CHAPTER ONE

INTRODUCTION

Celiac disease CD is a syndrome characterized by damage to the mucosa of the small intestine caused by ingestion of certain wheat proteins and related proteins in rye and barley (Fasano and Catassi, 2001). The gliadins of wheat gluten contain protein sequences toxic to persons with celiac disease (Kagnoff *et al*., 1982). Recent work has also shown that glutenins of wheat contain toxic sequences (van de Wal *et al*., 1999 and Wieser *et al*., 2004). Modern screening studies showed that celiac disease is much more prevalent than previously thought; the average worldwide prevalence is estimated as high as 1:266 (Fasano and Catassi, 2001).

The cornerstone treatment for celiac disease remains the total lifelong avoidance of gluten ingestion, which means that wheat, rye, and barley have to be avoided, including durum wheat, spelt wheat, kamut, einkorn, and triticale (Kasarda and D'Ovidio, 1999 and Kasarda, 2001). In CD patients, ingestion of gluten leads to inflammation and mucosal damage of the small intestine. The typical lesion in the small intestinal epithelium is villous atrophy with crypt hyperplasia, leading to malabsorption of most nutrients including iron, folic acid, calcium, and fat-soluble vitamins (Catassi and Fasano, 2008). This can lead to associated diseases such as osteoporosis, anaemia and type I diabetes and skin disorders (Pruska-Kędzior, *et al*., 2008).

Historically, nutrition counseling for celiac disease has focused on the foods to avoid in a gluten free GF diet but they should be advised on the nutritional quality of gluten- free. There are growing concerns over the nutritional adequacy of the GF dietary pattern because it is often characterized by an excessive consumption of proteins, and fats, and a reduced intake of complex carbohydrates, dietary fibre, vitamins and minerals (Catassi and Fasano, 2008 and Thompson *et al*., 2005). As a consequence, the long life adherence to gluten free products has been associated to undernourished and also minerals deficiencies that could conduct to anemia, osteopenia or osteoporosis (Thompson *et al.,* 2005). Breads made from sorghum without added wheat, as all gluten-free breads, require a different technology. Gluten-free doughs are more fluid than wheat doughs and closer in viscosity to cake batters (Cauvain, 1998) due to the lack of a gluten network. These batter-type doughs have to be handled similarly to cake batters rather than typical wheat doughs. Furthermore, gas holding is more difficult and the uses of gums, stabilizers, and pregelatinized starch have been suggested as a means to provide gas occlusion and stabilizing mechanisms (Cauvain, 1998 and Satin, 1988). Only a limited number of studies have addressed wheat-free sorghum breads, and most have used extra ingredients like methylcellulose (Hart *et al.,* 1970), xanthan gum (Satin, 1988), carboxy methyl cellulose and skimmed milk powder (Cauvain 1998), egg (Keregero and Mtebe, 1994 and Cauvain, 1998), or rye pentosans (Casier *et al.,* 1977). Bread based on simply 70% sorghum and 30% cassava starch has been developed by Olatunji *et al.,* (1992).

Preliminary experiments in our laboratory with sorghum and ≤30% corn starch confirmed that it is possible to produce good sorghum bread without any of these extra ingredients. A simple recipe may help reduce costs, especially with regard to developing countries, and provide a healthy cereal-based bread without added ingredients (egg, milk powder), which might cause new problems for people with allergies. One problem in evaluating nonwheat breads is the lack of standardized baking tests. In wheat baking tests such as ICC Standard No. 131 (ICC, 2000), standardization of the water level to achieve constant dough consistency is a widely accepted technique. Dough consistency is then measured using standardized physical dough testing equipment such as the Brabender farinograph. For gluten-free batters, no standard methods for consistency measurements exist. Sanchez, *et al.,* (2002) used a cone penetrometer to adjust water so that gluten-free batters reached a fixed consistency. Although previous research has been conducted on producing bread from sorghum flour, virtually no work has been done to examine the differences in sorghum bread quality among sorghum hybrids. Such information is necessary to determine whether certain hybrids yield better quality bread than others, to identify the responsible physicochemical factors, and to begin to select sorghum hybrids specifically for bread production.

The objectives of this work were:

- ❖ The main objective:

1. To produce breads and biscuits from sorghum and maize flours supplemented with chickpea flour and improvers.

- ❖ Specific objectives:

1. To determine the *in vitro* protein and starch digestibilities of the final products.
2. To determine the nutritive value of the raw materials and final products.
3. To study the acceptability of gluten free products by celiac disease patients.
4. To study the acceptability of the gluten free breads and biscuits.

CHAPTER TWO
LITERATURE REVIEW

2.1. Sorghum:

Sorghum (*Sorghum bicolor L. Moench*), the world's fourth major cereal in terms of production, and fifth in acreage following wheat, rice, maize and barley, is a staple food crop of millions of poor in semi-arid tropics (SAT) of the world. It is mostly grown as a subsistence dry land crop by resource limited farmers under traditional management conditions in SAT regions of Africa, Asia and Latin America, which are frequently drought-prone and characterized by fragile environments. India grows the largest acreage of sorghum in the world followed by Nigeria and Sudan, and produces the second largest tonnage after the US. In most of the regions of India, it is cultivated both as a rainy- and post rainy-season crop.

The yield and quality of sorghum produced worldwide is affected by a wide array of biotic and abiotic constraints (FAO, 1995; ICRISAT, 2004; Nadia, *et al*., 2009). It sustains the lives of the poorest rural people and often referred to as "coarse grain" or "poor people crop." However, with increasing world population and decreasing water supplies, it is foreseen as an important future crop. Although sorghum is a major cereal crop and resembles corn in general composition, it is considered to be inferior to corn for food, feed and industrial use.

Sorghum based food products occupy a low position in a diet due to its poor quality attributes. The dark color, high fiber content, pronounced flavor, grittiness of flour and difficulty to cook into the soft products like bread, biscuit, cake and pastries, are some of the disadvantages. One way of using surplus sorghum is by way of producing starch and starch based sweeteners. The process is likely to be economical as sorghum is available on large scale with low cost. Sorghum grain contains starch ranging from 68-75% depending upon cultivar, region and climatic conditions. (Subramanian *et al*., 1994; Shinde, 2005 and Singh et *al*., 2009). Among carbohydrate polymers, starch is currently enjoying attention owing to its usefulness in different food products (Taylor *et al*., 2006). Sorghum, like other cereals, is rich in starch–a major storage form for carbohydrates–which makes up about 60-80% of normal kernels and has excellent potential for industrial applications (Zhang *et al*., 2003; Elmoneim *et al*., 2004 and Claver *et al*., 2010). Starch plays an important role in physical, chemical and nutritive attributes of the finished foods.

Sorghum is the fifth most important cereal in total world production (SernaSaldivar, *et al*., 1988). It is able to grow and produce in the warmer temperatures and tropical regions of the world. Sorghum is the chief cereal grain consumed in Asia and Africa. It is used to prepare foods for adults and childrens. In the tropical regions, baby foods are made from sorghum and maize gruels with addition of sugar (Obizoba, 1988). The protein quality of sorghum grain is poor because of the low content of essential amino acids such as lysine, tryptophan and threonine (Badi *et al.,* 1990). Malting improves protein quality of cereals because of an increase in lysine (Dalby and Tsai, 1976). Sorghum is poorly digested by infants (MacLean *et al*., 1981), but if it supplemented with foods high in lysine, can be a satisfactory weaning food (Badi *et al*., 1990). Sorghum proteins become less digestible after cooking (Actell *et al*., 1981; Eggum *et al*., 1983).

Sorghum, like the other cereals, is a good source of B vitamins such as thiamin, riboflavin, vitamin B6, biotin and niacin, but refining produces losses of all B vitamins (Hegedus *et al*., 1985). Mineral composition of sorghum is similar to that of millet (Hulse *et al*.,1980).The chief minerals present in sorghum grain are potassium and phosphorus,while calcium is low (Khalil *et al*., 1984). Sorghum contains polyphenolic compounds called condensed tannins, which are antinutritional factors. Condensed tannins decrease the nutritional value of the sorghum grain because they are able to bind to dietary proteins, digestive enzymes, minerals such as iron and B vitamins like thiamin and vitamin B6 (Wang and Kies ,1991). They are present in sorghums having a pigmented testa (Obizoba, 1988), and are absent in white and colored sorghums without a pigmented testa (Anglani, 1994). Snack foods can also be made from sorghum. The chief foods prepared from sorghum are tortillas, couscous, porridges and baked goods. Sorghum grain is mixed into a dough and baked as flat, unleavened bread to produce tortillas (Rachie,1969; Torres,1994). Tortillas are produced by cooking maize in alkali (calcium hydroxide), steeping and washing the cooked maize (nixtamal). The nixtamal is ground into masa, formed into flat dough pieces and cooked on a hot surface to form tortilla by the nixtamalization process (Bedolla *et al*., 1983).

Some workers (Bedolla et al.,1983; Choto *et al*.,1985) have reported that tortillas are made from sorghum and sorghum maize mixtures. Couscous is a major food staple in North Africa (Kaup and Walker, 1986). It is a steamed, agglomerated food which can be made from durum wheat semolina, sorghum, maize and pearl millet. Porridges are made from wheat (e.g. cream of wheat and farina), maize (grits or hominy grits), rolled or fermented oat meal, rice, sorghum, maize and pearl millet (Bello *et al*., 1990). Ogi, which is a fermented porridge, is the most important weaning food for babies (Dada and Muller, 1983) .The baby foods made from sorghum contains less ash, protein and fat than the corresponding baby foods prepared from millet (Badi *et al*., 1990). In sorghum and millet baby foods, the 80% extraction rate products contain less ash and more starch than the whole grain products. The decrease in ash is due to the reduction of mineral content which is associated to decortications (Badi *et al*., 1990).

2.1.1 Utilization of sorghum:

Sorghum *(Sorghum bicolor L.* Moench) has been consumed as a major food staple in Asia and Africa for centuries. However, in the United States, sorghum has been used mainly for livestock feed with only a small percentage used for food and industrial purposes (Rooney and Waniska 2000). Sorghum is the third major cereal crop in the United States with annual production of 13 million metric tonnes harvested from 4 million ha (Smith, 2000). Because sorghum is currently used mainly as animal feed, significant opportunities exist for increased utilization of sorghum. Sorghum is increasingly being used to produce fuel ethanol, especially in the sorghum growing regions. Sorghum also has potential for increased human consumption due to its high level of phytochemical components (Taylor and Belton, 2002; and Awika and

Rooney, 2004). Sorghum is also being used in the production of wheat-free food products suitable for consumption by people with celiac disease. Sorghum starch plays an important role in both the production of food products and the fermentation of sorghum to produce products such as fuel ethanol. During fermentation, it is the starch that is broken down into sugars, later to be converted into ethanol. Starch content has been positively correlated to ethanol yields in sorghum (Zhan *et al*., 2003). Sorghum starch plays an important role in the production of many sorghum-based food products, including bread (Schober *et al*., 2005).

2.1.2 Importance of sorghum:

Sorghum supplies important minerals, vitamins, proteins, and micronutrients essential for optimal health, growth, and development (Chan *et al*., 2007 and Salgueiro, *et al*. 2002). Determination of nutritional composition in sorghum is of paramount importance for improving malnutrition caused by lack of mineral elements, protein, and vitamins in food stuff (Feil *et al*., 2005; and Welch and Graham, 2004). Feil *et al*., (2005) reported that breeding for higher concentrations of minerals in food crops is an alternative method for improving the health of humans suffering from the consequences of mineral deficiency. Identifying and manipulating the available germplasm accessions can improve yield and quality of sorghum varieties. Ethiopia is well known for the diversity of its indigenous food crops, including sorghum. The chemical composition of sorghum landraces collected from the western regions of Ethiopia has not yet been adequately studied to meet the needs of growers, consumers, and traders of sorghum for both food and industrial uses. Therefore, exploration of available genetic variation in landraces for chemical composition requires the identification of sorghum accessions before inclusion in sorghum breeding programs. Selection of sorghum accessions for improved mineral elements, total starch, and protein content is dependent on the knowledge of the genetic variation expressed in a given environment.

2.1.3 Sorghum grain composition

2.1.3.1 Protein content

The second major component of sorghum grains is protein. The majority of these proteins are located in the kernel endosperm, divided between the protein bodies and the endosperm's protein matrix. About half of sorghum proteins are prolamin (alcohol soluble proteins). This prolamin fraction of sorghum is referred to as kafirin. Kafirin is also known to be high in glutamic acid and aspartic acid. Kafirins are made up of three fractions: α-, β-, and γ-kafirin, with α-kafirin being the prevalent form and is found in the innermost regions of the protein body (Rooney and Serna-Saldivar, 2000). The other protein fractions of sorghum are in the form of glutelins, albumins and globulins that make up enzymes, cell material, and other proteins needed for seed structure and plant development. Like most other cereal grains, sorghum's limiting amino acid is lysine (Rooney and Serna-Saldivar, 2000; Taylor and Dewar, 2001).

Sheorain, *et al.*, (2000) reported that sorghum composition starch ranged from 63 – 68%, moisture 9 -13%, protein 9 -11%, fat 1 – 1.5%, crude fiber 1.5 – 2%, ash 1 -2%, and others organics 8 – 12%.

2.1.3.2 Fats content

The crude fat content of sorghum is 3 percent, which is higher than that of wheat percent and rice percent but lower than corn percent. The germ and aleurone layers are the main contributors to the lipid fraction. The germ itself provides about 80 percent of the total fat (Rooney and Serna-Saldivar, 2000).

2.1.3.3 Carbohydrates content

Starch is the major storage form of carbohydrate in sorghum. The difference between sorghum starch and corn starch is their gelatinization temperature, with sorghum's being higher. Because of this, sorghum requires a longer cooking time as well as more thermal energy during processing to reach its starch gelatinization temperature (Rooney and Waniska, 2000). Lower starch digestibility in some varieties of sorghum is mainly due to the presence of tannins in the grain (Keregero and Mtebe, 1994).

2.1.3.4 Crude fiber content

According to Rooney and Waniska (2000), sorghum kernel contains 6.5 to 7.9 percent fiber with a majority of insoluble (86.2 percent). This fiber comprises most of the pericarp layer. It is made of cellulose, hemicelluloses, and small amount of lignin. Although in minority, soluble fibers are also present in sorghum especially under the form of β-glucan (Rooney and Serna-Saldivar, 2000).

2.1.3.5 Energy value (Kj/100g) content

Sorghum has an energy value of 96% that of corn and can be a complete replacement for corn in swine diets (Carter *et al.*, 1989); however in many recent trials, low-tannin sorghum with proper feed processing and diet formulation has been shown to result in equal pig performance to corn-based diets (Shelton *et al.*, 2004; Issa, 2009 and Benz, *et al.*, 2011).

2.1.3.6 Amino acid content

Mokrane *et al.* (2010) reported that values of essential amino acids in raw sorghum ranged from 3.69 to 4.27, 15.77 to 17.34, 4.47 to 5.07, 4.62 to 5.11, 4.49 to 6.26, 1.58 to 2.05, 1.06 to 1.42 and 2.69 to 3.19 g/100 g protein for isoleucine, leucine, threonine, valine, phenylalanin, lysine, methionine and tyrosine, respectively. whereas, Elhadi, et al., (2005) reported average of 3.64 to 3.71, 11.50 to 13.37, 2.99 to 3.45, 4.64 to 5.90, 4.54 to 4.81, 1.66 to 1.88, 1.00 to 1.76 and 3.53 to 3.76 g/100 g protein, respectively.

2.1.3.7 *In vitro* protein digestibility

The nutrient composition of sorghum indicates that it is a good source of energy, proteins, carbohydrates, vitamins and minerals (Dicko *et al.*, 2006; and Afify *et al.*, 2011). Cereals for human and monogastric animals, because of its anti-nutritional factors such as tannins and phytic acid, removal of these undesirable components is essential to improve the nutritional quality of sorghum and effectively utilize its potential as human food or animal feed (Soetan and Oyewole, 2009 and Kumar et *al.*, 2010). Interaction between tannins and sorghum proteins and starch reduces both protein and starch digestibility. This is important in both human and animal nutrition. The formation of complexes between sorghum proteins and tannins is thought to render the proteins indigestible as well as inhibit digestive enzymes. Proteins rich in proline bind more sorghum tannins than other proteins. In addition, a protein containing more proline repeats will bind more tannin than one with less such repeats (Medugu *et al*., 2010).

The low digestibility of sorghum proteins is presumably due to the high protein cross linking. Good quality proteins are those that are readily digestible and contain the essential amino acids in quantities that correspond to human requirements (Zhao *et al*., 2008 and El-Beltagi, *et al*., 2011). The *in vitro* pepsin digestion assays mimics the digestive system, and are widely used to study the structural changes, digestibility and release of food components under simulated gastrointestinal conditions. The most frequently used biological molecules included in the digestion models were digestive enzymes, bile salts, and mucin (Coles *et al*., 2005; and Hur *et al*., 2011).

2.1.3.8 *In vitro* starch digestibility

Cereal grains, tubers and legume seeds are staple foods in both developed and developing countries. All contain starch, but the starch digestibility is greatly influenced by plant type and depends on physicochemical characteristics of the starch and plant microstructure and composition, and is influenced by processing and storage conditions (Kingman and Englyst, 1994; Ring *et al.,* 1988). Most starch related foods are cooked before consumption and consequent starch gelatinization and retrogradation play important roles in the quality and digestibility of the many resultant food products.

There have been many reports on starch digestibility from different plant sources (Botham et al., 1996; Hu, *et al.,* 2004; Madhusudhan and Tharanathan, 1995; Van der Merwe *et al.,* 2001), but there is little information on the relationship between the starch digestibility, and the thermal and rheological properties of cereals, tubers and legumes grown in China. Glycemic index is greatly influenced by the starch digestibility (e.g. rate of starch digestion) in the food system. Resistant starch and slowly digestible starch result in low glycemic index in starch-based food products. In recent years, the glycemic index has been transformed from a potentially useful tool in planning diets for diabetic patients to a key player in the prevention of diabetes, hyperlipidemia, cardiovascular disease, and even certain types of cancer in the general population (Bjo¨rck and Asp, 1994).

However, no information is available on quickly digestible starch, slowly digestible starch and resistant starch in vitro in cereals, tubers and legumes grown in China. In addition, the retrogradation behaviour of selected plant flours has not been fully investigated and understood.

2.1.3.9 Minerals content

Sorghum is the second cheapest source of energy and micronutrients [after pearl millet (*Pennisetum glaucum*)]; and a vast majority of the population in Africa and central India depend on sorghum for their dietary energy and micronutrient requirement (Parthasarathy Rao *et al*. 2006). Limited studies indicated that mineral concentrations and bioavailability are limited in cooked sorghum grain (Kayode, *et al*. 2006); but this needs to be further validated. Micronutrient malnutrition, primarily the result of diets poor in bio-available vitamins and minerals, causes blindness and anemia (even death) in more than half of the world’s population, especially among women of reproductive age, pregnant and lactating women and preschool children (Underwood, 2000; Sharma, 2003; and Welch and Graham, 2004) and efforts are being made to provide fortified foods to the vulnerable groups of the society. Biofortification, where possible, is the most cost-effective and sustainable solution for tackling micronutrient deficiencies as the intake of micronutrients is on a continuing basis with no additional costs to the consumer in the developing countries in arid tropics and subtropics. Bio fortification of sorghum by increasing mineral micronutrients [especially iron (Fe) and zinc (Zn)] in the grains is of widespread interest (Pfeiffer and Mc Clafferty, 2007; Zhao, 2008 and Kumar *et al.,* 2009).

Evaluating foods in forms as eaten is the most reliable approach for determining bioavailability. Iron, zinc and copper content and extractable iron, zinc and copper in sorghum flour and as eaten in fermented bread (injera) were analyzed by Mohammed, *et al*. (2010). They reported the iron, zinc and copper content for sorghum flour as 2.24 mg/100 g, 0.75 mg/100g and 0.61 mg/100g and the extractable iron, zinc and copper as 34%, 52% and 34% respectively. For the fermented injera on a dry basis, the iron, zinc and copper content amounts were 3.95 mg/100 g, 0.64 mg/100g and 0.61 mg/100g and the extractable amounts were 34%, 62% and 38% respectively. These data are specific for the Tabat sorghum variety (Mohammed *et al*., 2010). Other varieties may show different mineral levels and bioavailability due to variety, geographic region cultivated and other methods of processing. However, Mohammed *et al*. (2010) found that fermentation may increase mineral bioavailability is useful in countries where fermented foods are widely eaten.

2.2 Maize:

Maize (*Zea mays* L.), the American Indian word for corn, means literally "that which sustains life". It is, after wheat and rice, the most important cereal grain in the world, providing nutrients for humans and animals and serving as a basic raw material for the production of starch, oil and protein, alcoholic beverages, food sweeteners and more recently, fuel. Presently world produces around 638.04 million tonnes of maize and is grown in an area of about 140 million hectares (Anon, 2004). Over 43 million ha of maize is grown in Asia producing 166 million tons with an average yield of 3.8 t/ha (Anon, 2004). India ranks eighth in terms of production and shares about 1.85 percent of the total maize production of the world. Other major maize producing countries are China, Brazil, Mexico, France, Argentina, Romania, Italy and Canada. In India the production of maize is 14.13 million tonnes and the total area under this crop is 7.55 million hectares (Anon., 2005). Major maize growing states are Bihar, Gujarat, Uttar Pradesh, Madhya Pradesh, Himachal Pradesh and Karnataka. World-wide with its high content of carbohydrate, fats, proteins, some of the important vitamins and minerals, maize has acquired a well deserved reputation as a poor man's nutricereal. It is estimated that several million people, particularly in the developing countries derive their protein and calorie (11.1 g and 342 Kcal/day) requirement from maize (Gopalan, *et al.*, 1999). Another estimate indicates that maize grain accounts for about 15 to 56 percent of the total daily calories in diets of people in about 25 developing countries (Prasanna, 2001). Besides this, it is also used as industrial starches and in pharmaceuticals as dextrose, maltose, ethanol and corn oil. A variety of maize based fast foods both the domestic and international super markets are found world over. Maize endosperm, consisting of approximately 9-12 per cent protein is however deficient in two essential amino acids. This leads to poor net protein utilization and low biological value of traditional maize varieties.

2.2.1 Utilization of Maize:

As indicated in previous sections, maize has three possible uses: as food, as feed for livestock and as raw material for industry. As a food, the whole grain, either mature or immature, may be used; or the maize may be processed by dry milling techniques to give a relatively large number of intermediary products, such as maize grits of different particle size, maize meal, maize flour and flaking grits. These materials in turn have a great number of applications in a large variety of foods. Maize grown in subsistence agriculture continues to be used as a basic food crop. In developed countries more than 60 percent of the production is used in compounded feeds for poultry, pigs and ruminant animals. In recent years, even in developing countries in which maize is a staple food, more of it has been used as an animal feed ingredient. "High moisture" maize has been paid much attention recently as an animal feed because of its lower cost and its capacity to improve efficiency in feed conversion. The by-products of dry milling include the germ and the seed-coat. The former is used as a source of edible oil of high quality. The seed-coat or pericarp is used mainly as a feed, although in recent years interest has developed in it as a source of dietary fibre (Earll, *et al.*, 1988; Burge and Duensing, 1989). Wet milling is a process applicable mainly in the industrial use of maize, although the alkaline cooking process used in manufacturing tortillas (the thin, flat bread of Mexico and other Central American countries) is also a wet milling operation that removes only the pericarp (Bressani, 1990).

2.2.2 Importance of maize:

Maize is a rare crop, which can be used at any stage of its growth and has very big market potential. Specialty corns (babycorn, sweetcorn, popcorn, high oil corn etc.) assume tremendous market potential not only in India, but in the International market as well (Venkatesh, *et al.*, 2003). For diversification and value addition as well as growth of food processing industries leading to the development of growing maize for vegetable purpose, which is known as 'Baby corn'. It can be eaten raw and included in the diet in number of ways as salads, chutney, vegetables, pickles, kheer, and other Chinese preparations. Baby corn is highly nutritive and its nutritional quality is on par or even superior to some of the seasonal vegetables. Besides protein, vitamins and iron it is one of the richest source of phosphorus. It is also free from residual effects of pesticides, as the young cobs are wrapped up within the husk and well protected from diseases, insects, fungicides and insecticides (Sain Dass, *et al.*, 2007).

