

DEVELOPMENT AND IMPROVEMENT OF SORGHUM-BASED GLUTEN-FREE  
DINNER ROLLS

by

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# Abstract

Despite the expansion of the gluten-free (GF) food market, some GF food items are still characterized by an overall mediocre quality. The effects of different types of egg ingredients (fresh, whites, dried) and carob germ flour (CGF) as well as par-baking technology on the quality of dough-based gluten-free sorghum dinner rolls were evaluated. Gluten-free rolls containing 30% of fresh shell eggs or equivalent of egg products and 10% of CGF on a flour basis were evaluated against a control (no egg, no CGF). The feasibility of partial baking of rolls was studied on control as well as fresh eggs and carob germ flour formulas during 5 baking times (0, 8, 10, 12 and 18 minutes). Breads were evaluated for crumb and crust color, specific volume, cell profile, Texture Profile Analysis (TPA) and consumer acceptability. Results showed that rolls containing egg ingredients had higher specific volumes than control ( $p < 0.05$ ) with an increase from  $1.45 \text{ cm}^3/\text{g}$  to  $1.96 \text{ cm}^3/\text{g}$ . Carob germ flour did not have a significant effect on specific volume. Eggs also improved cell elongation and produced significantly darker crust ( $p < 0.05$ ). CGF did not appear to have an effect on cell elongation but increased average cell number when combined with egg ingredients, and greatly impacted rolls texture. The combination of fresh eggs or egg whites with CGF reduced significantly ( $p < 0.05$ ) crumb hardness from 2,074 to 1,404 g and 1,468g of force respectively. Par-baked dinner rolls displayed similar color, volume, cell profile and texture trends to conventionally baked rolls. Sensory study revealed that acceptability, organoleptic characteristics and willingness to buy of par-baked dinner rolls could be similar to that of conventional wheat products. This research proved that the addition of eggs and CGF to a GF rolls formulation resulted in better overall quality of the product. Moreover, par-baking of the rolls showed great potential to provide safe, convenient and acceptable GF foods to celiac individuals.

Keywords: gluten-free, eggs, carob, partial baking, sensory, texture profile analysis.

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# INTRODUCTION

Wheat has been used for centuries as a major source of food in the human diet. This use is highly related to its protein content. Indeed, wheat proteins give the dough the ability to form strong visco-elastic properties when mixed with water. This dough traps leavening gas and produces light, aerated baked products. This ability is linked to the wheat storage proteins contained in the starchy endosperm. This protein fraction is called gluten and is contained in wheat, barley and rye (Hoseney, 2010).

Celiac disease, also known as celiac sprue or gluten-sensitive enteropathy, is an immune-mediated enteropathy triggered by the consumption of gluten proteins by genetically sensitive individuals causing severe damage to the intestinal tract. For such persons, the only treatment available is to follow a strict gluten-free diet (Sollid and Lundin, 2009).

As in most western developed countries, wheat and other gluten containing cereals account for the majority of the dietary caloric intake. Safe alternative grains and foods for celiac, gluten intolerant and wellness seeking individuals have been developed and introduced on the market. The lack of safety, availability, quality, and price accessibility of commercial gluten-free (GF) products are some of the factors pushing food companies to conduct research on production of acceptable food products.

Food scientists and students of the Food Science Institute (FSI) at Kansas State University (KSU) and the United States Department of Agriculture, Agriculture Research Service Center for Grain and Animal Health Research (USDA-ARS CGAHR) in Manhattan, Kansas, have recently successfully studied the use of sorghum for common GF food application (i.e. GF bread).

The present research is a follow-up study to previous work conducted in collaboration between the FSI of KSU and the USDA-ARS CGAHR. The subject is the development of sorghum based, gluten-free dinner rolls focusing on the impact of different type of egg ingredients and carob germ proteins on GF products quality. Assessment of product qualities and determination of the impact of the two additives on the rolls were performed by evaluation of texture, color, density, cell structure, and sensory analysis.

This report will be divided in various parts starting with a literature review covering the role of gluten in bakery and snack products, the different aspects of the gluten-related

celiac disease, the description of sorghum grain and its utilizations, the GF market in the USA, GF technology, and, finally, the functionality and uses of carob germ proteins and eggs in GF food applications. Following the literature review section, the materials and methods used in the research will be detailed. The next part will present results and interpretations of the different tests and analysis. Finally, the outcomes of the research will be detailed followed by a conclusion.

# **PART I: CONTEXT AND LITERATURE REVIEW**

## **I. Context and objectives of the study**

### **A. Study's context**

This Master's research was a collaborative work conducted jointly between the Food Science Institute (FSI) at Kansas State University (KSU) and the United States Department of Agriculture, Agriculture Research Service Center for Grain and Animal Health Research (USDA-ARS CGAHR) located in Manhattan, KS.

Gluten-free food research and development has attracted very little interest among food scientists compared to the more conventional gluten-containing counterparts. For instance, wheat-based products such as bread, cakes and other bakery products have been the subject of a myriad of very diversified and specific research. The United States of America has substantial production of sorghum. Yet, its main uses had been mostly confined to animal feed and industrial applications.

Therefore, cooperation between the FSI and the USDA on gluten-free projects came logically from a push by the Kansas Sorghum Commission in 2005, which was looking for novel usages of food grade sorghum grain. The collaborative research benefits the two institutions. While the graduate students gain access to the USDA facilities and equipment necessary to conduct their Master's or Ph.D. research, they are incorporated into a research team to support ongoing USDA projects. The research mainly focuses on studying sorghum uses in food products as well as gluten proteins and its substitutes. The research team is composed of three scientists at the USDA CGAHR, Dr. Thomas Herald, Dr. Scott Bean and Dr. Brennan Smith as well as Dr. Fadi Aramouni and a Master's student from the FSI. The studies are either the base for scientific publication and possibly oral presentation or for Master's or Ph.D. research projects. Research members often participate in annual scientific conventions such as the American Association of Cereal Chemists (AACC), or the Institute of Food Technologists (IFT) conventions to present their work.

