (EAA) (g amino acid/16 g N) pattern of the FAO/WHO standard protein:

Number in parentheses presents Amino acid scores (AA)which is calculated by the formula:

(FAO/WHO, 1985) reference pattern (Ref.Pat)

Meth=Methionine

Phenyl=Phenylalanine

**Table 3. 6 Treatment combination effects of the independent variables on the physical properties of control wheat flour and flour blends, (water activity and color values comparison).**

| <b>Means (std dev)</b>          | <b>a<sub>w</sub></b> | <b>L*</b>        | <b>a*</b>        | <b>b*</b>        |
|---------------------------------|----------------------|------------------|------------------|------------------|
| <b>Control (100%W)</b>          | 0.57a<br>(0.00)      | 90.73a<br>(0.34) | -0.07c<br>(0.02) | 13.98c<br>(0.08) |
| <b>Treatment 1 (90W-10C%)</b>   | 0.53b<br>(0.00)      | 86.20b<br>(0.62) | -0.36d<br>(0.07) | 14.50b<br>(0.39) |
| <b>Treatment 2 (90W-10D)</b>    | 0.48c<br>(0.00)      | 84.29c<br>(0.41) | 0.03b<br>(0.02)  | 10.28d<br>(0.05) |
| <b>Treatment3 (80W-20C)</b>     | 0.46d<br>(0.00)      | 83.48d<br>(0.09) | -0.68e<br>(0.04) | 16.81a<br>(0.01) |
| <b>Treatment4 (80W-20D)</b>     | 0.41e<br>(0.00)      | 82.21e<br>(0.01) | 0.19a<br>(0.03)  | 6.65e<br>(0.11)  |
| <b>Treatment5 (70W-20C-10D)</b> | 0.41e<br>(0.00)      | 80.7f<br>(0.00)  | -0.67e<br>(0.04) | 16.44a<br>(0.01) |
| <b>Treatment6 (70W-20D-10C)</b> | 0.41e<br>(0.00)      | 79.62g<br>(0.00) | 0.18a<br>(0.03)  | 6.65e<br>(0.11)  |

a<sub>w</sub>: Water activity; L\*:Brightness; a\*: Redness Vs Greenness, b\* Yellowness Vs Blueness

**Table 3. 7 Farinograph and Mixolab rheological data of all wheat control and FDDG and chickpea flour blends.**

| <b>Means (std)</b>                        | <b>Fwab<br/>/500FU%</b> | <b>Fdvt<br/>/min</b> | <b>Fstab<br/>/min</b> | <b>MTI<br/>/FU</b> | <b>Mwab<br/>/%</b> | <b>Mdvt<br/>/min</b> | <b>Mstab<br/>/min</b> |
|---|-------------------------|----------------------|-----------------------|--------------------|--------------------|----------------------|-----------------------|
| <b>Control<br/>(100%W)</b>                | 56.50g<br>(0.00)        | 4.70g<br>(0.14)      | 8.8a<br>(0.00)        | 29.860c<br>(0.00)  | 50.06g<br>(0.00)   | 4.65d<br>(0.81)      | 11.44a<br>(0.13)      |
| <b>Treatment 1<br/>(90W-<br/>10CP%)</b>   | 58.65f<br>(0.07)        | 5.90f<br>(0.00)      | 7.2b<br>(0.00)        | 30.12c<br>(0.00)   | 51.94f<br>(0.06)   | 5.70c<br>(0.64)      | 10.59ab<br>(0.27)     |
| <b>Treatment 2<br/>(90W-10D)</b>          | 60.60e<br>(0.14)        | 6.98e<br>(0.35)      | 6.9b<br>(0.99)        | 31.02c<br>(0.00)   | 53.70e<br>(0.00)   | 5.92bc<br>(0.62)     | 10.02b<br>(0.26)      |
| <b>Treatment3<br/>(80W-20CP)</b>          | 62.9d<br>(0.04)         | 8.02d<br>(0.06)      | 5.50c<br>(0.14)       | 34.50b<br>(6.36)   | 56.50d<br>(0.00)   | 6.08b<br>(5.54)      | 9.82b<br>(0.24)       |
| <b>Treatment4<br/>(80W-20D)</b>           | 65.42c<br>(0.07)        | 9.30c<br>(0.14)      | 5.2c<br>(0.06)        | 35.70b<br>(0.06)   | 60.11c<br>(0.13)   | 6.23b<br>(0.22)      | 9.6b<br>(0.23)        |
| <b>Treatment5<br/>(70W-20CP-<br/>10D)</b> | 67.52b<br>(0.00)        | 11.99b<br>(0.08)     | 4.90c<br>(0.00)       | 38.46a<br>(0.28)   | 63.90b<br>(0.00)   | 7.37a<br>(0.81)      | 8.54c<br>(1.15)       |
| <b>Treatment6<br/>(70W-20D-<br/>10CP)</b> | 69.60a<br>(0.00)        | 13.75a<br>(1.06)     | 4.70c<br>(0.21)       | 39.50a<br>(2.12)   | 66.55a<br>(0.07)   | 7.53a<br>(0.05)      | 8.14c<br>(1.58)       |

W: wheat flour, all-purpose flour (APF), CP: chickpea flour, D; FDDG, F: Farinograph, M: Mixolab, dvt: development time, stab: stability, MTI: mixing tolerance index:



**Table 3. 8 Texture analysis data of wheat, chickpea, and FDDG on studies dough and pita breads**

| <b>Sample</b>                            | <b>Rmax<br/>(gm)</b> | <b>Emax<br/>(mm)</b> | <b>Burst<br/>distance<br/>(mm)</b> | <b>Burst<br/>force<br/>(gm)</b> | <b>Tug<br/>distance<br/>(mm)</b> | <b>Tug force<br/>(gm)</b> |
|--|----------------------|----------------------|------------------------------------|---------------------------------|----------------------------------|---------------------------|
| <b>Control<br/>(100%W)</b>               | 14.10f<br>(0.17)     | 43.44a<br>(3.34)     | 19.9a<br>(0.00)                    | 353.4c<br>(0.00)                | 5.09a<br>(0.00)                  | 237.2d<br>(0.00)          |
| <b>Treatment 1<br/>(90W-<br/>10CP%)</b>  | 17.78e<br>(0.55)     | 31.18b<br>(1.68)     | 18.7a<br>(0.00)                    | 398.3c<br>(0.00)                | 4.98a<br>(0.00)                  | 251.5d<br>(0.00)          |
| <b>Treatment 2<br/>(90W-10D)</b>         | 20.11d<br>(0.00)     | 28.06c<br>(0.00)     | 16.6ab<br>(0.00)                   | 455.3c<br>(0.00)                | 4.74a<br>(0.00)                  | 385.9c<br>(0.00)          |
| <b>Treatment3<br/>(80W-20C)</b>          | 23.31c<br>(0.43)     | 21.94d<br>(0.00)     | 14.6b<br>(0.00)                    | 540.1b<br>(0.00)                | 4.68a<br>(0.00)                  | 356.3c<br>(0.00)          |
| <b>Treatment4<br/>(80W-20D)</b>          | 25.6b<br>(0.00)      | 16.77e<br>(0.00)     | 13.5b<br>(0.00)                    | 599.1b<br>(0.00)                | 4.40ab<br>(0.00)                 | 686.1b<br>(0.00)          |
| <b>Treatment5<br/>(70W-20C-<br/>10D)</b> | 28.18a<br>(0.26)     | 11.49f<br>(0.20)     | 14.9b<br>(0.00)                    | 725.2a<br>(0.00)                | 3.92b<br>(0.00)                  | 633.7b<br>(0.00)          |
| <b>Treatment6<br/>(70W-20D-<br/>10C)</b> | 28.63a<br>(1.00)     | 11.87f<br>(0.52)     | 10.8c<br>(0.00)                    | 742.3a<br>(0.00)                | 3.76b<br>(0.00)                  | 753.3a<br>(0.00)          |

W: wheat flour, APF; all-purpose flour, CP: chickpea, D:FDDG, Rmax: dough strength, Emax: dough extensibility, Burst distance: pita extensibility Burst force; pita strength, Tug distance: pita extensibility, Tug fore: pita chewability; (gm) grams; (mm) millimeter.

**Table 3. 9 physical properties of pita breads (color)**

| <b>Sample</b>                             | <b>L*</b>        | <b>a*</b>       | <b>b*</b>        |
|---|------------------|-----------------|------------------|
| <b>Control<br/>(100%W)</b>                | 91.73a<br>(0.34) | 0.36e<br>(0.07) | 13.98d<br>(0.08) |
| <b>Treatment 1<br/>(90W-<br/>10CP%)</b>   | 90.06b<br>(0.08) | 0.09b<br>(0.02) | 9.03f<br>(0.07)  |
| <b>Treatment 2<br/>(90W-10D)</b>          | 89.28c<br>(0.10) | 0.66f<br>(0.06) | 15.50b<br>(0.14) |
| <b>Treatment3<br/>(80W-20CP)</b>          | 88.44d<br>(0.12) | 0.19a<br>(0.03) | 6.65g<br>(0.11)  |
| <b>Treatment4<br/>(80W-20D)</b>           | 86.20e<br>(0.62) | 0.68f<br>(0.04) | 16.81a<br>(0.01) |
| <b>Treatment5<br/>(70W-20CP-<br/>10D)</b> | 84.29f<br>(0.41) | 0.03c<br>(0.02) | 10.28e<br>(0.05) |
| <b>Treatment6<br/>(70W-20D-<br/>10CP)</b> | 83.48g<br>(0.09) | 0.07d<br>(0.02) | 14.50c<br>(0.39) |

W= wheat, All Purpose Flour; CP=chickpea; D= FDDG; L\*:Brightness; a\*: Redness Vs Greenness, b\* Yellowness Vs Blueness

**Table 3. 10 Sensory analysis of pita bread enriched with 10 to 20% Chickpea and Distillers Dried Grains and combinations of chickpea and FDDG.**

| <b>Samples</b> | <b>Ingredients</b>  | <b>Color<br/>(<math>\mu \pm SD</math>)</b> | <b>Aroma<br/>(<math>\mu \pm SD</math>)</b> | <b>Taste<br/>(<math>\mu \pm SD</math>)</b> | <b>Texture<br/>(<math>\mu \pm SD</math>)</b> | <b>Overall<br/>(<math>\mu \pm SD</math>)</b> |
|----------------|---------------------|--|--|--|--|--|
| <b>Control</b> | 100%W               | 3.82 B<br>$\pm 0.98$                       | 4.14 A<br>$\pm 0.65$                       | 4.12 A<br>$\pm 0.81$                       | 4.00 A<br>$\pm 1.09$                         | 4.30 A<br>$\pm 0.96$                         |
| <b>T1</b>      | 90%W-10%CP          | 4.13A<br>$\pm 0.69$                        | 3.66 B<br>$\pm 0.63$                       | 3.46 B<br>$\pm 0.65$                       | 4.08 A<br>$\pm 0.86$                         | 3.96B<br>$\pm 0.85$                          |
| <b>T2</b>      | 90%W-10%D           | 3.60 c<br>$\pm 0.66$                       | 4.04AB<br>$\pm 0.72$                       | 4.10 A<br>$\pm 0.54$                       | 4.06 A<br>$\pm 0.32$                         | 3.54C<br>$\pm 0.18$                          |
| <b>T3</b>      | 80%W-20%CP          | 4.14 A<br>$\pm 0.85$                       | 3.68 B<br>$\pm 0.98$                       | 3.42 B<br>$\pm 1.16$                       | 4.12 A<br>$\pm 0.77$                         | 3.50 C<br>$\pm 0.92$                         |
| <b>T4</b>      | 80%W-20%D           | 3.52 c<br>$\pm 1.20$                       | 4.21 A<br>$\pm 0.63$                       | 4.07 A<br>$\pm 0.72$                       | 4.18 A<br>$\pm 0.71$                         | 3.78 BC<br>$\pm 1.02$                        |
| <b>T5</b>      | 70%W-20%CP<br>10%D  | 4.00 A<br>$\pm 0.86$                       | 4.04 AB<br>$\pm 0.86$                      | 4.07 A<br>$\pm 0.77$                       | 3.88 A<br>$\pm 1.08$                         | 4.29 A<br>$\pm 0.66$                         |
| <b>T6</b>      | 70%W-20%D-<br>10%CP | 4.10 A<br>$\pm 0.79$                       | 4.03 AB<br>$\pm 0.69$                      | 3.96 A<br>$\pm 1.00$                       | 3.98 A<br>$\pm 1.09$                         | 4.26 A<br>$\pm 0.68$                         |

Means followed by similar letters for a given dependent variable within columns are not significantly different at  $P < 0.05$ , LSD. Different letters for a given dependent variable denotes significant difference ( $\alpha = 0.05$ ) across treatment conditions for that independent variable.

SD= standard deviation

W=wheat flour

D=food grade DDG

CP=chickpea flour

T=treatment

(1=poor, 2=fair, 3=acceptable, 4=good, 5=excellent).

**Table 3. 11 Visual monitoring of mold growth of bread during a 6-day storage at room temperature (25±1°C).**

| Samples              | Day 1 |       | Day 2 |       | Day 3 |       | Day 4 |       | Day 5 |       | Day 6 |       |
|----------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|                      | 12hrs | 12hrs | 12hrs | 12hrs | 12hrs | 12hrs | 12hrs | 12hrs | 12hrs | 12hrs | 12hrs | 12hrs |
| <b>CONTROL</b>       | -ve   | -ve   | -ve   | -ve   | -ve   | +ve   | --    | --    | --    | --    | --    | --    |
| <b>(100%W)</b>       |       |       |       |       |       |       |       |       |       |       |       |       |
| <b>90W-10CP%</b>     | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | +ve   | --    | --    | --    | --    | --    |
| <b>90W-10D%</b>      | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | +ve   | --    | --    | --    | --    |
| <b>80W-20CP%</b>     | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | +ve   | --    | --    | --    |
| <b>80W-20D%</b>      | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | +ve   | --    | --    |
| <b>70W-20CP-10D%</b> | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | +ve   | --    |
| <b>70W-20D-10CP%</b> | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | -ve   | +ve   |

DDGS (D): Dried Distillers Grains. W= wheat, (APF) All Purpose Flour CP=chickpea  
D= DDG

+ve= visual growth of mold, -ve=no visual growth of mold; -- = product was discarded after visual mold growth

**Table 3. 12 Chemical and physical properties of pita bread fortified with chickpea and food grade distillers grains**

| <b>Sample</b>              | <b>Moisture%</b> | <b>WA</b> | <b>Protein%</b> | <b>TDF%</b> |
|----------------------------|------------------|-----------|-----------------|-------------|
| <b>CONTROL<br/>(100%W)</b> | 40.27a           | 0.96a     | 14.78g          | 5.21g       |
| <b>90W-10CP%</b>           | 38.64b           | 0.96a     | 15.69f          | 7.21f       |
| <b>90W-10D%</b>            | 34.21c           | 0.90b     | 16.7e           | 10.4e       |
| <b>80W-20CP%</b>           | 31.97d           | 0.84c     | 17.30d          | 11.74d      |
| <b>80W-20D%</b>            | 30.98e           | 0.80d     | 18.06c          | 13.05c      |
| <b>70W-20CP-<br/>10D%</b>  | 30.11f           | 0.76e     | 19.57b          | 15.64b      |
| <b>70W-20D-<br/>10C%</b>   | 31.01g           | 0.72f     | 20.89a          | 17.44a      |

WA: water activity

TDF: total dietary fiber

Means within the same row with the same letters are not significantly different ( $P < 0.05$ ).

**Table 3. 13 Total Phenolic Content, Total Carotenoids, and Antioxidant Activity of Pita Bread Ingredients.**

| <b>Ingredient</b> | <b>TP (mg TAE/100 g)</b> | <b>Carotenoids <math>\mu\text{g}/100\text{g}</math></b> | <b>AA%</b> |
|-------------------|--------------------------|---|------------|
| <b>APF (W)</b>    | 142.4c                   | 22c   | 123.5c     |
| <b>CP</b>         | 1390.2b                  | 1382,3b   | 566.2b     |
| <b>FDDG</b>       | 2062.9a                  | 2021.6a   | 789.7a     |

TP: total phenolic content

AA%: antioxidant activity

TC: total carotenoids

Means within the same row with the same letters are not significantly different ( $P < 0.05$ ).

**Table 3. 14 Total Phenolic Content, Total Carotenoids, and Antioxidant Activity of Pita Breads.**

| <b>Pita bread</b>          | <b>TPC</b> | <b>AA%</b> | <b>TC</b> |
|----------------------------|------------|------------|-----------|
| <b>CONTROL<br/>(100%W)</b> | 234.75g    | 155.88g    | 0.19g     |
| <b>90W-10CP%</b>           | 240.70ef   | 208.82f    | 1.14f     |
| <b>90W-10D%</b>            | 335.98e    | 229.41e    | 1.80e     |
| <b>80W-20CP%</b>           | 383.62d    | 260.29d    | 2.22d     |
| <b>80W-20D%</b>            | 419.35c    | 275c       | 2.71c     |
| <b>70W-20CP-10D%</b>       | 529.52b    | 377.94b    | 3.82b     |
| <b>70W-20D-10C%</b>        | 770.7a     | 425a       | 4.92a     |

DDGS (D): Dried Distillers Grains.

W= wheat, (APF) All Purpose Flour

CP=chickpea

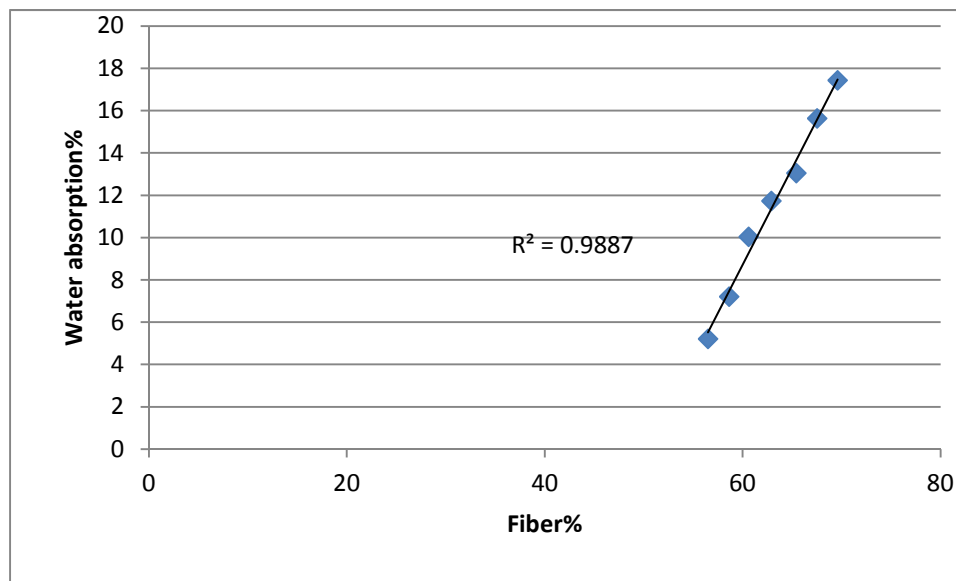
D= DDG

TP: total phenolic content

AA%: antioxidant activity

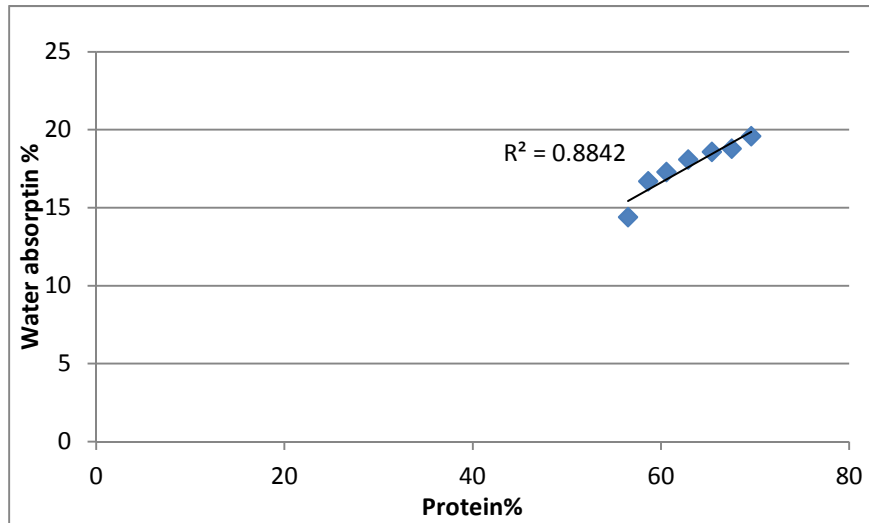
TC: total carotenoids

Means within the same row with the same letters are not significantly different ( $P < 0.05$ ).