Maize as a source of starch is the third most important cereal in the world after rice and wheat. Its utilization includes food uses, for industrial processing as a raw material and for animal feed formulation (Kent and Evers, 1994). However, utilization of maize for food production is the most common in developing countries as against industrial usage in the developed countries (Mejia, 2005).

2.2.3Maize grain composition

2.2.3.1 Protein content

The protein content of untreated maize and lentil was found to be 10.90 and 26.10 %, respectively(Alonos *et al*., 1998). Ijabadeniyi and Adebolu, (2005) found the protein content of three maize varieties grown in Nigeria in the range of 10.67 – 11.27% for the maize grains. Ikram *et al*. (2010) stated that the protein content was found in the range of 7.71 – 14.60%. The corn kernel is made up of four main structures: the pericarp (bran), endosperm, germ (embryo), and the tip.

Corn contains 8 to 11% of protein with lower lysine content (usually less than 30 mg/100g protein) than other cereal grains such as rice or wheat (Shewry, 2007). While most corn protein (75%) comes from the endosperm, it is in the germ that the proteins with the best amino acid profile are concentrated. Those proteins present about three times more albumin, twice as much globulin, and ten times less zein than the whole kernel (Gupta and Eggum, 1998 and Shewry, 2007).

In the industrial processing of corn, the kernel is degermed and the amylaceous endosperm, which is of the greatest interest to the food industry, is separated from the other fractions. The most important fraction is made up of the germ with pericarp, generally used for oil extraction and animal feed due to its high density of nutrients, particularly lipids, proteins, and fibers (Gupta and Eggum, 1998; Watson and Ramsted, 1999). Sheorain, *et al*., (2000) reported that sorghum composition starch ranged from 60 – 64%, moisture 8 – 11%, protein 9 -11%, fat 3 -5%, crude fiber 1.5 – 2%, ash 1 -2%, and others organics 7 – 9%.

2.2.3.2 Fats content

Ijabadeniyi and Adebolu, (2005) determined the fat content of three maize varieties grown in Nigeria to be in the range of 4.77 - 5.00 % for the maize grains.

2.2.3.3 Carbohydrates content

Carbohydrates are the major chemical component of the maize grains. It was found to be in the range of 69.659 – 74.549%. Ijabadeniyi and Adebolu, (2005) reported slightly lower values (65.63 – 70.23%) of the carbohydrate content for the maize varieties grown in Nigeria.

2.2.3.4 Crude fiber content

Ikram, *et al*. (2010) reported that the percent of crude fiber in the maize grains was found in the range of 0.80 to 2.32%. Ijabadeniyi and Adebolu, (2005) reported slightly higher values (2.07 to 2.77%) of the fiber content for the maize varieties grown in Nigeria.

2.2.3.5 Caloric value (Energy) content

Calculated energy values of maize varieties grown in Pakistan varied from 307.047 (Kcal/100g) to 394.066 (Kcal/100g) in dry matter basis (Ikram *et al*., 2010). Kouakou, *et al*. (2008) showed the energy level of maize grains as 387.7 (Kcal/100g). In another study Ejigue *et al*. (2005) found the energy value of 447 (Kcal/100g) for yellow maize.

2.2.3.6 *In vitro* protein digestibility

In vitro protein digestibility of untreated maize was found to be 29.03% while that of untreated lintel was found to be 92.27%. Cooking was found to increase *in vitro* protein digestibility of maize to 96.25%. Cooking of seeds was found to increase in vitro protein digestibility for both maize and lentil to 34.25% and 96.10%, respectively. The improvement of protein digestibility after cooking could be attributable to the reduction of anti nutrients such as phytic acid and condensed tannins and polyphenols, which are known to interact with protein to form complexes (Awada *et al*., 2005).

2.2.3.7 *In vitro* starch digestibility

Starch is classified into rapidly digestible starch, slowly digestible starch and resistant starch RS according to the rate of glucose release and its absorption in the gastrointestinal tract (Englyst *et al*., 1992). Rapidly digestible starch is the portion digested within 20 minutes and slowly digestible starch is digested between 20 and 120 minutes. Slowly digestible starch is believed (Englyst *et al*., 1992) to be slowly but completely digested, leading to a slower entry of glucose into the blood stream and lower glycemic response. RS cannot be digested in the small intestine and is left in the colon. Englyst *et al*., (1982) defined resistant starch as that starch that remained after enzymic hydrolysis, resists digestion in the stomach and small intestine, and ferments in the large intestine. The concept was improved by EURESTA (Asp, 1992) as the total amount of starch, and the products of starch degradation that resists digestion in the small intestine of healthy people. The amount of rapidly digestible starch is positively correlated with the glycemic index of food products (Englyst *et al.*, 1999). It has been suggested that the content of rapidly digestible starch and slowly digestible starch can be used to predict the glycemic index of cereal-based food products (Englyst *et al*., 2003).

Glycemic index represents the level of the postprandial glucose rise in blood as compared to a reference food or glucose (Jenkins *et al*, 1981). Long-term intake of foods with a high glycemic index has been shown to be associated with obesity and related chronic diseases of diabetes and cardiovascular disease (Ludwig, 2000). Dietary carbohydrates, such as starch, effect on human health are important, because they provide 45-65% of the total caloric intake (Dietary Guidelines for Americans, 2005). Thus, starch in food is important for healthy diets, and the starchy food with less refined and less processed should be increased in the diet since it leads to a low glycemic index value. The digestion process of starch is catalyzed by amylolytic enzymes which are comprised of pancreatic α-amylase and the intestinal brush border glucoamylases, maltase-glucoamylase, and sucrose-isomaltase (Nichol *et al.,* 2003). The activities of these enzymes affect the rate of starch digestion. Slowly digestible starch is slowly digested and is related to its substrate property. Ferguson *et al*., (2000) revealed that some native cereal starches with semicrystalline A-type structure contain high levels of slowly digestible starch, more than 50% in maize and sorghum starches. A number of studies on raw 2 cereal starches showed that the slow digestion property is affected by their biosynthesis (James *et al*., 2003), structure (Buleon, *et al*., 1998 and Tester *et al*., 2004), physicochemical properties (Oates, 1997), and enzymatic hydrolysis (Tetlow *et al*., 2004). Zhang *et al*., (2006) reported the side-by-side digestion mechanism and layer-by-layer digestion pattern of slow digestion property of native cereal starches. The crystalline and amorphous regions of starch granules were evenly digested through a mechanism of side-by-side digestion of layers of semi crystalline shells of native starch granules. Enzymatic hydrolysis requires the binding of amylolytic enzymes to starch molecules. After slow digestion, resistant starch is left in the colon and fermented by colonic bacteria. Hence, resistant starch has potential for prebiotic applications and has physiological benefits which are associated with disease prevention. Fermentation of resistant starch produces short-chain fatty acids (SCFA), such as acetate, propionate, and butyrate. These products lower the overall pH of the colon, induce chemoprotective enzyme activity, and hinder growth of harmful colonic bacteria. Thereby, RS plays a role in protecting against colorectal cancer (Burns and Rowlands, 2000; Ferguson, *et al*, 2000; Topping and Clifton, 2001; and Wollowski, *et al.*, 2001). Other benefits of RS consumption include lowering of plasma cholesterol and blood lipids, as well as improved glucose tolerance (Vanhoof and De Schrijver, 1998 and Voragen, 1998).

Rendon-Villalobos, *et al.* (2002) reported a similar pattern, with higher digestible starch value in “masa” (79.6 g/100 g) than tortilla (72.9 g/100 g). In addition, a similar value of digestible starch for tortilla made with commercial “masas” was reported (70.1–76.0 g/100 g) (Agama-Acevedo, 2005) and those made with commercial dry masa flour (70.6–74.9 g/100 g) (Agama-Acevedo, 2004). Tortilla prepared with commercial white maize flour (63.5 g/100 g) (Hernández-Salazar,2006) and commercial tortilla (65.2 g/100 g) (Sáyago-Ayerdi, 2005) had lower digestible starch content. The addition of bean to tortilla decreased digestible starch content in approximately 15.6%, this pattern due to the amount of bean added and the lower digestible starch level in this legume. Mexican “taco” (Sáyago-Ayerdi, 2005) (a mixture of tortilla and bean 60:40) was studied in its digestible starch content, showing a digestible starch content of 52.6 g/100 g; the ratio maize: bean and the method utilized for the preparation of the samples could explain such differences. In commercial maize-bean tortilla digestible starch content of 60.3 g/100 g was reported, but the ratio maize-bean is not declared (Hernández-Salazar, 2006).

2.2.3.8 Minerals content

Hassan, *et al*. (2009) determined the mineral contents of two maize varieties grown in Sudan and showed the level of Na in the range of 15-18 ppm, K 93- 108ppm, Ca 212 -162 ppm, Fe 18 ppm and Zn 5ppm. Hussaini, *et al*. (2008), determined mineral content of dry season maize K content in the range of 3400-3600 ppm, Ca 350-360 ppm and Mg 1060-1120 ppm, and also Feil, *et al*. (2005) showed the concentration of K in the range 3930-3710ppm, Mg 1120-1130 ppm, Ca 82-137 ppm, Zn 23.1-25 ppm and Cu 2.21-2.36 ppm.

2.3 Nutritional quality and health benefits of chickpea (*Cicer arietinum L.*)

Chickpea contains 29% protein, 59% carbohydrate, 3% fiber, 5% oil and 4% ash. Chickpea protein is rich in lysine and arginine but most deficient in sulphur-containing amino acids methionine and cystine (Iqbal, *et al*., 2006). Chickpea is also a good source of absorbable Ca, P, Mg, Fe and K (Christodoulou, 2005).

The protein content in chickpea significantly varies as percentage of the total dry seed mass before (17-22%) and after (25.3-28.9%) dehulling (Badshah, *et al*., 2003). The seed protein content of eight annual wild species of genus *Cicer*, ranged from 168 g/ kg-1 in *Cicer cuneatum* to 268 g /kg-1 in *Cicer pinnatifidum* with an average of 207 g /kg-1 over the eight wild species (Ocampo, *et al*., 1998). Chickpea protein quality is better than some pulse crops such as black gram [*Vigna mungo* L.], green gram [*Vigna radiata* L.] and red gram [*Cajanus Cajan* L.](Kaur *et al*., 2005). Additionally, there is no significant difference in protein concentration of raw chickpea seed compared to some pulses such as black gram, lentils, red kidney bean and white kidney bean (Rehman and Shah, 2005).

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

(Yang *et al*., 2007). Chickpea supplementation in the diet resulted in increased satiation and fullness (Murty *et al*., 2010).

Total dietary fiber content (DFC) in chickpea is 18-22 g/100-g of raw chickpea seed (Aguilera, 2009; Tosh and Yada, 2010) and it has higher amount of DF among pulses . Soluble and insoluble DFC is about 4-8 and 10-18 g/100-g of raw chickpea seed respectively (Dalgetty and Baik, 2003; and Rincón *et al*., 1998). The DFC of chickpea seed is equal to or higher than other pulses like lentils [*Lens culinaris]* and dry peas [*Pisum sativum*](Tosh and Yada, 2010). The desi types have higher total DFC and insoluble DFC compared to the kabuli types. This could be due to thicker hulls and seed coat in desi (11.5 % of total seed weight) compared to the kabuli types (only 4.3-4.4 % of total seed weight) (Rincón et al. 1998).

2.3.1 *In vitro* Protein Digestibility:

The *in vitro* protein digestibility (IVPD) of raw chickpea seeds varies from 34-76% (Khalil *et al*., 2007; Khattak *et al*., 2008; Clemente *et al*., 1998). Chitra, *et al.* (1995) found higher IVPD values for chickpea genotypes [65.3-79.4%] compared to those of pigeon pea [*Cajanus Cajan*; 60.4 to 74.4%], mung bean [*Vigna radiata*; 67.2 to 72.2%], urd bean [*Vigna mungo*; 55.7 to 63.3%] and soybean [*Glycine max*; 62.7 to 71.6%]. The digestibility of protein from kabuli type is higher than the protein from desi types (Sánchez-Vioque *et al*., 1999; and Paredes-López *et al*., 1991).

2.3.2 *In vitro* starch digestibility:

The total starch content of chickpea seeds is reported to be ~ 525 g/ kg-1 dry matter, about 35% of total starch is considered to be resistant starch (RS) and the remaining 65% as available starch (Aguilera, 2009; and USDA, 2010). Cereals such as wheat have higher amount of starch compared to chickpea (USDA, 2010), but the chickpea seeds have higher amylose content [30-40% versus 25% in wheat] (Williams and Singh, 1987; Guillon and Champ, 2002). The *in vitro* starch digestibility values (ISDV) of chickpea vary from 37-60% (Zia-Ul-Haq *et al*., 2007; Khalil *et al*., 2007) are higher than other pulses like black grams, lentils and kidney beans (Rehman and

Shah, 2005). However, the ISDV of pulses in general are lower than cereals due to higher amylose content (Madhusudhan and Tharanathan, 1996).

2.3.3 Health benefits:

Chickpea seed oil contains different sterols, tocopherols and tocotrienols (Akihisa *et al*., 2000; Akihisa *et al*., 1992). These phytosterols are reported to exhibit anti-ulcerative, anti-bacterial, anti-fungal, anti-tumoric and anti-inflammatory properties coupled with a lowering effect on cholesterol levels ((Murty *et al*., 2010; Arisawa *et al*., 1985). Δ7-Avenasterol and Δ5-avenasterol, phytosterols present in chickpea oil have antioxidant properties even at frying temperatures (Wang *et al*., 2002). Carotenoids like lutein and 19 zeaxanthin, the major carotenoids in chickpea seeds, are speculated to play a role in senile or age-related macular degeneration (AMD). Though there are some epidemiological and association studies suggesting a beneficial effect of lutein and zeaxanthin on AMD, evidence from RCTs on the effect of carotenoids on AMD is not presently available (Mozaffarieh *et al*., 2003). Carotenoids are reported to increase natural killer cell activity (Santos et *al*., 1998).

Chickpea seeds have been used in traditional medicine as tonics, stimulants and aphrodisiacs (Pandey and Enumeratio, 1993). Further, they are used to expel parasitic worms from the body (anthelmintic property), as appetizers, for thirst quenching and reducing burning sensation in the stomach (Zia-Ul-Haq *et al*., 2007). In the Ayurvedic system of medicine chickpea preparations are used to treat a variety of ailments like throat problems, blood disorders, bronchitis, skin diseases and liver or gall bladder related problems [biliousness] (Sastry and Kavathekar, 1990). In addition to these applications, the chickpea seeds are also used for blood enrichment, treating skin ailments, ear infections, and liver and spleen disorders (Warner, *et al*., 1995). Uygur people of China have used chickpea in herbal medicine for treating hypertension and diabetes for over 2500 yrs (Zhang, 2007).

2.4 Celiac disease:

Celiac disease is an immune mediated condition affecting the small intestine that is triggered in genetically susceptible individuals by the consumption of the gliadin fraction of gluten (Fasano, *et al*., 2003). The villi of the intestinal mucosa become flattened, reducing the production of disaccharidases and peptidases necessary for digestion. This deficiency in digestive enzymes and the reduced surface area of the small intestine results in malabsorption of virtually all nutrients. Depending on the extent of damage to the small intestine, patients may be relatively symptom-free or may have significant GI distress, malabsorption, and malnutrition. Some patients lack intestinal symptoms, and instead initially present with symptoms such as type I diabetes mellitus, anemia, osteoporosis, arthritis, infertility, or Down syndrome (Fasano *et al*., 2003).

Work has been done to determine the gliadin peptide sequence that results in the celiac immune response. All gliadins appear to be active in causing epithelial damage, and the most immunoreactive amino acid sequences in gliadins are not well characterized (Lähdeaho *et al*., 1995). Tu_ková, *et al*. (2002) digested gliadin and tested the different fractions for the degree of immune response elicited; finding that the peptide sequence FQQPQQQYPSSQ produced the highest immune response. In examining gliadins, Ensari, *et al*. (1998) determined that the octapeptide sequence PQQPFPQQ is important in the immunopathology of celiac disease. Lähdeaho and 22 colleagues (1995) identified two peptides associated with celiac disease: peptide 9 (QPYPQPQPFP) in gliadins and peptide 42 (LGQGSFRPSQ) found only in gliadin. While this work may one day create the possibility of genetically modified wheat that is safe for celiac patients, all sources of gluten must currently be avoided Gluten-Free Diet. The only treatment for celiac disease is the lifelong adherence to a gluten-free diet. Consumption of wheat, rye, and barley, as well as less common cereals such as spelt, kamut, einkorn, and triticale must be avoided. A growing body of research strongly suggests that oats can be safely consumed by celiacs, but there is concern about contamination by wheat during processing (Thompson, 2001). Sorghum, flax, corn, rice, millet, buckwheat, amaranth, quinoa and teff are all grains that can be safely included in the gluten-free diet (Mechanic-Schlossmann *et al*., 2003). Tubers such as potatoes and cassava, beans, and oilseeds are also gluten-free. However, because wheat is ubiquitous in the food supply, its elimination from the diet presents a significant challenge to celiac patients and usually results in decreased quality of life (Lee and Newman, 2003). The Codex Alimentarius standard for gluten-free foods requires that the nitrogen content of foods derived from gluten-containing grain cannot exceed 0.05 g per 100 g grain on a dry matter basis. It is estimated that wheat starch meeting the Codex standard may contain 40 to 60 mg gluten per 100 g, which is equivalent to 200 to 300 ppm gliadin (Thompson, 2001). Currently, a draft revised standard for gluten-free foods is being developed by the Codex Committee on Nutrition and Foods for Special Dietary Uses in order to re-define the amount of gluten that is allowed in gluten-free foods. In 23 the new definition, foods made from naturally gluten-free ingredients may not contain more than 20 ppm gluten (10 ppm gliadin). Gluten-free foods made from ingredients that contain gluten (such as wheat, rye or barley) may not contain more than 200 ppm gluten (100 ppm gliadin). Currently, approval of the revised definition is pending until more information regarding tolerance levels to gluten can be determined (Joint FAO/WHO Food Standards Program, 2004).

Standards for the gluten-free diet vary by country. In the United States and Canada, the diet contains no gluten, and is based on naturally gluten-free grains such as rice and corn. However, in the United Kingdom and Scandanavia, foods such as wheat starch that have been rendered gluten-free are included in the diet. Because the minimum dose of gliadin required to elicit an immune response in celiac patients is unknown, dietitians in the United States advise against the use of gluten-free wheat starch (Thompson, 2001). Until recently, celiac disease was thought to be rare in the United States. However, Fasano *et al.* (2003) conducted that the largest multicenter epidemiologic study ever performed to establish the prevalence of celiac disease in the United States. This study indicated that the prevalence of celiac disease is 1:133 in patients who are considered not-at-risk, 1:22 among first-degree relatives of celiac patients, and 1:39 among second-degree relatives. Affecting 3 million Americans, celiac disease is the most common autoimmune disease in the United States.

However, only 15% of the 3 million celiacs in the U.S. are currently diagnosed (Fenster, 2004). Increased awareness of the prevalence of celiac disease by physicians will lead to 24 an increased diagnosis rate and an increase in demand for gluten-free products, particularly high quality gluten-free breads.

2.4.1Celiac patient:

Sorghum is often recommended as a safe food for celiac patients because it is more closely related to maize than to wheat, rye, and barley (Kasarda, 2001). Sorghum might therefore provide a good basis for gluten-free bread. However, the bulk of studies dealing with leavened breads containing sorghum have focused on composite breads from wheat and sorghum, in which a maximum of only 30% low-tannin sorghum are regarded as acceptable (Munck, 1995). While such breads have been found acceptable by consumers (Carson *et al*., 2000), they are inappropriate for celiac patients.

In the Sudan out of the 172 patients investigated for celiac disease, only 128 patients were confirmed positive for celiac disease. The remaining forty four negative patients were excluded from further analysis. The titers of antibodies were higher in the anti-tissue transglutamase tests than the anti-gliadin tests. The commonest presenting symptom was chronic diarrhea (20.3%) followed by weight loss (14%). The third common presentation was noted to be stunted growth (13.3%). Males and females were nearly equally affected (M:F = 1:0.97). All age groups were affected with a peak incidence between 5 to 10 years (41.4%). The over whelming majority of celiac disease is among the Benne Amir tribe (63.3%), followed by the Hadandwa tribe (14.1%) and the Northern Sudan tribes (12.5%). (Ageep, 2012). Celiac disease pateints distributed according to Celiac Disease Support Association, ibn seina hospital 3000, followed by Omdurman hospital165 and gafar ibn aoof hospital 153patient suffer from celiac disease. Some patients undiagnosed for years till to now and others have has special clinics for follow up.

We believe that the prevalence of CD in the Red Sea state may be under estimated due to lack of awareness and low suspicion of the disease. Unfortunately, some doctors, especially in the rural areas, still do not know when to suspect celiac disease. Many physicians still do not realize there is no “typical” celiac disease patient. Celiac disease can begin at any age, persists for life and can affect multiple organs. People with celiac disease can be thin, obese or have normal weight. In both children and adults the symptoms of celiac disease can be extremely variable or there may be no symptoms at all. Unfortunately owing to the heterogeneity in the clinical. Presentation and lack of a standard clinical profile in CD, some patients go undiagnosed for years together despite several consultations with different doctors. (Ageep, 2012). Currently the only treatment for celiac disease is a gluten free diet. (Fasano, *et al.*,2003). Dietary avoidance of gluten leads to symptom improvement in 70% of patients within 2 weeks. (Pink and Creamer, 1967). However, complete histological resolution of small bowel inflammation may take up to 2 years in some individuals.(Grefte, *et al.*,1988). It can be challenge to avoid gluten in eastern Sudan. Poverty and ignorance are the major obstacles in such developing regions. As the pathophysiology of celiac disease gets clearer, new methods of treatment are being developed including orally active drugs.(Shan, *et al.*,2002).

2.5 Anti-nutritional factor in cereal

2.5.1 Tannins

Many plant components have the potential to precipitate adverse effects on the productivity of farm livestock (D’Mello, 2000). These compounds are present in the foliage and/or seeds of virtually every plant that is used in practical feeding. These compounds are often called anti-nutritional factors. Anti-nutritional factors are also those generated in natural feed stuff by the normal metabolism of the specie from original materials and by different mechanisms exert effects contrary to optimum nutrition (Chubb, 1982). Anti-nutritional factors may be grouped according to their mode of action as follows;

* Substances depressing digestion or metabolic utilisation of protein e.g. protease inhibitors, lectins (haemagglutinins), saponins and polyphenolic compounds

* Substances reducing or interfering with the utilization of mineral elements e.g. Phytic acid, oxalic acids, gluccosinolates and gossypol

* Substances inactivating or increasing the requirements of certain vitamins e.g. Anti-vitamins A, D, E and K, anti-thiamine, nicotinic acid, pyridoxine and cyanocobalamin.

Some anti-nutritional factors may however exhibit more than one mode of activity (Chubb, 1982). Sorghum [*Sorghum bicolour (*L) Moench] is widely grown in the semi-arid and savannah regions of Nigeria. Maunder (2002) reported that sorghum is a traditional crop of much of Africa and Asia and an introduced and hybridized crop in the western hemisphere. It benefits from an ability to tolerate drought, soil toxicities and temperature extremes effectively than other cereals. Sorghum grains contain about 92.50% dry matter, 3270.00 kcal/kg metabolisable energy, 9.50% crude protein, 2.55% ether extract, 2.70% crude fibre, 1.25% ash and 76.60% nitrogen free extract (NFE). Its protein is slightly higher than maize but as with most cereals deficient in lysine and tryptophan. More importantly, some varieties of sorghum grain have been reported to contain anti-nutritional factors chiefly tannin which binds proteins and impair digestion (Aduku, 1993; Tacon, 1995; Ngoka, 1997; Aletor, 1999. Aduku, 2004; and Etuk and Ukaejiofo, 2007).

Atteh (2002) reported that sorghum, especially the brown variety contains high levels of tannins. Etuk and Ukaejiofo (2007) reported 0.42% tannin content in brown coat coloured sorghum while Subramanian and Metta (2000) reported that the local Indian sorghum variety and ICSV 112 variety developed by ICRISAT and grown in India contain no tannins. 0.40% tannin was reported for samsorg 17, a variety previously coded (SSV) -3 (SK5912) and developed from local collections of Kaura through mutation breeding at the Institute of Agricultural Research (IAR), Samaru, Nigeria. ICSV 400, released by ICRISAT in 1996 recorded tannin value of 0.69% (Etuk, 2008).