Academic research are entirely conducted by the students under the supervision and mentorship of their professors. This research group's main objective is to study, develop and

improve gluten-free products using food grade sorghum and functional ingredients such as carob germ proteins.

Obtaining financial support for such scientific projects is both complicated and necessary. The United Sorghum Checkoff Program (USCP), which is a national organization for sorghum growers and producers, has been the major financial sponsor over the past few years. Every year, there is an annual call for proposals based on the agenda set by the USB. Grants are then allotted to researchers on a competitive basis. Additional financial support can come from other entities such as the American Egg Board (AEB) and government agencies such as the Kansas Department of Agriculture (KDA) and the USDA. The AEB, is a United States marketing board, which focuses on marketing and promotion of eggs for human consumption. Because eggs were used to improve gluten-free bakery products, the research was also partially funded by the AEB. This present research follows many others studies on sorghum and gluten-free food products conducted at KSU. It is a logical follow-up study as it is based and inspired by the relevant findings of these scientists.

The first study conducted by Fernholz (2008) focused on sorghum grain as well as flour characterization of four sorghum hybrids based on physical, chemical, textural, and sensory analysis of flour tortillas. In 2009, Frederick examined the effects of sorghum flour composition and particle size on functionality in gluten-free batter bread. The principal finding was that sorghum flours with lower amounts of fiber and a smaller particle size produced more satisfactory batter-based breads. The study by Marston (2009) evaluated the effects of heating and ozone treatments on sorghum flour functionality in gluten-free bread and cake. Smith (2009), characterized the biochemical, physical and baking properties of caroubin (main carob germ protein). Smith analyzed the physical properties of carob germ protein-maize starch dough and found that the dough's functionality was dependent on disulfide bonded protein networks, similar to what is found in wheat gluten. Furthermore, when baked into a bread carob germ proteins decreased staling in gluten-free breads.

Finally in 2012, two studies were conducted by KSU Master's students. Bize (2012) studied the effect of fresh shell eggs as well as an antistaling agent on the overall quality and staling rate of batter-based gluten-free sorghum bread. It was determined that eggs contributed in delaying staling and better overall quality and acceptability of the bread. Lastly, Pruett (2012) compared the glycemic index of sorghum muffins with that of muffins made from other commonly consumed grains.

## **B. Study's objectives**

Awareness of Celiac disease and gluten intolerance has increased by consumers and food companies in the USA. Because the only known treatment to cure this genetically induced illness is a strict gluten-free diet, numerous GF products have been developed (Lazaridou *et al.*, 2007; Schober *et al.*, 2007; Smith, 2009; Bize, 2012; Sciarini *et al.*, 2012).

However, according to many sources, some improvements still need to be made to have more palatable GF products launched on the market. Because the main challenge in GF technology is the absence of gluten, egg products and carob germ flour were used as they were proven in previous studies (Mine, 2002; Bengoechea *et al.*, 2008; Smith, 2009; Bize, 2012) to provide desirable textural properties to GF breads. Partial baking technology was employed to determine if it was reliable to produce acceptable GF goods.

The main objective of this study was to develop, improve and make readily available gluten free dinner rolls using egg ingredients and carob germ protein. The study consisted of two major phases. Firstly, common physical characteristics were tested as indicators of the product's quality and included texture profile, color, specific volume, and crumb cell profile analysis. These characteristics were tested on fully baked and eventually partially baked products. Secondly, sensory testing using a large consumer panel was performed in order to determine the acceptance of the products.

The hypothesis regarding this study was that egg proteins and carob germ proteins would, by synergy, help obtain dough-like intermediary products that eventually could be easily shaped into desired final products. Partial baking and freezing would then allow alleviating the strong staling phenomenon occurring in all GF bakery products.

## **II. Gluten proteins and Celiac disease**

### **A. Gluten proteins**

#### **1. Description**

The main protein fraction in wheat related to visco-elastic dough formation is commonly called gluten (prolamin proteins rich in glutamine and proline). The wheat gluten proteins correspond to the major storage proteins (80-90% of the total proteins in flour) that are deposited in the starchy endosperm cells of the developing grain. These form a continuous proteinaceous matrix in the cells of the mature dry grain and are brought together to form a continuous viscoelastic network when flour is mixed with water to form dough (Shewry *et al.*,

2002). With the exception of rye that shares at a lower level this ability, there are no other cereal flours that can be mixed with water to reproduce this viscoelasticity.

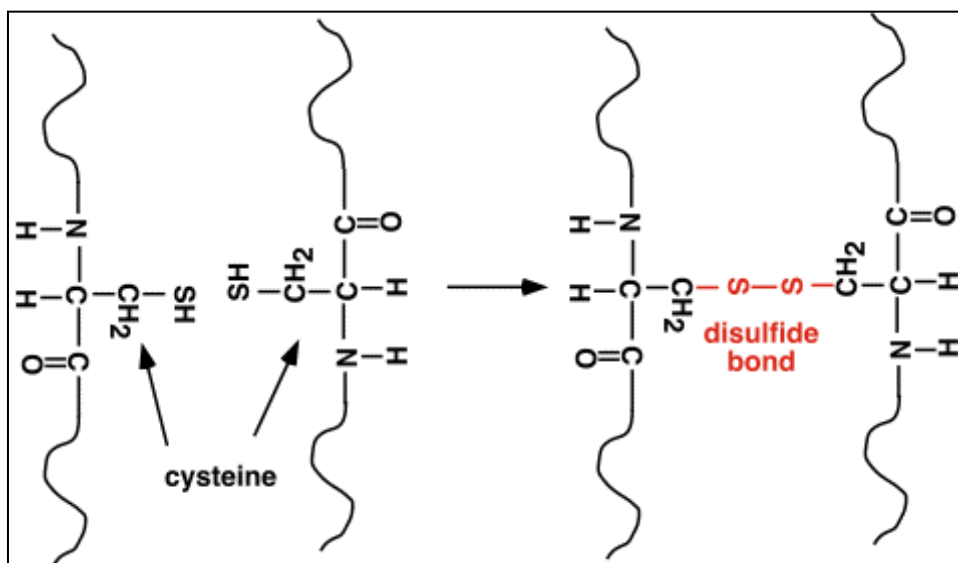
Cereal scientists (Lafiandra *et al.*, 2004; Dexter *et al.*, 2006; Wieser, 2006; Wang *et al.*, 2007; Hoseney, 2010) affirmed that the gluten proteins are responsible for this cohesive and viscoelastic ability of wheat flour dough as well as its properties to retain gas during the fermentation step and to set the dough during baking. The gluten proteins are made of a highly complex mixture of proteins (Hoseney, 2010). They are easy to isolate in relatively pure form due to their high insolubility in water. In 1745, Beccari was the first cereal scientist to describe wheat gluten isolation: the water soluble components and the starch can be removed from the gluten by an appropriate working of the dough under a continuous stream of water, after this washing, a rubbery aggregate of gluten is obtained. According to Marconi *et al.* (2003), gluten, also commonly referred to as vital wheat gluten, is used to improve the technological and rheological performances of flour and more precisely weak flours.