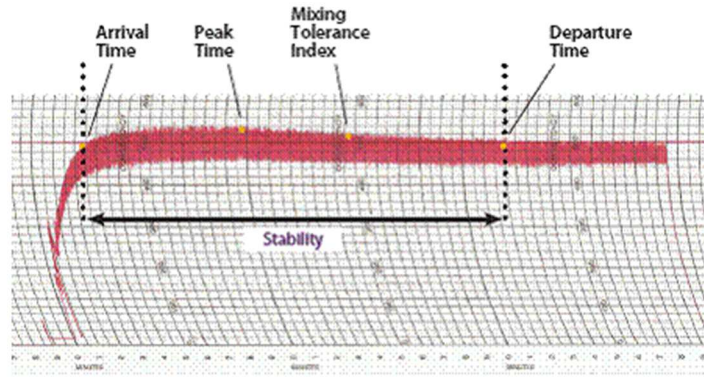


**Figure 3. 1 Correlation between fiber % and water absorption.**



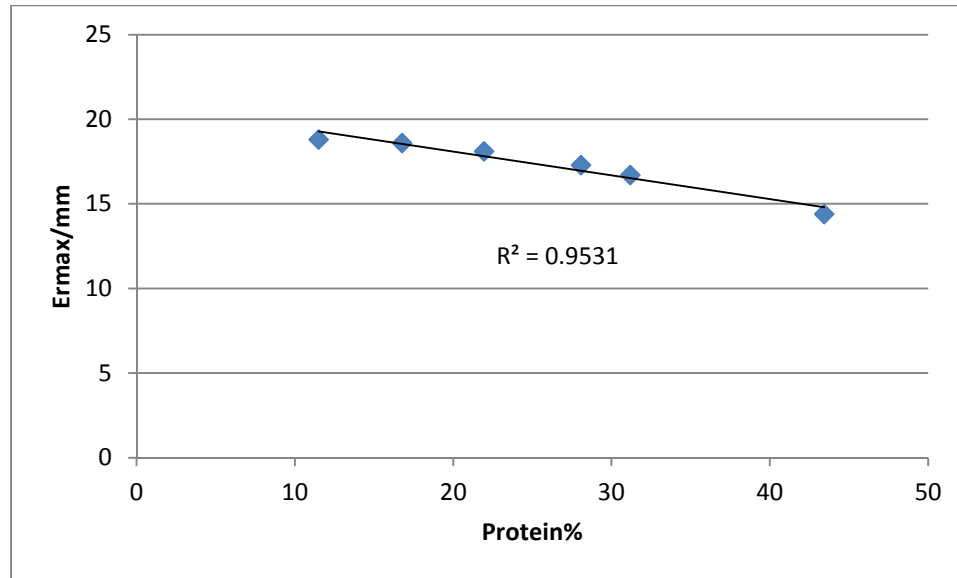


**Figure 3. 2 Correlation between protein% and water absorption.**

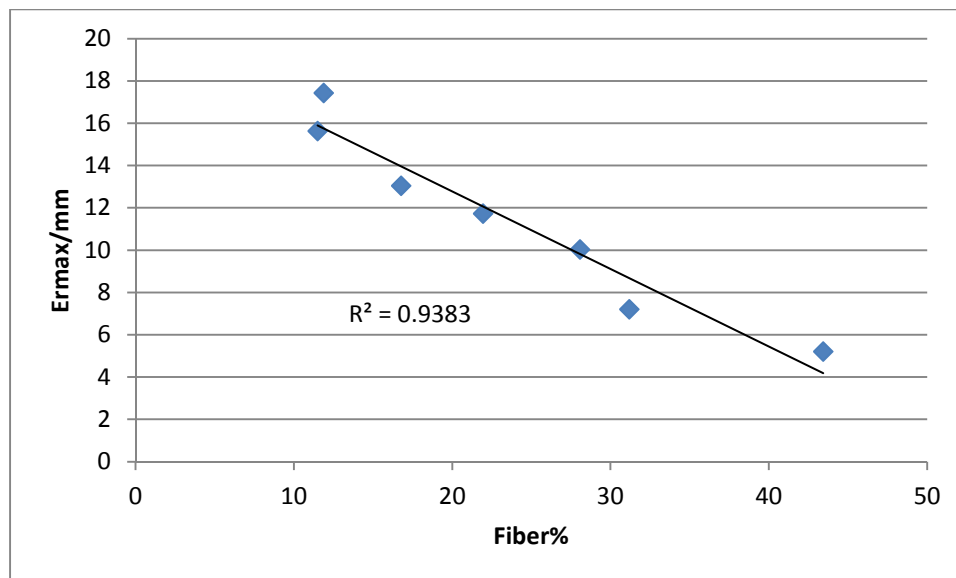


**Strong Gluten Flour**

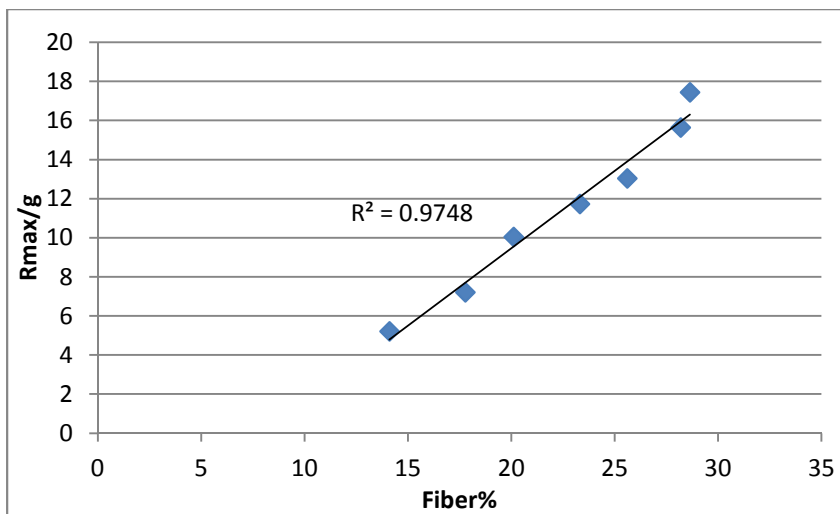
**Figure 3.3 Typical Frainger profile**



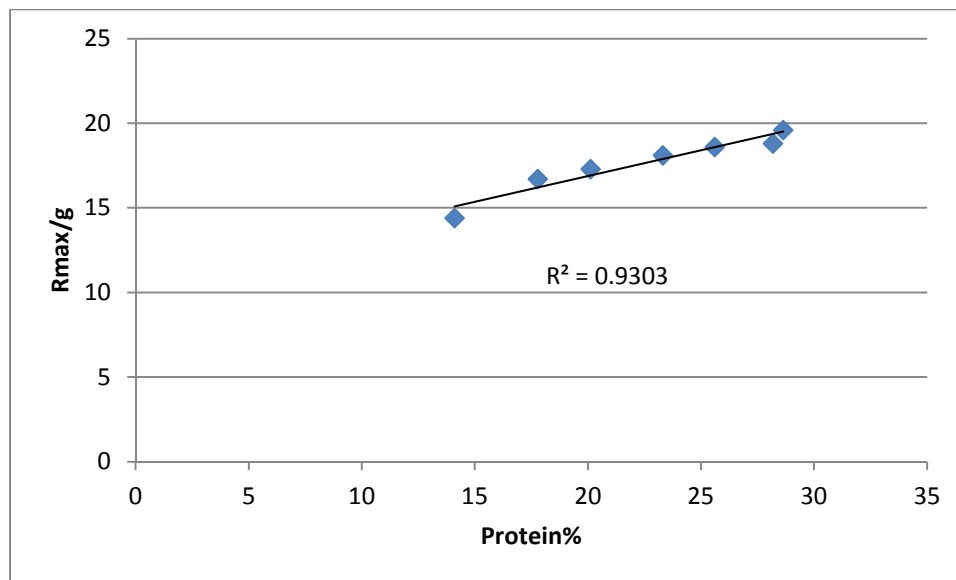
**Figure 3. 4 Correlation between dough extensibility and protein.**



**Figure 3. 5 Correlation between dough extensibility and fiber.**



**Figure 3. 6 Correlation between dough strength and fiber.**



**Figure 3.7 Correlation between dough strength and protein.**

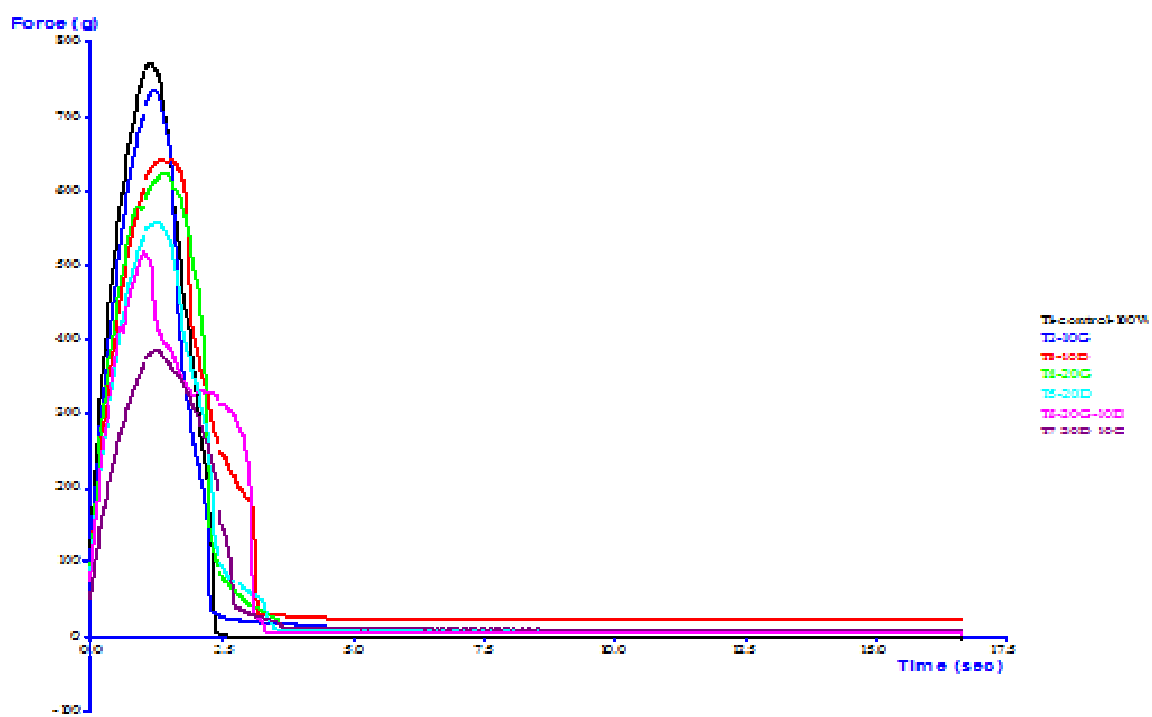
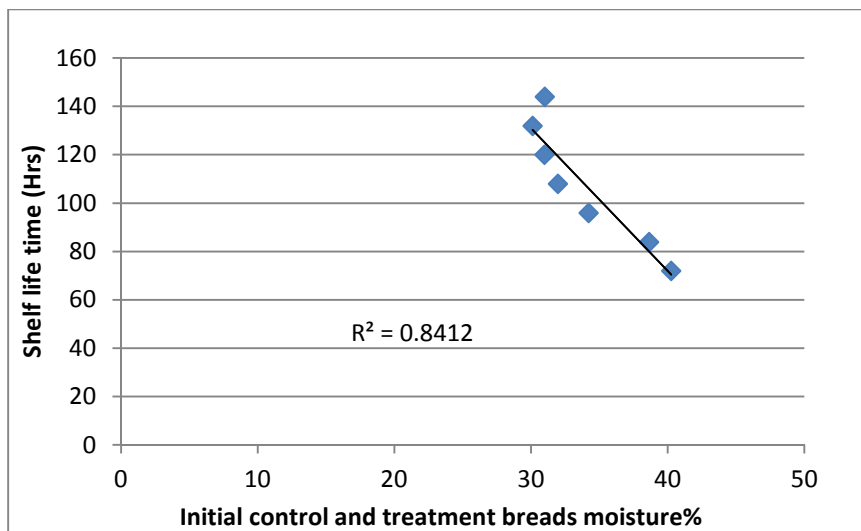
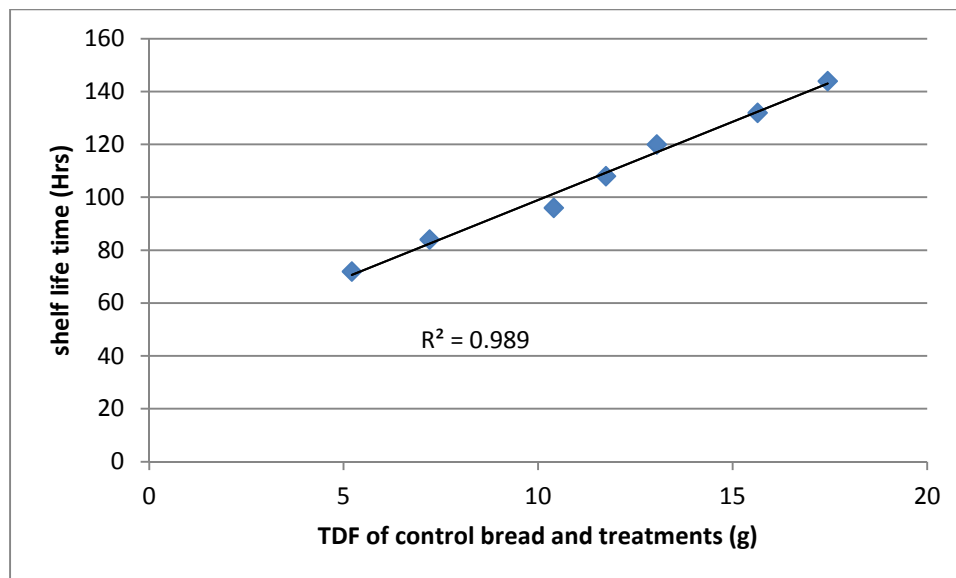


Figure 3. 8 Tug Fixture analysis of Pita bread

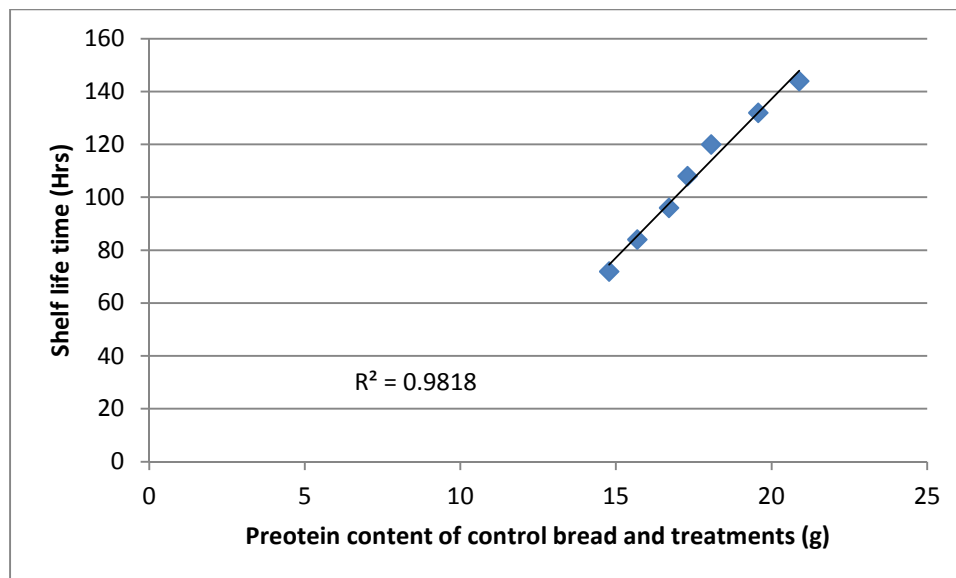


**Figure 3. 9** Molds growth in hours in relation to initial water content of control bread and treatments.

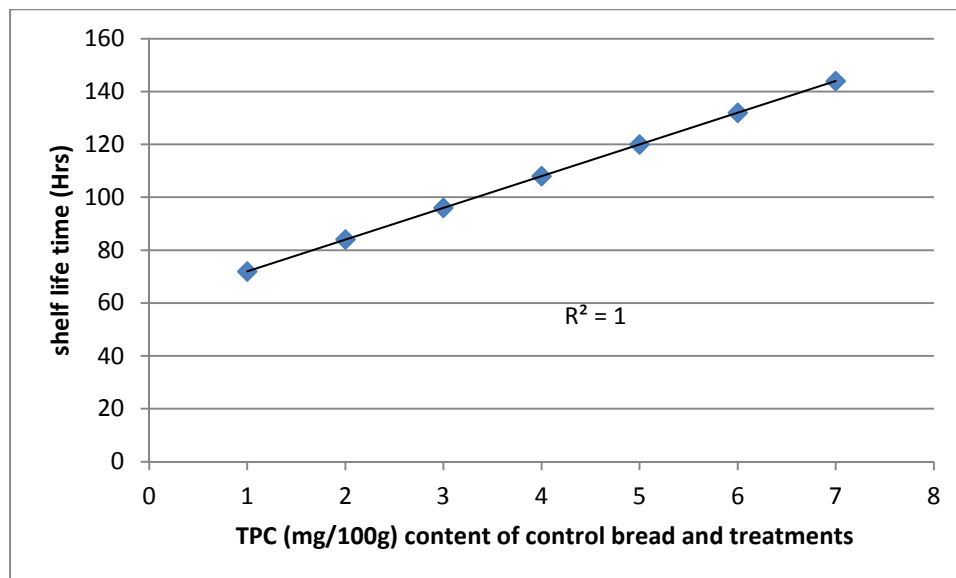




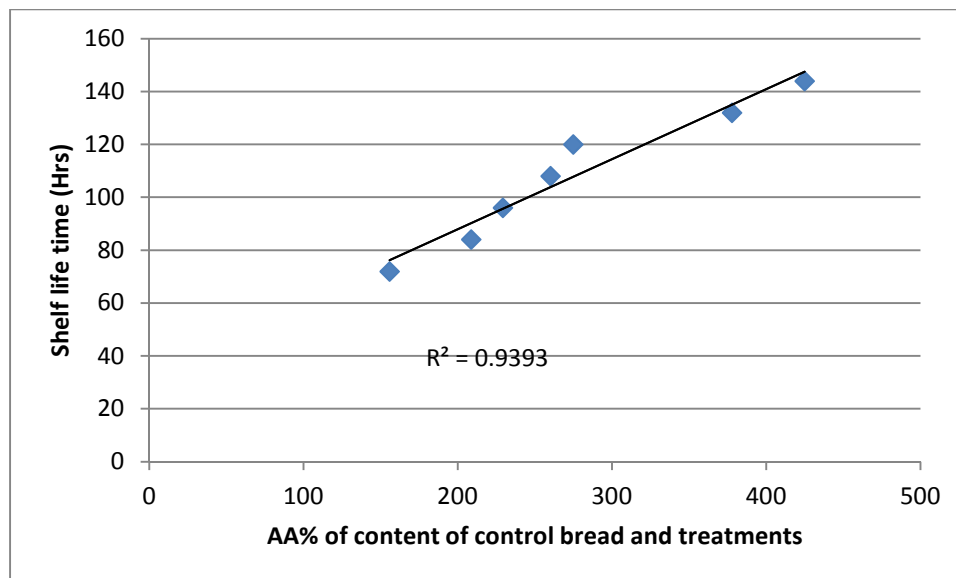
**Figure 3. 10** Molds growth in hours in relation to TDF content of control and treatment breads.



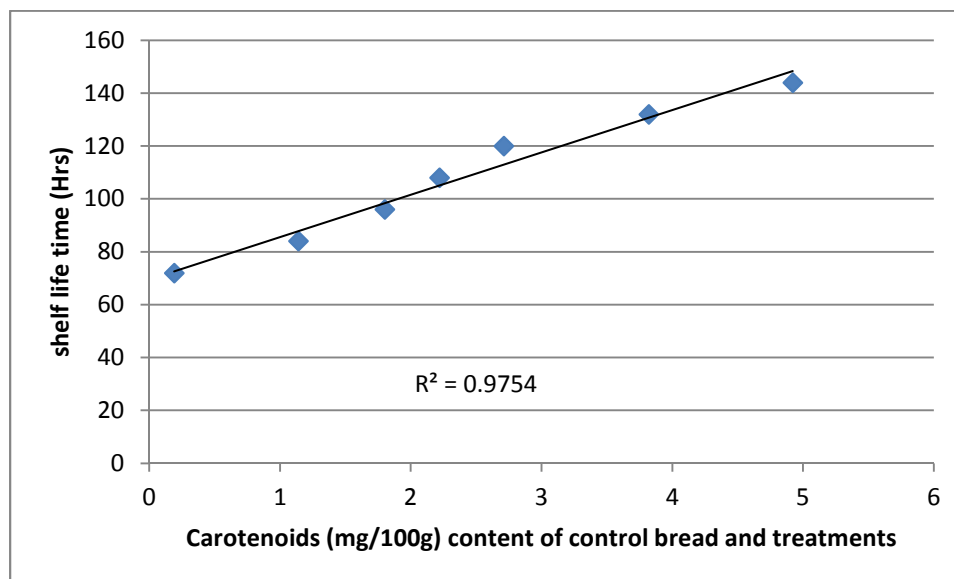
**Figure 3. 11 Molds growth in hours in relation to protein content of control and treatment breads.**



**Figure 3. 12** Molds growth in hours in relation to initial phenolic content of control and treatment breads.



**Figure 3. 13** Molds growth in hours in relation to initial AA% content of control and treatment breads.



**Figure 3. 14** Molds growth in hours in relation to initial carotenoid content of control and treatment breads.

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## CHAPTER 4

### **Effects of chickpea and distiller's dried grains (FDDG) fortified pita breads on glycemic response in humans**

#### **Abstract**

Consumption of low-glycemic index (GI) foods, have been shown to improve glucose tolerance in human subjects. The estimated cost of diabetes in the US is \$245 Billion (ADA, 2013), and it is expected to raise by 53% to more than \$622 billion dollars between the years 2015-2030 (Rowley, Bezold, Arikan, Byrne, and Krohe, 2017). While the consumption of low glycemic response foods (LGR) has increased in recent years (Riccardi, G., Rivellese, A. A., & Giacco, 2008), there is a need for a more diverse range of such foods in the market that are also affordable. High protein and high fiber ingredients such as chickpea (CP) and food grade distillers grain (FDDG) may be helpful in the formulation of low glycemic foods. Our objective was to compare the glycemic response (GR) in human subjects to consumption of foods prepared with combinations of wheat flour (W), chickpea (CP) and FDDG (D). Wheat-based pita breads were prepared employing flour blends prepared in the following ratios: Control W (100 %), W:CP (90:10 & 80:20), W:D (90:10 & 80:20), and W:CP:D (70:20:10 & 70:10:20). The experiment design was a single blind, randomized controlled, cross-over design with a convenience sample of twelve panelists, where the subjects served as their own control. Following overnight fasting, subjects followed a diet where they consumed each bread type. Serving size were regulated in order to achieve 50g of available carbohydrates.