Tannins reduce protein digestibility through the formation of complexes and the inhibition of activities of proteolytic enzymes in digestive secretions (Ahn *et al.*, 1989). The affinity of tannins for protein has been observed to increase with increase in molecular size of tannins. However tannins with extremely large molecular weight lose their affinity for protein and become insoluble (Kumar and Horigome, 1986). Proteins with high proline content impart an open structure which contains readily accessible sites for hydrogen bond formation with tannins (Hagerman, 1989). The polyphenols in brown sorghum may have a binding effect on minerals (Aningi, *et al.*, 1998). Recent studies also revealed that polyphenols of the procyanidins (CT) have an antioxidant property (Corder, 2006) while tannic acid has anti-bacterial, anti-enzymatic and astringent property as well as constringing action upon mucous tissues. The ingestion of tannic acid causes constipation so it can be used to treat diarrhoea in the absence of inflammation (Phytolab, 2007).

2.5.2 Phytic acid:

Phytic acid (C6H18O24P6) also known as inositol hexaphosphate (IP6) or phytate as a salt, is the storage form of Phosphorous in all grains and oil seeds (Jacela, *et al.*, 2010). The accumulation site of phytic acid in monocotyledonous seeds (wheat, millet, barley, rice, etc.) is the aleurone layer, particularly the aleurone grain. Corn differs from other cereals as more than 80% of phytic acid is concentrated in germ. Phytic acid content of cereals varies from 0.5 to 2.0%. Phytate is most known as a substance known to decrease mineral absorption however; it has also been looked at as a possible beneficial vitamin-like substance (Okazaki and Katayama, 2005). Phytic acid has a strong ability to chelate multivalent metal ions, specially zinc, calcium, iron and as with protein residue. The binding can result in very insoluble salts with poor bioavailability of minerals (Zhou and Erdman, 1995).

Sandberg (1995) had reported that the precipitation and ion-exchange methods are not specific as they do not separate inositol hexaphosphate from lower inositol phosphates and thus overestimate the phytate content in processed foods. The HPLC method determines the inositols in processed foods. The phytic acid in unprocessed products mainly appears as inositol hexaphosphate (IP6); since the precipitation methods are useful to measure the phytic acid content in unprocessed products.The amount of phytate present in plant seeds and grains ranges from 0.5-5% (Loewus, 2002).

Researchers provided two groups of people with bread. One group with bread with phytates and one with a control bread without phytates. Researchers then studied participants' mineral absorption via stool samples. Without phytic acid, participants absorbed about 30% of magnesium and zinc. With phytic acid, participants absorbed only 13% of their magnesium and 23% of their zinc (Egli *et al*., 2002; Bohn *et al*., 2004). The zinc-and iron-blocking effects of phytic acid can be just as serious as the calcium-blocking effects (Hallberg *et al*., 1989) showed that a wheat roll containing 2 mg phytic acid inhibited zinc absorption by 18%; 25 mg phytic acid in the roll inhibited zinc absorption by 64% and 250 mg inhibited zinc absorption by 82%. The growing children run into severe problems in a phytate-rich diet, their bodies will suffer from the lack of calcium and phosphorus with poor bone growth, short stature, rickets, narrow jaws and tooth decay and for the lack of zinc and iron with anemia and mental retardation. Onomi, *et al.* (2004) found that phytic acid at a level of 0.035% may protect against a fatty liver resulting from elevated hepatic lipogenesis and that the anti-nutrient effect of phytic acid on mineral absorption will only occur at 10 fold higher levels. As chelator phytates bind to extra iron or toxic minerals and remove them from the body. As with all anti-nutrients, phytates may play a therapeutic role in certain cases. Heart disease is a leading cause of death in the Western countries but it is low in Japan and developing countries. Elevated plasma cholesterol or elevated LDL-cholesterol concentrations have been shown to be one of the risk factors. It has been suggested that dietary fibre or more specifically phytate which is a component of fibre can influence the aetiology of heart disease (Potter, 1995). Jariwalla, *et al.* (1990) showed that dietary phytate supplementation resulted in the lowering of serum cholesterol and triglyceride levels. This effect accompanied the decrease in serum Zn level and Zn-Cu ratio. This is because coronary heart disease appears to be caused by an imbalance of Zn-Cu metabolism.

2.6 Importance and need for gluten-free products

Celiac suffering patients are not the only market for gluten-free products. In fact, there is a growing segment of the population choosing to follow a gluten-free diet for nonmedical reasons. These people may have family or friends with gluten intolerance or they may simply feel better on a gluten-free diet. There is not a lot of information on these people choosing to follow a gluten-free diet for nonmedical reasons. However, estimates of this population segment range from 2 million to as high as 10 million people in the U.S. (P F, 2011).

Additionally, Packaged Facts conducted an online nationwide survey of 1,881 adults in fall 2010 including 277 consumers of gluten-free products. Survey results showed that nearly half of people (46 percent) who buy gluten-free foods and beverages did so based on a perception that they are “generally healthier”. Thirty percent of gluten-free consumers said they did so in an effort to manage their weight and 22 percent said they thought gluten-free products were “generally higher quality”.Only about 10 percent of gluten-free consumers said they bought gluten-free products because they or a member of their household has celiac disease or has intolerance to gluten, wheat or other ingredients. As a result of this situation, there even are some speculations that gluten-free products may become accepted as a superior dietary alternative in the mass market.

2.7 Market growth of gluten free products

“The growth of the gluten-free market is incredible”, (MGNP, 2010) Database, which tracks new product development trends around the world, stated in (2010) report. In this report, Mintel states that the most common conditions currently impacting the market are celiac disease and lactose intolerance. Moreover, it was declared that in the United States, gluten-free is thought to be the dominant player in the “free-from” marketplace. Furthermore, MGNP (2010) also explained that the popularity of gluten-free products was particularly boosted in 2003 and 2004, paralleling the low-card diet boom. Hence, according to the report, some consumers seeking low-carb or low glycemic index products go "one step further" and seek out gluten-free products.

More recently, APFI (2010) reported that gluten-free was one of the top trends predicted and the rise of the sector continues to strengthen every year. Product launches has also gone up by two-fold since 2005, as more manufacturers join in the haste.

Finally, PF (2011) declared that the U.S. market for gluten-free foods and beverages enjoyed an annual growth rate of 30 percent over the 2006-2010 period of time. This led to a market worth U.S. $2.6 billion in 2010. While Packaged Facts expects this growth to slow, it still projects gluten-free sales to exceed U.S. $5 billion by 2015.

2.8 Anti-staling additives in food industry

Certain groups of ingredients retard the staling process or minimize its effect. Among all the additives that have been studied for their potential role in retarding staling in bread, the ones that seem to have the greatest effect are surfactants (complexing agents), enzymes, and hydrocolloids/gums (Gray and Bemiller, 2003).

2.8.1 Emulsifiers:

Emulsifiers, also referred to as food surfactants or surface active agents, are considered as optional additives and are used as dough and bread improvers. Indeed, emulsifiers are commonly included in bakery products' formulations to enhance the structure by increasing dough strength or crumb softness. The main characteristic of emulsifiers is their amphiphilic nature, which allows molecules to migrate to interfaces between two physical phases lowering surface tension and forming dispersions (Nunes, *et al*., 2009). Whether surfactants actually decrease the rate of firming or produce softer breads that then stale at the same rate as the control, has been debated. Kulp and Ponte (1981) concluded that a surfactant's ability to retard firming is more important than an initial softening of crumb in freshly baked bread.

In a study by Pisesookbunterng and D'Appolonia (1983), the same conclusion was reached. The authors studied the effects of surfactants on moisture migration from the crumb to the crust of bread as well as firmness values of bread crumb. They concluded that surfactants did not noticeably have an impact on firmness of fresh bread; however, they did slow the firming rate on bread crumb during storage.

Finally, Knightly (1988) confirmed this theory by showing that the anti-staling abilities of emulsifiers come mainly from their interaction with starch: they inhibit the process of amylopectin re-crystallization. Knightly (1988) explained that the formation of an emulsifier and amylose complex would contribute to a decrease in the initial firmness of the crumb, while the formation of a complex with amylopectin would result in a distinct reduction in the rate of firming during storage. Because emulsifiers mainly interact with the amylopectin part of starch, their action as anti-staling agents is thus about reducing the firming rate of the crumb. Application of emulsifiers in gluten-free products is still a non-widely studied point of interest. Nevertheless, some studies have been conducted on this subject. Nunes, *et al*. (2009) studied the effect of the addition of several emulsifiers on gluten-free breads. The emulsifiers tested were lecithin, diacetyl tartaric ester of monoglycerides (DATEM), distilled monoglycerides and sodium stearoyl lactylate (SSL). This study demonstrated that addition of distilled monoglycerides and SSL at significantly high levels reduce the staling rate of the crumb. Yet, retrogradation of starch over a five-day period did not seem to be affected by the addition of emulsifiers.

Nunes, *et al*. (2009) also showed that although emulsifiers retard bread staling, they also can have a positive or negative impact on the overall gluten-free bread quality. Indeed, crumb structure (cell size and distribution) was greatly affected by the presence of lecithin and DATEM. It was concluded that with high level addition of DATEM, bubble size decreased and a more homogeneous crumb was obtained. Overall, this study showed that emulsifiers had a positive effect on the quality of gluten-free breads and that they can greatly enhance these kinds of breads.

2.8.2 Diacetyl tartaric acid esters of mono- glycerides (DATEM):

Typically, mono- and diacetyl tartaric acid esters of mono-glycerides are used as dough conditioners for all baked products, particularly yeast-leavened products

(Gaupp and Adams, 2004). Diverse authors have studied the effects of DATEM as anti-stalling agents. Krog, *et al*. (1989) established that DATEM surfactants were as effective anti-stalling agents as SSL over five days of storage but less effective in reducing retrogradation of amylopectin. DATEM impact on crumb firming reduction was also proved. It was suggested that the anti-firming properties of DATEM may be due to changes in cell wall thickness and elasticity. It was further reported that optimal reduction in firmness increase over extended periods of storage can be achieved when DATEM is used in combination with monoglycerides (Gray and Bemiller, 2003).

Recently, in their review on DATEM, Gaupp and Adams (2004) explained that the most important effects induced by the addition of DATEM are the stabilization of a soft crumb which will have as consequence a delay in starch retrogradation as well as an improvement of dough performance during manufacturing (tolerance towards raw material quality, mechanical resistance, sticking to manufacturing equipment, mixing and fermentation tolerance). To sum up, the application of DATEM has a lot of advantages as a bread improving ingredient. Also, the economics of its use is considered even more important than its organoleptic effects (Gaupp and Adams, 2004).

2.8.3 Enzymes:

One strategy to reduce the rate of bread staling is to add enzymes to the bread formulation. Enzymes will participate in the production of a more thermostable amylase-lipids complex. Moreover, these enzymes will inhibit amylopectin retrogradation and therefore impact on the crumb firming rate: it will decrease (León *et al*., 2002).

2.8.3.1 α-amylases:

The most useful enzymatic approach to staling rate reduction has been the use of α-amylases, which catalyze a small amount of hydrolysis of the starch. Several studies about the role of α-amylases have been conducted (Morgan *et al.,* 1997; and León *et al*., 2002). As a result, it has been shown that the addition of these enzymes to the dough retard crumb firming. It has been demonstrated that α-amylases have most certainly an indirect effect. The anti-stalling effect of this enzyme is thus due to in-situ formation of starch dextrins and/or maltodextrins. Indeed, the role of amylases is to partially hydrolyze starch during baking into a mixture of smaller dextrins (shorter-chains). Like the α-amylases, enzymes cannot access intact starch granules, they mainly hydrolyze damaged starch. This is done by the enzyme attacking α-(1,4) linkages along starch chains. This action is stopped at α-(1,6) branch points of amylopectin. So, the addition of α-amylases enzymes results in the formation of increased amounts of dextrins. These dextrins are characterized by a particular low degree of polymerization (DP 3–9) and are presumably responsible for the anti-firming effect. So, because of their lower molecular weight, branched dextrins have a decreased ability to retrograde or interfere with retrogradation in any manner, and so they can reduce the extent of firming (León, *et al*., 2002; Gray and Bemiller, 2003; Pateras, 2007).

2.8.3.2Transglutaminase:

Regarding gluten-free breads, some recent studies focused on the use of transglutaminase. Moore, *et al*. (2006) acknowledged that one of the main problems associated with gluten-free bread is obtaining a good structure. Transglutaminase, an enzyme that catalyzes acyl-transfer reactions through which proteins can be cross-linked, could be an efficient additive to improve this poor structure of gluten-free breads. Therefore, in their study, authors tested the influence of various proteins sources (skim milk powder, soya flour, and egg powder) in combination with the different addition levels of transglutaminase on gluten-free bread quality (percent bake loss, specific volume, color, texture, image characteristics, and total moisture). The results showed that the application of transglutaminase in gluten-free systems modified the viscoelastic properties of the batters, improving the quality of the resulting gluten-free breads by promoting a protein network. Thus, the authors hypothesized that it is possible to form a protein network in gluten-free bread with the addition of transglutaminase. However, they specified that the efficiency of the enzyme is dependent on both the protein source and the level of enzyme concentration. Renzetti, *et al*. (2008) confirmed these results by demonstrating that transglutaminase can be successfully applied to gluten-free flours to improve their bread making potentials by promoting network formation. However, as Moore, *et al*. (2006) did, they emphasized the fact that the protein source is a key element determining the impact of the enzyme.

2.8.4 Hydrocolloids:

Hydrocolloids are water soluble polysaccharides with a range of functional properties that make them very useful in food technology. They are indeed widely used as additives in the food industry especially for their action in modifying the rheology and texture of aqueous suspensions. However, a different approach is the use of hydrocolloids as anti-stalling agents (Guardia, *et al*., 2004). Hydrocolloids' action as anti-stalling agents is linked to moisture content loss that occurs during staling. Certainly, all hydrocolloids interact with water, reducing its diffusion and presence (Anton and Artfield, 2008).

Different studies about diverse hydrocolloids demonstrated that breads containing hydrocolloids showed lower loss of moisture content, hence higher water retention in the crumb. So, hydrocolloid addition reduces the dehydration rate of crumb samples during storage. It was also demonstrated that out of all the hydrocolloids tested (xanthan gum, carboxy methylcellulose (CMC), guar gum, carrageenan, locust bean gum alginates, and hydroxyl propyl methyl cellulose (HPMC)), HPMC at a level of 0.5% (flour basis) was the one with an improvement effect on all the parameters tested which were: specific volume index, width/height ratio, and crumb hardness. Moreover, breads with HPMC showed good sensory properties for visual appearance, aroma, flavor, crunchiness, and overall acceptability, and produced a better effect as anti-stalling agent (Guardia, *et al*., 2004; Barcenas and Rosell, 2005; Lazaridou, *et al*., 2007).

Besides being applied as gluten-substitutes in gluten-free breads, hydrocolloids have also been used in foods to improve texture, to slow down the starch retrogradation, to increase moisture retention and to extend the overall quality of the product during time (Rojas *et al*., 1999). The use of hydrocolloids in the formulation of a gluten-free baked good to retard staling has been widely studied. As a result of these studies, xanthan gum and HPMC are now the most used hydrocolloids. They appear to be the best in mimicking the gluten properties, and the most promising in regards to water retention and the quality of the final product (Lazaridou, *et al*., 2007; Anton and Artfield, 2008; Sumnu, *et al*., 2010).

2.9 Nutritional aspect of gluten-free cereal foods:

Only a few studies have been conducted on the nutritional quality of gluten-free foods. One such study conducted by Thompson (1999) assessed thiamin, riboflavin and niacin contents of gluten-free cereal foods to determine how they compare nutritionally to enriched gluten-containing products they were intended to replace. The conclusion reached was that many gluten-free cereal products do not provide the same levels of thiamin, riboflavin or niacin as their enriched wheat-based counterparts.

Another study by Thompson (2000) assessed the folate, iron and dietary fiber contents of gluten-free flours, breads pastas, and ready-to-eat breakfast cereals. It was concluded that gluten-free cereal products generally provide lower amounts of folate and iron than their enriched wheat-based counterparts. The study also indicated that gluten-free cereal products contain generally more dietary fiber than their refined gluten-counterparts.

Finally, it is important to specify that gluten-free cereal foods are generally not enriched and are frequently made with refined flour and/or starch. As a result, they may not provide the same nutritional value as wheat-based foods, especially if we consider that wheat-based foods are often whole grain or enriched. This is why recommendations have been made that persons with celiac disease should be advised to consume more nutrient dense gluten-free cereal foods in the form of whole grains or enriched products and that manufacturers should produce more of these kinds of products (Thompson, 2009). Concerning the glycemic index of gluten-free foods, a controversy exists in the literature. Some research have pointed out that gluten-free breads have a higher glycemic index than regular wheat breads (Schober, 2009). However they did not look at specific grains to determine whether or not some gluten-free grains have lower or higher glycemic indexes.

2.10 Baked goods:

Perten, (1983) reported that the volume of bread made with sorghum or millet, was always smaller than that of bread made with wheat flour, but many consumers preferred it. The bread crumb was less elastic, drier and darker in breads made with sorghum or millet. Those authors suggested that sorghum flour could not be considered bread making flour because it did not produce the elastic dough needed to obtain a large bread volume. Incorporation of 30% sorghum flour to wheat flour can give a larger bread volume than that made from 100% wheat flour of poor quality. Bread made with 30% sorghum flour and 70% wheat flour of 72% extraction rate was evaluated as good to excellent by acceptability test (Perten, 1983). Breads made from sorghum bran contain more starch, sugar and dietary fiber and less ash and protein than breads made from wheat **(**Badi, 1990). Sorghum flours were also used to produce biscuits, granolas and snack foods such as crisps, chips and sham dates (Perten, 1983).

Breads made from sorghum without added wheat, as all gluten free breads, require a different technology. Gluten-free doughs are more fluid than wheat doughs and closer in viscosity to cake batters (Cauvain, 1998) due to the lack of a gluten network. These batter-type doughs have to be handled similarly to cake batters rather than typical wheat doughs. Furthermore, gas holding is more difficult and the use of gums, stabilizers, and pregelatinized starch has been suggested as a means to provide gas occlusion and stabilizing mechanisms (Cauvain, 1998 and Satin, 1988). Milk powder has also been described to have positive effects on gluten free breads (Gallagher *et al*., 2003, 2004) and has been suggested for use in gluten free sorghum bread (Cauvain, 1998). Only a limited number of studies have addressed wheat-free sorghum breads, and most have used extra ingredients like methyl cellulose (Hart *et* *al*., 1970), xanthan gum (Satin, 1988), carboxy methyl cellulose and skimmed milk powder (Cauvain, 1998), egg (Keregero and Mtebe, 1994 and Cauvain, 1998), or rye pentosans (Casier *et al*., 1977). Bread based on simply 70% sorghum and 30% cassava starch has been developed by Olatunji et *al*. (1992).

Preliminary experiments in our laboratory with sorghum and ≤30% corn starch confirmed that it is possible to produce good sorghum bread without any of these extra ingredients. A simple recipe may help reduce costs, especially with regard to developing countries, and provide a healthy cereal-based bread without added ingredients (egg, milk powder), which might cause new problems for people with allergies. One problem in evaluating non wheat breads is the lack of standardized baking tests. In wheat baking tests such as ICC Standard No. 131 (ICC, 2000), standardization of the water level to achieve constant dough consistency is a widely accepted technique.

Dough consistency is then measured using standardized physical dough testing equipment such as the Brabender farinograph. For gluten-free batters, no standard methods for consistency measurements exist. Sanchez *et al*., (2002) used a cone penetrometer to adjust water so that gluten-free batters reached a fixed consistency.

Developing baked products without gluten is difficult and the degree of difficulty is closely associated with how functional gluten is in the particular product system. For instance, Engleson and Atwell (2008) explained that good quality gluten-free cookies are available in the market. However, they insisted on the fact that batter-based products are good but generally not at parity with their gluten containing counterparts. Finally, breads are significantly inferior to those made from wheat flour. Indeed, baked products from gluten-free ingredients are generally of poor quality and this is due to the lack of the gluten network (Arendt, 2009). In wheat, gliadins (prolamins) are responsible for dough’s cohesiveness, while glutenins (glutelins) are apparently responsible for the dough’s resistance to extension. The combination of these two proteins, which results in the gluten complex, confers the dough unique viscoelastic properties and the ability to retain gases, resulting in good quality breads. Such properties are not found in proteins from gluten-free flours (Hoseney, 1994). In addition, the option for good quality, gluten-free bakery products in the marketplace is very limited and the cost associated with even low quality baked products is excessive (Engleson and Atwell, 2008).

The development of good-quality gluten-free bread is a serious task. Currently, many gluten-free types of bread available on the market are of a low quality, exhibiting a dry crumbling crumb, resulting in poor mouthfeel and flavor (Gallagher *et al*., 2003).

Studies on the rheological properties of gluten-free doughs as well as reports addressing the possible relation between those properties and the quality attributes of the end product are limited. However like specified by Lazaridou and Biliaderis (2009), it was demonstrated that doughs produced from gluten-free formulations do not have the cohesive and elastic characteristics obtained from wheat flour, because of the absence of gluten. Gluten-free doughs are more fluid than wheat doughs and, due to the lack of gluten network, are closer to cake batter in viscosity and rheological behavior. Accordingly, many researchers use the term batter to characterize gluten-free doughs used for breadmaking. Therefore, these batter-type doughs have to be handled like cake batters rather than typical bread doughs. Obviously, this makes hand kneading no longer appropriate.

Furthermore, many researchers have investigated the substitution of gluten by other ingredients able to mimic its functional properties. Several hydrocolloids are used such as xanthan gum and hydroxypropylmethylcellulose (HPMC) (Moore *et al*., 2004; Ahlborn *et al*., 2005; and Lazaridou *et al*., 2007) for obtaining high-volume and soft crumb texture breads. Different non-gluten proteins as soybean, pea, egg, and dairy proteins have been included in gluten-free formulations to provide structure and gas-retaining properties to the dough and to improve simultaneously the nutritional quality of these breads (Gallagher *et al*., 2003; Moore, *et al*., 2004; Ribotta *et al*., 2004). Enzymes are also used (Moore *et al*., 2006; and Renzetti *et al*., 2008). In addition to the use of these ingredients, processes have also been investigated. Thus, sour dough, in particular, has been studied and shown some improvement on gluten-free breads quality (Schober *et al*., 2007; and Arendt, 2009).

2.11 Starch-based breads

Starch breads are the simplest gluten-free breads. Schober (2009) explained that acceptable breads with good volumes can be made from pure starches with the appropriate formulation (this is with the addition of ingredients such as hydrocolloids, emulsifiers, shortening).

One of the main disadvantages of these kinds of breads is the nutritional aspect. Obviously, starch breads lack dietary fiber, micronutrients and protein. However, some solutions exist. Concerning micronutrients, enrichment is possible and dietary fiber may be added. In addition, another nutritional aspect of these starch-based breads is their undesirable quick and easy digestion of the starch (Schober, 2009). Another disadvantage is that gluten-free bakery products based on pure starch have dry, sandy mouth feel with a flat “starchy” aroma. In consequence these products are not very appealing to consumers (Lazaridou and Biliaderis, 2009).

A possibly less expensive and more natural way of achieving more nutritionally balanced and better tasting bread is the use of raw materials, which are less refined than starch. For instance naturally gluten-free cereals flours, like sorghum flour, can be used in gluten-free bread making (Schober, 2009).

2.12 Sorghum breads:

Most of the studies dealing with leavened breads containing sorghum have focused on composite breads from wheat and sorghum. Composite bread is defined as ‘a combination of wheat and non-wheat flours for the production of leavened breads, other baked products, and pastas’ (Dendy, 1992). Dendy (1992) explained that overall, the researches on sorghum-wheat breads concluded that up to 30 percent sorghum flour can be used with wheat flour to obtain a decent quality bread. While such breads have been found acceptable by consumers, they are inappropriate for celiac patients.

However, only a limited number of studies have addressed wheat-free sorghum breads. Most have used extra ingredients that are generally used in gluten-free breads to improve quality. Bread based on simply 70 percent sorghum and 30 percent cassava starch has also been developed by Olatunji *et al*. (1992). Experiments done by Schober *et al*. (2005) confirmed that it is possible to produce good sorghum bread with sorghum flour and up to 30 percent corn starch. Besides, Schober (2009) indicated that all the formulations found in the literature have in common some isolated starch used in addition to sorghum flour.