Gluten is separated in two main groups according to their solubility in aqueous alcohols: gliadins and glutenins. These two fractions play an important role in rheological properties of wheat dough even if their functions are not similar at all. Gliadins are a large group of single chained proteins that essentially contribute to the viscosity and extensibility of the dough system. Glutenins proteins are larger polymers than gliadins and play a crucial role in the bread-making process (Shewry *et al.*, 2002).

## **2. Disulfide bonds and role on structure and properties of gluten proteins**

To understand what disulfide bonds are, it is important to know the basic properties of proteins. Proteins are naturally occurring polymers that can be found in all living organisms including cereals (Hoseney, 2010). They are formed by amino acids linked together with peptides linkages. Protein molecules are classified into twenty-one different amino acids (Fox and Cameron, 1986). All amino acids have an amino and acid group while the R group (functional group) structure differs. Each amino and acidic group of amino acids is part of a peptide bond and form the backbone of the protein. The R group is not involved in the peptide bond. The difference between amino acids R groups allows classification. This sequence of amino-acids forming a protein is called the primary structure. These peptides bonds are relatively flexible which allows the polypeptide to twist or curl when subject to molecular interaction. Secondary and tertiary structure formation follows (Hoseney, 2010).

Disulfide bondage is one of these molecular interactions (covalent intra-molecular and inter-molecular bond). One amino-acid plays a major role in disulfide bondage: cysteine. Cysteine amino acids present sulfhydryl group that can react with another cysteine residue and form a disulfide bond (Figure 1). This covalent bond that joins the polypeptidic chain gives the protein its secondary structure. The disulfide bonds can allow the protein to form a loop when the two cysteine residues are on the same protein; or to link two proteins together when sulfhydryl groups are located on two different proteins.



**Figure 1: The formation of the disulfide bond (Shewry *et al.*, 2002).**

Lavelli *et al.* (1996), showed intra- and inter-molecular disulfide bonds within gluten proteins are important in forming the gluten matrix in dough. Spectroscopic studies (Shewry *et al.* 1992) of high molecular weight glutenin subunits and of model peptides based on the repeat motifs suggests that non-covalent hydrogen bonding between glutenin subunits and polymers may also be important.

### **3. Characterization of the two different gluten protein fractions**

The gluten proteins can be divided in two groups according to their solubility in different solvents (Osborne fractionation of proteins, 1903). According to Hosene (2010), the two types of proteins can be easily separated by solubilizing the gluten in a diluted acid. Ethyl-alcohol is then added to make this solution 70% alcohol. Enough base reagent is added



for neutralization of the solution and cause glutenin proteins to precipitate. The gliadin fraction is left behind in the solution.

#### **a) Gliadin fraction**

According to Uthayakumaran *et al.* (2001), gliadins represent more than a half of the gluten proteins. Hoseney (2010) defined gliadins as being a large group of protein having similar properties. The average molecular weight of the single chain structure is about 40,000 daltons and the protein is very sticky when hydrated. They have almost no resistance to extension and are believed to be responsible for the cohesiveness of dough.

Most gliadins are present as monomers; they were initially classified into four groups ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\omega$ ) on the basis of mobility at low pH in gel electrophoresis (Wieser, 2006). Within each fraction, structural variations and differences are small due to substitution, deletion, and insertion of single amino acid residues (Wieser, 2006). The  $\alpha$ ,  $\beta$  and  $\gamma$  gliadins are sulfur-rich and the  $\omega$ -gliadins are sulfur-poor (Shewry and Tatham, 1997). Gliadins mostly interact by hydrogen bonding and hydrophobic interactions.

Gliadins contribute to give extensibility to the dough, allowing it to flow during fermentation and baking (Uthayakumaran *et al.*, 2000). Gliadin-supplemented doughs generally have a shorter mixing time, greater resistance to breakdown, lower maximum resistance to extension, and a decreased loaf volume (Wieser, 2006). These claims were confirmed by experiments conducted by Uthayakumaran *et al.* (2000) and showed that all gliadin fractions reduced mixing time, peak resistance, maximum resistance to extension, and loaf height, while resistance to breakdown and extensibility were increased. According to this same study, the  $\omega$ -gliadin fraction had the most effect on loaf height and on breakdown resistance. Some other cereal scientists also tended to say that gliadins are complementary to glutenins and can reinforce the effects of glutenins in bread-making processing (Uthayakumaran *et al.*, 2000; Lafiandra *et al.*, 2006; Wieser 2006).

#### **b) Glutenin fraction**

Glutenin consist of polypeptides that are cross-linked by interpolypeptide disulfide bonds and are the most important fraction related to bread-making quality (Johansson and Svensson, 1995). The glutenin fraction comprises aggregated proteins linked by interchain disulphide bonds; they have varying size ranging from about 500,000 to more than 20 million daltons (Wieser, 2006). Thus, a part of glutenins belong to the largest proteins in nature. The

molecular weight distribution of glutenins has been recognized as one of the main determinants of dough properties and baking performance.