Blood samples were collected at 30 min intervals and glycemic response curves were constructed.

The incremental area under the Curve (IAUC) was calculated. Control Pita (W) yielded an IAUC of 94.84mmol.min/L). Pita bread containing 10% CP yielded an IAUC of 85.46 mmol.min/L while the 20% CP showed IAUC of 56.32mmol.min/L. FDDG pita breads with 10% FDDG showed IAUC of 81.21 mmol.min/L while the 20% FDDG pita bread resulted in an IAUC of 46.23 mmol.min/L. Moreover, IAUC for (70W-20CP-10D) was 40.06 mmol.min/L, and 36.53 mmol.min/L for (70W-20D-10CP). Inclusion of CP and FDDG in wheat flour, separately and in combinations (70:20:10 & 70:10:20), brought about improvements in the GR when compared to control wheat pita. This study demonstrated the efficacy of high fiber, protein, fat, and antioxidants (phenolic compounds and carotenoids ingredients such as chickpeas and food grade distiller's grains in the development of low glycemic response foods.

#### **4.1 Introduction**

The total estimate of diabetes cost in 2012 was \$245 billion, which was a 41% increase from the \$174 billion cost in 2007 (American Diabetes Association, 2013). A potential solution to manage diabetes cost is to consume foods that have a low GI. Low GI diets are more expensive than the higher GI equivalents, which affect the consumer buying behavior and food choice (Cleary, J., Casey, S., Hofsteede, C., Moses, R. G., Milosavljevic, M., & Brand Miller, J. 2012). Foods with a low glycemic index (GI) have been shown to reduce fats and lipid concentrations in the blood of diabetic and healthy individuals (Gray, 2015). GI is a rating system that ranks food into three categories (high, medium and low). Food products with high GI include bread and breakfast cereal (GI of

70 or greater relative to pure glucose) whereas products like fruits, legumes, pasta and dairy products have low GI (between 0 and 55, relative to glucose). GI is determined by the digestive and absorptive effects of carbohydrates in the respective foods. Goni & Valenti'n-Gamazo (2003) reported that the digestive rate of various carbohydrates such as starch, in particular, triggers multiple physiological responses. Chronic diseases such as cardiovascular diseases, obesity, diabetes, and cancer are mostly due to unhealthy lifestyles and unhealthy eating habits (Chan & Woo, 2010). The superior quality food products fortified with glycemic response-reduction ingredients could address these challenges and could prevent nutrition-related chronic diseases. One of the biggest challenges of food research is to deliver a sustainable food supply endowed with excellent quality supplemented with functional ingredients, such as protein and fiber. Apart from providing desirable health benefits, these functional ingredients could mitigate diseases caused by nutritional deficiency. These ingredients may also lower the risk of diabetes and other life-style related diseases. A great number of research activities in the field of health related dietary aspects have demonstrated a significant link between the regular intake of fiber and diabetes (Wang et al., 2012). Among the low glycemic foods, legumes have received special attention among researchers owing to their ability to reduce blood glucose level. This ability of legumes, chickpea in particular, can be attributed to its high total dietary fiber content (Leonora et al., 1995). Because Chickpea is a rich source of protein, complex carbohydrates, vitamins and minerals, it is one of the most important crops worldwide (Abou Arab et.al, 2010). Chickpea accounts for an important share of overall pulse production. India is the largest producer of chickpea (70%), however the US has increased its production in the last decade. Chickpea is

expected to play a major role in the northern states of USA (Tulbek, 2006). It is an excellent inexpensive source of vitamins, minerals and bioactive compounds with potential to reduce risk of chronic diseases. This has led to the acceptance of chickpea as a functional food (Jukanti, Gaur, Gowda, & Chibbar, 2012). The consumption of foods with reduced energy content has escalated in recent years. As a result, the food industry is creating new initiatives to develop food products, especially those that provide fiber in foods. In legumes, the highest proportion consists of the carbohydrates at around 50–60% dry matter. The largest components of the carbohydrates are the starch and non-starch polysaccharides dietary fiber (DF), with the former at between 22–45%. There has been an increased use of legumes in different countries around the world to develop dietary formulas that prevent diabetes, heart-related diseases, colorectal cancer, and hypercholesterolemia. A previous report by the Agricultural Marketing Resources Center (AgMRC) shows that the U.S chickpea consumption per capita in 2014 was 0.7 lbs which will nearly double within the next 5 years . A more current report by AgMRC reported estimates the consumption of chickpeas has increased to 1.85 pounds per person in 2017, which is up from 2016 by 1.21 pounds per person. Similar to DF, resistance starch (RS) which is considered to be part of DF found in legumes is limited in energy and consequently has the same physiological effects (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, Pérez-Álvarez, 2010). RS digestibility occurs in the colon through microbial fermentation, affecting the aforementioned physiological functions. Recently, there have been promising attempts to control diabetes through the alteration the glucose impact of the carbohydrates consumed. All forms of legumes contain significant amounts of RS. This explains why the intake of legumes is associated with the slow digestion and

release rates of starch. The slow digestion rate of the starch in legumes is due to the presence of high dietary fiber, which prevents complete starch breakdown (Utrilla-Coello, Osorio-Diaz, & Bello-Perez, 2007).

Dried legume seeds inhibit the rapid increase of post-meal blood glucose levels. This important process is caused due to the rigidity of the legume cell walls, the reduced enzyme action of some of the legume content, e.g., starch, and the presence of other highly indigestible compounds, e.g., carotenoids, polyphenols,  $\alpha$ -amylase inhibitors, non-starch polysaccharides, and oligosaccharide in the diabetic patients. Moreover, legumes provide high levels of protein, although evidence reveals a decline in intake in the recent past (Goni & Valenti'n-Gamazo, 2003; Yudan liu, 2012).

There are various non-wheat adjuncts in the food market that are considered as excellent choices in fortifying wheat flour with protein and fiber. The list includes soy, chickpea, spelt, quinoa, amaranth, oat bran, rye, buckwheat, potato, flax, and varieties of nuts. DDG (Distillers Dried Grains) and DDGS (Distillers Dried Grains with Solubles) may be additional ingredients. These co-products from the processing of ethanol are potentially excellent good ingredients for diabetic subjects by virtue of their low starch content, high fiber content and high protein content. Research has revealed the applicability of the DDG and DDGS human foods production. During 1980s, multiple experiments had been conducted to examine DDG and DDGS in food products which included bread, dinner rolls, muffins, chili, pasta, and granola. However, Rosentrater and Krishnan (2006) reported a decline research in DDG in the 90's.

The sale of DDG and DDGS is crucially dependent on the functional and aesthetic quality of the flavor (Abbott et al., 1991; O'Palka et al., 1989; Rosentrater & Krishnan,

2006). Research has shown that ethanol processing co-products have variations in color, protein, fat, pH, and fiber, such products also have odor, taste, and color, which are considered unpleasant in food processing. However, technological advancements may enable the bleaching and deodorizing of DDG to nullify its adverse traits (Saunders, 2008).

Nutritionally, DDG and DDGS contain protein, fats, carbohydrate, starch, and dietary fiber at the ranges of 26.8-33.7%, 3.5-12.8%, 39.2-61.9%, 4.7-5.9%, and 24.2-39.8%, respectively (Rosentrater & Muthukumarappan, 2006). Dong and Rosco (1987) indicated that a cupful of DDGS can supply a subject with a whole day's dietary fiber requirement as well as proteins.

Based on the above information, DDG can be termed as excellent addition to grains and cereal due to their high nutritional value. It is anticipated that food made with DDGS will have a lower glycemic response compared to products made completely with either all-purpose flour or whole wheat flour.

There is a paucity of information on glycemic response to DDG in food products. Bechen (2008) studied the effects of three types of porridge, including all-purpose flour, wheat flour and DDGS (20 g each, in order to achieve 15 g of available carbohydrate) on glycemic response of 10 healthy subjects. The results of this study revealed an inhibitive property of DDGS which yielded the lowest glucose response while all-purpose flour demonstrated the highest glucose response (Bechen, 2008). As an illustration, baked bread contains high carbohydrates content, high glycemic index, low protein content, low amount of resistant starch, and small amounts of dietary fiber. Such refined carbohydrate foods combined with a sedentary life style may cause adverse effects on an individual's



health have highlighted the change and replacements of the bread food formulas. The use of legumes, seeds, non-wheat cereal flour, and dietary fibers as single or combined ingredients is vital to improving the nutritional value and taste of these bread (Dhinda et al., 2012).

Bread is a staple food and consumed worldwide in various forms. However, the glycemic response to bread varies widely according to the type of bread (Fardet et.al, 2006). Low glycemic responses are considered favorable to health, especially with subjects with diabetes. The literature shows that careful selection of raw ingredient (with known composition such as protein, fiber, fat, and antioxidants (phenolic compounds and carotenoids) used in food formulations is an essential in decreasing the glycemic response in breads, such as) (Fardet et.al, 2006; Dembinska-Kiec et.al, 2008; Spence et.al, 2010; Tundis et.al, 2011). Understanding of the mechanisms underlining such high variability in glycemic response of bread appears to be gaining importance (Fardet et.al, 2006). We hypothesize that incorporation of chickpea and FDDG alone or in combination will lower the glycemic response of the pita breads and both DDG and chickpea may have a therapeutic role within the diabetic diet.

## **4.2 Materials and methods**

### **4.2.1 Materials**

Corn distillers dried grains with solubles (DDGS) was obtained from a commercial ethanol plant and was stored at  $-80\pm 1^{\circ}\text{C}$  until further processing for food applications. Other ingredients for preparation of pita bread, such all-purpose flour, chickpea, salt, sugar, active dry yeast, and olive oil, were purchased from a local grocery.

## **4.2.2 Methods**

### **4.2.2.1 Sample preparation**

#### **4.2.2.1.1 Preparation of chickpea flour**

Chickpea flour was prepared by milling dry chickpea in a Retsch mill (Company: GmbH & Co. Germany, Model: KG 5657HAAN1) into a fine powder. The powder obtained after milling was sieved using 0.5mm sieve to get fine flour.

#### **4.2.2.1.2 Preparation of FDDG**

FDDG was processed specifically for food applications in this study. The DDGS obtained from commercial ethanol plant was placed in stainless steel trays lined with cheesecloth, and then washed extensively with absolute alcohol i.e. 99.5% pure ethanol to remove pigments and oil. De-fatted samples were then washed multiple times with distilled water to remove traces of ethanol. The samples were then freeze-dried for 3-4 days in a shelf freeze dryer (Company: Virtuis, Model: USM15). Freeze dried DDGS powder was milled in Retsch Ultra centrifugal mill (Company: GmbH & Co. Germany, Model: KG 5657HAAN1) at the centrifugal speed of 20,000 rpm. Using a 0.5mm sieve, the powder obtained after milling was sieved and then stored in air-tight glass jars and sterilized in an autoclave at 15 psi (per square inch) pressure for 15 minutes. Sterilized FDDG flour was stored in a freezer to ensure maximum quality.

#### **4.2.2.2 Preparation of flour blends**

Control flour containing 100 % wheat (W) and six treatment blends containing wheat, chickpea and FDDG blends containing varied proportions of chickpea and FDDG were prepared. The control consisted of a 100% All Purpose Flour (APF). The flour blends

were mixed to ensure homogeneity in a V-shaped twin-shelled dry blender (Company: Peterson Kelly Co. Inc. Stroudsburg, PA) at a constant speed for 45 minutes to ensure uniform mixing of the ingredients.

#### **4.2.2.3 Pita bread formulation**

Seven different types of pita bread, corresponding to the flour blends and differing in ingredient composition (W, CP and D) were prepared (table 4.1). These were control all-purpose wheat flour pita bread (W:100), chickpea-only wheat flour pita breads (10% or 20% replacement level, W90:CP10 & W80:CP20), FDDG-only fortified pita bread (10% or 20% replacement level, W90:D10 & W80:D20), and finally, chickpea-FDDG fortified wheat flour pita breads (W70:CP20:D10 & W70:CP10:D20).

The pita recipe and baking procedure were provided by a professional chef from a Mediterranean/Middle Eastern restaurant. This method of pita bread was followed consistently for the control and all 6 treatments. The basic formula for pita bread for 4-5 servings included 187.5 grams (g) flour, 14.3 g sugar, 59 ml (milliliter) lukewarm water, 1.2 g salt, 14.3 g yeast, and 4.8 g (5 ml) olive oil. In pita production, sugar, yeast and water were mixed and set aside for 10 minutes at room temperature for activation of yeast. Yeast growth was confirmed by liberation of bubbles from the mixture. The dough was prepared in an automatic dough mixer (Kitchen Aid, Model: KSMQO). First, flour was added in the mixer followed by yeast mix. The dough was mixed at a low speed for 1.5 min. Salt was added, followed by olive oil. Mixing was done at faster speed this stage. The dough was then covered and leavened at room temperature for 1.5 h in a proofing cabinet. The flour blends were mixed using a dough hook head using the Hobart mixer.

#### **4.2.2.3.1 Rolling and Shaping of the dough**

Rolling and shaping of the dough was done manually. Before dough handling, it is advisable to rinse the hands with cold water to prevent sticking of dough to hand. From each dough mix, 4-5 dough balls of equal size were made and spread on a table using dough roller. Before rolling, the table was sprinkled with flour to prevent sticking. After rolling, the flattened dough was laid on parchment paper and kept for re proofing for about 5 minutes before baking.

#### **4.2.2.3.2 Baking of pita bread**

The pita breads were baked in an oven at 525°Fahrenheit (274 °C) for 60-90 seconds. After the specified baking time, the bread was removed from oven and allowed to cool for 1-2 hours at room temperature 77° Fahrenheit (25±1°C). Each piece of pita bread was cut into 8 slices using a bread knife, sealed in plastic bags and refrigerated further analysis.

#### **4.2.2.4 Proximate analysis**

**Moisture:** Moisture content was measured using oven the drying method according to AACCI approved method 44-19.01 (AACC 2000).

**Fat:** Fat content was determined using AOAC method 920.39 (AOAC, 1990) in an automated Soxhlet extractor using petroleum ether as solvent (CH-9230, Buchi laborotechnik AG, Flawil, Switzerland).

**Protein:** Protein content of the pita bread samples was analyzed for using the Dumas combustion analysis method (AOAC 17th ed., method 968.06) using a Rapid N cube

(Elementar Analysen Systeme, GmbH, Hanau Germany). Nitrogen content was then multiplied by a conversion factor of 6.25 to calculate Crude Protein % (CP).

Ash: Ash content of the pita bread samples was determined using incineration (Method. 08-03, AACC, 2000) in a muffle furnace (Company: Model: Box furnace, 51800 series). The dried pita bread samples were ashed at 525°C for 12 hours in muffle furnace to estimate inorganic content (minerals) in the bread.

Total Dietary Fiber (TDF): Fiber content was analyzed by enzymatic gravimetric method employing AOAC method (Method 30-25) for non-digestible fibers. The Megazyme assay test kit was used.

Resistance starch: (RS) was analyzed by enzymatic digestion using AOAC Official Method 2002.02 (Resistant Starch in Starch and Plant Materials).

Carotenoids: Total Carotenoids was analyzed using AOAC method 970.64-1974 in dried plant materials and mixed feeds (spectrophotometer).

Total Phenolic Content: Association of Official Analytical Chemists (AOAC method 952.03, AOAC, 1990) was used to measure the total phenolic content (TPC).

Antioxidant activity: The free radical scavenging activity that was measured by the Mellors and Tappel method (1996).

Carbohydrates: The (CHO) in pita bread samples was calculated by difference [100%-(protein%, + fat%+ ash%, + moisture%)].

#### **4.2.2.5 Glycemic response**

Assessment of the postprandial glucose response was determined by calculating the incremental area under the curve (IAUC) as described by Food and Agriculture Organization (FAO), and (Marinangeli et.al, 2009).

Experiment design: single blind randomized controlled cross over design.

The objective of the study was to compare the glycemic response by human subjects who were fed control wheat pita bread and 6 types of pita bread containing varied proportions of wheat, chickpea and distiller's grains.

Tables 4.2 and 4.3 summarize information on subjects such as height and weight which were used to calculate (BMI) body mass index. The BMI was calculated using a smart tool on the official web page of national heart, lung, and blood institute using the link below.

[https://www.nhlbi.nih.gov/health/educational/lose\\_wt/BMI/bmicalc.htm](https://www.nhlbi.nih.gov/health/educational/lose_wt/BMI/bmicalc.htm)

As shown in table 4.2, 12 subjects participated in the study with an average age of 24.25 (+3.56) and BMI of 22.8 (+1.94) with 75% participants being females and 25% being males. Table 4.3 demonstrates the average age of female subjects, which was 23.77 (+4.08), and that of males, which was 25.66 (+2.3). The average BMI for female subjects was 22.27 (+1.9) and that of the males was 24.36 (+1.1).

#### **4.2.2.5.1 Individual nutritional and physical instructions**

After volunteers were selected, an email was sent to each subject individually providing instructions on nutrition before and during the experiment. Subjects were advised to stop eating 12 hours prior to blood collection in order to obtain fasting blood glucose levels. They were also asked to limit the intake of chickpea and chickpea products for at least two weeks as a wash-out period. Lastly, subjects were told not to consume any kind of alcohol or its products, or do any type of exercise for 48 hours prior to any of the blood test dates.

#### 4.2.2.5.2 Measurement of Glycemic response of pita bread

The glycemic response to consumption of pita bread was measured in twelve healthy participants who volunteered and signed the informed consent forms. Subjects in the age group of 18-30 years and having fasting blood glucose levels between 70-100 mg/dL were selected for the test. The height and weight of the participants were recorded prior to the test to calculate BMI (Body Mass Index). Patients with similar BMI were included in the test (Table 4.2). Participants were required to fast for 12 hours and limit their physical activity for 48 hours prior to testing. Following fasting, each participant was given 50 g of available carbohydrates of the pita bread (one at a time) to ingest them in a random order. After each ingestion, blood samples were collected from each participant using the simple finger-prick test to measure the individual's glucose levels at 0, 30, 60, 90, and 120 minutes (Figure 4.1). Available carbohydrates are defined as the fraction of carbohydrates that human enzymes can digest. It can be calculated either by difference once all other nutrients are known or it can be analyzed directly. In our study it was calculated by differences method. To calculate available carbohydrate by difference, the following formula was employed:  $100 - (\text{weight in grams} [\text{protein} + \text{fat} + \text{water} + \text{ash} + \text{alcohol} + \text{dietary fiber}] \text{ in } 100 \text{ g of food})$ .

Available carbohydrates were calculated for all different types of pita breads used in the glycemic response test. The weight of each type of pita bread fed to subjects corresponded to the amount needed to yield 50 grams of available carbohydrates.

#### 4.2.2.5.3 Nutrient Profile of test food

Physico-chemical properties such as moisture, protein, total dietary fibers, fat, ash, and carbohydrates were determined for the control and 6 treatments of pita bread.

All seven types of pita bread were freeze-dried for 3-4 days in a shelf freeze dryer (Company: Virtis, Model: USM15) prior to milling in Retsch mill (Company: GmbH & Co. Germany, Model: KG 5657HAAN1) at the centrifugal speed of 20,000 rpm. The powder obtained after milling was sieved using a 0.5mm sieve to obtain homogenous fine flour.

#### 4.2.2.5.4 Dietary Energy density

Food energy values are based on theoretical calculations rather than from direct energy measurements. The Food and Drug Administration (FDA) allows calorie content to be calculated using the Atwater method for nutrition labeling of food products. This method provides calories per gram values for protein (4 calories), fat (9 calories), available carbohydrates (4 calories) and total dietary fiber (2 calories). Composite protein, fat, and carbohydrates calorie factor were calculated using values per 100 grams of protein, fat, and carbohydrates for each ingredient using the formula:

$$\text{Energy (kcal/100g EP)} = \text{protein (g/100g EP)} \times 4 + \text{fat (g/100g EP)} \times 9 + \text{available carbohydrates (g/100g EP)} \times 4 + \text{dietary fiber (g/100g EP)} \times 2 + \text{alcohol (g/100g EP)} \times 7.$$



## **4.3 Results and discussion**

### **4.3.1 Proximate analysis**

#### **4.3.1.1 Nutritional composition of the raw ingredients**

Table 4.4 provides the nutritional composition for the raw starting materials used in the pita bread production, namely all-purpose flour, chickpea flour and food grade distiller's grains. These materials varied considerably in their content of moisture, protein, fats, minerals and carbohydrates as reflected by their composition. Their diversity thus provided for unique properties in the finished products when they were brought into the pita bread formulations in fixed ratios described earlier in table 4.1. Food Grade DDG was composed of protein (31.0%), TDF (30.9%), fat (5.1%), and ash (3.1%) in composition. Chickpea flour in contrast to all-purpose flour, had almost twice the amount of protein (22.3%), about four times higher TDF (21.1%) and ash content (2.6%), and the fat content was almost doubled (3.2%).