Furthermore, the kind of sorghum flour used plays an important role in the end product. Schober *et al*. (2005), showed that clear differences were found between various sorghum hybrids in their potential to produce gluten-free leavened bread. In their results they explained that crumb structure differed most characteristically, whereas volume and height did not show significant differences among the samples evaluated. Kernel hardness and damaged starch seem to be key elements in these differences. The flour starch damage will depend on the milling technique and on the sorghum grain. Thus, flour with low starch damage might more likely require adding pregelatinized starch or a hydrocolloid to promote water binding in the batter than flour with high starch damage. This confirms the results of Rooney *et al*. (1986) which specified that endosperm hardness plays a role in the food quality. Like mentioned in previous part about sorghum, these authors demonstrated that the flour of soft-endosperm sorghum is highly preferred for preparation of bread. These findings were also verified by Fernholz (2008). In that study, it was shown that sorghum hybrids can differ in kernel and flour properties; and that smaller particle size and higher damaged starch flour produced a better end product. Frederick (2009) also established that sorghum flour composition and particle size have an effect on the quality of gluten-free bread. This study also validated the impact of starch damage on bread performance. Additionally, Marston (2009) revealed that treating sorghum flour with ozone and heat affect the quality of gluten-free bread. When sorghum flour was ozonated, the bread produced was characterized by an extremely poor structure. However, heat treatment showed positive effects on the quality of gluten-free bread. When sorghum flour was heated to 125°C prior to use, bread volume, was improved probably due to the oxidation of sulfhydryl units. This volume improvement also led to an amelioration of both the crumb structure and the texture.

Finally, Schober (2009) also explained that the typical bread making procedure for sorghum bread was simply mixing, followed by a final proof in bake pans and baking. Studies on which he based his review all agree that higher water levels than for regular breads were required for good results; however, excessively high water levels reduced bread quality in term of volume and structure.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1Food grains

Sorghum grains sample (Tabat) and chickpea grains (season 2013) were obtained from the local market. White maize grains were obtained from Khashm Algirba Research Station.

3.1.2 Chemicals and reagents

Other material and chemicals were obtained from the National Food Research Center. Ascorbic acid was obtained from a chemical company in the local market. Xanthan gum was imported from Golden Peanut Company in Germany.

3.2 Methods:

3.2.1 Samples preparation

Sorghum, maize and chickpea samples were cleaned from dust, husk, and other impurities, then sorghum and maize were decorticated to 90% extraction, then all the samples milled using laboratory Mill Type 120, No. 69444 Helsinki-Stockholm-Sweden and seiving through mesh (250 micrometer) into fine powder.

3.2.2 Chemical composition

Moisture, ash, crude protein and fat contents were determined for decorticated sorghum flour, decorticated maize flour, chickpea flour and its supplemented products according to AOAC (2000) standard methods. Curde fiber according to AACC (2000) methods analysis.

3.2.2.1 Moisture content

Two grams of well-mixed sample were weighed in a clean aluminum dish of a known weight. The uncovered samples were kept in an air oven at 130°C for 1 hr., and then the dish was covered and transferred and placed in a desiccator and weighed after it reached room temperature. The loss of weight was calculated as percent of sample weight and reported as moisture content.

$$\text{Moisture content (\%)} = \frac{\text{Moisture loss} \times 100}{\text{Original wt. of sample}}$$

3.2.2.2 Ash content

Three grams of well-mixed sample were weighed into porcelain crucible and ignited in a temperature controlled muffle-furnace at 550°C for 3 hrs. When light gray ash resulted, the crucible was transferred and placed in a desiccator, then reweighed after it reached room temperature. The ash content calculated as percentage on dry basis according to the following equation:

$$\text{Ash content (\%) DM} = \frac{\text{Weight of residue} \times 100 \times 100}{\text{Weight of sample} \times (100-M)}$$

Where:

M = moisture content of the sample

DM: Dry matter

3.2.2.3 Protein content

Protein content was determined for sorghum, maize, chickpea and the supplemented products. A 0.2 gm of well-mixed sample was weighed in micro-Kjeldahl flask; 0.4 gm catalyst (Cupric Sulfate or Sodium Sulfate) and 3.5 ml of concentrated Sulfuric acid were added. Samples in Kjeldahl flask were digested on an electrical heater for 2 hours; the digested material was cooled and then placed in a distillation apparatus. Twenty milliliters of 40% Sodium hydroxide were added, the mixture was distilled. The Ammonia evolved was received in 10 ml 2% Boric acid solution until 50 ml were collected, the trapped ammonia was titrated with 0.02N hydrochloric acid using indicator (Bromocresol green plus Methyl-red in alcohol). The percentage of the protein content on dry basis was calculated according to the following equation:

$$\text{Protein content (\%)} = \frac{(T-B) \times N \times 14.0 \times F \times 100 \times 100}{W \times 1000\ (100-M)}$$

Where:

T = HCl titration volume in ml

B = Blank.

N = Normality of HCl = 0.02N.

1000 = to convert from mg to g.

14.0 = each ml of HCl is equivalent to 14 mg nitrogen.

F = Factor (5.7 for wheat and wheat products, 6.25 for other grains).

W = Weight of sample.

M = Moisture content of the sample.

3.2.2.4 Fat content

Fat content was determined for sorghum, maize, chickpea and the supplemented products. Three grams of sample were weighed and placed in an extraction thimble. The sample was extracted with hexane for 7 hours in Soxhlet apparatus. Then the solvent was evaporated to dryness in an air-oven at 105°C for 2 hr. The flask with oil was cooled and weighed. The percentage of fat content was calculated on dry basis according the following equation:

$$\text{Fat content (\%)} = \frac{\text{Weight of residue} \times 100 \times 100}{\text{Weight of sample}\ (100-M)}$$

Where:

M = Moisture content of the sample.

3.2.2.5 Crude fiber content

Fiber content was carried out on the samples according to the AACC (2000) methods.

The steps were as follows:

Two grams of an air dried fat-free sample were transferred to a dry 600 ml beaker. The sample was digested with 200 ml of 1.25% (0.26N) H_2SO_4 for 30 minutes, and the beaker was periodically swirled.

The contents were removed and filtered through Buchner funnel, and washed with boiling water. The digestion was repeated using 200 ml of 1.25% (0.23N) NaOH for 30 minutes, and treated similarly as above. After the last washing the residue was transferred to ashing dish, and dried in an oven at 105°C over night then cooled and weighed. The dried residue was ignited in a muffle furnace at 550°C to constant weight, and allowed to cool, then weighed.

The fibre percentage was calculated as follows:

$$\text{Crude fibre \%} = \frac{Wt_1 - Wt_2 \times 100 \times 100}{\text{Wt. sample} \times (100 - \%\ \text{moisture})}$$

Where:

Wt_1 = Weight of sample and dish.

Wt_2 = Weight of dish with ashed sample.

3.2.2.6 Available Carbohydrates content:

The available carbohydrates were calculated by difference. The sum of moisture, fat, protein, fiber and ash contents were subtracted from 100 as it was described by West *et al.* (1988).

3.2.2.7 Caloric value (Energy)

The caloric value content was calculated using Atwater calorie conversion factors, based on assumptions that each gram of carbohydrate, fat and protein will yield 17 kJ (4.0 kcal), 37 kJ (9.0 kcal) and 17 kJ (4.0 kcal), respectively (FAO 2003). The values were expressed in kJ.

1 kcal = 4.184 (kJ).

3.2.3 Minerals composition:

The standard method described by the AOAC, (2005) was used for mineral analysis. The samples were ashed at 550 °C. The ash was boiled with 10ml of 20% hydrochloric acid in a beaker and then filtered into a 100 ml volumetric flask. This was made up to the mark with deionized water. The minerals were determined from the resulting solution. Sodium (Na) and Potassium (K) were determined using the standard flame emission photometer. NaCl and KCl were used as standards (AOAC, 2005). Phosphorus (P) was determined calorimecally using the spectronic 20 (Gallenkamp, UK) (Kirk and Sawyer, 1991) with KH_2PO_4 as the standard. Calcium (Ca), Magnesium (Mg) and Iron (Fe) were determined using Atomic Absorption Spectrophotometer (AAS model SP9). All values were expressed in mg/100g.

3.2.4 *In vitro* protein digestibility (%):

In vitro protein digestibility of sample was carried out using enzymatic method of Moulishwar *et al*. (1993). Samples containing 100 mg protein were treated with 12.5 mg of pepsin in 50 ml of 0.1 N HCl at 37°C for 3 hours. After neutralization with 0.5 N NaOH, 6 mg of pancreatin dissolved in 25 ml of phosphate buffer (pH 8.0) was added and digestion continued for 24 hour at 37°C. The volume was made to 100 ml and 50 ml aliquot was treated with 10%Tri chloro-actic acid TCA, left overnight to precipitate the proteins. The suspensions were centrifuged at 4000 rpm. The undigested material was subjected to protein assay by micro kjeldahl method. Protein digestibility was calculated by difference.

3.2.5 *In vitro* starch digestibility

In vitro starch digestibility was carried out using the method, described by Moulishwar *et al*. (1993). The slurry of sample (2%) was cooked on a boiling water bath for 15 minutes. To a slurry sample of 50 ml, 30 ml of 0.2 M glycine-HCl buffer (pH 2.0) containing 10 mg of pepsin was added. It was incubated at 37°C for 2 hours and neutralized with 0.2 N NaOH and the volume was made to 100 ml. To an aliquot of 10 ml of this sample 5 ml of 0.5 M phosphate buffer containing 15 mg of pancreatin and 15 mg amyloglucosidase was added and incubated for 2 hours at 37°C. The reaction was stopped at desired intervals (3 hours) by heating the samples for 5 minutes in boiling water bath. Aliquots of 0.5 ml of these samples were mixed with 2 ml of *dinitrosalicylic* acid reagent for determining reducing sugars. Glucose was used as a standard, while starch equivalent was calculated using the conversion factor of 0.9.

3.2.7 Tannins

Tannic acid was determined by the method described by Ranganna (1986). Colorimetric estimation of tannic acid is based on the measurement of the blue color formed by the reduction of molybdic acid by tannin like materials in alkaline solution.

3.2.7.1 Standard solutions

[A] Folin and Dennis reagent

Sodium tungstate (100 g) was added to 750 ml distilled water followed by 20 g phosphomolybdic acid. Then 50 ml of 85% of O-phosphoric acid were added and the mixture was refluxed for 2 hours, cooled to 25°C made up to volume in a 1000 ml volumetric flask.

[B] Standard anhydrous sodium bicarbonate:

Anhydrous sodium bicarbonate (350 g) was dissolved in 750 ml distilled water, heated at 70°C and cooled overnight then completed to one litre, the solution was filtered before use.

[C] Tannins standard solution:

Tannic acid (0.1 g) was dissolved in one litre distilled water, prepared freshly for each determination (0.1 mg/ml).

3.2.7.2 Tannins standard curve:

Standard tannic acid solution (10 ml) was pippeted into a series of 100 ml volumetric flasks containing 75 ml distilled water. To each flask was added 5 ml of Folin and Dennis reagent and 10 ml of anhydrous sodium bicarbonate then made up to volume in a 100 ml volumetric flask mixed well and left for 30 minutes then read in spectrophotometer (JENWAY-6400 spectrophotometer) at 760 nm.

3.2.7.3 Test sample preparation:

Sample (one gram) was added to 40 ml water boiled at 100°C for 30 minutes then transferred to a 100 ml volumetric flask, completed to volume, well shaken and filtered.

Calculation:

$$\text{Tannins} = \frac{\text{mg tannic acid x dilution}}{\text{MI taken for color development x w x 1000}}$$

Where:

W = weight of sample MI = standard tannin acid from curve

3.2.8 Phytic acid:

The phytic acid content in the different samples was determined as described by Wheeler and Ferrel (1971).

3.2.8.1 Principle

After extraction of phytic acid in a low acid medium, phytic acid is precipitated as ferric phytate by addition of Sodium hydroxide (NaOH). The Ferric hydroxide is then dissolved as Ferric nitrate [Fe $(NO_3)_3$] after addition of Nitric acid (NHO_3) and the concentration of the Ferric nitrate is measured spectrophotometrically at 480 nm. The phytate phosphorous is calculated assuming a ratio of 4:6 for iron and phosphorous, respectively.

Procedure

A sample of 2.0 g±1 mg was weighed in a 125 ml conical flask. Then, 50 ml of 3% trichloro-acetic acid (TCA) were added and the mixture was shaken mechanically (Gallen Kamp, England) for 3 hours. The suspension was then centrifuged for 5 minutes at 2500 rpm (Fisher, USA) and 10 ml from the clear supernatant was transferred into a test tube (40 ml) with 4 ml of $FeCl_3$ solution (2 mg Fe^{++} ions/ml TCA). Then, the tube was heated in a boiling water path (Electrothermal, England) for 45 minutes, cooled and centrifuged again at 2500 rpm for 15 minutes. After decantation, the precipitate was washed, transferred into a test tube containing 25 ml TCA (3%) and dispersed well in a water-bath for 15 minutes at 100°C. Then, the tube was cooled, centrifuged, and the remaining precipitate was washed once again and dispersed well in distilled water with 3 ml of NaOH (1.5N). The total volume was made up to 30 ml and the sample was kept in the water-bath for 30 minutes at 100°C and immediately filtered through a filter paper [Whatman (2) No. 308463, Balston]. The remaining precipitate was washed again with hot distilled water and quantitatively transferred into a 100 ml volumetric flask with 40 ml hot NHO_3 (3.2N) and the final volume was made up to 100 ml. Then, 0.5 ml aliquot was taken into a 10 ml volumetric flask and 2 ml of potassium thiocyanate (1.5N) was added and the volume was made up to the mark with distilled water and immediately read in a spectrophotometer (Jenway, 6305 UV/VIS) at a wavelength of 480 nm. A standard curve was prepared under the same condition by using different concentrations of Ferric nitrate [$Fe(NO_3)_3$].

Calculation

Phytate concentration [mg/100 g sample]

$$= \frac{6 \times A \times mk \times 20 \times 10 \times 50 \times 100}{4 \qquad 1000 \times 2}$$

Where:

A = Optical density   mk = Mean value of Fe^{+++} conc./A.

3.2.9 Functional properties

3.2.9.1 Bulk density (BD)

The bulk density was determined by the method of Wang and Kinsella (1976). Ten gm of sample was placed in a graduated cylinder (25 mL), and packed by gently tapping the cylinder on the bench top (10 times) from reasonable height (~5 – 8 cm). The final volume of sample was recorded and the bulk density is expressed as gram sample per milliliters volume occupied.

3.2.9.2 Water retention capacity (WRC)

The WRC was measured for the flours (raw materials) by the method of Lin *et al.* (1974) with modification described by Qunin and Beuchat (1975). A 10% H_2O suspension (3gm/ 30ml) was stirred in a 50ml centrifuge tube using a glass rod for 2min. after 30min. equilibrium, then centrifuged for 20min at 4400 rpm. The freed water was carefully decanted into graduated cylinder, and the volume was recorded. The WRC was expressed as milliliters of water retained by 100gm sample.

3.2.9.3 Fat absorption capacity (FAC)

The Fat absorption capacity (FAC) of samples was measured by a modified method of Lin *et al.* (1974). Four gm material was treated with 20 ml of refined edible oil (specific gravity 0.9) in a 50 ml centrifuge tube. The suspension was stirred with glass rod for 5min. and the contents were allowed to equilibrate for further 25min. the suspension was then centrifuged at 4400 rpm for 20min. and the volume of the fat was measured. The FAC was expressed as milliliters of fat–absorbed by 100gm sample.

Calculation: $$\text{FAC} = \frac{\{\text{oil used (ml) (20) - recoverd oil (ml)}\}\text{x } 100}{4}$$

3.2.10 Processing of biscuits samples:

Biscuits were prepared according to Vatsala and Harids Rio (1991) method. Control sample of decorticated sorghum flour (90% extraction) and decorticated maize flour (90% extraction) (Non-wheat flour) had been blended with chickpea flour substituting in ratio 0, 10, 20 and 30%. The formula used in biscuit:

Ingredients	Quantity (gm)
Biscuit flour	100
Sugar powder	30
Shortening	30
Skim milk powder	2
Sodium chloride	1
Sodium bicarbonate	0.4
Ammonium bicarbonate	1.5
Glucose	2
Water	15ml

The ingredients were weighed for 400gm of flour. Sugar powder, shortening, skim milk powder, and glucose were creamed in Hobart N-50 mixer with a flat beater for 3 min. at 60 rpm. Salt, ammonium bicarbonate and sodium bicarbonate were dissolved separately in part of required water added to the cream. Mixing was continued for 8min. at 125rpm to obtain homogenous cream. Finally, flour was added and mixed for 3min. at 60 rpm, and then the dough was sheeted to a thinness of 3.5mm with the help of an aluminum plate form and a frame. The piece dough was transferred to an aluminum tray. The biscuits were baked in electronic oven maintained at 205 °C for 8.5 min., the baked units were cooled, packed in polyethylene bags and stored for further analysis.

3.2.10.1 Biscuit spread ratio

Biscuits were evaluated for the spread ratio according to the following equation:

$$\text{Spread ratio} = \frac{\text{Width of 5 biscuits}}{\text{Thickness of 5 biscuits}}$$

3.2.10.2 Sensory evaluation of biscuits:

Sensory evaluation of biscuits made from sorghum and maize flours with and without chickpea flour were carried out. Fifteen panelist assessors provided coded samples (Appendix) and asked to evaluate the general appearance, color, after taste, texture, and overall quality of the biscuits according to the scoring (Hedonic) scale of 5 point describe by Ihekoronye and Ngoddy (1985). A key table was given to the panelists guided them to score according to it.

3.2.11Tin bread procedure

The procedure described by Badi *et al.* (1978) was modified for this type of bread. Decorticated sorghum and maize flour were incorporated with chickpea flour at 10, 20, and 30% levels to make bread. Dry ingredients (flour 250gm, dry yeast 6.25 gm, salt 6.25gm, sugar 3gm, xanthan gum 6.25gm and 10 ml oil) were mixed for 1 min. using hobert laboratory dough mixer. Water was added (based on the optimum absorption) and mixed for 3 min at medium speed. After mixing the dough was allowed to rest for 10 min. at room temperature (38˚C ± 2˚C), scaled to three portions of 150 g each, and put in pans and transferred into the fermentation cabinet for 45 min. The fermented dough's were then baked in Simon Rotary baking oven at 250˚C for 15 min.

3.2.11.1 Physical characteristics of tin bread

The loaves were left to cool for 1 hr at room temperature (38±2°C).

3.2.11.1.1 Bread weight

The weight of the loaf was taken in gm.

3.2.11.1. 2 Bread volume

The loaf bread volume was determined by the seed displacement method according to Pyler (1973). The loaf was placed in a container of known volume into which small seeds (millet seeds) were run until the container is full. The volume of seeds displaced by the loaf was considered as the loaf volume.

3.2.11.1.3 Tin bread specific volume

The specific volume of the loaf bread was calculated according to the AACC method (2000) by dividing volume (CC) by weight (gm).

3.2.11.2 Sensory evaluation of tin bread

The loaves were sliced with an electric knife and prepared for sensory evaluation at the same day. The sensory evaluation of bread samples (colour, odour, taste, crumb texture, crumb grains, and general acceptability) was carried out by 10 trained panelists. The surrounding conditions were kept the same all through the panel test.

3.2.12 Balady bread procedure

Decorticated sorghum and maize flour were incorporated with chickpea flour at 10, 20, and 30% % levels separatly. Dry ingredients (250gm flour, 6.25gm yeast, 6.25gm salt, 6.25gm xanthan gum and 10ml oil) the Ingredients mentioned made into dough by adding 64% water in a hobert laboratory dough mixer and mixed for 3 minutes at medium speed. The dough was placed in a fermentation cabinet. After removal from the fermentation cabinet, the dough was divided into three pieces of 150gm each and formed into balls by hand, then, rested for 5 minutes at the same conditions, then dusted with flour and shaped into a round flat form by hand. The flattened dough pieces were returned to the fermentation and proofed for 10 minutes, then baked in a commercial oven at 400°C for 3 minutes. The bread was left to cool for 10 minutes, then kept closed in polyethylene bags at room temperature for sensory evaluation and physical characteristics.

3.2.12.1 Physical characteristics of gluten-free balady bread

The loaves were left to cool for 1 hr at room temperature (38±2°C).

3.2.12.1.1 Balady Bread weight

The weight of the loaf was taken in gm.

3.2.12.1.2 Balady Bread volume

The volume of balady bread was determined according to the following equation

Volume $=\pi . r^2$. Height.

Where: $\boldsymbol{\pi} = 22/7$, $r^2 = (½ \text{ diameter})^2$

3.2.12.1.3 Balady bread specific volume

The specific volume of the loaf was calculated according to the AACC method (2000) by dividing volume (CC) by weight (gm).

3.2.12.2 Sensory evaluation of gluten-free balady bread

The balady bread pieces were sliced with an electric knife and prepared for sensory evaluation same day. The sensory evaluation of bread samples (colour, odour, taste, crumb texture, crumb grains, and general acceptability) was carried out by 10 semi trained panelists. The surrounding conditions were kept the same all through the panel test.

3.3Products samples

The products samples were dehydrated and milled into fine powder and stored in plastic bag for chemical analysis.

3.4 Statistical analysis

The analysis of variance was performed to examine the significant effect in all parameters measured. Duncan Multiple Range Test was used to separate the means according to Steel *et al.,* (1997).

CHAPTER FOUR

RESULTS AND DISCUSSION

4. 1 Proximate composition of raw materials

4.1.1Decorticated sorghum

Proximate analysis of decorticated sorghum flour was presented in Table 1. The moisture content of decorticated sorghum flour was 7.16%. This result was within the range of 5.7% to 10% reported by Yousif and Magboul, (1972) for Sudanese sorghum cultivars, but higher than 6.3% as found by Elshewaya, (2003). Ash content of decorticated sorghum flour as shown in Table 3 was 1.65%. This result was higher than 1.32% reported by Ranya *et al.* (2013). This value of ash obtained was found to be within the range of 1.1% - 2.7% reported by Shephered *et al.* (1970).

Protein content of decorticated sorghum flour was 12.15%. The result was higher than the value reported by Ranya *et al.* (2013), for decorticated sorghum was 10.62%. Fat content of decorticated sorghum was 3.38%. The value obtained was agreement with Shephered *et al.* (1970) and El-Tinay, (1979). Who reported that fat content of decorticated sorghum flour ranged between 2.5- 5.1% and 2.5 – 3.5%, respectively.

Fiber content of decorticated sorghum was 1.64%. This value was agreed with Mustafa *et al.* (2003), who reported that fiber content of three Sudanese varieties ranged from 1.64 to 2.26%. Carbohydrates content of the decorticated sorghum flour was presented in Table 3. The carbohydrate content was 74.0%. The result obtained was inagreement with Osman (2004) who recorded carbohydrates content of three Sudanese local cultivars (Tabat, Mugud and Feterita) ranging between 71.33 and 78.78%. Energy content of decorticated sorghum flour was 1589.61 kj/100gm. Tannin content of decorticated sorghum flour was 41.6 mg/100mg. Jambunthan and Mertz (1973) who reported that tannin content of high tannin sorghum is 2690mg/100gm and for low tannin sorghum is 500mg/100gm.

Table 1 Nutritional charactersitics and caloric value of raw materials:

Samples	Moisture %	Ash %	Protein %	Fat %	Fiber %	Available carbohydrates %	Energy kj/100gm	Protein digestibility%	Starch digestibility%	Tannins mg/100gm	Phytic acid mg/100gm
Decorticated Sorghum	7.16	1.65	12.15	3.38	1.64	74.00	1589.61	62.11	62.07	41.60	75.03
Decorticated Maize	5.05	1.58	11.12	4.66	3.00	74.56	1628.98	63.74	63.76	80.00	117.01
Chickpea	6.54	3.17	23.18	3.80	6..50	56.74	1499.24	60.78	60.82	305.00	299.65

Raw materials = Decorticated sorghum, decorticated maize and chickpea flours.

Whereas, Awadelkareem *et al.* (2009) who reported that tannin content of Sudanese and Indian sorghum cultivars ranged between 80mg/100gm to 1710mg/100gm. Phytic acid content decorticated sorghum flour was 75.03mg/100gm. In sorghum as in other cereals and oil seeds, phytic acid is the major storage form of phosphorous. Phytic acid ranged from 875.1 to 2211.9mg/100gm in sorghum. The important nutritional implication of phytic acid is that it chelates di and trivalent cations particularly Fe, Ca, Na, Mg, and Zn and decreases their bioavailability. Phytic acid forms insoluble compounds with mineral element including Ca, Fe, Mg, and S. (Makokha, *et al.*, 2002).