Moreover, a certain amount of these polymers remain unextractable in various extracting systems. Those unextractable polymeric proteins appear also to be correlated with baking performance (Kleiber *et al.*, 2001). In addition, Gupta *et al.* (1992) showed that the unextractable polymer quantity is more directly linked with certain technological parameters (especially those correlated with mixing) than the total glutenin quantity. The proportion of unextractable polymer fraction among the glutenin polymers appears to be an important ratio for technological response.

According to their relative mobilities on electrophoresis gels (Dong *et al.*, 2009), glutenins are classically divided into high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS). The separation can be done using the technique of the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) which separates proteins according to electrophoretic mobility (Weber and Osborn, 1969).

i. *Low Molecular Weight Glutenin subunits (LMW-GS)*

LMW-GS are present in gluten at about three times the quantity of the HMW-GS, but their distribution amongst other polypeptides, such as gliadins, makes them hard to analyze with SDS gel electrophoresis (Kleiber *et al.*, 2001). Their weight ranges from 10,000 to 70,000 daltons (Gianibelli *et al.*, 2001). LMW-GS tend to form intermolecular disulfide bonds to reinforce the gluten matrix during bread-making. But, LMW-GS present only a total of eight cysteine residues (important parameter to consider as they can be at the origin of the formation of intra-molecular and inter-molecular disulfide bonds).

Masci *et al.* (1998) study using DNA sequencing affirmed there are two main types of LMW-GS defined on the basis of N-terminal amino acid sequences: LMW-s types and LMW-m types. The LMW-s types are the most present in wheat gluten. They tend to have higher molecular weights, in the approximate range of 35,000 to 45,000 daltons. The LMW-m types, seem to fall into a wider molecular-weight range of about 30,000 to 45,000 daltons (Masci *et al.*, 1998). LMW-s and LMW-m types are both coded by genes present at the complex *Glu-3* loci (Kleiber *et al.*, 2001).

On the basis of Masci *et al.* (1998) experimentations, and those in literature, it seems possible that both LMW-m and LMW-s types have two cysteine residues available for the formation of intermolecular disulfide bonds. Nevertheless, classification of LMW-GS as the

LMW-m and LMW-s types probably has no essential importance in itself with regard to dough quality.

Yet LMW-GS play an important role in gluten structure. However, this role has attracted relatively little interest in the literature, mostly due to the difficulty of studying this quantitatively important family of gluten proteins. This is mostly related to the fact that LMW-GS derive from many more genes than HMW-GS. For Masci *et al.* (1998), if both main types of LMW-GS operate as chain extenders and just two cysteine residues are available for intermolecular disulfide-bond creation, then the positive correlation between the relative abundance of the LMW-s types and the good end-use quality of flours for bread-making has to be attributed to their higher level in good-quality flours rather than to intrinsic structural characteristics. They also suggested the longer repeated regions in LMW-GS improve the contributions to dough strength and elasticity relative to subunits with smaller-sized repeated-sequence domains. Thus, the two cysteine residues available for intermolecular disulfide bond formation seem to determine how these similar types of subunits affect properties significant to good quality.

#### ii. *High Molecular Weight Glutenin subunits (HMW-GS)*

The emphasis on the HMW-GS initially arose from their accessibility for analysis (even if they are minor components in terms of quantity). The weight of HMW-GS range from 80,000 to 200,000 daltons (Gianibelli *et al.*, 2001) and are only present in glutenin polymers. Indeed, they appear on the top of the electrophoresis gel and are extremely well separated from all the other polypeptide bands. The research focus on the HMW-GS has proved to be justified, as they tend to be particularly important components of the gluten complex (Shewry *et al.*, 1992; Shewry *et al.*, 2002; Lafiandra *et al.*, 2004).

In the context of improving protein quality in wheat flours (to provide elasticity and a good strength to the dough), research has shown the importance of glutenin proteins with an emphasis on subunits of high molecular weight (HMW) subunits, particularly those controlled by the D genome (Gianibelli *et al.*, 2001).

#### c) **General role of glutenin proteins in bread-making quality**

Gluten proteins are able to form a continuous protein matrix in the cells of the mature dry grain and can be brought together when water is added to wheat flour and mixed to form a continuous viscoelastic network: wheat flour dough. These viscoelastic properties are widely used in bread-making and other food processing. The precise changes that occur during

dough mixing are still not totally understood, but an increase of dough stiffness is generally considered to result from an optimization of interactions between proteins within the gluten network (Shewry *et al.*, 2002). This optimization may include some exchange of disulphide bonds with aeration because oxygen and nitrogen impart different effects on the sulphydryl and disulphide contents of wheat dough (Lafiandra *et al.*, 2006).

Uthayakumaran *et al.* (2000) conducted rheological experiments on wheat flours doughs. It appeared that the elastic elongation and strength of the dough is related to the amount of glutenin proteins contained in the dough. The presence of higher quantities of glutenins in the dough serves as a greater reservoir for the three-dimensional protein matrix development and imparts the gas retaining properties of the dough necessary for good baking quality. One fraction of gluten proteins, HMW subunits of glutenin, is extremely important in conferring high levels of elasticity and dough strength. These proteins are present in HMW polymers that are stabilized by disulphide bonds and are considered to form the elastic backbone of gluten.

The HMW glutenin subunits have been reported to account for only 12% of the total grain protein, corresponding to 1 to 1.7% of the flour dry weight (Johansson and Svensson, 1995). However, variation in the amount of HMW subunits and the properties of expressed subunits have been reported to account for between 45 and 70% of the variation in bread-making performance (Shewry *et al.*, 2002, Don *et al.*, 2006). For Shewry *et al.* (1992), two features of HMW subunit structure may be relevant to their role in glutenin elastomers: the number and distribution of disulphide bonds and the properties and interactions of the repetitive domains due to non-covalent bondings.