Table 4.5 provides the proximate composition of pita bread samples. The results showed that fortification levels of 10 and 20% of chickpea and FDDG individually, or as a combination of the two, resulted in significant increases in protein, fat, ash, and TDF contents while, moisture content and carbohydrates content were reduced.

#### **4.3.1.2 Nutritional composition of pita bread**

##### **4.3.1.2.1 Moisture content**

Table 4.5 shows that as the fortification levels of chickpea and FDDG increased, moisture content in the pita bread, decreased. Control pita bread with all wheat flour had the highest level of moisture while the breads containing 70% wheat flour showed the

lowest moisture content (30%). Other workers have reported reductions in moisture content in baked goods such as naan breads, cookies, and pizza fortified with DDG (Staudt and Zeigler, 1973; Ahmed 1997; Arra, 2011; Tsen et.al,1983; Maga and Van Everen,1988; Parmar, 2012; and Saunders et.al,2014). Differences in the initial moisture levels in the ingredients may explain this phenomenon. Initial ingredient moisture content of FDDG was 7.2% while All Purpose flour had a moisture content of 12%. The reduction of pita bread moisture content could be also due to the high protein and fiber content of FDDG. FDDG fiber content was 30.9% when compared to that of APF (5.24%), and FDDG protein content was 31.0% where as that of APF was 12%.

In the present study, an increase in Chickpea supplementation led to a decrease in pita breads moisture content. This result is consistent with earlier reports (Shehata et.al, 1970, Hefnawy et.al, 2012). The decrease in moisture could be attributed to the inherent low moisture content of chickpea flour (8.6%), compared to the wheat flour (12%). It could be also due to the high fiber content of chickpea flour which was (21.1%) when compared to APF (5.24), and CP protein content was (22.3%) where APF was (11.95%). Several studies have reported that high fiber content flour would lead to higher absorption of free water, thus decreasing the moisture content of the final baked product (Kurek & Wyrwicz, 2015; Parmar,2012; Dreese and Hosenev 1982). Incorporation of dietary fibers to food products such as bread imparts functional properties such as increased water holding capacity (Sivam, Sun-Waterhouse, Quek, & Perera, 2010). This mechanism may lead to reduced pita bread moisture content owing to greater non-gluten ingredients such as fiber and protein that tie up moisture in the final product.

#### 4.3.1.2.2 Fat content

Table 4.5 shows that, in general, there were significant differences between the fat content of pita bread. Overall however, fat content was less than 1% in the pita breads and ranged, 0.11% to 0.28% on a dry weight basis. This low fat content shows pita bread to be an inherently low fat food entrée in accordance to FDA labeling regulations.

Results showed that since FDDG had higher fat content than chickpea (table 4.5) pita bread with FDDG generally was higher in fat content in comparison to the pita bread having chickpea as an ingredient. All treatments, with the exception of 10% CP pita breads, were higher in fat content in comparison with the all-wheat control pita bread. It is thus shown that as FDDG fortification level increased, fat content increased correspondingly. These results agreed with findings of previous researchers who fortified different types of food items, breads, and different baked products (cookies, Naan, Lavash, pizza, and steamed bread) with different levels of DDG. (Joseph et.al, 1988; Arra,2011; Pourafshar, 2011; Parmar, 2012; Tsen et.al, 1983). The phenomenon of increased fat content may be due to the initial higher fat content of FDDG (5.10%) compared to all-purpose flour (1.89%). Another reason for this perhaps was the lower level of gluten in the dough network which contributed to reduced interactions of protein and lipid and reduced fat retention in dough compared to that of the control sample (Pourafshar, 2011). The result of our study demonstrated that incorporating chickpea flour into wheat flour increased fat content as well. Similar results were concluded by (Yousseff et.al, 1976; Hallab et.al 1974; Dhinda et.al, 2012). Chickpea flour was endowed with higher fat content (3.2%) than the all-purpose flour (1.89%).

#### 4.3.1.2.3 Total Dietary Fiber Content (TDF)

Table 4.5 demonstrated that all pita bread samples were found to be significantly different from each other in TDF content. With a range of 5.21g-17.44g/100g, it can be concluded that as the fortification level increased, TDF% increased as well. Fortification with 10%D yielded double the amount of TDF (7.21%). And fortification with (20%D) increased amount of TDF by two and half times (13.05%) when compared to control (5.21%). Similar result where found by Li et.al, 2016 in an unpublished paper where they fortified steamed bread with FDDG. Fairly similar results were reported by different researches where they fortified different types of food items, breads, and different baked products with different levels of DDG. (Joseph et.al, 1988; Arra, 2011; Pourafshar, 2011; Parmar, 2012; Tsen et.al, 1983; Wu et.al, 1987). Where they concluded that increased in the Neutral detergent and crude fiber levels at the higher substitution levels of DDG. This was because DDG had higher fiber levels compared to the all-purpose flour itself. Fortification with (10%CP) increased the TDF by 50% (7.21%), whereas fortification with 20%CP doubled the TDF content (11.74%) when compared to the control (5.21%). Similar results were concluded by different study in the literature when they fortified different types of breads with chickpea flour (Yousseff et.al, 1976; Hallab et.al 1974; Dhinda et.al, 2012). The reason behind increased TDF is that both chickpea and FDDG fiber content were higher (21.10%) and (30.90%) when compared to control (5.24%).

#### 4.3.1.2.4 Protein

From Table 4.5, it can be observed that there were significant differences in protein content among all treatments when compared to the all-wheat control. It was noted that as

the level of FDDG in the pita bread increased, the protein content of the pita bread also increased. These results agreed with results from several studies where they fortified food items, particularly, breads, and different baked products with different levels of DDG (Joseph et.al, 1988; Arra, 2011; Pourafshar, 2011; Parmar, 2012; Liu et.al, 2011; Tsen et.al, 1983; Li, Wang, and Krishnan, 2016 unpublished paper. This increase occurred owing to the fact that FDDG has almost three times the protein content (31.0%) when compared to all-purpose flour (12%). It was also found in our current study that as chickpea fortification level increased, protein level increased as well. The results are in agreement with the work of others (Eissa et.al 2007; Yousseff et.al, 1976; Hallab et.al.,1974; and Dhinda et.al, 2012). These workers showed that the increase in protein content was the direct result of the appreciably higher protein content of chickpea flour in foods.

#### **4.3.1.2.5 Carbohydrates (CHO)**

In our study, available carbohydrates as opposed to total carbohydrates was employed as called for in the glycemic response protocol. Available carbohydrates was calculated by a formula described earlier in the methods section. From table 4.5, it can be observed that as the substitution level of FDDG increased, the available carbohydrates in the pita bread decreased. That could be due to the fact that our FDDG had lower initial carbohydrates content (24.1%) when compared to all-purpose flour (68.3%). Several DDG fortification studies in the literature also are in agreement with our findings, where the authors concluded that increasing the fortification of DDG in the wheat products decreased carbohydrates content. (Tsen et.al, 1983; Liu et.al, 2011; unpublished paper by Li et.al, 2016). Diminished available carbohydrates in FDDG fortified porridge was also

reported in the work of Bechen (2008) who was the earliest to show diminished available carbohydrates in FDDG and potential for glycemic response reduction by Distillers Dried Grains.

When related to chickpea fortification, it was also shown that as chickpea fortification level increased, carbohydrates content decreased proportionately. This was due to the fact that chickpea had lower carbohydrates content (42.2%) in contrast to All Purpose Flour (68.3%).

Similar results were found by (Garg and Dahiya,2003; Hefnawy et.al 2012; Goni and Valentin-Gamazo, 2003; Dhinda et.al,2012; Yousseff et.al, 1976; Hallab et.al, 1974; Utrilla-Ceollo et.al, 2007). These workers fortified different types of breads and pasta with chickpea flour at different fortifications levels.

#### **4.3.2 Antioxidants**

Table 4.6 provides data on the total phenolics, antioxidants activity, and total carotenoids content of wheat flour control and wheat pita bread flour blends containing chickpea and FDDG. As the fortification levels of the two enrichment ingredients (chickpea and FDDG) increased in the pita breads, total phenolics (TPC), antioxidant activity (AA), and total carotenoids content increased significantly at each level of fortification. Each treatment was significantly different over the control, and when compared to each other in relation to TPC, AA, and Carotenoids content.

#### **4.3.3 Glycemic response**

As shown in table 4.5 all seven pita bread samples contained significantly different fat, protein, moisture, ash, fiber, and carbohydrates content. The enrichment of wheat-based

pita breads with chickpea and FDDG as protein and fiber adjuncts brought about significant improvements in both dietary fiber and protein contents. The differences in composition in the 6 pita bread treatments was attributed to the two ingredients (FDDG and chickpea) used in the formulation of the pita bread. These ingredients were each significantly different from each other in nutrient composition. The levels (0, 10 or 20%) and the type of ingredient (chick pea versus FDDG) had significant effects on the nutritional content of the finished product, namely pita bread. The addition of chickpea flour and FDDG and combinations of the two ingredients, increased fat, protein, fiber content of pita bread while the amount of carbohydrates decreased significantly. Different serving sizes were fed to subjects to ensure that each subject consumed 50g of available carbohydrates. Available carbohydrates are used as a criterion in the glycemic response study as opposed to total calories. Serving sizes of pita bread corresponding to 50g of available carbohydrates were consumed by test subjects and their blood sugar was monitored at 30 minute intervals (figure 4.1).

Table 4.7 shows the results for incremental area under the curve (IAUC) and GI values for control and all 6 treatments. Control (all-wheat pita bread) yield the highest value for IAUC (94.4 mmol.min/L) while pita bread from treatment 6 (70W-20D-10CP%) yielded an IAUC of (36.5 mmol.min/L). Similarly, GI values followed a similar pattern with the Control all-wheat pita bread yielding the highest GI and 70W-20D-10CP yielding the lowest GI. It can be concluded from this information that as fortification level of FDDG only, or chickpea only, or FDDG combinations increased in the pita breads, both the IAUC and GI values decreased correspondingly.

Figure 4.2 shows graphically, the glycemic response of subjects who were fed the various pita breads corresponding to the different treatments. Each treatment (namely DDG or Chickpea) and the varied doses of the two ingredients showed distinctly different glycemic responses in the test subjects (N=12). As shown in the figure 4.2, treatment 6, namely, the pita containing (70W-20D-10CP%) had the most dramatic effect of lowering blood glycemic response, whereas control all-wheat pita bread (100%APF) showed the smallest lowering effect on blood glycemic response. It can be concluded from figure 4.2 that as fortification level of FDDG increased in the pita bread, the glycemic response depression increased correspondingly. Bechen et al, (2008) establish this relationship between DDG feeding and glycemic response reduction in our laboratory. Bechen compared glycemic response for 3 different porridges made with APF, whole wheat flour, and FDDG. Her results showed that DDG porridge compared to wheat and APF produced the lowest glycemic response. Her conclusion was that the depressing effect on glycemic response was caused by the higher levels of protein, TDF, and fat content of FDDG in contrast to low levels of those constituents found in APF pita breads. Major factors that have the capability to reduce postprandial glucose response include the amount of fiber, type of fiber, protein, and fat content available in the food products (Marques et al., 2007; Marinangeli et al., 2009). Another factor that has the capability of reducing glycemic response includes the starch. Moghaddam, Vogt & Wolever, (2006) reported results consistent with their hypothesis that proteins reduce blood glucose response through amino acid mediated effects on human body insulin secretion. Various mechanisms have been postulated to explain the mode of action of dietary constituents in lowering glycemic response. Insoluble fibers exert their effects on decreasing the



digestion and rate of carbohydrates absorption (Higgins, 2012) which in turn, will reduce postprandial glycemic response. Starch digestion can be obstructed by dietary fibers (hemicellulose, cellulose, and lignin) that will prevent digestive enzymes access to their substrate which will thus cause reduced glycemic index (Reyes-Pérez, Salazar-García, Romero-Baranzini, Islas-Rubio & Ramírez-Wong, 2013). The presence of high levels of fiber and protein can depress blood glycemic response (Marques et al., 2007; Marinangeli et al., 2009; Dhinda et.al, 2012; Utrilla-Coello, et.al 2007). The depression of glycemic response that is caused by high fiber high protein, is a result of delayed gastric emptying which in turn slows down carbohydrates digestion (Meynier, Goux, Atkinson, Brack & Vinoy, 2015). A diet with high fat increases the power action of oral glucose on the gastric inhibitory of polypeptide secretion and influences gastrointestinal transit, explaining glucose lowering effect of fat is influenced by constant fat intake (Moghaddam & Wolever, 2006). The work of previous researchers on protein and fiber effects on starch digestion and absorption provides plausible mechanisms and modes of action for DDG and chickpea observed in our study. All-purpose flour pita with the lowest protein content and lowest fiber content, may have had the lowest effect in mitigating the rise in blood glucose as previously mentioned in the literature by (Bechen,2008; Bloomgarden,2004; Gretebech et.al., 2002; Mayod, 2005; Miller-Jones, 2002; Ostman, 2006; Schulze et al.,2004).

It was also noted from figure 4.2 that as chickpea fortification level increased, the glycemic response depression increased as well. However, the glycemic response reduction was not as dramatic as that produced by FDDG. Nestle et.al, (2004) compared the effects on insulin sensitivity of chickpea-based and wheat-based foods when these

foods were eaten as single meals or over 6 weeks. These workers concluded that when compared with a wheat-based meal, a single chickpea-based meal led to a diminished glycemic response in plasma glucose and insulin concentrations due to delayed gastric emptying.

Mollard et.al, (2014) studied the effect of 4 different pulses (chickpeas, yellow peas, navy beans, lentils) on blood glucose levels and appetite on 15 healthy men. These workers conducted a cross-over design with an iso-caloric (300 kcal) treatment with different amount of serving for each treatment. The weights of serving of chickpea, lentils, navy beans, yellow peas were 222.8g, 332.9g, 240.59g and 375.6g, respectively. Fasting blood samples (10-12h) were drawn, then after consuming the various legumes at 15, 30, 45, 75 and 135 minutes. Their (AUC) results showed that all treatments except nave beans had significant differences when compared to control (white bread). They concluded that blood glucose levels were lower after consuming lentils and chickpea when compared with white bread.

Panlasigui, Panlilio, & Madrid (1995) studied the glycemic response of five different legumes (chickpea (100g), pigeon pea (107.7), black bean (99.5g) , mung bean (93.8), and white bean (110.3g) in healthy subjects. Different portion sizes of the previous five beans were giving to achieve 50 grams of available carbohydrates in order to follow the glycemic response protocol. Fasting blood test were taken at fifteen minute intervals after consuming tested foods. The area under the curve was calculated and compared for all tested foods in relation to the control (bread). Their results showed that blood glucose response was significantly lower than control (bread) with chickpea having the lowest value. The reason behind that was due to the higher amount of fat which

caused delayed gastric emptying, higher amount of fiber especially soluble fibers, the amylose content in chickpea which forms a rigid gel that makes starch less accessible to hydrolytic enzymes.

Jenkins, Wolever, Taylor, Barker & Fielden (1980) tested blood glucose response to dried beans compared to other carbohydrates foods. Twenty-five healthy volunteers consumed different types of beans, grains, breads and pasta, breakfast cereals, biscuits, and tubers. Blood glucose test were done at fasting and 15,30,45,60,90,120 minutes) after consuming test foods. Area under the curves were then calculated and compared. The authors reported that dried legumes yielded significantly lower glucose response below the mean curves for other food groups. These workers credited the results to the fiber and resistant starches of legumes that are resistant to enzymatic breakdown.

Thompson, Winham, & Hutchins, (2012) compared rice to beans, and rice and bean mixed meals to test glycemic response in adults with type 2 diabetes. Seventeen men aged 35-70 were asked to consume 4 different test meals: white long grain rice (control), pinto beans with rice, black beans with rice, red kidney beans and rice to achieve 50 g available carbohydrates diet. Meals were consumed as breakfast on 4 different days after 10-12 hours of fasting. Blood glucose values were taken at fasting, and the 30 minute intervals after consuming test foods. The work of these authors showed that glucose response curves for the three combined rice and beans meals were significantly lower than control curve. They concluded that the cause of the glycemic curve depression could be due to the specific fiber fraction in the three kinds of beans. These beans contained soluble fibers and resistant starch which are known to reduce glycemic response.

Our results was also in agreement with a study was done by Utrilla-Coello, et.al (2007) who reported that chickpea fortified bread showed a lower glycemic response than wheat flour bread. The authors postulated that the dietary fiber present in chickpea exerted significant effects on the starch digestion and absorption rate of the breads. This rate may be reduced by the starch type and gelatinization degree, indigestible polymer, amylose lipid complex and indigestible protein. Dhinda et.al, (2012) tested the effects of ingredients on rheological, nutritional, and quality properties of high protein high fiber, low carbohydrates breads. These workers fortified wheat flour with 20%, 40%, and 60% SPOBCP blend (soy protein, oat bran, and chickpea). They used the in vitro starch hydrolysis method and found that breads fortified with SPOBCP had significantly lower starch hydrolysis when compared to control wheat flour bread. They concluded that the slow release of glucose in the fortified bread maybe attributed to the higher fiber, resistance starch (RS), and  $\beta$ -glucan. Gon and Valentín-Gamazo (2003) produced pasta fortified with chickpea flour using in vitro starch hydrolysis and in vivo glycemic response methods on 12 healthy females. The in vitro results showed lower degree of starch hydrolysis in chickpea (25%) fortified spaghetti compared to control (100%) wheat spaghetti. The in vivo results showed that postprandial rises in blood glucose for subjects who consumed chickpea fortified spaghetti was smaller than those given control. The authors speculated that chickpea contains non digestible constituents such as resistance starch, oligosaccharides, polyphenols and lectins. They concluded that the indigestible fraction (IF) was higher in fortified pasta which could be a reason for glycemic response depression. IF contains non-starch polysaccharides, lignin, compounds like non-digestible oligosaccharides stachyose, and resistant protein. These compounds are

resistant to digestion enzymes, which interfere with normal starch breakdown. The authors concluded that pasta fortified with chickpea presented a lower glycemic response compared to control wheat pasta. Thus, chickpea could help broaden the range of low-GI foods that are available to the consumer.

Our work in this study, to our knowledge, is the first study to test the statistical relationships between glycemic response (IAUC) and macronutrients (protein, fat, and fiber). Figures 4.3, 4.4, and 4.5 track the IAUC in relation to total protein, fat, and fiber. The graphs show strong coefficients of determination ( $R^2$ ) between key variables. A strong negative correlation was obtained between glycemic response (IAUC) and protein (-0.89); Glycemic response and fat (-0.69); and Glycemic response and fiber (-0.93).

#### **4.3.3.1 Antioxidant and GR**

Another reason for the glycemic response depression could be due to the carotenoid, phenolics, and antioxidant activity that is presented in FDDG and chickpea.

From table 4.6 it can be demonstrated that as FDDG and chickpea fortification level increased, carotenoids, phenolics, and antioxidant activity increased as well. Similar results were found by Vergara-Valencia, Granados-Pérez, Agama-Acevedo, Tovar, Ruales, & Bello-Pérez, (2007) where they fortified bread and cookies with mango dietary fibers (MDF) which are rich in carotenoids and polyphenols. They concluded that bakery products added with MDF showed higher TDF than respective controls, and the products maintained significant antioxidant capacity and low predicted glycemic indices. These ingredients may thus be used as dietary aids by people with special low caloric. This particular review brings to light the recent interests in nutrition and disease prevention

that may drive a consumer demand for functional bread with enhanced fiber and phenolic antioxidant contents.

Our work in this study, is to our knowledge the first study to test the statistical relationships between glycemic response (IAUC) and total phenolics, total carotenoids, and antioxidant activity percentage. Figures (4.6, 4.7, and 4.8) tracks the IAUC in relation to total phenolics, AA, and carotenoids. The graphics show strong coefficients of determination ( $R^2$ ). A strong negative correlation was obtained between glycemic response (IAUC) and TPC (0.74); Glycemic response and AA% (0.83); and Glycemic response and carotenoids (0.87). The high  $R^2$  also show that there is a strong positive correlation between both carotenoids (0.92) and TPC (0.98) values when related to antioxidant activity.

Antioxidant potential and thermal stability of chickpea proteins containing heavily albumin fraction. Chickpea contains high protein content and have low amount of toxic and anti-nutritive factors. Due to their nutritive value and functional properties, proteins are used as ingredients in different food systems. Plant (legumes and cereals) proteins have been reported to possess antioxidant activity. The proteins owe their antioxidant activity to their constituent amino acids such as aromatic, sulfur containing and basic amino acids which are capable to donate protons to free radicals (Arcan and Yemenicioglu, 2010)

Phenolics and carotenoids from legumes can inhibit carbohydrate breakdown and control of glycemic index of food products. Therefore, utilization of legume flours in the development of functional foods with increased therapeutic value would be a significant step toward disease prevention and management through diet. Chickpea with lower a-

amylase and higher  $\alpha$ -glucosidase inhibitory activities could be used as food ingredients and in composite flours for the delayed absorption of dietary carbohydrates in the meal, leading to suppression of an increase in postprandial blood glucose level without adverse effects (Ghiassi et.al 2012; Sreerama et.al, 2012).