Table 1 showed the *in vitro* protein digestibility of decorticated sorghum was 62.11%. Upon wet cooking, sorghum protein digestibility range from 36.4 to 74.0% as reported by Henley *et al.* (2010). Arguing that a pepsin digestion model was preferred, Mertz *et al.*, (1984) reported digestibility values up to 79%for decorticated/extruded sorghum. The result obtained was lower than Mertz *et al.* (1984) observed. Chavan *et al.* (1979) reported that significant lowering in vitro protein digestibility in a high tannin cultivar. The in vitro protein digestibility of high tannin grain was found to be improved to the level of low tannin grains upon de-hulling the grain. The minimum level of tannin requires showing the growth depressing effects in rats was ranged from 0.64 to 0.84% sorghum tannin (Fuller *et al.*, 1996). In vitro starch digestibility of decorticated sorghum is presented in Table 1. Starch digestibility of decorticated sorghum was 62.07%. Several works have been conducted to study the kinetics of starch digestion from different grains by α-amylase (Goni *et al*,. 1997; Frei *et al.*, 2003; Ezeogu *et al.*, 2005). Uncooked and cooked sorghum grains had been reported to have a lower starch digestibility when compared to maize and other cereal grains, such as rice and wheat (Ezeogu *et al.*, 2005). (Wong *et al*,. 2009) studied the starch *in vitro* digestibility in sorghum by comparing the level of reducing sugar per hour, they found 40–47 mg reducing sugar/h (without pepsin pretreatment) and 60–85 mg reducing sugar/h (with pepsin pretreatment) in sorghum meal. Several studies suggested that the endosperm protein is almost responsible for low starch digestibility of sorghum grain (Zhang and Hamaker, 1998; Elkhalifa *et al.,* 1999; Ezeogu *et al.,* 2005). Moreover, in vitro digestibility of starch was affected by the endosperm texture, structure of starch, and non starch components (Ezeogu, *et al.*, 2005; Benmoussa, *et al.*, 2006).

4.1.2Decorticated maize and chickpea flours

Moisture content of decorticated maize flour and chickpea flour was presented in Table 1. The results showed moisture content of decorticated maize was 5.05%. Aisha and El-Tinay (2004) found the moisture value in 12 corn genotypes to be in the range of 4.3 – 6.7% which is in close agreement with these results. Whereas, chickpea flour moisture content was 6.54%. The moisture contents of raw 3 chickpea cultivars ranged from 4.6 – 6.7 % (El-Tinay, *et al*, 1989). The result agreed with ElTinay *et al.*, (1989) and Aisha and ElTinay (2004).

Ash content of decorticated maize flour and chickpea flour were 1.58 and 3.17%, respectively. Aisha and El-tinay (2004) investigated the ash value in the range of 1.0 – 2.0% which is in close consistency with present results of our study. Protein content of decorticated maize and chickpea are presented in Table 1. The results obtained were 11.12% and 23.18% for decorticated maize and chickpea, respectively. Ijabadeniyi and Adebolu (2005) found the percent of protein content of three maize varieties grown in Nigeria in the range of 10.67 - 11.27% for the maize grains. This notion is inagreement with the result of the present study. Chickpea grains contains between 14.9 and 30.6% crude protein as reported by Chavan *et al.,* (1986). The result obtained was within the range of Chavan et *al.,* (1986), mentioned.

Fat of decorticated maize and chickpea flour were 4.66 and 3.80%, respectively. The value of maize obtained within the range of 3.21% to 7.71%, reported by Ikrany *et al.*, (2010). Fiber content of decorticated maize and chickpea flour are presented in Table 3. The result showed fibers content were 3.0% and 6.50% for decorticated maize and chickpea flour, respectively. Maize result value was higher than 2.07 – 2.77% reported by Ijabadeniyi and Adebolu (2005). Chickpea fiber content is about 4 – 8 and 10 – 18 % of raw chickpea grains, reported by Dalgetty and Baik, (2003) and Rincon *et al*. (1998), respectively. The result is slightly low than the range of 4 – 8 % reported by Dalgetty and Baik (2003).

Carbohydrates content of decorticated maize and chickpea flour are shown in Table 1. The results obtained were 74.56 and 56.74% for decorticated maize and chickpea flour, respectively. The maize result obtained is higher than the result obtained by Ijabadeniyi and Adebolu (2005) who reported slightly lower values (65.63 – 70.23%) of the carbohydrate content for maize varieties grown in Nigeria. Chickpea flour carbohydrate value obtained is lower than the value of 59% obtained by Iqbal, *et al.*(2006). Table 1 showed the energy values of decorticated maize and chickpea flour were 1628.98 kj/100gm and 1499.58 kj/100gm, respectively. Kouakou *et al.*, (2008) showed the energy level of maize grains as 1622.14 kj/100gm. This notion is inagreement with the results of the present study. Ejigue *et al.*, (2005) found the energy value of 1870.25 kj/100gm for yellow maize, which is higher than the value obtained. Tannin content of decorticated maize and chickpea flour are shown in Table 3 tannin content of decorticated maize and chickpea flour were 80.0 and 305.0 mg/100gm, respectively. Phytic acid content was 117.01 and 299.65 mg/100gm, for decorticated maize and chickpea flour respectively. Phytic acid result of maize studied by Awada *et al.*, (2005) showed that phytic acid content of untreated grains were 473.0 and 817.0 mg/100gm for maize and lentil, respectively.

The *in vitro* protein digestibility of decorticated maize and chickpea flour are presented in Table 1. The *in vitro* protein digestibility of decorticated maize and chickpea flour were 63.74% and 60.78%, respectively. The *in vitro* protein digestibility of maize is higher than Awada *et al.*, (2005) results. Therefore, the *in vitro* protein digestibility of chickpea agreed with Khalid *et al.*, (2007); Khattak *et al.*,

(2008) and Clemente *et al.*, (1998) who stated that the in vitro protein digestibility of raw chickpea grains variety from 34 -76%.

Table 1 showed *in vitro* starch digestibility of decorticated maize and chickpea flours were 63.76 and 60.82%, respectively. Starch digestibility value of chickpea flour is higher than Zia-ul-Haq, *et al.* (2007) and Khalid, *et al.* (2007) who reported that in vitro starch digestibility values of chickpea vary from 37-60 %.

4.1.3Mineral Content of gluten - free raw material:

Mineral composition of decorticated sorghum, decorticated maize and chickpea flour are shown in Table 2. The analysis shows the level of Ca (1.60, 1.69, and 1.57 mg/100gm), Fe (1.51, 1.39, and 1.48mg/100gm), Zn (0.069, 0.060, and 0.058 mg/100gm), Mg (3.69, 3.54, and 3.72 mg/100gm), P (8.66, 8.54, and 8.46 mg/100gm), K (14.9, 14.7, and 14.8 mg/100gm), Na (15.00, 15.50, and 14.70 mg/100gm) and Mn (0.078, 0.076, and 0.070 mg/100gm) for decorticated sorghum, decorticated maize and chickpea flours, respectively. The results mentioned that values of Na element were the most dominant compared with others elements, followed by the values of K and P element. Whereas, the values of Mn element were the lowest values noticed.

4.1.4 Functional properties of gluten - free flours:

4.1.4.1Bulk density:

The bulk density properties of the samples were studied. The bulk density is a measure of heaviness of flour samples. The results showed Table 3 no significant differences ($P \leq 0.05$) were observed between all samples studied. Bulk density of the decorticated sorghum flour incorporated with 10, 20, and 30% chickpea flour ranged from 0.45 to 0.50gm/ml^3 while, decorticated maize blended with 10, 20, and 30% chickpea flour ranged from 0.51 to 0.57 gm/ml^3.

Table 2 Mineral content of gluten - free raw material (mg/100gm):

Samples	Na	Ca	K	P	Mg	Fe	Zn	Mn
Decorticated Sorghum flour	15.00	1.60	14.90	8.66	3.69	1.51	0.069	0.078
Decorticated Maize flour	15.50	1.69	14.70	8.54	3.54	1.39	0.060	0.076
Chickpea flour	14.70	1.57	14.80	8.46	3.72	1.48	0.058	0.070

Raw materials = Decorticated sorghum, decorticated maize and chickpea flours.

4.1.4.2Water retention capacity

Water retention capacity of decorticated sorghum and maize flour incorporated with 10, 20, and 30% chickpea flour are presented in Table 3. Water retention capacity ranged from 210.0 to 270.0 ml/100gm. Sathe and Salunkhe (1981) observed a water retention capacity value of 273gm/100gm for great Northern bean and Egwu (1984) also obtained 260 gm/100gm for Nigeria red groundnut. Generally the results showed significant increasing trend with increasing the level of incorporation of chickpea flour with decorticated sorghum flour and decorticated maize flour. The addition of chickpea flour with sequential 10, 20, and 30%caused significant (P≤0.05) increasing in water retention capacity in sorghum and with chickpea flour and also maize with level of 30% chickpea flour. Water binding capacity is a useful indication of flour or isolate whether it can be incorporated into aqueous food formulations especially those involving dough handling (Okerie and Bello, 1988; Giami, 1993). Water retention capacity gives an indication of the amount of water available for gelatinization. Lower absorption capacity is desirable for making thinner gruels.

4.1.4.3 Fat absorption capacity

The trend of the fat absorption capacity of decorticated sorghum and maize flour incorporated with 10, 20, and 30% chickpea flour are presented in Table 4**.** The range of fat absorption capacity was 205.0 to 240.0 ml/100gm. Decorticated maize samples showed marginally high fat absorption capacity of 240.0 ml/100gm. The treated maize samples showed the values decreased significantly (P≤0.05) with increasing level of chickpea flour, compared with maize flour. These blends did not show significant (P≤0.05) differences between them. The high absorption of fat by food products improves the mouth feel and flavor retention. The flour which absorbs oil would be found to be suitable in formulating certain food products such as cakes, breakfast cereals and some others. Thus, the results showed that the blends would be useful in bakery products processing where hydration to improve handling is desired.

Table 3: Functional properties of decorticated sorghum, maize and supplemented with chickpea flour (0, 10, 20, and 30%)

Sample	Bulk density gm/ml	Water retention capacity ml/100gm	Fat absorption capacity ml/100gm
DSF	0.50^{a} ±0.00	210.00^{d} ±10.00	225.00^{b} ±5.00
DSF90% + 10% CPF	0.45^{a} ±0.00	215.00^{cd} ±5.00	208.30^{cd} ±10.41
DSF80% + 20% CPF	0.46^{a} ±0.00	220.00^{cd} ±10.0	220.00^{bc} ±0.00
DSF70% + 30% CPF	0.50^{a} ±0.00	230.00^{bc} ±10.00	220.00^{bc} ±0.10
DMF	0.57^{a} ±0.00	240.00^{b} ±0.00	240.00^{a} ±0.00
DMF90% + 10% CPF	0.51^{a} ±0.00	215.00^{cd} ±5.00	215.00^{bcd} ±5.00
DMF 80% + 20% CPF	0.54^{a} ±0.00	210.00^{d} ±10.00	213.30^{bcd} ±5.77
DMF70% + 30% CPF	0.54^{a} ±0.00	270.00^{a} ±10.00	205.00^{d} ±5.00
Lsd$_{0.05}$	$0.2567^{n.s}$	14.35^{**}	10.89^{*}
SE±	0.08563	4.787	3.632

Mean(s) values SD having different superscript(s) in the column are significantly different ($P \leq 0.05$) according to DMRT.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

4.2 Quality charactersitics of gluten - free biscuits

4.2.1 Proximate composition

Table 4 shows proximate composition of biscuits prepared from sorghum and maize supplemented with different ratio of chickpea flour (10, 20 and 30% level). Data are expressed on dry matter bases (per 100gm materials). The moisture content of biscuits was assessed and found to be ranged between 5.58 to 5.11%. These values are inagreement with that reported by Omoba and Omogbemile (2013). Moisture content of sorghum biscuit enriched with defatted soy flour ranged from 3.2 - 6.1%. These values are comparable to values (5 –10%) set by the Protein Advisory Group (Sanni *et al*., 2006). The lower the moisture contents of a product, the better the shelf stability of such product (Sanni *et al*., 2008). Hence, low moisture ensures higher shelf stability in dried products. However, low residual moisture content in confectionaries is advantageous; resulting in a reduction in microbial proliferation and prolonged storage life if stored inside appropriate packaging materials under good environmental condition. The ash content Table 4 of the biscuits ranged from 1.52 to 1.95%, respectively. These results are significantly ($P \leq 0.05$) lower than the range of 2.3 to 3.5% stated by Omoba and Omogbemile (2006). Decortication process was the main cause for lowering ash content in sorghum and maize. The fat content of biscuits ranged from 18.68 to 22.30%. These values are considered higher than the range of 12.0 – 18.1% stated by Omoba and Omogbemile (2006). The fat content plays a role in determining the shelf life of the food. A high amount of fat could accelerate spoilage by promoting rancidity which could lead to the production of off flavours and odours.

The protein content of biscuits is given in Table 4 results, however, showed values ranged from 11.14 to 15.89% , highest value observed in DSF + 30% CPF, and the lowest value gained by DMF biscuit (without chickpea flour). Protein content significantly increased ($P \leq 0.05$) gradually after chickpea inclusion in sorghum and maize flour with 10, 20, and 30% level.

Table 4 Proximate composition of gluten - free biscuits:

Sample	Moisture content (%)	Ash content (%)	Fat content (%)	Crude protein (%)	Crude fibre (%)	Available Carbohydrate (%)	Energy (kj/100gm)
DSF	5.36^{c} ±0.02	1.64^{d} ±0.03	20.60^{d} ±0.01	12.84^{f} ±0.03	1.20^{e} ±0.01	58.35^{b} ±0.06	1972.61^{c} ±0.68
DSF90% + 10% CPF	5.52^{ab} ±0.02	1.75^{c} ±0.02	21.93^{b} ±0.01	13.43^{e} ±0.16	1.43^{d} ±0.05	55.60^{e} ±0.54	1984.85^{ab} ±8.61
DSF80% + 20% CPF	5.57^{a} ±0.01	1.81^{b} ±0.01	21.44^{c} ±0.01	14.79^{c} ±0.19	1.78^{c} ±0.08	54.60^{f} ±0.26	1972.91^{c} ±1.10
DSF70% + 30% CPF	5.29^{c} ±0.02	1.85^{b} ±0.05	21.40^{c} ±0.43	15.89^{a} ±0.08	1.92^{b} ±0.06	53.65^{g} ±0.35	1973.87^{c} ±9.38
DMF	5.30^{c} ±0.01	1.52^{e} ±0.02	21.46^{c} ±0.01	11.14^{g} ±0.28	1.14^{e} ±0.05	59.44^{a} ±0.26	1993.88^{a} ±1.22
DMF90% + 10% CPF	5.58^{a} ±0.01	1.62^{d} ±0.04	22.30^{a} ±0.02	12.86^{f} ±0.06	1.39^{d} ±0.00	56.25^{d} ±0.03	1999.96^{a} ±1.07
DMF 80% + 20% CPF	5.11^{d} ±0.07	1.73^{c} ±0.03	19.40^{e} ±0.01	14.40^{d} ±0.01	1.78^{c} ±0.06	57.57^{c} ±0.12	1941.47^{d} ±1.90
DMF70% + 30% CPF	5.46^{b} ±0.09	1.95^{a} ±0.02	18.68^{f} ±0.01	15.50^{b} ±0.12	2.03^{a} ±0.03	56.37^{d} ±0.07	1913.13^{e} ±1.20
Lsd$_{0.05}$	0.07741^{*}	0.05474^{*}	0.2625^{*}	0.2448^{*}	0.07741^{*}	0.4612^{*}	8.012^{*}
SE±	0.02582	0.01826	0.08756	0.08165	0.02582	0.1538	2.673

Mean(s) values SD having different superscript(s) in a column are significantly different ($P \leq 0.05$) according to DMRT.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

The fiber content analysis of the biscuits Table 4 showed the values obtained ranged between 1.14 – 2.03%, high fiber values were observed in DSF 70%+ 30% CPF biscuit and DMF 70%+ 30% CPF biscuit, respectively. These values favorably agree with the recommended value of 2.0 – 3.8% by Omoba and Omogbemile (2006). Carbohydrate content of biscuit viewed in Table 4 ranged from 53.65 – 59.44%. The results showed significant ($P \leq 0.05$) decrease in carbohydrates in sorghum and maize biscuits with increasing the levels of chickpea flour.

The calculated energy values of biscuits ranged from 1913- 1999.96 kj/100gm. The energy density of biscuits in the study was enhanced by inclusion of fat in the formulation. The results obtained of energy meet the recommended minimum value of 1674kj/100gm (FAO/WHO, 1994) for supplementary food for young children. High dietary energy is important for sparing protein for body building and repairing body tissues avoiding diversion to provide energy (Stipanuk, 2006).

4.2.2 *In vitro* protein digestibility of gluten - free biscuits

In vitro protein digestibility of biscuits prepared from decorticated sorghum and maize supplemented with chickpea flour (10, 20, and 30% level) is shown in Table 5. *In vitro* protein digestibility was found to be ranged from 43.51 – 60.29%. Lowest value was obtained by sorghum biscuit without chickpea flour (DSF),whereas, DMF + 30% CPF biscuit gained the highest value 60.29%.These results showed *in vitro* protein digestibility increase significantly ($P \leq 0.05$) with increasing the level of chickpea flour in sorghum and maize biscuits.

Generally, protein digestibility values of sorghum biscuits seemed to be low. These results obtained agreed with Mertz *et al*. (1984) and Maclean *et al*.(1981) who reported effect of cooking on protein digestibility at the three level of organization, cooking caused a significant reduction in protein digestibility for both sorghum varieties (high and low tannins).

Maize biscuits *in vitro* protein digestibility values increased significantly ($P \leq 0.05$) with increasing the level of chickpea flour from 46.59 – 60.29 %, respectively. The relatively low protein digestibility maybe attributed to the influence of anti-nutrients such as enzyme inhibitors, tannins, and phytates which inhibit protein digestion and also due to presence of protein structures that resist digestion (Mrooj, 2011).

4.2.3 *In vitro* starch digestibility of gluten - free biscuits

Table 5 showed the *in vitro* starch digestibility of sorghum biscuits with chickpea flour (10, 20, and 30% levels). Starch digestibility ranged from 46.81 to 60.36%. The results obtained shows significant ($P \leq 0.05$) increase in starch digestibility with increasing level of chickpea flour. Most starch related foods are cooked before consumption and consequently starch gelatinization and retrogradation play important role in the quality and digestibility of many produced food products (Hu *et al*., 2004).

On the other hand, starch digestibility of maize blending with chickpea flour (10, 20, and 30% level). Table 6 the results showed starch digestibility increased significantly ($P \leq 0.05$) from 62.65 to 67.16% with the addition of chickpea flour. These results are higher than Hernandez-Salazar (2006) results who stated that commercial maize – bean tortilla digestible starch content of 60.3gm/100gm was reported, but the ratio is not declared.

4.2.4 Mineral content of gluten - free biscuits

Table 6 shows minerals content of biscuit prepared from decorticated sorghum and maize incorporated with chickpea flour with different levels 0, 10, 20 and 30% in the formulation. The results obtained showed Ca, Fe, and Mg values varied from 2.19 to 5.73, 0.055 to 0.175 and 1.05 to 1.825 mg/100gm, respectively. The results showed that calcium element values increased significantly ($P \leq 0.05$) with increasing the level of chickpea flour in the formulation of biscuit in both sorghum and maize.

Table 5 *In vitro* protein and starch digestibilities of gluten - free biscuits

Samples	**Protein digestibility (%)**	**Starch digestibility (%)**
DSF	43.51^{h} ±0.02	46.81^{h} ±0.03
DSF90% + 10% CPF	45.49^{g} ±0.02	48.81^{g} ±0.03
DSF80% + 20% CPF	47.13^{e} ±0.03	50.63^{f} ±0.03
DSF70% + 30% CPF	58.60^{b} ±0.03	60.36^{e} ±0.03
DMF	46.59^{f} ±0.02	62.65^{d} ±0.03
DMF90% + 10% CPF	48.13^{d} ±0.02	63.56^{c} ±0.21
DMF 80% + 20% CPF	50.20^{c} ±0.02	65.30^{b} ±0.03
DMF70% + 30% CPF	60.29^{a} ±0.02	67.16^{a} ±0.04
$Lsd_{0.05}$	0.0005474^{*}	0.1341^{*}
SE±	0.0001826	0.04472

Mean(s) values SD having different superscript(s) in a column are significantly different (P≤0.05) according to DMRT.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

Further more, no significant differences were noticed among iron values. The Mg elements values increased significantly ($P \leq 0.05$) with increasing the level of chickpea in the formulation in both sorghum and maize biscuits products. The results could be noticed that P, K, and Na elements were significantly difference in different blends of chickpea. In general, P, K, and Na elements values considered higher compared with other micro elements. Highest value obtained by Na element and varied from 17.65 to 19.50mg/100gm, followed by K element and varied from 6.96 to 8.76mg/100gm, and P element varied from 2.965 to 4.275mg/100gm, respectively.The results obtained showed P, K, and Na element values increased significantly ($P \leq 0.05$) with increasing the levels of chickpea flour in the formulation of sorghum and maize biscuits. The Zn and Mn elements values ranged from 0.0091 to 0.051 mg/100gm and 0.0012 to 0.0205mg/100 gm, respectively. The values of Zn and Mn elements considered lowest values compared with values of others elements. From the current results the values of Zn and Mn showed no significant difference.

4.2.5 Physical characteristics of gluten - free biscuits:

Table 7 shows the physical properties of sorghum and maize biscuits supplemented with chickpea flour (10, 20, and 30% level). The results observed appeared a significant difference ($P \leq 0.05$) in diameter, thickness, and spread ratio of sorghum and maize biscuits supplemented with chickpea flour. The diameter values ranged from 5.24 – 5.50cm.the least diameter observed in DSF90% + 10% CPF. Whereas, the high diameter obtained by DMF 80%+ 20% CPF. Thickness of biscuits made from sorghum and maize incorporated with chickpea flour (10, 20, and 30% level) varied from 0.065 – 0.75cm. Spread ratio of the biscuits ranged from 7.10 to 8.21cm. Biscuit having higher spread ratio are considered the most desirable (Sanni *et al.*, 2006).

Table 6 Minerals content (mg/100gm) of gluten-free biscuits:

Sample	Na	Ca	K	P	Mg	Fe	Zn	Mn
DSF	17.90^{c} ±0.14	2.35^{c} ±0.07	7.09^{cd} ±0.04	3.045^{d} ±0.06	1.095^{e} ±0.01	0.065^{a} ±0.01	0.0094^{a} ±0.001	0.0012^{a} ±0.0001
DSF90% + 10% CPF	18.30^{b} ±0.14	2.65^{c} ±0.07	7.26^{c} ±0.04	3.190^{c} ±0.01	1.270^{d} ±0.03	0.075^{a} ±0.01	0.0980^{a} ±0.003	0.00235^{a} ±0.0002
DSF80% + 20% CPF	18.85^{ab} ±0.21	3.06^{b} ±0.05	7.98^{b} ±0.02	3.875^{b} ±0.04	1.680^{ab} ±0.03	0.105^{a} ±0.01	0.0111^{a} ±0.0001	0.01045^{a} ±0.001
DSF70% + 30% CPF	19.50^{a} ±0.14	5.73^{a} ±0.10	8.76^{a} ±0.06	4.275^{a} ±0.11	1.825^{a} ±0.04	0.175^{a} ±0.01	0.051^{a} ±0.001	0.0205^{a} ±0.002
DMF	17.65^{c} ±0.07	2.19^{c} ±0.01	6.96^{e} ±0.05	2.965^{e} ±0.06	1.050^{ef} ±0.04	0.055^{a} ±0.01	0.0091^{a} ±0.0001	0.00155^{a} ±0.00
DMF90% + 10% CPF	17.80^{c} ±0.14	2.35^{c} ±0.07	7.16^{cd} ±0.05	3.150^{cd} ±0.04	1.225^{de} ±0.05	0.065^{a} ±0.01	0.0097^{a} ±0.0001	0.00245^{a} ±0.0002
DMF 80% + 20% CPF	18.25^{b} ±0.07	2.75^{c} ±0.07	7.88^{b} ±0.09	3.785^{b} ±0.02	1.545^{c} ±0.06	0.100^{a} ±0.00	0.01025^{a} ±0.0003	0.01045^{a} ±0.0002
DMF70% + 30% CPF	19.05^{a} ±0.07	5.47^{a} ±0.03	8.61^{a} ±0.03	4.085^{a} ±0.09	1.680^{ab} ±0.03	0.145^{a} ±0.02	0.0450^{a} ±0.001	0.0185^{a} ±0.001
Lsd$_{0.05}$	1.7456**	1.8291**	0.3871**	0.2746*	0.0428*	0.5249^{NS}	0.0254^{NS}	0.0567^{NS}
SE±	0.8741	0.7425	0.0406	0.0518	0.0173	0.0038	0.0083	0.0092

Mean(s) values ±SD sharing same superscript(s) in a column are not significant different (P>0.05) according to Duncan's multiple range test.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

The DMF80% + 20% CPF is therefore considered as the most desirable. The spread ratio increased significantly ($P \leq 0.05$) with increasing the level of chickpea flour in biscuits for both sorghum and maize. Spread ratio could have been affected by the competition of ingredients for the available water and other functional properties of proteins and fat. Invariably, this might affect the texture and eating quality of the biscuits. There is a relationship between the spread ability, thickness of differently biscuits, the thinner the biscuit the lesser its ability to with stand stress/load.