Non-covalent bondings like hydrogen bondings in HMW-GS are also at the origin of the elastic backbone of gluten and dough. Various researchers (Shewry *et al.*, 1992; Johansson and Svensson, 1995; Lavelli *et al.*, 1996; Shewry *et al.*, 2002), have shown dry proteins are disordered and have little regular structure, but when hydrated, their mobility increases and  $\beta$ -sheet structures form. More modifications occur if hydration continues because the proteins are made more mobile and turn-like structures are formed at the expense of  $\beta$ -sheets. These observations led to the development of a loop and train model (Shewry *et al.*, 2002). This proposes that the low hydration state presents a lot of protein-protein interactions via hydrogen bonding of glutamine residues in the  $\beta$ -spiral structures. As the hydration level increases the system tends to become plasticized, allowing the orientation of  $\beta$ -turns in adjacent  $\beta$ -spirals to form structures that look like an interchain  $\beta$ -sheet. Further hydration

leads to the breaking of some of the interchain hydrogen bonds in favor of hydrogen bonds between glutamine and water, which then leads to the formation of large loop regions.

However, interchain hydrogen bonds are not completely replaced since the number of glutamine residues is high and the probability of all the interchain bonds breaking simultaneously stays quite low. The result is an equilibrium between hydrated loop regions and hydrogen-bonded chain regions. The ratio between these is completely dependent on the hydration state. The equilibrium between loops and trains may also contribute to the elasticity of glutenin. Extension of wheat dough will result in stretching of the loops and “unzipping” of the “trains”. The resulting formation of extended chains may be a mechanism by which elastic energy is kept in the dough (Shewry *et al.*, 2002; Hernandez *et al.*, 2012), thus providing an explanation for the increased resistance to extension that occurs during dough mixing. Extended chains also play a determinant role in determining wheat bread loaf volume and level of aeration of the finished bread (Johansson and Svensson, 1995).

Hence, chemical bonds linking in HMW-GS have a role on dough elasticity and strength prevent over-inflation and the collapse of dough (Shewry *et al.* 2002; Don *et al.*, 2006; Hernandez *et al.*, 2012). The effects of HMW-GS on dough strength were determined using a mixograph. This instrument measures the energy input during the mixing of dough and is commonly used for wheat flour quality testing globally. Don *et al.* (2006), made the observation that when the dough is mixed the resistance increases up to a certain level, after which it decreases. The increase in resistance may result from a limited exchange of disulfide bonds and formation of the most stable patterns of hydrogen bonding (formation of extensive train regions). In contrast, the subsequent decrease in resistance is supposed to result from disruption of these interactions by overmixing.

Another factor that can be important in determining the role of glutenins in bread-making is the glutenin-to-gliadin ratio. Indeed Uthayakumaran *et al.* (2000) have shown that this criterion could be very important for bread processing.

## **B. Celiac disease**

### **1. Description and mode of action**

Celiac disease (CD) develops from an autoimmune response to specific foods containing gluten (Sollid and Lundin, 2009). This inflammatory affliction is mainly located in the small intestinal mucosa and more precisely in the *lamina propria* area (Green, 2009). Celiac disease patients suffer from various adverse effects and especially damages of the

intestine and malabsorption symptoms, which are related to specific gluten peptide sequences. In addition, gluten ingestion has increasingly been found to be associated with other conditions not usually correlated with gluten intolerance and are called gluten associated diseases (Helms, 2005).

Destruction of the intestinal villi caused by CD promotes malabsorption, with signs and symptoms including diarrhea and fatty stools as well as abdominal pain and distention (Helms, 2005). Similarly, a number of other malabsorption issues can occur in function of the severity of the damage, including growth retardation in children, osteoporosis, and iron deficiency (Hamer, 2005).

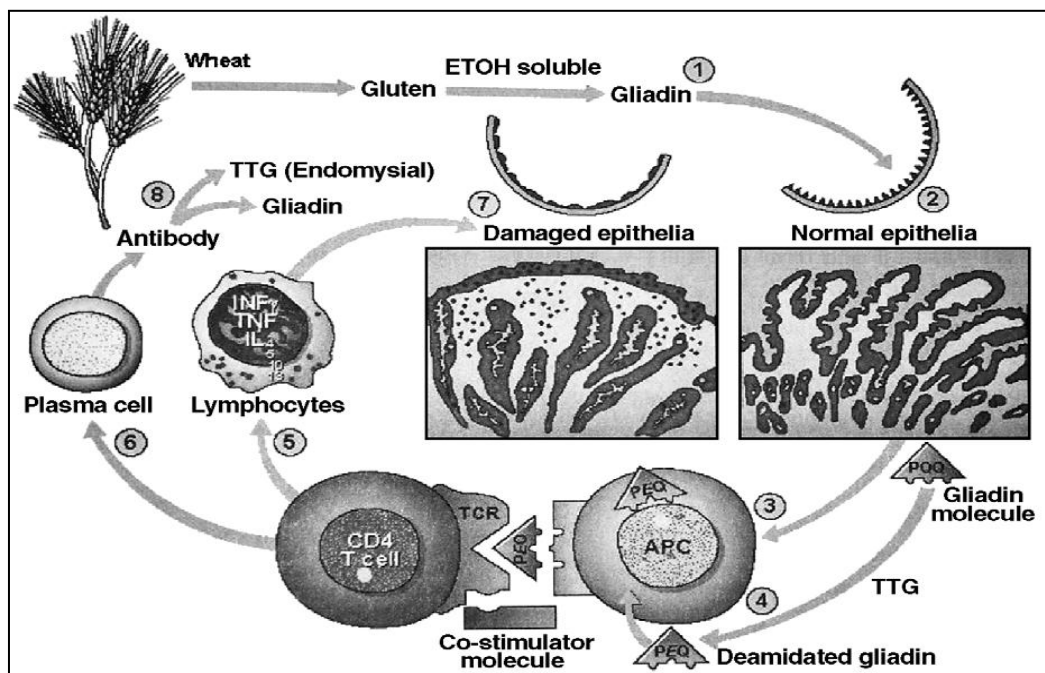
The gluten content of different grains is classified by gliadins (alpha, beta, gamma, omega) or glutenin (high and low molecular weight), with varying concentrations among plant species. The immunogenicity of some gliadins is related to the formation of glutamic acid metabolites from an abundance of proline (P) and glutamine (Q) residues during digestion. Hence, gliadins are believed to produce the strongest immune response in susceptible individuals and have been the subject of the majority of research (Helms, 2005).