DDGS on the other hand is a rich source of phenolic antioxidants. DDGS from corn contain almost three times more phenolic content than corn Luthria et.al, (2012). This may be of great interest to corn processors, ethanol manufacturers, and DDGS users since phenolic acids have potential health benefits to diabetic patients. These could be to factors such as the non-digestible constituents presented in chickpea, such as, oligosaccharides, RS, polyphenols and lectins. Other factors can contribute, such as cell walls rigidity of cotyledon, the intrinsically low enzyme susceptibility of legume starches, and the presence of polyphenols and other  $\alpha$ -amylase inhibitor. Moreover, a high proportion of non-digestible carbohydrates, such as RS, non-starch polysaccharides and oligosaccharides, contribute to a low glycemic response (Gon and Valenti'n-Gamazo, 2003;)

Major factors that have the capability to reduce postprandial glucose response are the total amount of fiber, type of fiber, protein, fat content available in the food products (Marques et al., 2007; Marinangeli et al., 2009). Other factors include starch and protein interactions (Jenkins et al., 1987; Hutchins et.al, 2012), as well as the presence of specific anti-nutrients and bioactive components, such as phytochemicals (tannins, phenolic acids, flavonoids and phytic acid (Champ, 2002; Hutchins et.al, 2012; Yudan liu,2012).

Another reason for low blood glucose response of the treatments when compared to control could be partially attributed to the high fat content associated with the fortified

pita breads. Presence of fat could reduce glycemic response by prolonging gastric emptying time (Leonora et.al, 1995; Moghaddam et.al, 2006; Marinangeli et.,al 2009). Other significant factor that has been widely investigated in legumes for its role in lowering the rate of digestion and blood glucose response is the amount of dietary fibers especially the soluble ones. Amylose content of the legumes was reported as one of the several factors in lowering the glucose response. In general, legumes contain 30-40 % of amylose and 60-70% of amylopectin in the starch granules (Leonora et.al, 1995). The significant lower plasma glucose and insulin concentration after the single chickpea meals might be due to higher amylose content of chickpeas. It can be corroborated from the findings that starch digested and absorbed more slowly in the small intestine from chickpeas than from wheat (Nestel et al., 2004; Hutchins et.al, 2012).

#### **4.3.3.2 Maillard and caramelization reactions in relation to GR**

Another reason behind the glycemic response suppression of pita breads could be due to Maillard reaction. Bakery products such as breads show a strong Maillard reaction (Sadd and Hamlet 2005). Baking has been reported to increase the antioxidant activity of whole meal bread compared with its flour and that the crust of white bread contained slightly more phenolic compounds than the crumb, because of the Maillard reaction (Yu & Nanguet, 2013). Bread products that exhibited browning reactions, especially caramelization intermediates, show antioxidant capacities (Sivam, Sun-Waterhouse, Quek, & Perera, 2010).

The study by Capuano, Garofalo, Napolitano, Zielinski & Fogliano, (2010) reported that antioxidant activity increased during toasting as a consequence of antioxidant Maillard reaction product formation. Their data suggested that the rate of



Maillard reaction were higher in whole flours owing to their higher free amino acids and protein content.

#### **4.4 Conclusion**

Many factors in this study were identified as capable of suppressing blood glucose response including the amount of fiber, protein, and fat as well as antioxidants presented in the tested food. It was shown that as the fortification level of chickpea and FDDG alone or in combination increased, glycemic response depression increased. Chickpea flour and FDDG can be both used as functional ingredients to produce unique low glycemic foods. The study findings have revealed that both chickpea and FDDG fortified pita breads showed significant depression in the glycemic response compared to the control bread. Results of the present study bolster the idea of using of chickpea flour and FDDG as a tool for scientists, health care practitioners and consumers in developing more nutritious, tasty, healthy, low glycemic foods that could assist in preventing and managing modern day life-style related diseases such as diabetes. Therefore, these findings suggest that chickpea could be added to the list of foods for diabetic's prone patients and consumption of legume related products in larger amounts should be recommended. Also introducing the use of FDDGS to be used as a new good source of high TDF, protein, and antioxidant which can be used in fortifying baked products to achieve lower glycemic response, which allows its uses within the diabetic diet. The mixture of these two ingredients could help broaden the range of low-GI foods available to the consumer.

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**Table 4. 1 Experimental design showing proportions of All Purpose wheat Flour (W), Chickpea (CP) and Distillers Dried Grains in control and treatment blends.**

| Treatment (T)<br>APF:CP:FDDGS | Fortification Level      |                        |                        |
|-------------------------------|--------------------------|------------------------|------------------------|
|                               | All-purpose flour<br>(W) | Chickpea flour<br>(CP) | Food grade DDGS<br>(D) |
| <b>Control</b>                | 100                      | 0                      | 0                      |
| <b>T1(90:10:0)</b>            | 90                       | 10                     | 0                      |
| <b>T2 (90:0:10)</b>           | 90                       | 0                      | 10                     |
| <b>T3(80:20:0)</b>            | 80                       | 20                     | 0                      |
| <b>T4(80:0:20)</b>            | 80                       | 0                      | 20                     |
| <b>T5 (70:20:10)</b>          | 70                       | 20                     | 10                     |
| <b>T6 (70:10:20)</b>          | 70                       | 10                     | 20                     |

DDGS (D): Dried Distillers Grains.

W= wheat, (APF) All Purpose Flour

CP=chickpea

D= DDG

**Table 4. 2 Demographics data for Individual participants in the Glycemic Response study**

| <b>Participant</b> | <b>Education</b> | <b>Gender</b> | <b>Race</b> | <b>Age</b> | <b>Height (cm)</b> | <b>Weight (kg)</b> | <b>BMI (kg/m<sup>2</sup>)</b> |
|--------------------|------------------|---------------|-------------|------------|--------------------|--------------------|-------------------------------|
| <b>1</b>           | UG               | Female        | h/I         | 24         | 167                | 65.5               | 23.5                          |
| <b>2</b>           | G                | Female        | Asian       | 26         | 158.5              | 55.7               | 22.2                          |
| <b>3</b>           | UG               | Female        | Asn         | 19         | 166                | 68.2               | 24.7                          |
| <b>4</b>           | G                | Male          | Asn         | 27         | 166                | 63.6               | 23.1                          |
| <b>5</b>           | G                | Female        | Mde         | 30         | 160                | 59.1               | 23.1                          |
| <b>6</b>           | UG               | Female        | Wht         | 22         | 161                | 50.4               | 19.4                          |
| <b>7</b>           | UG               | Female        | Wht         | 22         | 162                | 50.8               | 19.4                          |
| <b>8</b>           | UG               | Female        | Wht         | 21         | 163                | 57.3               | 21.6                          |
| <b>9</b>           | UG               | Female        | Wht         | 20         | 164                | 59.9               | 22.3                          |
| <b>10</b>          | UG               | Female        | Mde         | 30         | 181                | 79.7               | 24.3                          |
| <b>11</b>          | UG               | Male          | Blk         | 23         | 179                | 80.4               | 25.1                          |
| <b>12</b>          | G                | Male          | Asn         | 27         | 167                | 69.5               | 24.9                          |
| <b>Average</b>     |                  |               |             | 24.25      |                    |                    | 22.8                          |
| <b>Std. Dev.</b>   |                  |               |             | (3.56)     |                    |                    | (1.94)                        |

UG: Undergraduate, G: Graduate, Asn: Asian, Blk: Black, h/I: Hispanic Mde: Middle-east, Wht: White, Std. Dev.: Standard deviation

**Table 4. 3 Gender-based demographic data of participants.**

| <b>Gender</b>    | <b>Age</b> | <b>Height<br/>(cm)</b> | <b>Weight (kg)</b> | <b>BMI</b> |
|------------------|------------|------------------------|--------------------|------------|
| <b>Female</b>    | 23.3       | 163.2                  | 59.1               | 22.1       |
| <b>Std. Dev.</b> | 3.53       | 2.43                   | 7.12               | 2.16       |
| <b>Male</b>      | 27.3       | 179.3                  | 77.5               | 24.1       |
| <b>Std. Dev.</b> | 3.78       | 1.52                   | 4.48               | 1.16       |

BMI: Body mass Index, Std. Dev.: Standard Deviation

**Table 4. 4 Proximate composition of raw ingredients used in pita breads employed in the glycemic response study**

| Nutrient        | %                        |                        |                        |
|-----------------|--------------------------|------------------------|------------------------|
|                 | All-Purpose flour<br>(W) | Chickpea flour<br>(CP) | Food grade DDGS<br>(D) |
| <b>Moisture</b> | 12.0a                    | 8.60b                  | 5.80c                  |
| <b>Protein</b>  | 12.0c                    | 22.30b                 | 31.0a                  |
| <b>Fat</b>      | 1.89c                    | 3.20b                  | 5.10a                  |
| <b>Ash</b>      | 0.61c                    | 2.60b                  | 3.10a                  |
| <b>TDF</b>      | 5.24c                    | 21.1b                  | 30.9a                  |
| <b>CHO</b>      | 68.3a                    | 42.2b                  | 24.1c                  |
| <b>Total</b>    | 100%                     | 100%                   | 100%                   |

DDGS: Dried Distillers Grains TDF: Total dietary fibers, CHO: Carbohydrates.  
Means with the same letter within rows are not significantly different ( $P < 0.05$ ).

**Table 4. 5 Total Phenolic Content (TPC), antioxidant activity (AA%) and Carotenoids content of pita bread enriched with chickpea and distillers dried grains.**

| <b>Pita breads</b>     | <b>TPC(mg TAE/100 g)</b> | <b>AA%</b> | <b>Carotenoids µg/100g</b> |
|------------------------|--------------------------|------------|----------------------------|
| <b>CONTROL (100W%)</b> | 234.8g                   | 155.9g     | 41.9g                      |
| <b>90W-10CP%</b>       | 240.7ef                  | 208.8f     | 251.3f                     |
| <b>90W-10D%</b>        | 336.0e                   | 229.4e     | 396.8e                     |
| <b>80W-20CP%</b>       | 383.6d                   | 260.3d     | 489.4d                     |
| <b>80W-20D%</b>        | 419.4c                   | 275.0c     | 597.5c                     |
| <b>70W-20CP-10D%</b>   | 529.5b                   | 377.9b     | 842.2b                     |
| <b>70W-20D-10C%</b>    | 770.7a                   | 425.0a     | 1084.7a                    |

TPC: total phenolic content, AA: antioxidant activity. CP=chickpea D= DDGW= wheat, All Purpose Flour

**Table 4. 6 Physico chemical properties of pita breads enriched with 10 to 20% chickpea or Distillers grains and 30% flour replacement with combinations of DDG and chickpea (dry basis)**

| <b>Nutrients</b>                              | <b>Control<br/>100W%</b> | <b>T1<br/>90W-<br/>10CP%</b> | <b>T2<br/>90W-<br/>10D%</b> | <b>T3<br/>80W-<br/>20CP%</b> | <b>T4<br/>80W-<br/>20D%</b> | <b>T5<br/>70W-20CP-<br/>10D%</b> | <b>T6<br/>70W-20D-<br/>10CP%</b> |
|---|--------------------------|------------------------------|-----------------------------|------------------------------|-----------------------------|----------------------------------|----------------------------------|
| <b>Protein</b>                                | 14.8g<br>(0.05)          | 16.7f<br>(0.06)              | 17.3e<br>(0.09)             | 18.1d<br>(0.11)              | 18.6c<br>(0.10)             | 18.9b<br>(0.02)                  | 19.6a<br>(0.13)                  |
| <b>Fat</b>                                    | 0.11f<br>(0.00)          | 0.11f<br>(0.00)              | 0.12e<br>(0.00)             | 0.13d<br>(0.00)              | 0.16c<br>(0.00)             | 0.21b<br>(0.00)                  | 0.28a<br>(0.00)                  |
| <b>Ash</b>                                    | 0.59g<br>(0.00)          | 0.6f<br>(0.00)               | 0.61e<br>(0.00)             | 0.62d<br>(0.00)              | 0.72c<br>(0.00)             | 0.97b<br>(0.00)                  | 1.06a<br>(0.00)                  |
| <b>Moisture</b>                               | 40.3a<br>(0.25)          | 38.6b<br>(0.50)              | 34.2c<br>(0.28)             | 32.0d<br>(0.05)              | 31.e<br>(0.09)              | 30.1f<br>(0.16)                  | 30.0f<br>(0.20)                  |
| <b>TDF</b>                                    | 5.21g<br>(0.31)          | 7.21f<br>(0.31)              | 10.04e<br>(0.28)            | 11.74d<br>(0.31)             | 13.05c<br>(0.22)            | 15.64b<br>(0.54)                 | 17.44a<br>(0.81)                 |
| <b>Kcal /100<br/>g</b>                        | 267.50                   | 263.0                        | 254.1                       | 247.00                       | 234.00                      | 212.5                            | 201.0                            |
| <b>Av<br/>(CHO) in<br/>100 g</b>              | 49.2                     | 45.3                         | 41.0                        | 37.5                         | 33.0                        | 25.9                             | 21.4                             |
| <b>Amt. ser.<br/>TA/50 g<br/>Av<br/>(CHO)</b> | 101.5                    | 110.4                        | 122.1                       | 133.4                        | 151.4                       | 192.8                            | 234.0                            |

TDF: Total Dietary Fibers, Kcal: Kilocalories, g: grams, Amt.: Amount, ser.: served, TA: to achieve, Av: available, CHO: Carbohydrates W=wheat flour, D=food grade DDGS, G=garbanzo/chickpea flour

Means across rows with the same letter are not significantly different (P<0.05)



**Table 4. 7 Total phenolic, Carotenoids, and Antioxidant activity for ingredients**

| <b>Ingredient</b>            | <b>TPC (mg TAE/100 g)</b> | <b>AA%</b> | <b>Carotenoids <math>\mu\text{g}/100\text{g}</math></b> |
|------------------------------|---------------------------|------------|---|
| <b>APF (W)</b>               | 142.5c                    | 123.5c     | 22c   |
| <b>Chickpea (CP)</b>         | 1390.0b                   | 566.2b     | 1382.3b   |
| <b>Distillers grains (D)</b> | 2062.9a                   | 789.7a     | 2021.6a   |

Means with the same letter within columns

TPC: total phenolic content, AA: antioxidant activity.

CP=chickpea

D= DDG

W= wheat, (APF) All Purpose Flour

**Table 4. 8 Incremental Area under the Curve (IAUC) and Glycemic Index (GI,) of test subjects consuming pita bread containing varied ratios of wheat, chickpeas and distillers grains.**

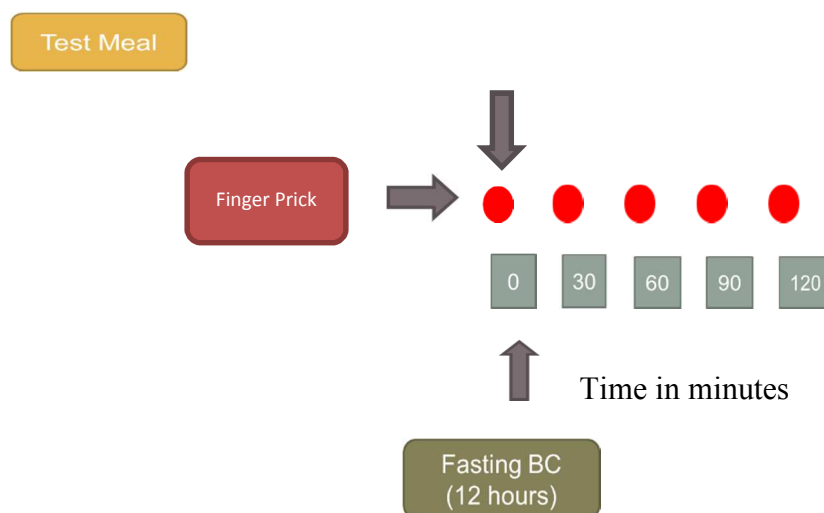
| <b>Pita breads</b>     | <b>IAUC<br/>mg.min/dl</b> | <b>IAUC<br/>mmol.min/L</b> | <b>GI</b> | <b>Reduction%</b> |
|------------------------|---------------------------|----------------------------|-----------|-------------------|
| <b>Control (100%W)</b> | 1708.86                   | 94.84                      | 100       | 0                 |
| <b>90W-10CP%</b>       | 1539.89                   | 85.46                      | 90.10     | 9.9               |
| <b>90W-10D%</b>        | 1463.28                   | 81.21                      | 85.62     | 14                |
| <b>80W-20CP%</b>       | 1014.92                   | 56.32                      | 59.38     | 40                |
| <b>80W-20D%</b>        | 833.06                    | 46.23                      | 48.74     | 51                |
| <b>70W-20CP-10D%</b>   | 721.92                    | 40.06                      | 42.23     | 57                |
| <b>70W-20D-10C%</b>    | 658.22                    | 36.53                      | 38.15     | 61                |

IAUC: incremental area under the curve (measured by FAO method calculating area under the curve for triangles and trapezoid GI: glycemic index, GI= IAUC for tested food / IAUC for control\*100

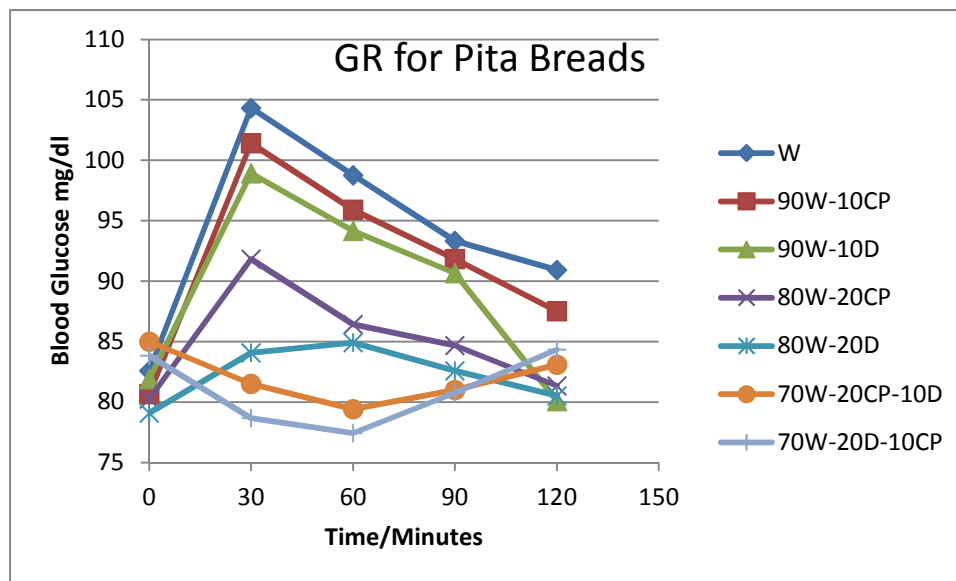
Reduction%= 100-GI

**Table 4. 9 Glucose response (mg/dL.min) of subjects monitored at 30 minute intervals after consuming pita bread from different treatments**

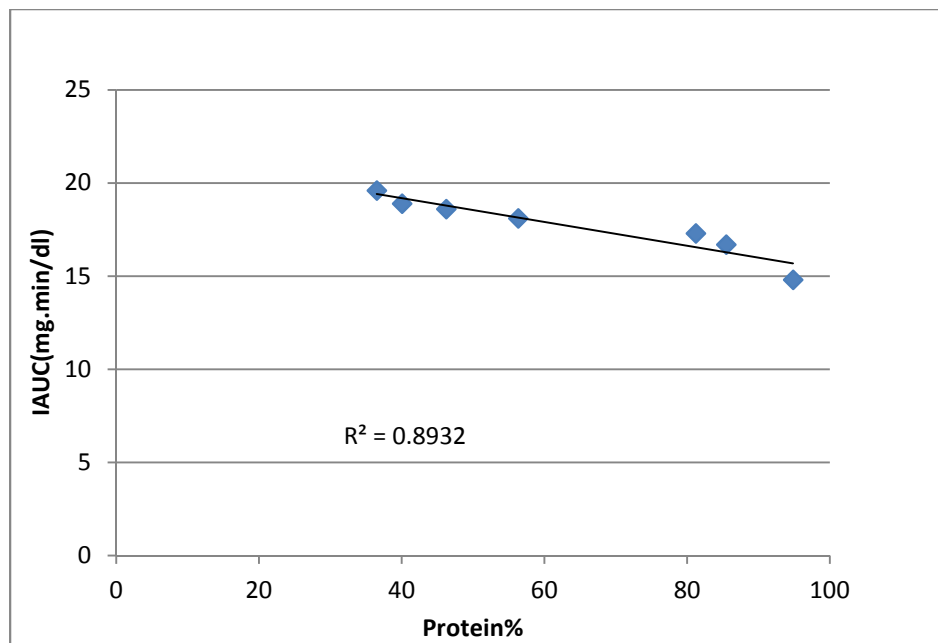
| Treatment (T)             | Time(minutes) |                |               |                |       |
|---------------------------|---------------|----------------|---------------|----------------|-------|
|                           | 0             | 30             | 60            | 90             |       |
| <b>Control 100w%</b>      | 82.58±6.33 ab | 104.33±5.26a   | 98.75±5.86a   | 93.33±6.27a    | 90.9  |
| <b>T1 (90w-10CP%)</b>     | 80.67±7.78 ab | 101.42±11.24 a | 95.92±7.61 a  | 91.83±8.20 a   | 87.50 |
| <b>T2 (90w-10D%)</b>      | 81.92±7.18ab  | 98.92±9.07 a   | 94.17±10.99 a | 90.67±13.61 ab | 88.08 |
| <b>T3 (80w-20CP%)</b>     | 80.25±4.88 ab | 91.08±68b      | 86.42±3.80b   | 84.67±2.67bc   | 81.3  |
| <b>T4 (80w-20D%)</b>      | 79.08±4.87b   | 84.08±5.98c    | 84.92±5.58b   | 82.58±4.48 c   | 80.5  |
| <b>T5 (70w-20CP-10D%)</b> | 85.00±4.86a   | 81.5±5.81c     | 79.42±5.48c   | 81.00±5.67c    | 83.0  |
| <b>T6 (70w-20D-10CP%)</b> | 83.83±6.13 ab | 78.67±5.25 c   | 77.42±3.92c   | 80.75±2.90c    | 84.33 |