4.2.6 Sensory characteristics of gluten – free biscuits:

The organoleptic properties of the biscuit including colour, odour, taste, texture, and general acceptability were assessed by 10-member panelists who are familiar with the product, nine point hedonic scale with 1 representing the least score (poor) and 9 the highest score (excellent), analysis of variance (ANOVA) was performed on the data to determine differences, while the Duncan multiple range test was used to separate means where significant difference existed.

The organoleptic properties of biscuits are presented in Table 8 and plate 1. Colour scores of biscuit samples made from sorghum and maize flour with and without chickpea flour with different levels (10, 20 and 30%) no significant ($P \leq 0.05$) differences were observed. The results appeared that sorghum substitution with 30% level of chickpea flour gained 5.47 score, while, sorghum with the addition of 10% and 20% levels of chickpea flour gained 4.20 and 5.27 scores, respectively. The lowest score was observed to be biscuits made from sorghum without chickpea. On the other hand, results of maize biscuit with and without inclusion of chickpea flour in the formulation gained 6.27, 7.33, 6.60 and 6.80 scores, respectively. No significant ($P \leq 0.05$) differences were noticed, these results were considered better than the results of sorghum biscuits

Table 7 Physical characteristics of gluten - free biscuits:

Samples	Width (cm)	Thickness (cm)	Spread ratio (cm)
DSF	5.26^{f} ±0.03	0.75^{a} ±0.02	6.99^{c} ±0.20
DSF90% + 10% CPF	5.24^{h} ±0.01	0.73^{d} ±0.01	7.15^{c} ±0.05
DSF80% + 20% CPF	5.36^{b} ±0.02	0.74^{b} ±0.01	7.21^{c} ±0.14
DSF70% + 30% CPF	5.31^{d} ±0.01	0.65^{h} ±0.00	8.16^{a} ±0.02
DMF	5.25^{g} ±0.02	0.74^{c} ±0.02	7.10^{c} ±0.19
DMF90% + 10% CPF	5.30^{e} ±0.00	0.70^{e} ±0.00	7.57^{b} ±0.00
DMF 80% + 20% CPF	5.50^{a} ±0.00	0.67^{g} ±0.02	8.21^{a} ±0.21
DMF70% + 30% CPF	5.35^{c} ±0.01	0.69^{f} ±0.02	7.80^{b} ±0.17
$Lsd_{0.05}$	0.0005474^{*}	0.0005474^{*}	0.2567^{*}
SE±	0.0001826	0.0001826	0.08563

Mean(s) values ±SD having different superscript(s) in a column are significantly different ($P \leq 0.05$) according to DMRT.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

Table 8 showed that odour scores of biscuits produced from decorticated sorghum and maize flours with and without substituted chickpea flour with different levels (10, 20 and 30%) . the results showed that odour scores of sorghum flour and its treatments levels ranged between 3.60 and 4.93 scores. Maize flour with and without chickpea flour, scores ranged between 6.67 and 5.93. From the results no significant ($P \leq 0.05$) difference was found between DSF70% + 30% CPF and maize flour with and without chickpea flour in all levels studied. These results are better than those obtained by DSF, DSF90% + 10% CPF and DSF 80%+ 20% CPF biscuits. Table 8 showed the taste scores of biscuits produced from sorghum flour and maize flour with and without substitution with chickpea flour with different levels (10, 20 and 30%).

Taste scores of biscuits made from DSF 70%+ 30% CPF and maize with and without chickpea flour with 10, 20 and 30% levels gained highest scores 5.40, 6.73, 6.87, 6.27 and 6.33 scores respectively, while, no significant ($P \leq 0.05$) differences were observed. Whereas, sorghum with zero, 10 and 20 % levels gave the lowest scores 3.40, 3.60, and 4.47, respectively. No significance ($P \leq 0.05$) differences were noticed. Table 8 showed that texture scores results of biscuits produced from decorticated sorghum and maize with and without incorporation of chickpea flour with 10, 20 and 30% level indicated that highest scores obtained were 6.60, 6.73, 6.07 and 5.87 from maize with and without chickpea flour in the formulation, respectively. No significant ($P \leq 0.05$) differences were noticed among maize and incorporated levels of chickpea, so, there were no significant ($P \leq 0.05$) differences between maize and sorghum at 30%level of chickpea. From results the least scores were 4.13 and 4.07 obtained by biscuits made from sorghum with zero and DSF90%+10%CPF, respectively. General acceptability scores of biscuits made from decorticated sorghum and maize with and without incorporated of chickpea flour with different levels 10, 20 and 30%. The results showed that maize with and with out levels of chickpea flour gained the highest scores 6.87, 6.93, 6.27 and 6.5, respectively.

Table 8 Sensory characteristics of gluten - free biscuits:

Sample	Colour	Odour	Taste	Texture	General acceptability
DSF	3.07^{e} ±2.15	3.60^{c} ±2.13	3.40^{c} ±2.26	4.13^{d} ±2.13	3.47^{d} ±2.36
DSF90% + 10% CPF	4.20^{de} ±2.18	3.73^{c} ±1.83	3.60^{c} ±2.03	4.07^{d} ±1.83	3.67^{d} ±1.84
DSF80% + 20% CPF	5.27^{cd} ±2.12	4.33^{c} ±1.88	4.47^{bc} ±2.20	4.60^{cd} ±1.80	4.67^{cd} ±1.95
DSF70% + 30% CPF	5.47^{bcd} ±1.30	4.93^{bc} ±1.28	5.40^{ab} ±1.50	5.20^{bcd} ±1.32	5.47^{bc} ±1.36
DMF	6.27^{abc} ±1.91	6.67^{a} ±1.63	6.73^{a} ±1.53	6.60^{ab} ±1.55	6.87^{a} ±1.60
DMF90% + 10% CPF	7.33^{a} ±1.23	6.40^{a} ±1.92	6.87^{a} ±1.55	6.73^{a} ±1.75	6.93^{a} ±1.62
DMF 80% + 20% CPF	6.60^{abc} ±0.91	5.93^{ab} ±1.79	6.27^{a} ±1.53	6.07^{ab} ±1.87	6.27^{ab} ±1.44
DMF70% + 30% CPF	6.80^{ab} ±1.61	6.00^{ab} ±2.04	6.33^{a} ±1.99	5.87^{abc} ±2.10	6.53^{ab} ±1.88
$Lsd_{0.05}$	1.257^{*}	1.323^{*}	1.339^{*}	1.311^{*}	1.289^{*}
SE±	0.4487	0.4722	0.4778	0.468	0.46

Mean values ±SD (s) sharing same superscript(s) in a column are not significantly different (P≤0.05).

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

No significant differences were obtained. Lowest scores were observed by sorghum with 0, 10 and 20% level of chickpea flour. The results indicated that no significant ($P \leq 0.05$) differences were noticed between sorghum and sorghum with different levels of chickpea.

4.2.7 Acceptability of gluten- free biscuits among celiac disease patients

The mean scores given by the sensory evaluation for colour, odour, taste, texture and general acceptability of biscuits are presented in Table 9 and plate 1 showed the organoleptic properties of biscuits evaluated by celiac disease patients (group testing).

Colour scores of biscuits samples made from decorticated sorghum and maize supplemented with different levels of chickpea flour 0, 10, 20, and 30%. The scores ranged between 7.30 and 8.40. The results showed no significant differences observed. That means biscuits colour had been accepted. Odour scores of biscuits produced ranged from 5.90 to 7.80. The panelists assessed odour of all samples as very good. That means odour of biscuits had been accepted compared with control. The results obtained showed no significant differences were noticed. Taste scores of biscuits ranged between 5.90 and 7.40. The results appeared had been accepted by the celiac disease patients panelists. The results of taste indicated that decorticated sorghum and maize supplemented with chickpea flour in the biscuits formulation were so good. Therefore, chickpea flour included in biscuits formulation is better for the celiac disease patients, because it enhanced the nutritional value of biscuits produced. Texture scores of biscuits ranged from 6.10 to 7.50. The results showed no significant differences were observed. The results obtained occured that texture values were accepted by the celiac patient's panelists. The scores obtained for general acceptability showed no significant ($P \leq 0.05$) differences were noted. The scores ranged from 5.90 to 7.60. The results indicated that general acceptability of biscuits samples were appreciated and accepted by the celiac disease patient's panelists.

Table 9 Acceptability of gluten free biscuits among celiac disease patients

Sample	Colour	Odour	Taste	Texture	General acceptability
	Scores				
DSF	8.00^{a} ±1.25	7.50^{ab} ±1.51	7.20^{a} ±1.48	6.10^{a} ±2.23	7.20^{a} ±1.55
DSF90% + 10% CPF	7.30^{a} ±1.34	5.90^{b} ±1.97	5.90^{a} ±1.97	6.30^{a} ±2.11	6.10^{a} ±1.66
DSF80% + 20% CPF	8.00^{a} ±1.41	6.60^{ab} ±1.71	6.70^{a} ±1.70	6.80^{a} ±1.48	6.60^{a} ±1.84
DSF70% + 30% CPF	7.40^{a} ±1.71	6.40^{ab} ±1.90	6.60^{a} ±1.84	6.80^{a} ±1.48	5.90^{a} ±2.28
DMF	7.30^{a} ±2.11	7.10^{ab} ±2.28	6.80^{a} ±2.49	7.00^{a} ±1.76	6.80^{a} ±1.81
DMF90% + 10% CPF	8.30^{a} ±0.67	7.20^{ab} ±1.23	7.40^{a} ±0.84	7.30^{a} ±1.34	7.60^{a} ±1.26
DMF 80% + 20% CPF	8.30^{a} ±0.82	7.60^{ab} ±1.17	6.70^{a} ±1.34	7.20^{a} ±1.32	7.10^{a} ±1.29
DMF70% + 30% CPF	8.40^{a} ±0.84	7.80^{a} ±1.14	7.20^{a} ±2.44	7.50^{a} ±2.12	7.50^{a} ±1.78
Lsd$_{0.05}$	1.203^{ns}	1.481*	1.637^{ns}	1.574^{ns}	1.527^{ns}
SE±	0.4268	0.5254	0.5806	0.5583	0.5416

Mean(s) values ±SD sharing same superscript(s) in a column are not significantly different (P>0.05) according to DMRT.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

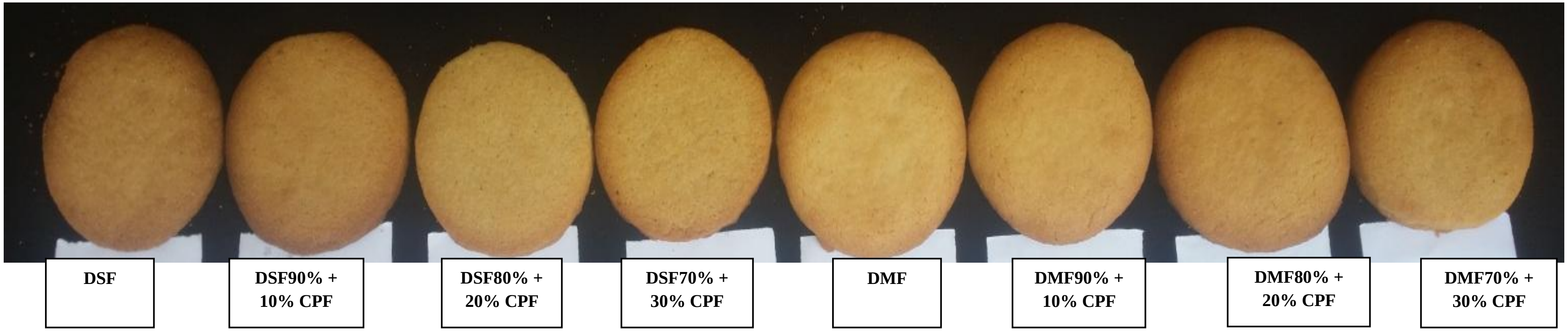

Plate (1): Biscuit made from decorticated sorghum and maize supplemented with 0, 10, 20, and 30% chickpea in the biscuit formulation.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

4.3 Quality charactersitics of gluten - free balady bread

4.3.1Proximate composition

Table 10 and Plate No 2 showed proximate analysis of balady breads prepared from incorporating sorghum and maize with different levels of chickpea (0, 10, 20, and 30% level). The results mentioned that moisture content of balady bread was assessed between 10.27 to 11.63%. Highest value noticed in sorghum baladi bread with 30%chickpea flour DSF 70%+ 30% CPF. Whereas, the lowest value appeared in maize balady bread without chickpea flour DMF. Ash content of balady breads made from decorticated sorghum and maize supplemented with different level of chickpea 0, 10, 20, and 30% was presented in Table 10. The results showed large variations were observed in ash content, ranging from 2.93 to 4.46%. The DSF70% + 30% CPF gained the highest value, mainly derived from the level of chickpea and salt added. Whereas, lowest value was observed by maize balady bread (DMF). From the results ash content increased significantly ($P \leq 0.05$) with increasing the levels of chickpea flour in balady bread from both sorghum and maize.

Fat content values ranged between 7.14 and 8.42%. The sorghum balady bread (DSF) presented the lowest value 7.14%, conversely, highest value noticed in DMF (8.42%), followed by DMF90% + 10% CPF (8.33%) and DMF80% + 20% CPF (8.21%). Protein content values of balady bread varied from 10.57 to 15.90%. The results obtained showed protein values increased significantly ($P \leq 0.05$) with increasing the level of chickpea flour in balady bread from both decorticated sorghum and maize samples tested. The DSF70% + 30% CPF gave highest value, whereas, the lowest value observed by DMF. From the results this increase in the protein content in balady breads must be associated to the presence of chickpea proteins in the formulation, since these ingredients are used as protein sources in gluten free breads (Marco and Rosell, 2008; Moore, *et al.*, 2004).

Table 10 Proximate composition of gluten - free balady bread:

Sample	Moisture content (%)	Ash content (%)	Fat content (%)	Crude protein (%)	Crude fibre (%)	Available Carbohydrate (%)	Energy (kj/100gm)
DSF	10.81^{b} ±0.05	3.09^{e} ±0.03	7.14^{d} ±0.12	11.26^{f} ±0.05	1.24^{e} ±0.07	66.46^{a} ±0.12	1586.09^{de} ±3.34
DSF90% + 10% CPF	10.63^{bc} ±0.08	3.38^{c} ±0.07	7.18^{d} ±0.11	12.96^{d} ±0.06	1.47^{d} ±0.06	64.39^{b} ±0.04	1580.52^{ef} ±4.18
DSF80% + 20% CPF	10.35^{e} ±0.04	3.60^{b} ±0.05	7.22^{d} ±0.11	14.78^{c} ±0.10	1.73^{c} ±0.08	62.31^{d} ±0.18	1577.89^{f} ±0.71
DSF70% + 30% CPF	11.63^{a} ±0.34	4.46^{a} ±0.00	7.26^{d} ±0.10	15.90^{a} ±0.02	2.31^{a} ±0.01	58.44^{g} ±0.23	1532.40^{g} ±7.64
DMF	10.27^{e} ±0.02	2.93^{f} ±0.10	8.42^{a} ±0.05	10.57^{g} ±0.02	1.40^{d} ±0.01	66.50^{a} ±0.22	1621.73^{a} ±2.97
DMF90% + 10% CPF	10.58^{cd} ±0.04	3.07^{e} ±0.02	8.33^{ab} ±0.05	12.71^{e} ±0.05	1.79^{c} ±0.03	63.52^{c} ±0.09	1604.24^{b} ±1.08
DMF 80% + 20% CPF	10.30^{e} ±0.05	3.20^{d} ±0.04	8.21^{bc} ±0.09	14.74^{c} ±0.13	2.20^{b} ±0.08	61.35^{e} ±0.06	1597.27^{c} ±2.04
DMF70% + 30% CPF	10.36^{de} ±0.02	3.45^{c} ±0.10	8.16^{c} ±0.06	15.74^{b} ±0.06	2.27^{ab} ±0.08	60.02^{f} ±0.25	1589.82^{d} ±1.53
Lsd$_{0.05}$	0.2189*	0.09481*	0.1548*	0.1224*	0.09481*	0.2896*	6.239*
SE±	0.07303	0.03162	0.05164	0.04082	0.03162	0.09661	2.081

Mean(s) values ±SD having different superscript(s) in the column are significantly different ($P \leq 0.05$) according to DMRT.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

The results showed fiber content of balady bread varied from 1.24 to 2.31%, upper value noticed in S_3, conversely, the lower value gained by DSF. the fiber content increased significantly ($P \leq 0.05$) with increasing the level of chickpea. Carbohydrates content varied from 62.12 to 72.34%. Yazynima *et al.* (2008) reported the nutritional composition of two kinds of gluten-free crispy breads, which contained 3.5 – 6.0 gm/100gm proteins, 3.0-6.5gm/100gm fats and 71.0-80.0gm/100gm carbohydrates. The present study shows that marketed gluten – free breads are carbohydrate based products. They have great variation in their protein, fat and mineral content, in contrast to the very narrow variation in proximate composition observed in wheat based bread products (Rosell, 2011). The carbohydrate content decreased significantly ($P \leq 0.05$) from 66.46 to 58.44% and from 66.50 to 60.02% in sorghum and maize breads, respectively. The energy values of balady breads ranged between 1532.40 and 1621.73 kj/100gm, highest value was noted in DMF, whereas, the lowest value obtained by DSF + 30% CPF.

4.3.2 *In vitro* protein digestibility of gluten - free balady bread:

Table 11 showed that incorporating sorghum flour with different levels of chickpea flour substantially increased *in vitro* protein digestibility significantly ($P \leq 0.05$) of balady bread compared with 100% sorghum bread. Replacement sorghum flour with 10, 20, and 30% chickpea flour increased protein digestibility from 39.41% for 100% sorghum balady bread to 41.47%, 44.63% and 46.20%, respectively. Interaction between tannins and sorghum proteins reduces both proteins and starch digestibility. This is important in both human and animal nutrition the formation of complexes between sorghum proteins and tannins is thought to render the proteins indigestible as well as inhibit digestive enzymes. Proteins rich in proline bind more sorghum tannins than others proteins. In addition, a protein containing more proline repeats well binds more tannin than one with less such repeats (Medugu *et al.*, 2010). The low digestibility of sorghum protein is presumably due to the protein cross – linking. Good quality proteins are those that readily digestible and contain the essential amino

Table 11 *In vitro* protein and starch digestibilities of gluten -free balady Breads:

Sample	Protein digestibility (%)	Starch digestibility (%)
DSF	39.41^{h} ±0.05	62.17^{b} ±2.92
DSF90% + 10% CPF	41.47^{g} ±0.08	61.76^{b} ±0.09
DSF80% + 20% CPF	44.63^{f} ±0.07	63.37^{b} ±0.10
DSF70% + 30% CPF	46.20^{e} ±0.03	65.35^{a} ±0.07
DMF	47.70^{d} ±0.04	65.57^{a} ±0.14
DMF90% + 10% CPF	49.30^{c} ±0.03	66.57^{a} ±0.04
DMF 80% + 20% CPF	53.63^{b} ±0.03	66.85^{a} ±0.05
DMF70% + 30% CPF	56.38^{a} ±0.06	67.23^{a} ±0.06
$Lsd_{0.05}$	0.09481^{*}	1.791^{*}
SE±	0.03162	0.5975

Mean(s) values ±SD having different superscript(s) in a column are significantly different ($P \leq 0.05$) according to DMRT.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

acid in quantities that correspond to human requirements (Zhao *et al*., 2008; El – Beltagi *et al*., 2011). Protein digestibility of balady bread made from decorticated maize flour compositing with chickpea flour was presented in Table 11.

The result of investigation revealed that *in vitro* protein digestibility was influenced by processing treatments employed. *In vitro* protein digestibility of maize balady bread was 47.70%. In vitro Protein digestibility of maize balady bread with10, 20, and 30% chickpea flour in the formulation increased significantly (P≤0.05) with increasing the level of chickpea flour in final product from 47.70 to 49.30%, 53.63, and 56.38%, respectively.

4.3.3 *In vitro* starch digestibility of gluten - free balady bread:

Starch digestibility of balady bread made from incorporation of decorticated sorghum and maize breads with different levels of chickpea flour in the formulations are presented in Table 11. The results showed that starch digestibility ranged from 61.76 to 67.23% for DSF80% + 20% CPF and DMF70%+30%CPF**,** respectively. It's clear that no significant differences were noted between DSF, DSF + 10% CPF and DSF + 20% CPF balady breads, respectively. But generally, starch digestibility increased significantly (P≤0.05) in DSF70% + 30% CPF, DMF, DMF90%+10%CPF, DMF80%+20%CPF, and DMF70%+30%CPF balady breads, respectively. Decortication increased starch digestibility for each type of sorghum. In the study reported by Bach Knudsen *et al*., (1988). According to Mc Neill *et al.* (1995) processing methods that alter the kernel structure by releasing the starch granules from the protein matrix will enhance their susceptibility to enzyme action and subsequent digestibility. It is also possible for gelatinized starches to form complexes with proteins, reducing the digestion of both (starch and protein). In addition, anti-nutritional factors such as tannins present in some sorghum have been shown to bind to protein and reduce starch digestion by inhabiting some enzyme systems (Waniska and Rooney, 2000). However, the lower overall digestibility of the decorticated white sorghum porridge compared to the corn porridge was influenced by extrinsic factors. Cooking has shown to significantly reduce protein digestibility of sorghum but not of corn, because of formation of disulfide cross links within, and possible between, protein bodies (Zhang and Hamaker, 1998). These protein complexes could interfere with starch digestibility. On the other hand, the kinetic constants of whole white, whole tannin, and decorticated tannin porridges were significantly different, suggesting that the differences in digestibility were due to the innate properties of the starches in the products. (Angelina, 2006).

4.3.4 Mineral content of gluten - free balady bread:

Table 12 shows that minerals content of balady bread made from decorticated sorghum and maize incorporated with different levels of chickpea flour (0, 10, 20, and 30%).