Some studies showed that when classified according to their primary amino acid structure, the gliadins and glutenins, while being very different and heterogeneous, do have some homologous repetitive sequences that make them very similar. The relevance of these to CD is demonstrated by their capacity to be stimulatory in assays of immune activity in susceptible patients (Howdle, 2006; Kagnoff, 2007). In such tests, glutenin peptides appeared to be immunostimulatory similarly to gliadin peptides. Glutenin subunits are also more and more suspected to have a direct mucosal toxicity in CD. Components in gluten, both in gliadin and in glutenin, can be considered as responsible for precipitating the abnormalities characteristic of CD (Howdle, 2006).

In the past years, important progress has been made in identifying the mechanism of the toxic reaction in individuals carrying the CD susceptibility alleles HLA-DQ2 and HLA-DQ8 (Figure 2). First, when gluten peptides reach the intestinal epithelium, they are partly deamidated by tissue type transglutaminase (TG2) which is an enterocyte enzyme present in the intestinal mucosa. It is essential to gluten digestion because of the high proline and glutamine content of gluten that makes it resistant to proteolysis by other intestinal enzymes: gastric and pancreatic (Helms, 2005). This deamidation process causes these peptides to be more immunogenic (Metcalf *et al.*, 2003).

Deamidated peptides of gliadin or glutenin are able to bind to an antigen presenting cell (APC) in the subepithelial region of the small intestine (Kagnoff, 2007). The APC cell presents the antigen to a CD4 T-cell, which becomes activated and produces signals that activate both plasma cells (production of IgA-type antibodies and anti-tTG-antibodies) and the proliferation of T-cell. CD4 T-cells activation is thought to start a cascade of reactions leading to the damage of the intestinal epithelium (Hamer 2005; Kagnoff 2007). T-cell clones release tumor necrosis factor (TNF) and other pro-inflammatory cytokines. Cytokines induce a migration of lymphocytes into the intestinal epithelium, further formation of activated lymphocytes, macrophages and plasma cells in the *lamina propria* (Metcalf *et al.*, 2003).

This inflammatory response leads to damages of the structural support and the microcirculation within the villus, making it collapse. As a consequence, the finger shaped villi of the intestine are lost and the capacity to absorb nutrients is severely affected (Metcalf *et al.*, 2003). This mechanism is schematized in the Figure 2.



**Figure 2: The immune-response to gluten in celiac individuals (Metcalf, 2003).**

## 2. Genetic aspects

Celiac disease has been proved to be mainly associated with two human leukocyte antigens (HLA) haplotypes: DQ2 and DQ8 (Kagnoff, 2007; Green, 2009). HLA-DQ alleles encode for specific HLA-DQ2 heterodimers or HLA-DQ8 heterodimers; they are present on APCs and mainly allow binding and presentation of proline rich gluten derived peptides to CD4 T-cell and beginning of the harmful process of intestinal villi damage. The presence of genetic factors that predispose for the CD suggest that a person suffering from the disease might have family members affected by the same affliction (Green, 2009).

## 3. Diagnosis

Diagnosis of CD is not simple as problems related to the human gastro intestinal tract can have a variety of origins and also because CD can be asymptomatic. Patients suffering from the disease may present typical gastrointestinal symptoms such as diarrhea, fatty stools, and abdominal bloating and cramping. Celiac disease can also be the source of anemia, osteoporosis, short stature, infertility, neurologic problems or cutaneous infections (Setty *et al.*, 2008). Dermatitis Herpetiformis (DH) is one of these infections and is often considered to be characteristic of CD (Pietzak, 2012). Although these symptoms make CD diagnosis fairly easy in pronounced cases during early childhood, when there is mild disruption to the absorptive surface diagnosis can be more difficult, sometimes resulting in diagnosis being delayed until late adulthood; some atypical cases have been reported to present minimal or no gastrointestinal signs (Helms S., 2005). Hence clinical symptoms are not necessary to establish a diagnosis.

Nowadays, screenings for CD are mainly made through serum antibody assays testing for the presence of specific serologic markers. They can be of different kinds (Setty *et al.*, 2008):

- ELISA (enzyme-linked immunosorbent assay) test for anti-tissue transglutaminase antibody (TG2-IgA). This test is globally acknowledged as the first test of choice for screening as it displays the highest level of sensitivity (up to 98%). However, it is generally coupled with another assay test (e.g., EMA-IgA) as its specificity is not always sufficiently high.
- Indirect immunofluorescence (IF) test for anti-endomysial antibody (EMA-IgA). It is a highly specific marker for celiac disease.



- ELISA test for anti-deaminated gliadin peptide tests (DGP-IgA), which are used when TG2 or EMA test is negative and, also, in cases where a patient is IgA deficient.
- ELISA test for anti-gliadin antibody (AGA-IgA). It is not considered sensitive or specific enough for adults, but is used for children under two years old as TGA and EMA antibodies may not be present.

Noninvasive blood tests measuring antibodies to gluten and TGA are a considerable support for CD diagnosis but duodenal biopsy and tissue histology remain the only widely recognized method in CD diagnosis (Wieser and Koehler, 2008).

#### **4. Treatment**

The only known and undisputed treatment for CD is the adoption of a gluten-free diet (GFD). It requires the complete removal of any forms of wheat, barley, and rye from the diet (Hamer, 2005; Helms, 2005; Setty *et al.*, 2008; Verbeek *et al.*, 2008; Sollid and Lundin, 2009).