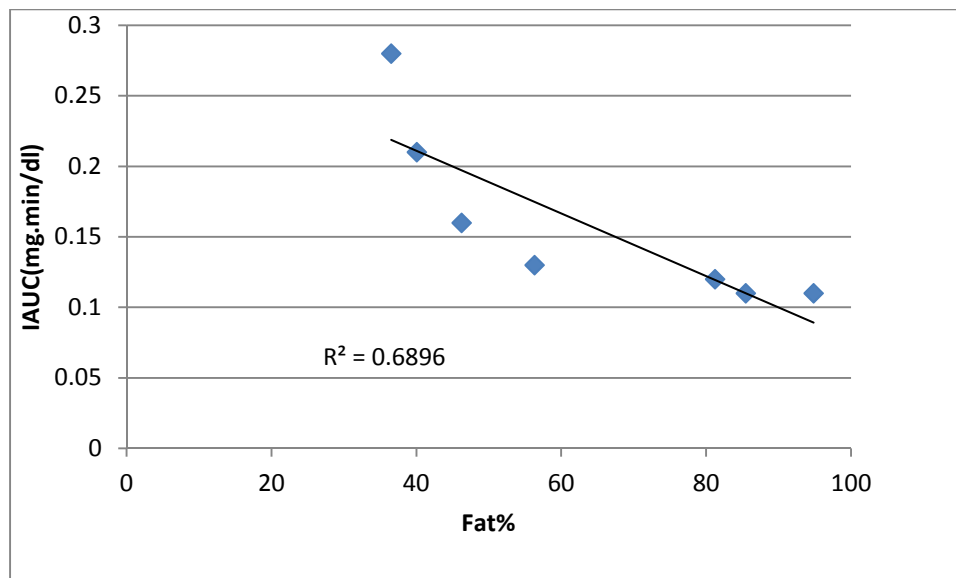
**Figure 4. 1: Graphic representation of Glycemic Response study for determination of effects of chickpea, ddg and wheat flour in pita bread on blood sugar. Experiment Design: (Marinangeli, Kassis, & Jones, 2009).**



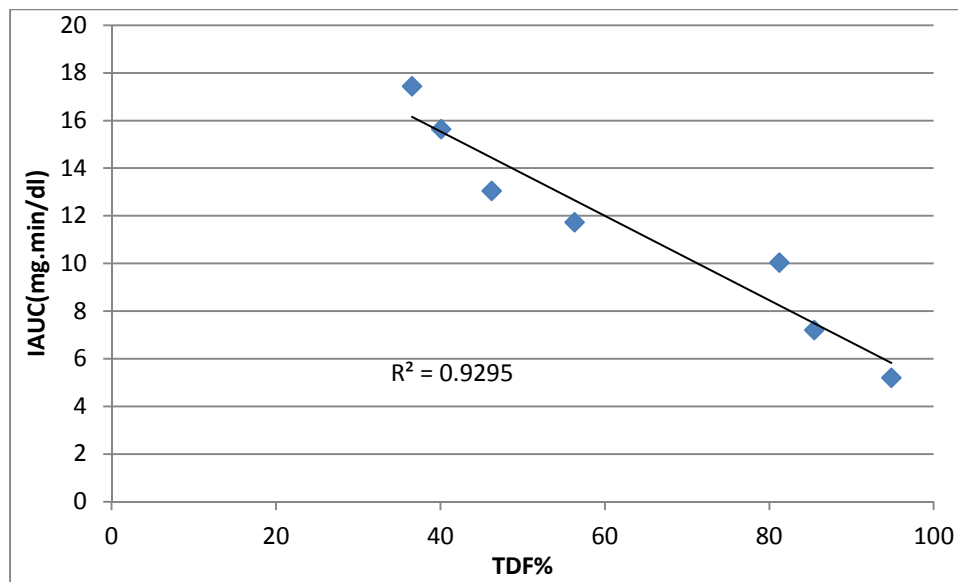
**Figure 4. 2 Glycemic response of subjects consuming control all-wheat pita bread (W), pita bread containing 10% to 20% chick pea or Distillers grains (90w-10cp, 90w-10d, 80w-20cp, 80w-20D), and wheat pita bread containing combinations of chickpea and DDG.(700W-10CP-20D & 70W-20CP:10D)**



**Figure 4. 3** Correlation coefficient between (IAUC) of n= 10 subjects and protein content of consumed pita breads.

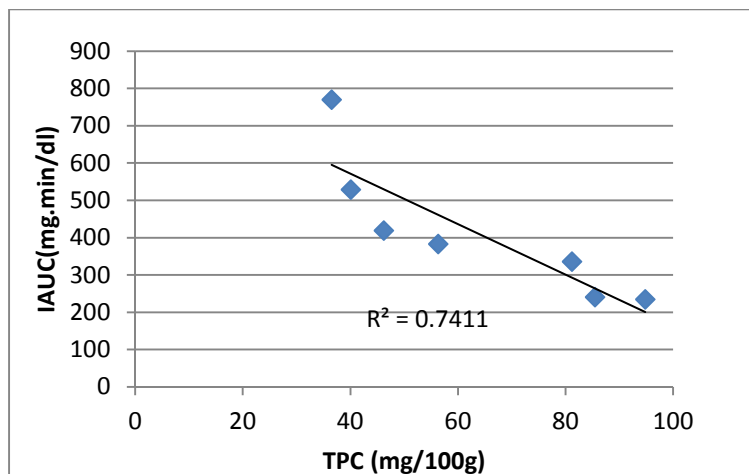


**Figure 4. 4 correlation coefficient between (IAUC) of n= 10 subjects and fat content of consumed pita breads.**

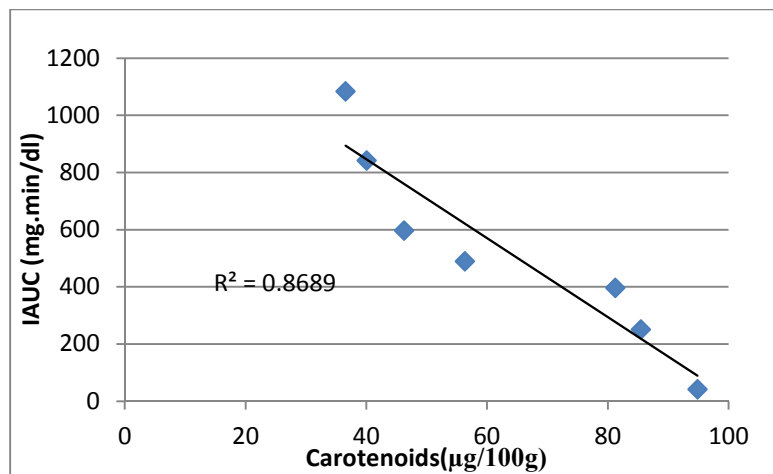


**Figure 4. 5 Correlation coefficient between (IAUC) of n= 10 subjects and fiber content of consumed pita breads.**

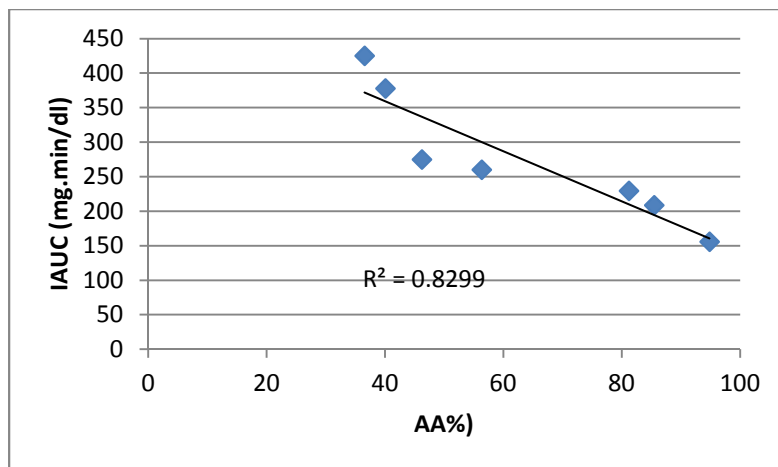




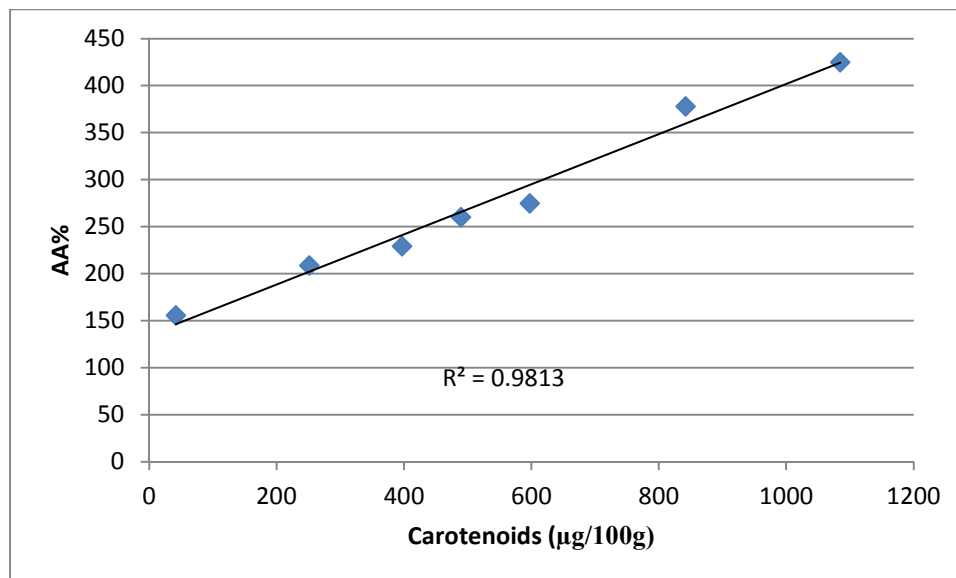
**Figure 4. 6** Correlation coefficient between (IAUC) and total phenolic content of consumed pita breads (N=10 subjects).



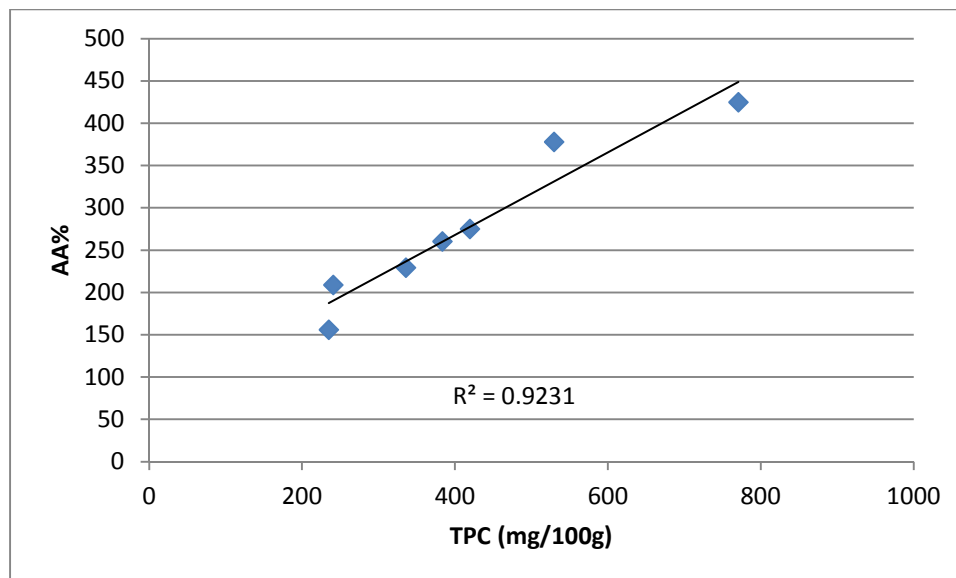
**Figure 4. 7 Correlation coefficient between (IAUC) of n=10 subjects, and total carotenoids content of consumed pita breads.**



**Figure 4. 8** Correlation coefficient between (IAUC) and total antioxidant activity percentage content of consumed pita breads (N=10 subjects).



**Figure 4. 9 correlation coefficient between total carotenoids and antioxidant activity percentage in consumed pita bread.**



**Figure 4.10** Correlation coefficient between total phenolics and antioxidant activity percentage in consumed pita bread.

## CHAPTER 5

### **Development and Optimization of High Energy Biscuits Containing High Protein Chickpea and Food Grade Distiller's Grains for International Food Relief Programs**

#### **Abstract**

High Energy Biscuits (HEB) are emergency food relief supplements used by humanitarian agencies (WHO, USDA, UNICEF, etc.) in international emergency food intervention programs. A wide variety of ingredients are used in making HEB including, wheat, corn, soy, milk, peanuts, coconut, etc. Two high protein and high fiber ingredients, namely, chickpea (CP) and food grade distillers dried grains (FDDG), were explored as functional ingredients in HEB. FDDG is a co product of ethanol production in the corn ethanol industry. Typically, HEB provide 400-450 Kcals per 100g serving, 3 to 8g protein, 26 to 53g carbohydrates, and 9 to 23g of fat. Wheat based HEB which served as control and four other treatments fortified with chickpea flour (25% and 50%) and FDDG (25% and 50%) were developed to improve taste, fiber content and protein content. FDDG reflects a high protein (38%) and high fiber ingredient (43% TDF) that can be used to enhance the nutritive value of emergency relief foods. Chickpea flour had a protein content of 22.3% and TDF content of 21.1%. All substitutions were based on the percentage of all-purpose wheat flour (APF), brown sugar and oil contents. Chemical, physical, and sensory evaluations were conducted to determine the efficacy of the fortification. Moisture content of control and CP and FDDG HEB ranged from 4.3 to 7.5% and was sufficiently low in moisture control to be conducive to extended shelf life. Increase in protein content was noticed in the 4 treatments in contrast to the control all-

wheat HEB made with APF. Caloric content of CP HEB and FDDG HEB were significantly higher than control all-wheat HEB. Increased total dietary fiber content (TDF%) and nutritional content were observed with the CP-FDDG fortified biscuits. Proximate analysis showed higher values for protein, fiber, carbohydrates, and fats in HEB containing CP and FDDG in contrast to unfortified all wheat HEB. Sensory scores of fortified HEB were acceptable as judged by panelists. HEB, particularly those containing 25% FDDG, 25% CP, and 50% CP, were highly enriched with nutrients and exceeded nutritional value as compared to the currently used HEB employed by food aid programs. HEB containing 50% FDDG had particularly high protein content (16.6g/100g). Overall sensory results showed that 50% CP fortified HEB has a moderately acceptable score (3.86), whereas 25% FDDG, 25% CP, and 50% FDDG HEBs received good scores of 4.0, 4.18, and 4.12, respectively, as rated by the panelists. These results show good potential for the use of CP & FDDG in High Energy Bars for emergency food programs

## **5.1 Introduction**

Malnutrition and hunger are two of the greatest challenges in the world. Poverty, hunger and malnutrition are all related terms. By definition, hunger is “A condition, in which people do not receive basic food intake to be provided by enough energy and nutrients for fully productive lives” (Behrman et al., 2004). Malnutrition, on the other hand, is a general term for medical conditions caused by an inadequate diet and poor nutrition. The United Nations Children’s Fund (UNICEF), World Food Supply and other organizations are trying to help malnourished children by providing food aid. However, this is not enough, as there are still many places in which food security does not exist. According to

the FAO organization, food security exists when all people, have physical and economic access to sufficient, safe and nutritious foods to meet their dietary needs (FAO, 1983). Malnutrition occurs not only in developing countries, but it can also occur world-wide owing to a variety of circumstances. Crises associated with man-made and natural disasters are a major cause of malnutrition and food insecurity, resulting in thousands of deaths each year. Natural disasters may occur suddenly or may develop over a period of time, and relief and rehabilitation responses may vary accordingly. Where resources and socio-economic conditions are favorable, rehabilitation may be short-lived because households can quickly regain food security. If an emergency occurs in conditions of chronic food insecurity, long-term assistance and a variety of interventions will be needed to support the affected people (Thompson et al., 2012).

In the 1990s, war and disaster affected 2 billion people and those individuals requiring food and humanitarian assistance tripled since the mid-1980s. In 2001, aid recipients stood at nearly 34 million, of which 13.7 million were refugees and 20.3 million were displaced persons (Brisske et al., 2006; Grobler-Tanner, 2001). In response to the increasing number of disasters (including natural and man-made disasters) and complex humanitarian emergencies requiring food relief operations, the United States Agency for International Development Bureau for Humanitarian Response sought to create specifications for an Emergency Ration Bar, also called an Emergency Food Product. A committee appointed by the Institutes of Medicine (IOM) of the National Academies of Science released a report outlining the specifications for an emergency relief bar (Brisske et al., 2006; IOM, 2002).



Increasingly widespread humanitarian emergencies that are associated with natural disasters and war, along with heightened interest in tackling poverty and hunger under the United Nations' Millennium Development Goals in September of 2008, have boosted calls for global action, including reform of food aid. Now more than ever, the international community needs an effective mechanism for governing food aid that minimizes disputes, enables rapid response to emergencies, and ensures appropriate resourcing for humanitarian and development objectives. The solution to help people in emergencies is to provide nutritious foods which are also inexpensive (Barrett and Maxwell, 2006). Energy-dense nutritional foods that can be packaged and stored for extended periods of time in any environment, presents a challenge to the processor. In a natural or man-made malnutrition emergency, these products must also meet the nutritional needs of all age groups from infants to adults and be sufficiently palatable to be consumed for up to two weeks as the sole food. Nutrient profiles for an emergency food product (EFP) can and have been developed, but the required useful life of the product will be met only through careful consideration and selection of ingredients, processing techniques, and packaging materials. Key considerations include microbiological and chemical safety, and ease of use.

A successful EPF considers five components namely, the EFP must be (1) safe, (2) palatable, (3) easy to dispense, (4) easy to use, and (5) nutritionally complete. The anticipated duration of use is 3 to 7 days, but the product may be used for up to 15 days. The EFP should provide the required energy (kcal), protein, vitamins, minerals, and other essential nutrients required for survival during this short time span. The EFP should also exhibit sensory appeal, as well as logistic and cultural convenience (IOM, 2002).

Microbiological safety, nutritional value maintenance, and oxidative stability are all important features for a product with extended shelf life under adverse conditions. All of these characteristics are influenced by water content and water activity (IOM, 2002). In addition, the sensory quality of the emergency bar must be acceptable in many cultures (Grobler-Tanner, 2001). To minimize microbiological spoilage, nutrient degradation, and oxidation, the moisture content of the bar should be below 9.5% with water activity of no more than 0.6 (IOM, 2002). Ideally, the final EFP should meet a minimum shelf life requirement of 36 months at 21°C. Each bar should contain approximately 233 kcal. Therefore, adults will need to consume between 9 and 10 bars each day (about 2100 kcal/d). Pregnant/lactating women and children will consume more or fewer EFPs, respectively, to meet their specified caloric needs. Per the IOM (2002), the primary source of protein could be in the form of a soy product (flour, concentrates, isolates, or textured vegetable protein); partially hydrogenated soybean oil and flaxseed oil will supply the lipid content of the EFP; and a cereal base, vitamin/mineral premix, sugars, and possibly baking and leavening agents will also be constituents of the bar. Fortification of cereal-based foods would be a great help, since cereals are the most highly consumed food products around the world. Cereal based products are a cheap source of energy and are available to almost everyone. There are many alternatives to choose from.

Urbanization has been responsible for the long-time existence of the bakery industry which had resulted in increased demand for ready to eat food products such as bread, cookies, cake, and biscuits. Supplementing of wheat flour with legume flours, especially chickpea flour has good potential for improving the nutritional value of the

flour and its products, particularly baked products. A number of studies have demonstrated the nutritional value of chickpea supplemented flour and food products such as breads (pita breads, chapatti, and toast); cookies, cakes, papads, and pasta (Singh et.al, 1991; Shehata et.al, 1970; Dhinda and Lakshmi, 2012) (Dodok et al., 1993; Eissa et al., 2007; Garg and Dahiya, 2003; Hallab et al., 1974; Yousseff et al., 2006). The supplementation of chickpea flour at 15 - 20 percent level in wheat flour biscuits has been reported to not only improve protein quality but also to improve dough texture and sensory attributes in the final product (Masur et al., 2009).

The nutritional value of wheat flour can be also enhanced using a variety of alternative flours and co-products of different industries such as distillers dried grains with soluble's (DDGS) and chickpea flour. DDGS is a major co-product of the ethanol industry (Singh and Muthukumarappan, 2014b; Singh and Muthukumarappan, 2014a). The starch from cereals serves as the yeast energy source during the fermentation process. Due to the loss of starch, the protein and fiber components are concentrated thus making the dried residue a potentially nutritious food for humans (Singh, 2016). Previous studies have reported on the incorporation of DDGS in various cereal-based products, such as breads (chapatti, naan, corn breads, toast, pita breads), cookies, pizza, tortillas (Arra, 2011; Parmar, 2012; Pourafshar, 2011; Tsen et al., 1983) where the results showed increased/enhanced nutritional potential.

Fortification, which is the use of available, nutritious and cost-effective nutrient sources to increase both chemical and physical properties of the original food, is one means of combating both macro and micro nutrient deficiencies. Fortification of cereals can be done by using different ingredients which are rich in vitamins and minerals such

as alternative non-traditional flours. Co-products from the ethanol processing industry may also be appropriate for use as enrichment ingredients in view of their nutritional, health-promoting and food functional attributes.