The results indicated that the Ca element values of decorticated sorghum and maize breads incorporating with chickpea flour in the formulations substantially varied from 1.48 to 1.62 mg/100gm. Highest value noticed in DMF + 10% CPF, whereas, the lowest value obtained by DMF + 20% CPF. It could be noticed that Ca element values were significantly difference in different chickpea blends. In general, Ca element values were increased in sorghum. The Fe element values of sorghum and maize breads with different levels of chickpea flour in the formulations varied from 1.40 to 1.60 mg/100gm. The results of the analysis of the bread produced showed Mg, P, K, and Na elements values varied from 3.45 to 3.73, 8.42 to 8.67, 13.90 to 15.30 and 14.90 to 16.00mg/100gm, respectively. Therefore, the results appeared that Zn and Mn elements content ranged between 0.055 to 0.07 and 0.079 to 0.0910 mg/100gm, respectively. Minerals are vital to the functioning of many body processes. They are critical players in the functioning of the nervous system, other cellular processes, water balance and structural (e-g skeletal) systems (Ameh *et al.*, 2013). Inadequate intakes of micronutrients (minerals) have been associated with severe malnutrition,

Table 12 Minerals content of gluten –free balady bread(mg/100 g)

Sample	Na	Ca	K	P	Mg	Fe	Zn	Mn
DSF	15.90^{ab} ±0.13	1.52^{de} ±0.02	14.40^{d} ±0.09	8.60^{b} ±0.04	3.73^{a} ±0.01	1.40^{de} ±0.01	0.070^{a} ±0.01	0.090^{a} ±0.02
DSF90% + 10% CPF	15.00^{e} ±0.11	1.49^{e} ±0.02	14.00^{e} ±0.08	8.55^{bc} ±0.01	3.52^{de} ±0.03	1.51^{bc} ±0.03	0.055^{a} ±0.02	0.082^{a} ±0.01
DSF80% + 20% CPF	15.80^{b} ±0.12	1.55^{c} ±0.03	15.20^{ab} ±0.10	8.67^{a} ±0.02	3.45^{f} ±0.02	1.47^{c} ±0.02	0.059^{a} ±0.01	0.087^{a} ±0.02
DSF70% + 30% CPF	14.90^{ef} ±0.10	1.60^{ab} ±0.07	14.80^{c} ±0.07	8.44^{d} ±0.02	3.66^{bc} ±0.03	1.59^{a} ±0.01	0.062^{a} ±0.01	0.079^{a} ±0.02
DMF	16.00^{a} ±0.14	1.58^{b} ±0.05	14.20^{de} ±0.06	8.59^{b} ±0.02	3.57^{d} ±0.04	1.60^{a} ±0.03	0.063^{a} ±0.01	0.091^{a} ±0.02
DMF90% + 10% CPF	15.70^{bc} ±0.10	1.62^{a} ±0.04	15.30^{a} ±0.09	8.42^{d} ±0.03	3.49^{e} ±0.01	1.48^{c} ±0.01	0.059^{a} ±0.02	0.089^{a} ±0.00
DMF 80% + 20% CPF	15.30^{d} ±0.11	1.48^{e} ±0.01	13.90^{ef} ±0.06	8.50^{c} ±0.01	3.70^{ab} ±0.02	1.53^{b} ±0.00	0.070^{a} ±0.03	0.082^{a} ±0.01
DMF70% + 30% CPF	14.80^{f} ±0.07	1.50^{d} ±0.02	14.20^{de} ±0.07	8.56^{bc} ±0.02	3.68^{b} ±0.03	1.44^{d} ±0.02	0.068^{a} ±0.01	0.090^{a} ±0.02
$\mathrm{Lsd}_{0.05}$	0.1952^{**}	0.0258^{*}	0.1259^{**}	0.4581^{**}	0.0267^{*}	0.0316^{*}	0.0519^{NS}	0.0437^{NS}
SE±	0.0871	0.0171	0.0846	0.2607	0.0194	0.0078	0.0087	0.0065

Mean(s) values ±SD sharing same superscript(s) in a column are not significant different (P>0.05) according to Duncan’s multiple range test.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

increased disease conditions and mental impairment (Abulude, 2005). Breads contain zinc ranged from 0.77 to 2.0 mg/100gm.(Al-Mussali and Al-Gahri, 2009). Diagnosis of celiac disease is often accompanied with observed deficiencies in mineral components, caused by the damage of intestinal epithelium. It is usually most evident in the levels of calcium and iron. Although the adherence to gluten-free diet restores proper absorption of minerals, they could be lacking in many of gluten-free products (Wild, *et al*., 2010). Supplementation of gluten-free bread seems to be a good way to provide appropriate levels of minerals (Kiskini *et al*., 2012; and Gambuś *et al*., 2009).

4.3.5 Specific volume of gluten- free balady bread

Specific volume of gluten free balady breads produced from decorticated sorghum and maize supplemented with chickpea flour in the formulation are presented in Table 13. Sorghum balady bread weight ranged between136.63, 138.57, 136.60, and 137.23cm for DSF, DSF90%+10%CPF, DSF80%+20%CPF, and DSF70% +30%CPF, respectively. The results showed no significant ($P \leq 0.05$) differences observed, compared with sorghum without chickpea in bread formulation. On the other hand, maize balady bread weight increased significantly ($P \leq 0.05$) with increasing the level of chickpea in the formulation from 133.90 to 137.55, 142.20, and 142.60gm for DMF, DMF90%+10%CPF, DMF80%+20%CPF, and DMF70%+30%CPF, respectively. The results obtained explained that weights of balady bread were high. This may be due to the increment of water which was absorbed by the samples studied. These high weights of balady bread reduced the specific volume. Volume of balady bread produced ranged from 304.12 to 370.59cm^3. The results showed no significant differences ($P \leq 0.05$) noted.

Specific volume of balady bread produced from sorghum and with chickpea in the bread constituents were 2.72, 2.47, 2.56, and 2.32cc/gm. for DSF, DSF90%+10%CPF, DSF80%+20%CPF, and DSF70%+30%CPF, respectively. Likewise, the specific volumes of balady bread produced from maize and it's treated with chickpea in the bread constituents were 2.24, 2.21, 2.14, and 2.20cc/gm. for DMF, DMF90%+10%CPF, DMF80%+20%CPF, and DMF70%+30%CPF, respectively. Specific volume of breads produced showed no significant differences ($P \leq 0.05$) were observed.

Balady breads (gluten free) circumferences are shown in Table13. Balady breads (gluten free) circumferences values ranged between 43.90 and 40.60 cm. Table 13 showed no significant differences in balady breads circumferences in all samples studied. These results indicated that chickpea flour percentages that were included in the bread ingredients have no effect on breads circumference.

4.3.6 Sensory characteristics of gluten - free balady bread:

The organoleptic evaluation of balady bread produced from decorticated sorghum and maize flours supplemented with chickpea flour (0, 10, 20, and 30% level) were presented in Table 14.

The result of color scores showed no significant differences ($P \leq 0.05$) amongst sorghum balady breads with and without chickpea flour and maize bread (DMF), and DMF90% + 10% CPF in the bread formulation. Highest score obtained by DMF70% + 30%CPF. Odor scores ranged between 3.90 to 5.50. The results showed no significant differences ($P \leq 0.05$) in all samples studied.

The mean scores given by the sensory panel for taste, crumb texture and crumb grains of the balady bread are presented in Table 16. Mean scores ranged from 4.20 to 6.20, 3.60 to 5.70 and 3.20 to 5.70 for taste, crumb texture and crumb grains, respectively. The results obtained did not differ significantly ($P \leq 0.05$) in quality attribute. General acceptability scores of balady bread ranged between 3.60 and 6.10. The results obtained showed no significant differences ($P \leq 0.05$) were noted.

Table 13 specific volume of balady bread:

Sample	Weight (gm)	Volume (cc)	Specific volume (cc/gm)	Circumference (cm)
DSF	136.63^{b} ±0.86	370.59^{a} ±6.41	2.72^{a} ±0.07	42.69^{ab} ±2.08
DSF90% + 10% CPF	138.57^{b} ±0.12	343.05^{ab} ±37.05	2.47^{abc} ±0.27	40.68^{b} ±2.33
DSF80% + 20% CPF	136.60^{b} ±0.87	349.41^{ab} ±31.15	2.56^{ab} ±0.23	43.90^{a} ±1.74
DSF70% + 30% CPF	137.23^{b} ±2.51	351.92^{ab} ±57.90	2.32^{bc} ±0.37	42.53^{ab} ±1.62
DMF	133.90^{c} ±0.00	306.92^{b} ±11.10	2.24^{bc} ±0.03	41.45^{ab} ±0.23
DMF90% + 10% CPF	137.55^{b} ±0.55	304.8^{b} ±0.71	2.21^{bc} ±0.01	41.43^{ab} ±0.14
DMF 80% + 20% CPF	142.20^{a} ±0.61	304.12^{b} ±6.04	2.14^{c} ±0.03	41.45^{ab} ±0.43
DMF70% + 30% CPF	142.60^{a} ±0.60	314.01^{b} ±11.76	2.20^{bc} ±0.09	41.27^{ab} ±0.02
$Lsd_{0.05}$	1.821*	47.72*	0.3238*	2.423*
SE±	0.6075	15.92	0.108	0.8081

Mean(s) values ±SD having different superscript(s) in a column are significantly different ($P \geq 0.05$) according to Duncan's multiple range test.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

The scores of general acceptability corresponded to a degree of acceptability from acceptable to highly acceptable and indicated that the bread samples were generally appreciated by the panelists.

The relatively high amount of shortening and sugar in bread recipe may have contributed to a pleasant taste. It is well recognized that addition of fat plasticizes dough and increase bread volume, typically by 10%. It makes bread stay soft and more palatable for a longer period of time (Delcour and Hoseney, 2010). With a relatively high percentage of fat and sugar, it is possible to increase the level of non – wheat flour considerably without significant changes in the bread characteristics (Aboaba and Obakpolor, 2010).

4.3.7 Acceptability of gluten free balady bread among celiac disease patients

Celiac disease patients evaluation of balady bread produced from decorticated sorghum and maize supplemented with chickpea flour 0, 10, 20, and 30%levels in the bread formulation which was done is presented in Table15 and Plate 2.

The organoleptic quality of balady breads which were assessed by celiac disease patients, to know the degree of their acceptance. It includes colour, odour, taste, crumb texture, crumb grain and general acceptability.

The results showed colour scores ranging between 7.30 and 8.30. No significant differences were noticed among the samples studied. That means all the samples tested had been accepted. Odour scores of balady bread ranged from 6.10 to 8.20. The results showed no significant differences were noted. From the current results, chickpea flour added in the balady bread formulation has substantially positive effect on the bread odour, and the product was accepted. Likewise, taste scores ranged from 6.20 to 8.40.

Table 14 Sensory characteristics of gluten - free balady bread:

Sample	Colour	Odour	Taste	Crumb texture	Crumb grains	General acceptability
DSF	4.00^{b} ±2.40	4.50^{a} ±2.46	4.20^{b} ±1.69	4.20^{ab} ±1.48	3.20^{c} ±2.15	3.60^{c} ±1.84
DSF90% + 10% CPF	4.40^{b} ±2.27	4.60^{a} ±1.90	4.50^{ab} ±2.32	4.40^{ab} ±1.84	3.70^{bc} ±2.06	4.30^{abc} ±2.41
DSF80% + 20% CPF	4.10^{b} ±1.97	4.20^{a} ±1.69	4.20^{b} ±1.75	3.60^{b} ±1.58	3.90^{abc} ±1.45	4.00^{bc} ±1.49
DSF70% + 30% CPF	4.90^{b} ±1.37	4.80^{a} ±1.23	5.50^{ab} ±1.08	4.80^{ab} ±1.69	4.90^{abc} ±0.99	5.00^{abc} ±1.49
DMF	5.70^{ab} ±1.77	5.00^{a} ±1.94	4.80^{ab} ±1.40	5.20^{ab} ±2.20	5.10^{ab} ±2.02	5.00^{abc} ±1.63
DMF90% + 10% CPF	5.10^{ab} ±1.37	3.90^{a} ±1.97	4.70^{ab} ±2.16	5.20^{ab} ±1.75	4.30^{abc} ±1.77	4.30^{abc} ±1.77
DMF 80% + 20% CPF	6.70^{a} ±1.42	5.50^{a} ±1.65	5.40^{ab} ±1.78	5.00^{ab} ±1.41	4.80^{abc} ±1.75	5.70^{ab} ±1.77
DMF70% + 30% CPF	6.80^{a} ±1.23	5.30^{a} ±1.25	6.20^{a} ±1.99	5.70^{a} ±1.70	5.60^{a} ±2.27	6.10^{a} ±2.18
Lsd$_{0.05}$	1.583^{*}	$1.606^{n.s}$	1.614^{*}	1.534^{*}	1.605^{*}	1.647^{*}
SE±	0.5613	0.5696	0.5724	0.5442	0.5694	0.584

Mean(s) values ±SD having different superscript(s) in a column are significantly different ($P \geq 0.05$) according to Duncan’s multiple range test.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

The results showed no significant differences ($P \leq 0.05$) were obtained. Taste quality was considered the most effective factor for the acceptability of breads produced (gluten free) which was evaluated by celiac disease patients, has been obtained required quality for taste. Therefore, taste is attractive factor or changing factor in the quality attributes of the food products. Hence, it has been accepted by the celiac disease patients. Crumb texture and crumb grain scores of the balady breads produced a range between 6.20 to 7.60, and 6.10 to 7.40, respectively.

The results obtained showed no significant difference ($P \leq 0.05$) observed compared with sorghum and maize breads scores. General acceptability scores of gluten free balady breads ranged from 6.30 to 8.40. No significant differences were observed. General acceptability of the samples of balady breads studied had been accepted by the celiac disease patient's panelists.

4.4 Quality charactersitics of gluten - free tin bread

4.4.1 Proximate composition

Proximate analysis is important for evaluating the nutritional content of the developed food products. The different chemical composition of the tin bread made from decorticated sorghum and maize incorporated with different ratios from chickpea flour **(**10, 20, and 30%) affects the nutritional quality of the final product. The protein, fiber, moisture, fat, ash, carbohydrates and energy content in the final bread product are shown in Table 16.

Moisture content of the bread product values ranged between 9.55 and 10.47%. Highest value noted in DSF+ 20% CPF, whereas, the lowest value observed in DMF + 20% CPF. High moisture content increases the microbial activity which may deteriorate the product during bad storage.

Table 15 Acceptability of gluten free balady bread among celiac disease patients

Sample	Colour	Odour	Taste	Crumb texture	Crumb grain	General acceptability
	Scores					
DSF	7.40^{a} ±1.71	7.10^{ab} ±1.45	7.20^{ab} ±1.87	6.20^{a} ±1.75	6.60^{a} ±1.43	7.20^{ab} ±1.62
DSF90% + 10% CPF	7.00^{a} ±1.70	6.10^{b} ±1.52	6.40^{b} ±1.65	6.20^{a} ±1.23	6.10^{a} ±1.52	6.40^{b} ±1.96
DSF80% + 20% CPF	7.30^{a} ±1.64	7.00^{ab} ±1.25	6.70^{b} ±1.42	6.20^{a} ±1.32	6.20^{a} ±1.32	6.90^{b} ±1.52
DSF70% + 30% CPF	7.30^{a} ±1.95	6.40^{b} ±1.78	6.20^{b} ±1.93	6.20^{a} ±1.03	6.10^{a} ±1.20	6.30^{b} ±1.83
DMF	7.70^{a} ±1.34	8.20^{a} ±0.79	8.40^{a} ±0.70	7.00^{a} ±1.83	7.40^{a} ±2.01	8.40^{a} ±0.52
DMF90% + 10% CPF	7.90^{a} ±1.29	7.20^{ab} ±1.23	7.00^{ab} ±1.41	6.60^{a} ±1.58	6.60^{a} ±1.35	7.60^{ab} ±0.97
DMF 80% + 20% CPF	8.30^{a} ±0.67	7.40^{ab} ±1.07	7.20^{ab} ±1.14	7.60^{a} ±1.07	6.90^{a} ±0.99	7.50^{ab} ±0.97
DMF70% + 30% CPF	8.30^{a} ±0.67	7.40^{ab} ±1.35	7.10^{ab} ±1.20	7.00^{a} ±1.49	6.90^{a} ±1.37	6.90^{b} ±1.29
Lsd$_{0.05}$	1.286^{ns}	1.19*	1.306*	1.283^{ns}	1.271^{ns}	1.257*
SE±	0.4562	0.422	0.4634	0.4552	0.4509	0.446

Mean(s) values ±SD having different superscript(s) in a column are significantly different ($P \geq 0.05$) according to Duncan's multiple range test.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

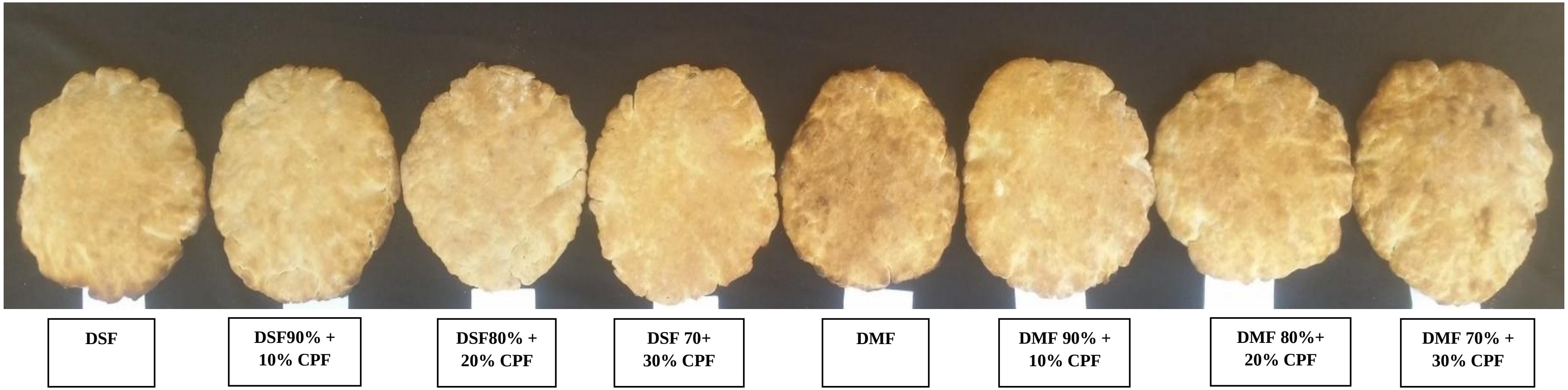

Plate (2): Balady bread made from decorticated sorghum and maize supplemented with 0, 10, 20, and 30% chickpea flour in the bread formulation.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

From the current result moisture content decreased significantly (P≤0.05) with increasing the level of chickpea flour in both sorghum and maize bread.

Ash content is composed of non-combustible, inorganic materials that are concentrated in the bran layer. Ash content can attributed to the mineral content in the samples. Ash content of Tin bread made from decorticated sorghum and maize flours with different ratios (10, 20, and 30%) of chickpea flour ranged from 1.40 to 2.45%.

Table 16 showed crude fat content of tin bread ranged from 7.34 to 8.62%, for DSF and DMF, respectively. Fat content showed no significance (P≤0.05) difference noticed among sorghum breads, but decreased significantly with increasing the level of chickpea in maize bread samples examined.

Besides providing essential nutrition, protein can also be related to finished product attributes like texture and appearance. The crude protein content of experimental bread from decorticated sorghum and maize mixed with different levels of chickpea flour ranged from 11.15 to 16.91%, for DMF and DMF70% +30% CPF, respectively. From the results protein content increased significantly (P≤0.05) in sorghum and maize bread with increasing the level of chickpea flour in the formulation. The crude fiber content of breads made from decorticated sorghum and maize supplemented with different levels (10, 20, and 30%) of chickpea flour in the formulation ranged between 0.85 to 2.10%. crude fiber increased significantly (P≤0.05) with increasing the addition of chickpea flour in the ingredients of sorghum bread and maize bread, respectively. No significant differences were noticed between bread from sorghum and maize when the addition of chickpea flour reached 30% in the bread formulation. The main role of fiber is to keep the digestive system healthy. Fiber has also been shown to benefit diabetes (Montonen *et al.*, 2003), blood cholesterol levels (Anderson *et al.*, 2004), reduces constipation, coronary heart disease (Lin *et al.* 1999), and obesity (Lairon *et al.*, 2005).

Carbohydrates content of breads made from decorticated sorghum and maize supplemented with different levels (10, 20, and 30%) of chickpea flour in the formulation are viewed in Table 16. The results of carbohydrates ranged between 60.86 and 67.84% highest values noted in DSF and DMF, and the lowest value obtained by DMF70%+30%CPF. The addition of chickpea flour in the formulation of breads resulted a significant ($P \leq 0.05$) decrease on carbohydrates with increasing the level chickpea flour. Energy value content of breads were in the range of 1607.15 to 1657.07 kj/100gm. Table 16 showed a significant difference observed on energy values, but no signficant differences ($P \leq 0.05$) among sorghum bread with chickpea flour in the bread formulation.

4.4.2 *In vitro* protein digestibility of gluten-free tin bread:

Table 17 showed that compositing of decorticated sorghum flour and decorticated maize flour with 10, 20, and 30%level of chickpea flour substantially increased significantly ($P \leq 0.05$) the in vitro protein digestibility of bread compared with 100% sorghum bread and therefore, 100% maize bread. Replacement of decorticated sorghum flour with 10, 20, and 30% chickpea flour increased *in vitro* protein digestibility from 43.35% to 45.51, 46.62, and 48.22%, respectively. These increments in digestibility of protein could be attributed to dilution of less digestible sorghum kafirins with more soluble chickpea globulins (Mac Lean *et al.*, 1981). The same authers reported 46% and 81% apparent digestibility for sorghum and wheat, respectively in young children. Improved protein digestibilities suggest potentially improved protein absorption and retention in humans.

The results showed that maize bread protein digestibility varied from 50.78 to 58.54%. It's clear that in vitro protein digestibility of maize bread was increased with increasing the level of chickpea flour in maize bread.

Table 16 Proximate composition of gluten - free tin Bread

Samples	**Moisture content (%)**	**Ash content (%)**	**Fat content (%)**	**Crude protein (%)**	**Crude fibre (%)**	**Available Carbohydrate (%)**	**Energy (kj/100gm)**
DSF	10.23^{b} ±0.04	2.45^{a} ±0.09	7.34^{d} ±0.12	11.26^{f} ±0.05	0.85^{e} ±0.03	67.84^{a} ±0.25	1616.62^{e} ±0.73
DSF90% + 10% CPF	10.47^{a} ±0.05	2.35^{ab} ±0.07	7.38^{d} ±0.11	12.44^{e} ±0.06	1.20^{d} ±0.10	66.16^{b} ±0.16	1607.99^{f} ±3.44
DSF80% + 20% CPF	10.10^{c} ±0.00	2.32^{b} ±0.07	7.42^{d} ±0.11	14.03^{c} ±0.13	1.77^{b} ±0.01	64.36^{d} ±0.06	1607.15^{f} ±3.31
DSF70% + 30% CPF	9.93^{d} ±0.01	2.00^{c} ±0.07	7.45^{d} ±0.08	16.89^{a} ±0.02	2.09^{a} ±0.08	61.64^{f} ±0.20	1610.65^{f} ±2.67
DMF	9.97^{d} ±0.02	1.40^{e} ±0.02	8.62^{a} ±0.05	11.15^{f} ±0.06	1.30^{cd} ±0.12	67.56^{a} ±0.21	1657.07^{a} ±1.42
DMF90% + 10% CPF	9.77^{e} ±0.05	1.85^{d} ±0.01	8.53^{ab} ±0.05	13.24^{d} ±0.08	1.38^{c} ±0.10	65.22^{c} ±0.05	1650.60^{b} ±1.77
DMF 80% + 20% CPF	9.55^{f} ±0.03	2.01^{c} ±0.04	8.45^{bc} ±0.06	15.00^{b} ±0.18	1.76^{b} ±0.06	63.23^{e} ±0.31	1642.42^{c} ±0.81
DMF70% + 30% CPF	9.72^{e} ±0.02	2.05^{c} ±0.12	8.36^{c} ±0.06	16.91^{a} ±0.09	2.10^{a} ±0.08	60.86^{g} ±0.34	1631.39^{d} ±2.91
$Lsd_{0.05}$	0.05474^{*}	0.1224^{*}	0.1448^{*}	0.1642^{*}	0.1341^{*}	0.3792^{*}	4.091^{*}
SE±	0.01826	0.04082	0.0483	0.05477	0.04472	0.1265	1.364

Mean(s) values ± SD having different superscript(s) in a column are significantly different ($P \leq 0.05$) according to DMRT.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

4.4.3 *In vitro* starch digestibility of gluten-free tin bread:

Table 17 shows that starch digestibility of tin bread produced from decorticated sorghum and maize compositing with different levels (0, 10, 20, and 30%) of chickpea flour in the bread formulations. Starch digestibility of sorghum bread ranged between 62.55 to 66.11%. The results appeared that the significant increment in starch digestibility resulted due to the inclusion of chickpea flour in sorghum breads formulations. The continuous increment in starch digestibility was resulted from the increment of chickpea level. Several intrinsic factors in various sorghums could influence digestion rates of starch and protein including phenolic compounds (other than condensed tannin), phytate and kafirin, the dominant protein fraction in sorghum. (Selle *et al.*, 2010). The value of starch digestibility of decorticated maize breads with chickpea flour in the formulation ranged from 66.77 to 67.43%. From the current results starch digestibility of maize breads increased significantly ($P \leq 0.05$) with addition of chickpea flour to decorticated maize breads. No significant differences were noticed between DMF90% +10%CPF, DMF80% +20%CPF and DMF70% +30%CPF. Bread is an open structure with many air holes. According to van der Merwe *et al*., (2001), the porous structure of bread increases the contact surface area of the sample with the enzymes during digestion. A great accessibility of starch in the bread contributes to the high rate of starch digestibility during the digestion period. During digestion, carbohydrates that break down quickly have high glycemic indexes (giving fast and high glucose responses). On the other hand, carbohydrates that break down slowly have low glycemic indexes (releasing glucose gradually into the blood stream). Lowering postprandial blood glucose (by consuming low GI foods) has positive health outcomes for both healthy subjects and patients with insulin resistance. These effects are summarized by Lang, (2004).