Oats are generally believed to be acceptable for patients with celiac disease. Scientific studies have clearly demonstrated that daily consumption of even high amounts oats for extended periods of time causes no harmful effect to patients (Salovaara *et al.*, 2009). However, the main concern against their consumption is the potential cross contamination from other grains containing gluten and especially barley (Ciclitira and Ellis, 2009; Immer and Haas-Lauterbach, 2009; Salovaara *et al.*, 2009). Individuals suffering from CD consume foods that can be separated into two categories: naturally occurring gluten-free (GF) foods (*e.g.* unprocessed meat, fruits or vegetables) and GF substitute foods in which GF grains replace wheat or other gluten containing cereals (Lee *et al.*, 2007).

Rice, corn, and potatoes used to be the traditional substitutes for gluten-containing grains. Further opportunities have been recently developed to enhance palatability and nutritional quality of GF foods based on the usage of several grains and seeds. These include sorghum, amaranth, millet, buckwheat, quinoa, flax, Indian rice grass, and teff (Arendt and Renzetti, 2009).

#### **5. Celiac disease incidence**

Celiac disease is one of the most common lifelong disorders in European countries or countries populated by individuals of European origin such as the United States of America. It is believed to be mainly related to the widespread consumption of gluten-containing cereal

foods (Catassi and Yachha, 2008). In these countries, it has been determined that approximately 0.9 to 1.2% of the overall population is affected by the CD (Fasano *et al.*, 2003; Hamer, 2005; Catassi and Yachha, 2008; Pietzak, 2012). The advances made during the past few decades in serological testing, including highly sensitive and specific tests for anti-gliadin antibodies (AGA), anti-endomysial antibodies (EMA) and anti-transglutaminase antibodies (tTg); allowed large scale screening among these populations but also the discovery of an unsuspected frequency of clinically atypical and even silent forms of CD (Fasano *et al.*, 2003; Catassi and Yachha, 2008).

Scientists introduced the idea of a “celiac iceberg” to explain why the minority of celiac cases are being diagnosed on a clinical ground and a larger portion remains undiagnosed unless they are dynamically searched for by the serological screening tests. The main causes for this under-diagnosis are the many possible forms of clinical symptoms and the unawareness of physicians and doctors. However, health specialists are becoming more and more effective in CD diagnosis (Green, 2009). It is now commonly accepted that the prevalence of celiac disease should be conceived as “the overall size of the iceberg” (Gallagher *et al.*, 2004). Celiac disease is not frequent only in developed countries and has become more and more reported in areas of North Africa, the Middle East, and India (Catassi and Yachha, 2008).

Gluten sensitivity or intolerance in non-celiac populations has been recently reported to represent a significant part of the population. Indeed, some patients having symptoms of irritable bowel syndrome (IBS), responded well to a gluten-free diet without having any markers of celiac disease (Biesiekierski *et al.*, 2011). It has been estimated that in the United States, about 10% of the population representing 31.1 million people are gluten intolerant or consider themselves as such (Cureton and Fasano, 2009; MINTEL, 2012).

### **III. Gluten-free marketplace and sorghum**

#### **A. Gluten-free: definition and labeling**

##### **1. Definition**

The latest official definition of the term “gluten-free” was established in November 2007 by the Codex Alimentarius Commission. Established in 1962 by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) the Codex Alimentarius Commission is a subsidiary organ of both organizations, and

has been charged with the creation of the Codex Alimentarius: a collection of uniformly defined food standards. The Codex Alimentarius is recognized by the World Trade Organization as an international reference point for the resolution of disputes concerning food safety and consumer protection (Masson-Mathee, 2007).

The term gluten-free was defined as the following:

a) “Consisting of or made only from one or more ingredients which do not contain wheat (i.e., all *Triticum* species, such as durum wheat, spelt, and kamut), rye, barley, oats or their crossbred varieties, and the gluten level does not exceed 20 mg/kg in total, based on the food as sold or distributed to the consumer, and/or

b) Consisting of one or more ingredients from wheat (i.e., all *Triticum* species, such as durum wheat, spelt, and kamut), rye, barley, oats or their crossbred varieties, which have been specially processed to remove gluten, and the gluten level does not exceed 20 mg/kg in total, based on the food as sold or distributed to the consumer.

Gluten-free products that have the same quality of wheat based counterparts are the most highly desired foods by celiac individuals, and yet are the most difficult to formulate.”

## **2. Label requirements**

Persons living with CD must rely on suppliers to provide convenient access to affordable, safe (i.e. gluten-free) and flavorful food. Over the years, one problem that consumers have faced is navigating new food labels, as wheat and ingredients containing wheat proteins are often not evident (Worosz and Wilson, 2012).

In 2007, the Food and Drug Administration (FDA), which is responsible for protecting and promoting public health through the regulation and supervision of food safety, proposed to define the term “gluten-free” for voluntary use in the labeling of foods. This implies that the food does not contain an ingredient that is any species of wheat, rye, barley, or a crossbred hybrid of these grains (referred to as “prohibited grains”); an ingredient that is derived from a prohibited grain and that has not been processed to remove gluten (e.g., wheat flour); an ingredient that is derived from a prohibited grain and that has been processed to remove gluten (e.g., wheat starch), if the use of that ingredient results in the presence of 20 parts per million (ppm) or more gluten in the food (Code of Federal Regulations 21CFR101, 2012).

As the rules for gluten-free labeling have yet to be formally codified, to make the best choices celiac consumers are forced to rely on manufacturers’ voluntary labeling and depend

on full disclosure and accuracy in labeling. The main points that will need to be addressed during this process are determining the threshold for consumption, defining what is “free” from gluten and finally how to effectively test for gluten concentration (Worosz and Wilson, 2012).