The specific objectives of this study were to develop formulations for a nutrient-dense energy bar containing wheat flour, chickpea flour, and FDDG and to determine proximate composition and sensory characteristics. Chickpea and FDDG are highly nutritious ingredients that were used as principal ingredients for development of extruded snacks (Singha et al., 2018; Singha, 2017). Therefore, it is hypothesized that cereal based foods can be effectively fortified with chickpea and FDDG to produce products of higher nutrient content that can be used in emergency food programs.

## **5.2 Materials and Methods**

From the literature there were few studies that have employed different types of ingredients for emergency aid programs (Table 5.1), but only 3 of them have used chickpea flour. To our knowledge, this is the first study to use food grade DDG in such formulations.

### **5.2.1 Materials**

Corn distillers dried grains with solubles (DDGS) was obtained from a commercial ethanol plant and was stored at  $-80 \pm 1^{\circ}\text{C}$  until further processing as a food ingredient. Ingredients for preparation of the HEB, such all-purpose flour, chickpea flour, brown sugar, canola oil, baking soda, and agave were purchased from a local grocery store.

## 5.2.2 Methods

### 5.2.2.1 HEB preparation

The recipe for HEB was adapted from several studies (Rawat and Darappa, 2014) (Masur, Tarachand, & Kulkarni, 2009). The study design contained a control (wheat flour only) and 5 different flour blends that were prepared using different proportions of wheat flour, chickpea flour, and FDDG. Tables 5.2 and 5.3 provide the experiment design and formulation of HEB.

The dry ingredients (wheat flour, FDDG or chickpea flour) were mixed using a twin-shell dry blender (Peterson Kelly Co. Inc. Stroudsburg, PA). This blender consists of a V-shaped mixing chamber, which rotates on its horizontal axis at a constant speed for 45 minutes to ensure uniform mixing of the ingredients. A reel oven (National. MEG.CO, model:16/32 Reel Oven: Lincoln, NE) was set to 180 °C (356°F). A large metal baking pan was sprayed using nonstick spray.

HEB dough was prepared in an automatic dough mixer (Kitchen Aid, Model: KSMQO). First, the sugar and canola oil were creamed together until smooth. Then, one half of the agave was gradually added while mixing. One half of the flour and baking soda were added gradually to the previous mix. Finally, the rest of the flour and the agave were added until a smooth batter is formed. Water was added as needed. The dough was covered and chilled for one hour or more for ease of rolling and prevention of stickiness.

For the 50% FDDG and chickpea fortified bar, 15g of water was added to the batter to achieve consistency. Also, about 10 more grams of agave were added to 50% FDDG and chickpea fortified bar to increase sweetness to mask bitterness and beany taste of FDDG and chickpea, respectively.

The batter was spread in the baking pan and gently pressed uniformly until the thickness was about 1.5 cm. Then it was placed in a convection oven and baked at 180 °C (356 °F) for 9-10 minutes until brown at edges and golden brown in the center. Then, the bars were cooled for two hours in a pan on wire rack. Finally, bars were cut into smaller bars that weighed 100g each. Additionally, to achieve a moisture level below 4.5%, the bars were placed in a drying oven overnight at 60-80 °C (AACC approved method 44-19.0, AACCI 2000).

#### **5.2.2.2 Proximate analysis**

Moisture content was measured using an oven drying method according to AACC approved method 44-19.0 (AACC 2000). Fat content was determined using AOAC method 920.39 (AOAC, 1990) using an automated Soxhlet extractor. Petroleum ether was used as a solvent (CH-9230, Buchi laborotechnik AG, Flawil, Switzerland). Protein content was analyzed using the Dumas combustion analysis (AOAC 17th ed., method 968.06), using the Rapid N Cube (Elementar Analysen Systeme, GmbH, Hanau Germany). Nitrogen content was then multiplied by a conversion factor of 6.25 to calculate protein percent.

Ash content was determined using incineration (Method. 08-03, AACC, 2000) in muffle furnace (Model: Box furnace, 51800 series). The dried pita bread samples were ashed at 525°C for 12 hours in muffle furnace to estimate inorganic content (minerals) in the bread.

Total Dietary Fiber (TDF) content was analyzed by an enzymatic gravimetric method using AOAC Method 30-25 to determine non-digestible fibers. The Megazyme assay test kit was used.

Available Carbohydrates (AVB CHO) was calculated by difference.

CHO = [100%-(protein%+ fat%+ ash%+TDF%+ moisture%)]. Sugar content was calculated by dividing total amount of sugar in the ingredient recipe by number of servings. Dietary energy density was calculated using the equation: Energy (kcal/100g EP) = protein (g/100g EP) x 4 + fat (g/100g EP) x 9 + available carbohydrates (g/100g EP) x 4 + dietary fiber (g/100g EP) x 2 + alcohol (g/100g EP) x 7. Mineral analysis was done using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

### **5.2.2.3 Sensory analysis**

The HEB were evaluated for overall acceptability, color, aroma, texture and taste. This was carried out using a 5-point hedonic scale rating (1=dislike extremely, 2=dislike moderately, 3= neither like or dislike, 4=like moderately, 5=like extremely). Products were judged to be acceptable if a score of 3 was assigned by the panelists. Thirty-two trained and un-trained judges consisting of undergraduate and graduate students, faculty, and staff members of South Dakota State University served as the sensory panel.

## **5.3 Results and discussion**

### **5.3.1 Proximate analysis**

#### **5.3.1.1 Nutritional composition of the starting raw materials**

Table 5.4 illustrates the nutritional composition of the starting raw materials used in the production of HEB, namely wheat flour (APF), chickpea flour (CP) and food grade distiller's grains (FDDG). These materials were significantly different from each other in their content of moisture, protein, fat, minerals and carbohydrates as reflected by their composition. Their diversity provided unique properties in the finished products when

they were brought into the HEB formulations in fixed ratios as described in Table 5.2 Proximate composition of HEB samples (table 5.4) demonstrated that 25% and 50% fortification levels of CP and FDDG resulted in significant ( $P<0.05$ ) increase in protein, fat, ash, and TDF contents and a significant ( $P<0.05$ ) decrease in the moisture and carbohydrates content as compared to the control.

### **5.3.1.2 Proximate analysis of HEB**

#### **5.3.1.2.1 Moisture content**

From Table 5.4, it can be seen that as the fortification levels of CP and FDDG increased, moisture content in the HEB finished product decreased. Control HEB with only APF had the highest level of moisture while the HEB containing 50% wheat flour showed the lowest moisture content (30%). Other researchers have also reported a reduction in moisture content in high energy biscuits, high energy bars, papads, and pita breads when fortified with chickpea (Garg and Dahiya, 2003; Naseem et al., 2013; Rawat and Darappa, 2014) (Shehata et.al, 1970; Hefnawy et.al, 2012). The decrease in moisture could be attributed to the inherent low moisture content of chickpea flour (8.6%), compared to the wheat flour (11.95%). It could also be due to the high fiber and high protein content of CP flour which were 21.1% and 22.3%, respectively, as compared to that of APF which were 5.24% and 12.0%, respectively. Many studies have reported that flours containing high fiber levels absorb more free water thus decreasing the moisture content of the final baked product (Kurek & Wyrwisz, 2015; Parmar,2012; Dreese and Hosney 1982). Also, it can be related to the interference of chemical compound such as phenols which lead to water binding (Peighamardoust & Aghamirzaei 2014). Incorporation of dietary fibers to food products such as bread imparts functional



properties such as increased water holding capacity (Sivam, Sun-Waterhouse , Young Quek , Perera, 2010). This mechanism may lead to reduced HEB moisture content owing to greater fiber and protein content that tie up moisture in the final product.

The result in our study is in line with reports of moisture content in baked goods such as naan breads, cookies, and pizza fortified with DDG (Staudt and Zeigler, 1973; Ahmed 1997; Arra, 2011; Tsen et.al,1983; Maga and Van Everen,1988; Parmar, 2012; and Saunders et.al,2014). The reason for the decrease in the moisture content can be due to the lower content of gluten in the dough in which DDGS was incorporated. Since the gluten content decreased, it could not contribute to the network to bind with water molecules unlike the control wheat dough (Pourafshar, 2011). Differences in the initial moisture levels in the ingredients may explain this phenomenon. Initial ingredient moisture content of FDDG was 7.2% while APF had a moisture content of 12%. The reduction of HEB moisture content could be also due to the high fiber and protein content in FDDG. The reduction of moisture could be due to incorporation of CP and FDDG which are both gluten free and could cause increased water holding capacity owing to lowering of gluten in the developed food compared to wheat.

Other workers have reported mixed results in relation to final moisture content of food product. Hallab et al. (1974) studied the nutritional value and organoleptic properties of white Arabic bread supplemented with (10%, 20%, 30%, 40% & 50%) of chickpea flour. They demonstrated that moisture of the final product decreased with increase in chickpea flour fortification level. Sharma et al. (2013a) conducted a study on chickpea fortified biscuits, and found that there was no significant differences in moisture content when wheat-based biscuits were fortified with 20, 40 and 60% chickpea.

### 5.3.1.2.2 Protein content

There were significant differences in protein content between all treatments when compared to the control (Table 5.4). The protein content in the HEBs ranged from 6.12 to 16.6 g/100g. When fortified with 25% CP, the protein content in the HEB increased by 33% and when fortified with 50% CP the protein content in the HEB increased by 119%. It may be concluded that as the fortification level of chickpea increased, protein content increased as well. These results are in agreement with the results from several studies where fortified HEB with different levels of chickpea flour were conducted and chickpea fortified biscuits had higher protein content than wheat control biscuits (Naseem et al., 2013; Rawat and Darappa, 2014; Sharma et al., 2013b) (Masur, Tarachand, & Kulkarni, 2009).

Supplementation of wheat flour with legumes especially chickpea, which is a richer source of protein, is one way to increase proteins in baked goods such as biscuits, cookies, and cakes (Masur et al., 2009). Our results are also in agreement with Eissa et al. (2007) who fortified Egyptian Balady bread with chickpea flour and found that incorporation of raw chickpea flour increased protein content compared to control wheat bread. The increase in protein content might be due to the appreciably higher protein content of chickpea flour (Eissa et al. (2007).

Yousseff et al. (2006) supplemented wheat flour bread with different ratios of chickpea flour. They found that as the fortification level increased, protein content in the bread increased as well. Hallab et al. (1974) studied the nutritive value and organoleptic properties of white Arabic bread supplemented with soybean and chickpea. They

demonstrated that protein content increased with increased chickpea flour fortification level in the final product.

Dhinda et.al, (2012) tested the effect of ingredients on rheological, nutritional and quality characteristics of high protein, high fiber and low carbohydrate bread. They fortified wheat flour with SPOBCP blend (soy protein, oat bran, and chickpea) in different fortifications levels. They demonstrated that increasing amount of SPOBCP in the blend significantly increased total protein value in the final product. This finding supports our findings with chickpea fortification.

When comparing the FDDG fortification factors (Table 5.4), fortification with 25% FDDG and 50% FDDG resulted in the protein content of 11.12% and 16.6%, respectively in contrast to 6.12 % protein in the control all wheat HEB. Hence, the protein content in the HEB increased 2-fold after fortification. Similar results were concluded by Tsen et al. (1982).

These results are in agreement with previous results from different researchers who fortified different types of baked products with different levels of DDG and found increases in the protein levels at the higher substitution levels of DDG (Arra, 2011; profushar, 2011; Parmar, 2012; Liu et.al, 2011; Brochetti et.al,1991; Li, Wang, Krishnan, 2016 unpublished paper) (Tsen et al., 1982). This occurred because DDG is a high protein cereal product when compared to all-purpose flour. The increased protein occurred owing to the fact that DDG has almost three times the protein content (31.0%) when compared to APF (11.95%), also chickpea flour has almost twice the protein content (22.3%) when compared to APF (11.95%).

### 5.3.1.2.3 Total Dietary Fiber (TDF)

As noted in Table 5.4, All HEB samples were found to be significantly different from each other in TDF content and when compared to control all-wheat flour, with a range of (3.2g-12.6g/100g) (Table 5.4). Fortification with 25% CP increased the amount of TDF to 4.9%, and fortification with 50% CP increased the amount of TDF two-fold (8.9%TDF) when compared to the control (3.2%TDF). It can be concluded that as the fortification level of chickpea increased, TDF% increased as well. Similar results were reported by several workers (Naseem et al., 2013; Rawat and Darappa, 2014; Sharma et al., 2013b; Masur et al., 2009) who fortified HEB with different levels of chickpea.

Hallab et al. (1974), Yousseff et al. (2006) and Dhinda et al. (2011) studied the chickpea fortification in bread and reported an increase in TDF. The reason for increased TDF content in the finished product is attributable to high TDF content in both chickpea (21.1% TDF) and FDDG (30.9%TDF) when compared to the all-wheat unfortified control (5.24%TDF).

Hallab et al. (1974) studied the nutritive value and organoleptic properties of white Arabic bread supplemented with soybean and chickpea. They demonstrated that fiber content increased with increased chickpea flour fortification level in the final product. Dhinda et.al, (2012) tested the effect of ingredients on rheological, nutritional and quality characteristics of high protein, high fiber and low carbohydrate bread. They fortified wheat flour with SPOBCP blend (soy protein, oat bran, and chickpea) in different fortifications levels. They demonstrated that increasing amount of SPOBCP in the blend significantly increased TDF value in the final product.

When comparing FDDG fortification levels, fortification with 25% FDDG increased the TDF content to 6.8% and fortification with 50% FDDG increased the level of TDF by four times (12.6%TDF). As the FDDG fortification level increased, dietary fiber increased as well. Other workers (Arra, 2011; Prouafshar, 2011; Parmar, 2012; Wu et.al, 1987) (Tsen et al., 1982) who fortified bread and different baked products with different levels of DDG have determined that fiber, as measured as neutral detergent and crude fiber levels, increased at the higher substitution levels of DDG. This was because DDG had higher fiber levels compared to the all-purpose flour. Li et.al, 2016 fortified steamed bread with DDG and found that with increase in the level of DDG, the TDF in the final product increased significantly. Brochetti et.al, (1991) fortified yeast bread with DDG and found that increasing DDG increased TDF values in the final product. The increase in TDF content of HEB in our study occurred because both chickpea (TDF = 21.1%) and FDDG (TDF = 30.90%) had an initial higher TDF content, while APF had a TDF content of 5.24%.

#### **5.3.1.2.4 Fat Content**

There were significant differences between the fat content of HEB treatments when compared to all-wheat HEB (Table 5.4). When analyzed separately, the data showed that key ingredients were distinctly different from each other with regard to fat content. FDDG had significantly higher fat content (5.10%) than chickpea flour (3.2%) when compared to APF (1.9%). HEB with FDDG generally was higher in fat content in comparison to the HEB fortified with chickpea. All treatments showed increased fat content in comparison with the all wheat control HEB. The results of our study demonstrated that incorporating chickpea flour into wheat flour HEB increased fat

content. Similar result was reported by (Naseem et al., 2013; Rawat and Darappa, 2014; Sharma et al., 2013b; Masur et al., 2009). Yousseff et al. (2006) supplemented wheat flour bread with different ratios of chickpea flour (10 and 15%) and found that as the fortification level increased, fat content in the bread increased as well. Hallab et al. (1974) studied the nutritive value and organoleptic properties of white Arabic bread supplemented with soybean and chickpea. They demonstrated that the fat content increased with increased chickpea flour fortification level in the final product. Dhinda et.al, (2012) tested the effect of ingredients on rheological, nutritional and quality characteristics of high protein, high fiber and low carbohydrate bread. They fortified wheat flour with SPOBCP blend (soy protein, oat bran, and chickpea) in different fortifications levels. They demonstrated that increasing amount of SPOBCP in the blend significantly increased fat value in the final product. Chickpea flour was endowed with higher fat content (3.2%) than the all-purpose flour (1.89%).

It can be demonstrated that as the proportion FDDG fortification level increased in the formula, fat content increased as well. These results agreed with results from previous researchers who fortified different types of food items, breads, and different baked products (cookies, Naan, Lavash, pizza, and steamed bread) with different levels of (Tsen et al., 1982; Arra, 2011; Parmar, 2012; Pourafshar, 2011). The phenomenon of increased fat content may be due to the initial higher fat content in FDDG (5.10%) compared to all-purpose flour (1.89%). Another reason for this perhaps was the lower level of gluten in the dough network which contributed to reduced interactions of protein and lipid and reduced fat retention in dough compared to that of the control sample

(Pourafshar, 2011). The amount of lipid in DDG increased up to 1.4-2.4 times when compared to whole grain wheat.

#### **5.3.1.2.5 Ash Content**

There were significant differences between the ash content of HEB, with a range of 1.14-2.93g/100g (Table 5.4). It was found that as the fortification level of chickpea increased in HEB the ash content increased as well in between treatments and when compared to control all-wheat flour HEB. Similar results were concluded by other workers who fortified HEB with chickpea flour (Masur et al., 2009; Naseem et al., 2013; Rawat and Darappa, 2014; Sharma et al., 2013b). and different types of wheat fortified chickpea food products such as (pita bread, bread, papads) (Garg and Dahiya, 2003; Hallab et al., 1974; Yousseff et al., 2006). This could be due to the fact that chickpea as a pulse is good source of minerals like folate, iron, phosphorous, magnesium, potassium, calcium, and zinc (Dodok et al., 1993; Garg and Dahiya, 2003; Iqbal et al., 2006; Liu, 2012).

Significant increase in ash content as the fortification proportion of FDDG substitution increased between all treatments and when compared to control. These results agreed with the result from several other studies (Maga and Van Everen, 1989; Tsen et al., 1982; Arra, 2011; Davis, 2001; Pourafshar, 2011; Rasco et al., 1990; Reddy et al., 1986). In these studies the researcher fortified different types of breads, baked products, and pasta with different amount of DDG and found increased ash content as DDG increased. The reasons for increased ash amount is probably attributed to the soluble solids which were added to the distillers dried grains during processing. DDG soubles are a source of various vitamins and minerals. Ash content is directly related to the type of flour used in the production of bread. Also, neither total ash nor the content of

any of the mineral elements are directly related to the reported degree of refinement of the flour (Czerniejewski et al, 1964).

#### **5.3.1.2.6 Carbohydrate Content**

Carbohydrate content ranged between 48% and 77% in the HEBs. From Table 5.4, it can be observed that as the substitution level of chickpea increased, the carbohydrate content increased as well for HEB treatments. Chickpea and FDDG had lower carbohydrate content of 42.2% and 24%, respectively, in contrast to APF (68.3%). Similar results were reported by (Naseem et al., 2013; Sharma et al., 2013b; Rawat and Darappa, 2014; Masur et al., 2009), when they fortified HEB with different levels of chickpea flour. Garg and Dahiya (2003) fortified papads with chickpea flour, and concluded that as the fortification level increased, carbohydrate content decreased. Also, Hefnawy et.al (2012), reported the same finding when they added chickpea flour to wheat flour to toasted bread. Dhinda et.al, (2012) fortified wheat flour breads with different ingredient and different levels such as soy protein isolate, oat bran, and chickpea flour. It was found that chickpea flour had a lower carbohydrates content than wheat flour. Yousseff et al. (2006) supplemented bread with chickpea flour with at the 5, 10, 15, 20, and 25 % flour replacement levels. They found that as the fortification level of chickpea flour increased, carbohydrates level decreased in their final products. Utrilla-Ceollo et.al, (2007) fortified wheat flour breads with (20%, and 40%) of chickpea flour. They reported that carbohydrates in breads decreased as the fortification level of chickpea increased. Liu et.al, (2011) fortified cornbread with different fortification level of DDGS. They concluded that as DDGS fortification level increased the carbohydrates level decreased in the final products. In an un unpublished paper by Li et.al, (2016), they fortified steamed



bread with different fortification level of DDG. It was shown that increased DDG fortification level decreased carbohydrates content in steamed breads.

#### **5.3.1.2.6 Minerals content**

Mineral contents of wheat flour, chickpea, and FDDG were investigated. Table 5.5 provides minerals content of raw ingredients which were used in formulating the HEB. From table 5.5 it was demonstrated that major and minor elements in both chickpea and food grade DDG were greater than those found in all-purpose flour. It also demonstrated that chickpea had higher values of calcium, copper, manganese, and potassium than FDDG. However, FDDG was higher than chickpea in content of iron, magnesium, phosphorus, sodium, sulfur, and zinc. It is known that chickpea as a pulse is good source of vitamins and minerals such as folate, iron, phosphorous, magnesium, potassium, calcium, and zinc (Tulbek, 2006; Abou Arab et.al, 2010; Yudan liu,2012; Garg and Dahiya, 2003; Dodok et.al, 1993; Iqbal et.al, 2006). Another reason could be due to the soluble solids which were added to the distillers dried grains during processing, which are a source of vitamins and minerals. DDG contains high amounts of most minerals such phosphorus, sodium, and sulfur (Lim & Yildirim-Aksoy, 2008). Mineral analysis of HEB from wheat flour and different forms of chickpea and FDDG are presented in table 5.6. The result indicated that as the level of replacement of either chickpea or FDDG increased, minerals values in HEB increased as well. Similar results were found by Abou Arab et.al, (2010), when they fortified spaghetti with 10, 15, 20, 25, & 30% of chickpea. The highest minerals values where found in the products made at the 50% FDDG replacement level.