Table 17 Protein and starch digestibilities of gluten-free tin breads:

Sample	Protein digestibility (%)	Starch digestibility (%)
DSF	43.35^{h} ±0.05	62.55^{f} ±0.10
DSF90% + 10% CPF	45.51^{g} ±0.02	63.35^{e} ±0.09
DSF80% + 20% CPF	46.62^{f} ±0.09	65.24^{d} ±0.08
DSF70% + 30% CPF	48.22^{e} ±0.05	66.11^{c} ±0.05
DMF	50.78^{c} ±0.05	66.77^{b} ±0.15
DMF90% + 10% CPF	53.65^{c} ±0.03	67.54^{a} ±0.33
DMF 80% + 20% CPF	55.80^{b} ±0.04	67.43^{a} ±0.05
DMF70% + 30% CPF	58.54^{a} ±0.06	67.41^{a} ±0.11
$Lsd_{0.05}$	0.09481^{*}	0.2567^{*}
SE±	0.03162	0.08563

Mean(s) values ± SD having different superscript(s) in a column are significantly different ($P \leq 0.05$) according to DMRT.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

4.4.4 Minerals content of gluten-free tin bread:

Mineral content of tin bread samples produced from decorticated sorghum and maize incorporated with different levels of chickpea flour (0,10, 20, and 30%) in formulation. The micro elements (calcium, iron, magnesium, phosphorus, potassium, sodium, zinc and manganese) analysis was reported in Table 18. Calcium element values of the bread samples were appeared low and it ranged from 1.38 to 1.62mg/100gm for sorghum bread with 10% flour of chickpea (DSF 90%+10% CPF) and sorghum bread with 20% flour of chickpea (DSF80% +20% CPF), respectively. The low values of calcium element resulted because the decortications processes which were done conducted to this reduction.Iron values (Fe) of tin bread samples were ranging between 1.38 to 1.50 mg/100gm. whereas, magnesium values ranging from 3.48 to 3.68 mg/100gm. The tin bread analysis results appeared the elements values of P, K, and Na were the most dominant compared with others minerals results. The P, K, and Na elements values ranging between 8.48 to 8.61, 13.80 to 15.50, and 14.60 to 15.60 mg/100gm, respectively. The results showed zinc and manganese elements values varied from 0.054 to 0.070 and 0.077 to 0.089mg/100gm, respectively. It's clear that zinc and manganese elements values were the lowest values obtained compared with others elements.

4.4.5 Loaf specific volume of gluten-free tin bread:

Loaf specific volume of tin bread prepared from decorticated sorghum and maize with incorporation of chickpea flour with different levels (0, 10, 20, and 30%) are presented in Table 20.

Table 19 loaf volumes were 338.33, 336.67, 340.0 and 340.0 cm^3 for sorghum and its treated breads with (10, 20, and 30% chickpea flour), respectively. The results showed there were no significant differences ($P \leq 0.05$) in loaf volume in presence or absence of chickpea flour with decorticated sorghum bread and its treated breads with chickpea flour in different levels. On the other hand, loaf volume of maize bread and

Table 18 Minerals content (mg/100 g) of gluten-free tin bread

Samples	Na	Ca	K	P	Mg	Fe	Zn	Mn
DSF	15.60^{a} ±0.15	1.59^{b} ±0.02	14.00^{de} ±0.11	8.49^{d} ±0.03	3.66^{b} ±0.07	1.50^{b} ±0.04	0.068^{ab} ±0.02	0.077^{b} ±0.01
DSF90% + 10% CPF	15.20^{bc} ±0.14	1.38^{c} ±0.01	14.20^{d} ±0.12	8.60^{a} ±0.02	3.55^{e} ±0.05	1.44^{d} ±0.01	0.060^{c} ±0.01	0.079^{b} ±0.02
DSF80% + 20% CPF	15.30^{b} ±0.13	1.62^{a} ±0.03	15.50^{a} ±0.13	8.54^{c} ±0.04	3.68^{a} ±0.04	1.47^{c} ±0.01	0.058^{d} ±0.02	0.083^{a} ±0.01
DSF70% + 30% CPF	14.80^{d} ±0.11	1.58^{b} ±0.01	14.60^{c} ±0.14	8.60^{a} ±0.05	3.55^{e} ±0.03	1.53^{b} ±0.02	0.054^{de} ±0.01	0.080^{a} ±0.04
DMF	15.60^{a} ±0.10	1.44^{d} ±0.02	14.00^{de} ±0.10	8.48^{d} ±0.02	3.48^{f} ±0.02	1.59^{a} ±0.05	0.063^{c} ±0.01	0.089^{a} ±0.01
DMF90% + 10% CPF	15.30^{b} ±0.09	1.57^{c} ±0.01	15.20^{b} ±0.11	8.57^{b} ±0.01	3.68^{a} ±0.03	1.48^{c} ±0.03	0.070^{a} ±0.02	0.078^{b} ±0.02
DMF 80% + 20% CPF	14.80^{d} ±0.07	1.43^{d} ±0.03	14.00^{de} ±0.12	8.61^{a} ±0.02	3.62^{c} ±0.03	1.41^{e} ±0.04	0.069^{a} ±0.01	0.084^{a} ±0.01
DMF70% + 30% CPF	14.60^{de} ±0.10	1.39^{c} ±0.01	13.80^{f} ±0.13	8.54^{c} ±0.01	3.58^{d} ±0.02	1.38^{e} ±0.02	0.066^{ab} ±0.02	0.087^{a} ±0.02
Lsd$_{0.05}$	0.1961^{**}	0.0291^{*}	0.2904^{**}	0.0215^{*}	0.0272^{*}	0.0308^{*}	0.0135^{*}	0.0148^{*}
SE±	0.0054	0.0152	0.0181	0.0167	0.0193	0.0165	0.0021	0.0025

Mean(s) values ± SD having different superscript(s) in a column are significantly different ($P \leq 0.05$) according to DMRT.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

its treated breads with 10, 20, and 30%levels chickpea flour were 291.67, 273.33, 305.0 and 318.33cm^3, respectively.

The results showed loaf weight increased significantly ($P \leq 0.05$) in all samples with increasing level of chickpea flour(0,10,20 and 30%), whereas, loaf specific volume of breads produced was 2.79, 2.61, 2.55 and 2.54cm^3/gm for sorghum bread with chickpea flour, respectively. Likewise, bread specific volume was 2.43, 2.11, 2.30 and 2.46 cm^3/gm for maize bread with chickpea flour (0, 10, 20 and 30%), respectively. Loaf specific volume of breads produced decreased significantly ($P \leq 0.05$) with increasing chickpea flour in the bread formulation in both sorghum and maize. But DMF 70%+ 30% CPF loaf specific volume showed no significant ($P \leq 0.05$) difference, from the results the main reason was the high level of chicpea incorporated. Nunes, *et al.* (2009), showed that it did not have such a positive impact on loaf specific volume for gluten free breads. According to Sciarini, *et al.*(2010), gluten – free breads have a smaller specific volume, with an average of 2.32cm^3 /gm than wheat bread(average specific volume of 4.41cm^3/gm). Generally, our sorghum and maize breads and their treatments breads with chickpea flour had a higher loaf specific volume compared to the average gluten free breads reported by Sciarini, *et al.* (2010).

4.4.6 Sensory charactersitics of gluten-free tin bread

The organoleptic properties of gluten free bread (Tin Bread) produced from sorghum and maize incorporated with 0, 10, 20 and 30% level chickpea flour was presented in Table 20 and Plate 3. One of the limiting factors for the consumer acceptability is the organoleptic properties therefore; color, odor, taste, crumb texture, crumb grain and general acceptability were performed.

Table 19 Loaf specific volume of gluten – free tin bread

Samples	Loaf volume cc	Loaf weight gm	Loaf specific volume cc/gm
DSF	338.33^{a} (±7.64)	121.07^{b} (±0.76)	2.79^{a} (±0.06)
DSF90% + 10% CPF	336.67^{ab} (±5.77)	128.70^{a} (±1.18)	2.61^{b} (±0.05)
DSF80% + 20% CPF	340.00^{a} (±10.00)	132.93^{a} (±2.41)	2.55^{bc} (±0.06)
DSF70% + 30% CPF	340.00^{a} (±0.00)	133.83^{a} (±1.10)	2.54^{bc} (±0.02)
DMF	291.67^{de} (±14.43)	119.90^{b} (±1.21)	2.43^{c} (±0.11)
DMF90% + 10% CPF	273.33^{e} (±7.64)	129.63^{a} (±2.61)	2.11^{e} (±0.03)
DMF 80% + 20% CPF	305.00^{cd} (±17.32)	132.43^{a} (±3.93)	2.30^{d} (±0.11)
DMF70% + 30% CPF	318.33^{bc} (±12.58)	129.50^{a} (±5.41)	2.46^{c} (±0.08)
Lsd$_{0.05}$	18.53^{**}	4.816^{**}	0.1224^{*}
SE±	6.18	1.606	0.04082

Mean(s) values ±SD having different superscript(s) in a column are significantly different ($P \geq 0.05$) according to Duncan's multiple range test.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

Table 20 showed that the results of color scores of bread produced from maize incorporated with chickpea flour with different levels ranged between 6.14 and 6.79, and it had been accepted. No significant differences ($P \leq 0.05$) were obtained, but significant differences ($P \leq 0.05$) were noted comparing with decorticated maize bread without chickpea flour, decorticated sorghum bread and its treated bread with chickpea flour. Likewise, the entire sample was accepted. Odor scores ranged between 3.64 and 5.71 score no significant differences ($P \leq 0.05$) were noticed compared with sorghum and maize breads without chickpea flour. The bread products had accepted values of taste comparing with sorghum and maize bread without chickpea flour, no significant differences were observed. Crumb texture and crumb grain of breads produced had been accepted. The scores of crumb texture and crumb grain of breads produced showed no significant difference ($P \leq 0.05$) observed compared with sorghum and maize breads scores. General acceptability of breads produced showed no significant differences were noticed, that means all samples treated studied were accepted. The mean comparison of scores of different attributes like texture, flavor, taste, appearance, mouth feels and over acceptability were recorded and found to be non-significant differences with treatment group (Dhore, 2011).

4.4.7 Acceptability of gluten free tin bread among celiac disease patients

Sensory evaluation has been widely used in the field of organoleptic quality of food products. The evaluation of gluten free product (tin bread) has been done by the celiac disease patients to determine suitable constituents for high quality bread, for themselves. Sensory attributes evaluated included colour, odour, taste, crumb texture, crumb grain and general acceptability using a 9 point hedonic scale. Sensory evaluation of gluten free (tin bread) made from decorticated sorghum and maize supplemented with different levels of chickpea flour 0, 10, 20, and 30% in the bread formulation presented in Table 21 and Plate 3.

Table 20 Sensory characteristics of gluten – free tin bread

Samples	Colour	Odour	Taste	Crumb texture	Crumb grains	General acceptability
DSF	3.21^{b} ±2.42	4.57^{ab} ±3.11	4.29^{ab} ±2.95	3.86^{a} ±2.63	4.36^{c} ±2.73	4.50^{ab} ±3.25
DSF90% + 10% CPF	3.07^{b} ±1.98	3.64^{b} ±2.44	3.29^{b} ±1.64	4.21^{a} ±2.67	4.36^{bc} ±2.31	3.36^{b} ±2.37
DSF80% + 20% CPF	3.71^{b} ±2.13	3.71^{ab} ±2.09	3.71^{ab} ±1.86	4.07^{a} ±1.90	4.50^{abc} ±1.91	4.00^{ab} ±2.29
DSF70% + 30% CPF	4.14^{b} ±1.56	4.00^{ab} ±1.80	4.36^{ab} ±1.82	4.14^{a} ±1.61	4.64^{abc} ±1.65	4.29^{ab} ±1.90
DMF	6.79^{b} ±2.01	5.43^{ab} ±2.59	5.50^{a} ±2.35	5.43^{a} ±2.10	5.36^{ab} ±2.02	6.07^{a} ±2.27
DMF90% + 10% CPF	6.21^{a} ±1.97	5.21^{ab} ±2.15	4.93^{ab} ±2.09	5.00^{a} ±2.18	5.71^{abc} ±2.09	5.36^{ab} ±2.47
DMF 80% + 20% CPF	6.50^{a} ±1.61	5.71^{a} ±2.16	5.07^{ab} ±2.16	5.00^{a} ±2.39	5.64^{abc} ±2.10	5.64^{a} ±2.41
DMF70% + 30% CPF	6.14^{a} ±1.83	5.14^{ab} ±2.07	4.71^{ab} ±2.16	5.36^{a} ±2.50	5.43^{a} ±2.24	4.93^{ab} ±2.37
Lsd$_{0.05}$	1.466^{*}	1.748^{*}	1.62^{*}	$1.704^{n.s}$	$1.613^{n.s}$	1.83^{*}
SE±	0.5227	0.6234	0.5777	0.5442	0.5753	0.6525

Mean(s) values ±SD having different superscript(s) in a column are significantly different (P≥0.05) according to Duncan's multiple range test.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

Colour scores ranged from 5.56 to 8.56. The evaluating of celiac patients has a positive reflection in widely acceptance. Therefore, odour scores ranged between 4.11 and 7.56 and it is considered good. These results indicated that odour of gluten free, tin bread, has been accepted. Chickpea flour incorporated with the bread ingredients in the formulation has a positive effect on the final acceptance. Taste values of gluten free tin bread ranged from 4.67 to 7.67. The taste was accepted by the celiac patients. Taste was considered important factor in the process of evaluation. If it was accepted that lead to accept the final product. Likewise, crumb texture values of gluten free (tin bread) ranged between 5.33 and 7.89. The results showed that no significant differences were noted, compared with sorghum and maize breads with absence of chickpea flour in the bread formulation. It's clear that maize bread and its supplementations were more accepted than the sorghum bread and it's treated with chickpea flour in the bread formulation. Crumb grain values ranged between 4.22 to 8.0 score. The results showed no significant differences were noticed. From the results crumb grain was accepted by the celiac patients. On the other hand, general acceptability of bread produced from the gluten free values ranged between 4.22 to 8.22score. No significant differences were observed. The results indicated all the samples tested were accepted. Adding chickpea flour in the bread recipe of gluten free enhance the nutritional value of the final product, so that may help and encourages creating new products from gluten free cereals.

Table 21 Acceptability of gluten free tin bread among celiac disease patients

Sample	Colour	Odour	Taste	Crumb texture	Crumb grain	General acceptability
	Scores					
DSF	5.56^{c} ±2.60	4.11^{b} ±1.76	4.67^{b} ±1.22	5.44^{b} ±2.70	4.22^{c} ±1.99	4.67^{b} ±2.24
DSF90% + 10% CPF	5.56^{c} ±1.88	4.22^{b} ±2.05	4.67^{b} ±1.73	5.44^{b} ±2.35	4.44^{c} ±2.19	4.22^{b} ±2.59
DSF80% + 20% CPF	6.44^{bc} ±2.79	6.00^{ab} ±2.18	6.00^{ab} ±1.87	5.67^{ab} ±3.28	5.67^{bc} ±2.74	6.22^{ab} ±2.68
DSF70% + 30% CPF	6.89^{abc} ±1.62	4.67^{b} ±2.35	4.89^{b} ±2.71	5.33^{b} ±2.78	5.11^{bc} ±2.03	4.78^{b} ±2.28
DMF	8.22^{ab} ±0.83	7.56^{a} ±1.13	7.33^{a} ±1.12	7.11^{ab} ±1.69	7.78^{a} ±1.20	7.89^{a} ±1.05
DMF90% + 10% CPF	7.78^{ab} ±1.09	7.11^{a} ±0.33	7.67^{a} ±0.87	7.11^{ab} ±0.93	7.67^{a} ±0.50	7.89^{a} ±0.60
DMF 80% + 20% CPF	8.56^{a} ±0.73	7.22^{a} ±1.86	7.67^{a} ±1.00	7.89^{a} ±1.36	8.00^{a} ±0.71	8.22^{a} ±0.83
DMF70% + 30% CPF	8.33^{a} ±1.12	6.89^{a} ±2.42	6.89^{a} ±2.42	7.22^{ab} ±1.99	6.78^{ab} ±2.11	7.44^{a} ±2.35
$Lsd_{0.05}$	1.643^{*}	1.77^{*}	1.639^{*}	2.128^{*}	1.729^{*}	1.877^{*}
SE±	0.5817	0.6264	0.58	0.7531	0.6121	0.6643

Mean(s) values ±SD having different superscript(s) in a column are significantly different ($P \geq 0.05$) according to Duncan’s multiple range test.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

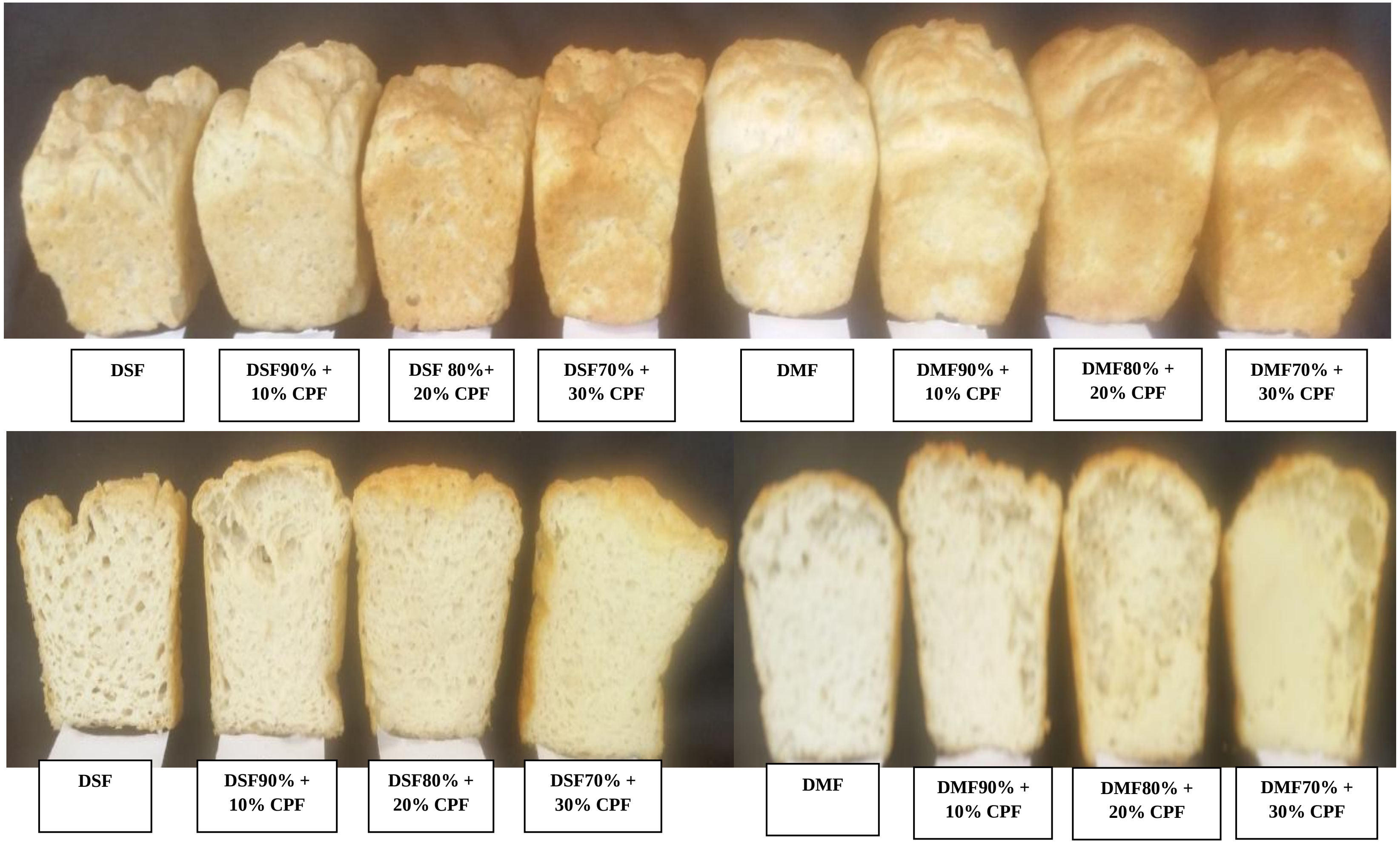

Plate (3): Tin bread made from decorticated sorghum and maize supplemented with 0, 10, 20, and 30% chickpea in the bread formulation.

Key: DSF= Decorticated sorghum flour/ DMF= Decorticated maize flour/ CPF= chickpea flour

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1Conclusions

- Gluten free products (biscuits and breads) were made from decorticated sorghum and maize supplemented with chickpea flour with different ratios (10%, 20%, and 30%) with maize being the best.
- Adding chickpea flour to the product formulation enhanced protein content, protein and starch digestibilities and some minerals.
- Sensory evaluation panalist's results showed all samples of decorticated maize and sorghum breads (balady and tin) and biscuits tested were acceptable.
- Celiac disease patient's acceptability results indicated that all biscuits, tin and balady breads samples were accepted.
- Specific volume of sorghum bread without chickpea flour was better than the other breads produced.

5.2 Recommendations

From this study it could recommended that:

- Acceptable gluten free biscuits and breads (tin and balady) could be made from decorticated sorghum or maize supplemented with chickpea.
- Further study is needed to investigate the safty of the products for celiac disease patients (in vivo).
- Further studies are needed to envestigated the keeping quality of gluten free products.
- Microbiological studies of gluten free products are demanded to determine the shelf life of the product.
- Further studies are recommended to investigate the utilization other local cereals and legumes to produce gluten free products.

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Appendices

1. Sensory evaluation of biscuits

Please examine the following samples of biscuits presented in front of you, and give values to attributes on the form sheet, taking: (8-9) as excellent, (6-7) as very good, (4-5) as good, (2-3) as fair and (1) as poor:

Evaluation

Sample	Colour	Odour	Taste	Texture	General acceptability
DSF					
DSF90% + 10% CPF					
DSF80% + 20% CPF					
DSF70% + 30% CPF					
DMF					
DMF90% + 10% CPF					
DMF 80% + 20% CPF					
DMF70% + 30% CPF					

2. Sensory evaluation of tin bread

Please examine the following samples of Tin Bread presented in front of you, and give values to attributes on the form sheet, taking: (8-9) as excellent, (6-7) as very good, (4-5) as good, (2-3) as fair and (1) as poor:

Evaluation

Sample	Colour	Odour	Taste	Crumb texture	Crumb grains	General acceptability
DSF						
DSF90% + 10% CPF						
DSF80% + 20% CPF						
DSF70% + 30% CPF						
DMF						
DMF90% + 10% CPF						
DMF 80% + 20% CPF						
DMF70% + 30% CPF						

3. Sensory evaluation of balady bread

Please examine the following samples of balady bread presented in front of you, and give values to attributes on the form sheet, taking: (8-9) as excellent, (6-7) as very good, (4-5) as good, (2-3) as fair and (1) as poor:

Evaluation

Sample	Colour	Odour	Taste	Crumb texture	Crumb grains	General acceptability
DSF						
DSF90% + 10% CPF						
DSF80% + 20% CPF						
DSF70% + 30% CPF						
DMF						
DMF90% + 10% CPF						
DMF 80% + 20% CPF						
DMF70% + 30% CPF						

Appendice 4 Balady bread from decorticated sorghum and maize supplemented with different level of chickpea flour (10, 20, and 30%).

Appendice 5 Balady bread from decorticated sorghum and maize supplemented with different level of chickpea flour (10, 20, and 30%).

Appendice 6 Balady bread from decorticated sorghum and maize supplemented with different level of chickpea flour (10, 20, and 30%).