### **5.3.2 Comparison between developed HEB and other biscuits**

Tables 5.4 and 5.7 provide nutritional composition data on HEB. While table 5.4 reports on composition of chickpea and FDDG fortified HEB, table 5.7 provides the nutritional contribution of HEB currently used in food aid programs. 25% CP fortified biscuits meets the IOM (2002) standards whereas the 25% and 50 % FDDG and 25 CP % fortified biscuits exceeded the

IOM (2002) standards in terms of nutritional value. HEB fortified with 50% FDDG had a significantly higher protein content (16.6%) than the ones reported by WFP (9%) and USDA (10%), and moderately higher protein content than the ones reported by UNICEF (10-15%). It can be concluded that all our five developed HEB have a good nutritional composition.

### **5.3.3 Sensory**

A panel of thirty-two members comprising males and females, trained and untrained judges performed the sensory analysis of the HEBs. Scores were presented on a scale of 1 to 5 (Table 5.8). The lowest score awarded by panelist was 3.54, where the highest score was 4.39. Scores of 3, 4, and 5 were designed acceptable, good, and excellent, respectively.

The sensory analysis provided scores for color, aroma, taste, texture and over all acceptability. Our results showed that there were no statistically significant differences between control HEB and both 25% and 50% chickpea-fortified HEB. Consumer panels could not distinguish color differences between the 25% or 50% chickpea-fortified HEB and control (made with all-wheat flour). Significantly lower scores were received for color for 25% and 50% FDDG fortified HEB as compared to the control all wheat HEB.

Fortification of HEB with chickpea did not affect color scores as noted earlier in this section. Fortification with chickpea flour had added a desirable salmon-white color, which was deemed by panelists to be desirable. Hefnawy et al. (2012) reported that adding chickpea flour to wheat flour in toasted bread improved color acceptance among their panelists. Fernandez and Beery (1989) fortified bread with 10% chickpea flour and found that chickpea fortified breads had higher color scores than the control bread. Yousseff et al. (2006), found that as the chickpea fortification levels increased to 5%, 10%, 15% substitution levels, the sensory scores for color also increased in wheat flour bread. These workers reported however that fortification at 20% ratio decreased the color score. These results indicated that wheat flour probably should not be replaced at a level higher than 20% in chickpea flour fortification where color is the sole criterion. Sensory scores after chickpea addition were not always desirable. Hallab et al. (1974) studied the nutritional value and organoleptic properties of white Arabic bread supplemented with 10 to 50% chickpea flour. They reported that color scores decreased with increased chickpea flour fortification level in the final product.

Fortification with FDDG resulted in darker products. The color scores of HEB reduced significantly with the increasing amounts of FDDG in the product. The dark brown colored HEBs were not liked by the panelists. Color scores for FDDG HEB were, however still considered acceptable with average score of 3,87 (25% CP) and 3,78 (50%CP) as seen in Table 5.8. Thus, chickpea flour and FDDG can be used in conjunction with other ingredients and to yield acceptable color in HEB.

Sensory data on Aroma (Table 5.8) also showed that chickpea fortified HEB (25% and 50% CP) had the lowest scores, whereas FDDG fortified HEB (25% and 50%

FDDG) had no significant difference from the all-wheat control. It can be concluded that HEB fortified with FDDG did not affect the aroma, whereas the blends made with chickpea scored lower for aroma. Dodok et al. (1993) reported similar finding when they fortified wheat flour bread rolls with 10%, 20% chickpea flour. The breads fortified at either levels (10 or 20%) had lower aroma scores than the control. In contrast, Fernandez and Beery (1989) who fortified bread with 10% chickpea flour, found that chickpea fortified breads had higher aroma scores than the control bread. When lower aroma scores are reported, they can be attributed to the beany odor that specific to chickpea flour. Beany odor of chickpea is considered one of the important factors that may influence the quality as well as acceptability of any food product that is fortified with chickpea or chickpea flour (Gonzales et.al., 2014).

Taste scores on HEB evaluated by the sensory evaluation panel are provided in Table 5.8. Sensory evaluation results of HEB revealed no statistically significant differences in taste scores between control and 25% FDDG fortified HEB. HEB with 25% CP and 50% FDDG were not significantly different from each other for taste scores. The latter two treatments received lower scores than the control. Finally, the lowest score was observed in the 50% chickpea fortified HEB. Incorporation of chickpea flour into HEB imparted a distinct bitter beany flavor, which could be the reason for the low taste scores. Some additives may be required to be added to mask the flavor of chickpea flour, for a more desirable food product. Hallab et al. (1974) who studied the nutritive value and organoleptic properties of white Arabic bread supplemented with soybean and chickpea found that taste scores decreased with increased chickpea flour fortification level in the final product. Fortification with lower percentage of FDDG (25%) did not affect taste

scores. However, 50% replacement of wheat flour in the formula with FDDG, lowered taste scores.

The texture scores from our study showed that there were no significant differences between all of the HEB treatments. While the treatments were not significantly different from each other, a range of 4.00 to 4.22 indicated an overall high sensory value for all HEB on a scale of 1 through 5. Yousseff et al. (2006) found that when wheat flour bread was supplemented with different ratios of chickpea flour (10% and 15%), the texture scores decreased. The findings of Yousseff et al. (2006) are also in agreement with the results by Hallab et al. (1974) who studied the nutritive value and organoleptic properties of white Arabic bread supplemented with soybean and chickpea. They demonstrated that texture scores decreased with increased chickpea flour fortification level in the final product. Maga and Van Everen (1989) fortified pasta with two levels of DDGS (25% and 50%), and found that as the DDGS levels in the formula increased, the texture score decreased. These results contrast with our result.

The sensory evaluations for overall acceptability indicated that the lowest scores were awarded for 50% chickpea fortified HEB, whereas there was no significant differences among scores awarded to all other HEB as well as the control. Lowered liking at the 50% replacement level using chickpea was owed to both beany flavor and odor of chickpea. These were considered important factors that influence the quality as well as acceptability of any food products that are fortified with chickpea or chickpea flour (Gonzales et.al., 2014).

Another reason for decreased overall acceptability scores could be the darker color of HEB which may have occurred due to increased Millard reaction during baking

due to the presence of lysine in chickpea flour. In the Millard reaction, reducing carbohydrates react with free amino acid side chains of proteins, mainly lysine that are present in chickpea flour, and lead to amino acid sugar reaction products (polymerized protein and brown pigments). (Hallén et al., 2004; Mohammed et al., 2012).

#### **5.4 Conclusion**

The world is facing extremely serious problems with hunger and malnutrition whether it is natural or man-made. Urgent action is needed, in many countries to alleviate the effects of hunger and malnutrition. One way to tackle the problem of malnutrition, is to develop fortified food staples and increase the availability of energy-dense nutritional foods, which can be consumed by most people in countries where malnutrition is imminent.

Our study demonstrated that high energy bars with high nutritional composition content can be prepared by partially substituting wheat flour with either or in combination of chickpea and FDDG. The final developed products were satisfactory in achieving the requirement nutritional value and, simultaneously with good sensory characteristics. The results showed that fortification with CP and FDDG increased protein, fiber, fat, and minerals content. Where the comparison illustrates the compatibility to the diversity range of biscuits that is used as food aid for emergency from different agencies. These products may have been valuable sources as food aid but their lower protein content for most of them and some disadvantages such as including soy and coconut ingredient which may cause allergy for some people may make them less suitable to be used.

To our knowledge this is the first study where we have reported the use of FDDG in HEB and one of very few studies which used chickpea to develop HEB. Our results are

important for the production of HEB with improved nutritional characteristics by the emergency food aid agencies. To this end FDDG and chickpea are promising ingredients to fortify food products like HEB as a solution to malnutrition that is developed from emergencies relied feeding programs.

**Table 5. 1 Fortified high energy biscuits (HEB) studies from literature.**

| References                   | Product & key ingredients   | Comments   |
|------------------------------|---|--|
| <b>Naseem et.al, (2013)</b>  | CP-fortified (5,10,15,20%) HEB  | HEB was developed for malnourished children in Pakistan. Supplementation increased protein, fat, fiber, iron, and zinc   |
| <b>Sharma et al. (2013a)</b> | CP (20,40,60%) fortified biscuit  | To develop rich protein and fiber source food. Supplementation increased protein, fiber, and ash.  |
| <b>Masur et.al, (2009)</b>   | CP-fortified (10,15,20,25) biscuit  | Increasing nutritional awareness among consumers. CP fortified high protein biscuit improved the nutritional and textural quality of biscuits                                    |
| <b>Young et al. (2007)</b>   | HEB fortified with eggs, soy oil, and dried milk.   | Developed to be used in feeding programs to prevent malnutrition after disaster. The adopted recipe was satisfactory in achieving nutritional values when compared to literature |
| <b>Briske et al. (2006)</b>  | Prototype nutrient-dense Bar, soy based, corn syrup, granulated sugar, high fructose corn syrup | Was developed as emergency product for refugees and displaced persons. Proximate composition met general specifications of IMO.  |

CP: chickpea flour, HEB: high energy biscuits, IMO: Institution of medicine



**Table 5. 2 Experimental design formulation for flour blends containing All Purpose Flour, Chickpea and Distiller’s Dried Grains.**

| <b>High Energy Biscuit</b> | <b>APF%</b> | <b>CP%</b> | <b>FDDG%</b> |
|----------------------------|-------------|------------|--------------|
| <b>Control</b>             | 100         | 0          | 0            |
| <b>75W-25CP%</b>           | 75          | 25         | 0            |
| <b>75W-25D%</b>            | 75          | 0          | 25           |
| <b>50W-50CP%</b>           | 50          | 50         | 0            |
| <b>50W-50D%</b>            | 50          | 0          | 50           |

HEB: High Energy Biscuit, APF; All-purpose flour, CP; Chickpea flour, FDDG; Food grade DDG

**Table 5. 3 Ingredient composition of All-wheat Control, Chickpea HEB (25% and 50%)  
and FDDG HEB (25% and 50%).**

| <b>HEB</b>               | <b>APF<br/>(g)</b> | <b>CP<br/>(g)</b> | <b>FDDG<br/>(g)</b> | <b>Water<br/>(g)</b> | <b>Brown<br/>sugar (g)</b> | <b>Canola<br/>oil (g)</b> | <b>Baking<br/>soda(g)</b> | <b>Agave<br/>(g)</b> | <b>Serving<br/>size<br/>(100g)</b> |
|--------------------------|--------------------|-------------------|---------------------|----------------------|----------------------------|---------------------------|---------------------------|----------------------|------------------------------------|
| <b>Control<br/>100W%</b> | 625                | 0                 | 0                   | 10                   | 450                        | 340                       | 10                        | 175                  | 16                                 |
| <b>75W-25CP%</b>         | 468.7              | 400               | 0                   | 10                   | 337.5                      | 225                       | 10                        | 131.2                | 16                                 |
| <b>75W-25D%</b>          | 468.7              | 0                 | 400                 | 10                   | 337.5                      | 225                       | 10                        | 131.2                | 16                                 |
| <b>50W-50CP%</b>         | 312                | 800               | 0                   | 10                   | 225                        | 170                       | 10                        | 87.5                 | 16                                 |
| <b>50W-50D%</b>          | 312                | 0                 | 800                 | 10                   | 225                        | 170                       | 10                        | 87.5                 | 16                                 |

W= all wheat, APF=All-purpose flour, CP= Chickpea flour, D=Food grade DDG, g= grams

**Table 5. 4 Nutritional composition of SDSU experimental HEB formulated with APF, CP and FDDG.**

| <b>SDSU HEB</b>  | <b>Moisture (%)</b> | <b>Protein (%)</b> | <b>Fat (%)</b> | <b>Ash (%)</b> | <b>TDF (%)</b> | <b>CHO (%)</b> | <b>Sugar (g)</b> | <b>Kcal</b> |
|------------------|---------------------|--------------------|----------------|----------------|----------------|----------------|------------------|-------------|
| <b>Control</b>   | 7.52a               | 6.12e              | 4.4e           | 1.14e          | 3.2e           | 77.62          | 28               | 381         |
| <b>100W%</b>     |                     |                    |                |                |                |                |                  |             |
| <b>75W-25CP%</b> | 5.51b               | 8.19d              | 6.6d           | 1.5d           | 4.9d           | 73.3           | 21               | 395         |
| <b>75W-25D%</b>  | 5.18b               | 11.12c             | 9.2c           | 1.96c          | 6.8c           | 65.74          | 21               | 404         |
| <b>50W-50CP%</b> | 4.62c               | 13.42b             | 12.43b         | 2.47b          | 8.9b           | 58.16          | 14               | 416         |
| <b>50W-50D%</b>  | 4.36c               | 16.6a              | 15.38a         | 2.93a          | 12.6a          | 48.13          | 14               | 423         |

APF: All-purpose flour, CP: Chickpea flour and D: Food grade DDG, Kcal: Kilocalories, CHO: Carbohydrates, TDF: Total dietary fiber Provide composition of CP, FDDG and APF. It does not make sense to provide composition of only some of the ingredients.

**Table 5. 5 Minerals content of ingredients used in FDDG and Chickpea fortified High Energy Biscuits.**

| <b>Mineral</b>         | <b>APF</b> | <b>CP</b> | <b>FDDG</b> |
|------------------------|------------|-----------|-------------|
| <b>Calcium (%)</b>     | .047       | 0.07      | 0.057       |
| <b>Copper (ppm)</b>    | 1.54       | 7.17      | 3.65        |
| <b>Iron (ppm)</b>      | 54.6       | 57.1      | 84.3        |
| <b>Magnesium (%)</b>   | .028       | 0.099     | 0.247       |
| <b>Manganese (ppm)</b> | 8.23       | 65.0      | 12.7        |
| <b>Phosphorus (%)</b>  | 0.12       | 0.386     | 0.596       |
| <b>Potassium (%)</b>   | 0.139      | 0.872     | 0.577       |
| <b>Sodium (%)</b>      | 0.004      | 0.010     | 0.042       |
| <b>Sulfur (%)</b>      | 0.109      | 0.199     | 0.498       |
| <b>Zinc (ppm)</b>      | 10.5       | 38.8      | 52.0        |

APF= all-purpose flour, CP= chickpea, FDDG= food grade DDG

**Table 5. 6 Mineral content of Chickpea and FDDG High Energy Biscuits.**

| <b>Mineral</b>             | <b>Control<br/>100W%</b> | <b>75W-<br/>25CP%</b> | <b>75w-25D%</b> | <b>50W-<br/>50CP%</b> | <b>50W-<br/>50D%</b> |
|----------------------------|--------------------------|-----------------------|-----------------|-----------------------|----------------------|
| <b>Calcium (%)</b>         | 0.013                    | 0.028                 | 0.023           | 0.055                 | 0.032                |
| <b>Copper (ppm)</b>        | 0.680                    | 2.14                  | 1.12            | 3.00                  | 1.7                  |
| <b>Iron (ppm)</b>          | 22.0                     | 27.9                  | 40.5            | 35.4                  | 48.5                 |
| <b>Magnesium (%)</b>       | 0.015                    | 0.052                 | 0.040           | 0.107                 | 0.079                |
| <b>Manganese<br/>(ppm)</b> | 3.28                     | 5.86                  | 7.26            | 9.46                  | 8.03                 |
| <b>Phosphorus (%)</b>      | 0.058                    | 0.073                 | 0.109           | 0.162                 | 0.188                |
| <b>Potassium (%)</b>       | 0.074                    | 0.294                 | 0.188           | 0.428                 | 0.344                |
| <b>Sodium (%)</b>          | 0.028                    | 0.058                 | 0.111           | 0.120                 | 0.165                |
| <b>Sulfur (%)</b>          | 0.038                    | 0.056                 | 0.086           | 0.104                 | 0.158                |
| <b>Zinc(ppm)</b>           | 4.94                     | 8. 28                 | 11.0            | 10.5                  | 17.9                 |

W=all wheat, APF= all-purpose flour, CP= chickpea, FDDG= food grade DDG

**Table 5. 7 Nutrients and Kcal specifications of biscuits designed for use as food supplements  
by world food and health agencies (100g serving size).**

| <b>Agency</b> | <b>Energy<br/>(kcal)</b> | <b>Protein<br/>(g)</b> | <b>Fat<br/>(g)</b> | <b>Sugar<br/>(g)</b> | <b>Fiber<br/>(g)</b> | <b>Moisture<br/>(%)</b> | <b>Minerals<br/>(g)</b> | <b>Ca<br/>(mg)</b> | <b>Mg<br/>(mg)</b>  | <b>Fe<br/>(mg)</b> | <b>I<br/>(µg)</b> |
|---------------|--------------------------|------------------------|--------------------|----------------------|----------------------|-------------------------|-------------------------|--------------------|---------------------|--------------------|-------------------|
| <b>WFP</b>    | 400                      | 9                      | 15                 | 10-19                | 2.3                  | 4.5                     | 3.5                     | 250                | 150                 | 11                 | 75                |
| <b>USDA</b>   | 462.2                    | 10                     | 12                 | 10-19                | 2.3                  | 4.5                     | 3.5                     | 250                | 150                 | 11                 | 75                |
| <b>UNICEF</b> | 450                      | 10-15                  | 15                 | 10-15                | 2.3                  | 4.5                     | 3.5                     | 212.5-<br>287.5    | 127.5<br>-<br>172.5 | 9.35-<br>12.65     | 63.75-<br>86.25   |

All values are based on a 100g serving size. World Food Program (WFP) website, Handbook. United States department of agriculture (USDA) website Handbook. United Nation children's funds (UNICEF) website Handbook.

**Table 5. 8 Sensory evaluation of High Energy Biscuits (HEB) prepared with wheat, chickpea flour and Food Grade DDG.**

| <b>HEB</b>       | <b>Color</b> | <b>Aroma</b> | <b>Taste</b> | <b>Texture</b> | <b>Overall</b> |
|------------------|--------------|--------------|--------------|----------------|----------------|
| <b>Control</b>   | 4.39a        | 4.36a        | 4.39a        | 4.22a          | 4.34a          |
| <b>W100%</b>     |              |              |              |                |                |
| <b>75W-25CP%</b> | 4.12a        | 3.72b        | 3.78b        | 4.00a          | 4.00a          |
| <b>75W-25D%</b>  | 3.87b        | 4.28a        | 4.28a        | 4.08a          | 4.18a          |
| <b>50W-50CP%</b> | 4.08a        | 3.66b        | 3.54c        | 4.18a          | 3.86b          |
| <b>50W-50D%</b>  | 3.78b        | 4.18a        | 3.87b        | 4.12a          | 4.12a          |

W=all wheat, APF=All purpose flour, C=Chickpea flour and FDDG=Food grade DDG.  
(N=32)

**Table 5. 9 Comparison of nutrient composition of commercial High Energy Biscuits (HEB).**

| <b>Biscuit</b>                                      | <b>Energy/<br/>kcal</b> | <b>Protein</b> | <b>Fat</b> | <b>CHO</b> | <b>TDF</b> | <b>Moisture</b> | <b>Reference</b>           |
|---|-------------------------|----------------|------------|------------|------------|-----------------|----------------------------|
| <b>DX3600F<br/>18 serving per<br/>packet</b>        | 200                     | 3g/7%          | 9gm/23%    | 26g/65%    | 2g         | 3.68%*          | product<br>label<br>(AIOL) |
| <b>Mainstay3600<br/>9 serving per<br/>container</b> | 400                     | 3g             | 23g/36%    | 46g/15%    | 2g         | 5.69%*          | product<br>label<br>(AIOL) |
| <b>ER bar 9<br/>serving per<br/>container</b>       | 410                     | 7g             | 19g/29%    | 52g/17%    | 2g         | 8.25%*          | product<br>label<br>(AIOL) |
| <b>SOS Bar 9<br/>serving per<br/>container</b>      | 410                     | 8g/16%         | 18g/28%    | 53g/18%    | 1g         | 4.2%            | product<br>label<br>(AIOL) |

Kcal: Kilocalories, CHO: Carbohydrates, TDF: Total dietary fiber,  
\*: was done in researchers lab, AIOL; analyzed in our lab



**Table 5.10 Comparison of commercial HEB biscuits that available in the market.**

| <b>Biscuit/bar</b>         | <b>CON</b> | <b>Manufacture</b>        | <b>Advantages</b>   | <b>Disadvantages</b>   | <b>Price</b>                               |
|----------------------------|------------|---------------------------|---|--|--|
| <b>DX3600F</b>             | USA        | Datrex inc                | Soft and Very easy to separate rapped into individual parts.  | -Contain coconut<br>-Only 7% protein<br>-no moisture content labeled | 9.95 USD                                   |
| <b>format Mainstay3600</b> | USA        | Mainstay products Inc     | - halal food<br>- well sealed<br>-easy to open  | -Contain soy<br>-One big piece<br>-hard<br>-3g protein               | 7.35 USD                                   |
| <b>ER bar</b>              | USA        | Vita-Life Industries, Inc | recommended by US homeland security   | -contain soy<br>- very hard<br>-expensive                            | 9.95 USD<br>Sometimes on sale for 4.95 USD |
| <b>SOS bar</b>             | USA        |                           |   |  |  |
| <b>NRG-5</b>               | Germany    |                           |   |  |  |
|                            |            | S.O.S food lab            | -us coast guard approved<br>- double side zip lock bag  | -not easy to open<br>-contain soy and coconut                        | 5.50 USD                                   |
|                            |            | MSI manufacture           |   | - contain soy<br>- expensive   | 7.4 USD                                    |
|                            |            |                           | -Contain guar gum<br>-In 5 universal languages<br>-14.5 g protein<br>-soft<br>-rapped into individual parts<br>-10 years shelf life |  |  |

Con; country of manufacture, Halal: foods permissible or lawful in traditional Islamic law

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