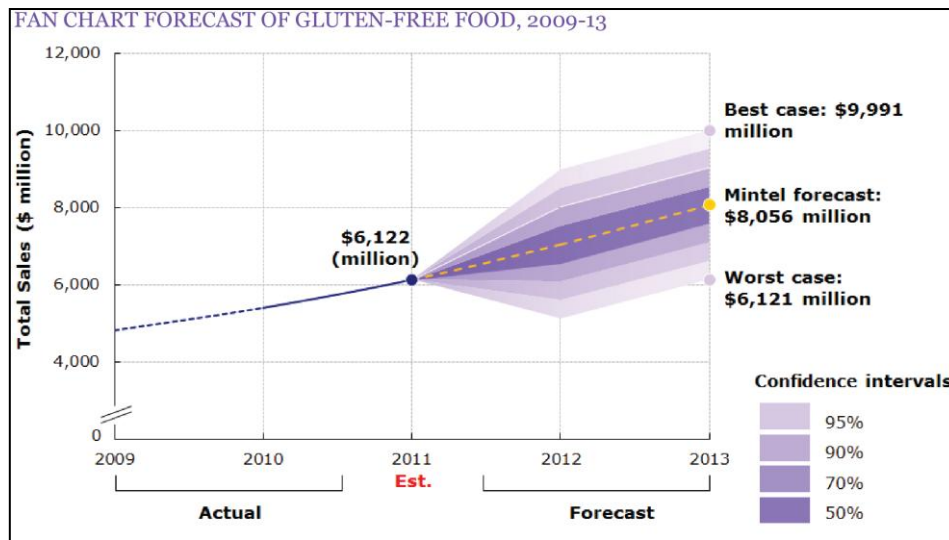
It is important to note that a food that bears the claim “gluten-free” in its labeling and fails to meet the above mentioned conditions and requirements would be deemed misbranded by the FDA (Ciclitira and Ellis, 2009). The European Food Safety Authority (EFSA) defined similar requirements for labeling products compatible with gluten-free diet (Official Journal of the European Union, 2007).

## **B. Market of gluten-free snacks and bakery product**

### **1. Market trends**

According to a study conducted by MINTEL (2012), retail gluten-free food market has grown from \$4.8 billion in 2009 to \$5.4 billion in 2010 to an estimated \$6.1 billion in 2011 (Figure 3). While this figure includes all food labeled gluten-free (including products that are inherently gluten-free such as meat or fruit and vegetable products), sales of gluten-free food products that are truly alternatives to gluten-containing, grain-based products (*e.g.*, snacks, cookies, and bakery products) have grown dramatically. Moreover, according to Cureton and Fasano (2009), the “free-from” market (*i.e.* gluten-free and lactose-free) has enjoyed a 300% growth between the years 2000 and 2007. Some conventional food manufacturers have made major investments and innovation in their gluten-free product lines and marketing in order to satisfy that growing demand (Worosz and Wilson, 2012).

Innovation in the American gluten-free category includes products such as soups, frozen entrees, pizzas, pudding/desserts, snacks, bread, bakery products and candy (Bogue and Sorensson, 2009).



**Figure 3: Evolution of the gluten-free market value in the U.S. (MINTEL, 2012).**

The MINTEL study, previously cited, estimated that several factors are driving this market growth. The improved quality, taste and prices of the new gluten-free products available have been the major component of the market boost. Over the past decade manufacturers have achieved tremendous improvement on their gluten-free food. Early gluten-free breads and other bakery products had poor baking qualities. These loaves, cakes, pastries or snacks used to be tough, crumbly, had a low specific volume, an unpleasant taste and a very short shelf-life due to fast molding (Stevens 2009). Food manufacturers achieved to produce gluten-free goods comparable to gluten containing food items. Stevens (2009), converged with the MINTEL study: for CD patients taste and cost are the most important factors when making purchasing decisions for gluten-free products. A study by Lee *et al.* (2007), on the evaluation of the economic burden of adhering to a gluten-free diet was conducted. Authors indicated that generally, in the US, the cost of gluten-free products was almost three times as much as their gluten-containing counterparts sometimes impacting negatively the compliance to the GFD.

More choices and better distribution of GF products have also lead to this dramatic market increase. In 2007, a study conducted by Lee *et al.* (2007), showed that in the U.S.A, common substitute GF products (e.g. pasta, bakery products, snack products...) are broadly carried by online stores while 94% of health food stores, 42% of upscale grocery and 36% of regular grocery stores carried the same products. Availability varied significantly across geographical regions: stores located on the east and west coast of the U.S. carried on average

more GF products than those located in the Midwest and mountainous areas. This study however argued that in the U.S. GF food is not readily available and is considerably more expensive than regular gluten-containing foods (Lee *et al.*, 2007).

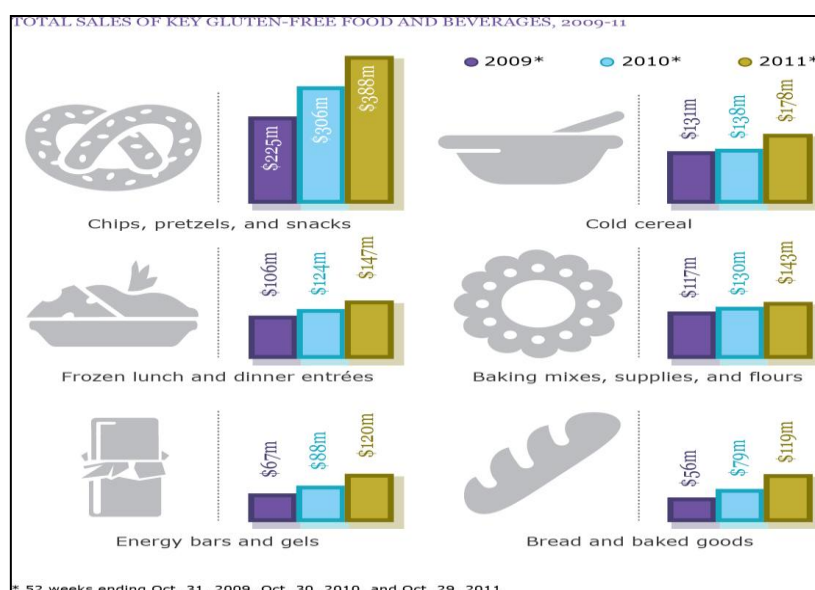
The rising incidence and prevalence of celiac disease due to better understanding and diagnosis has also helped the growth of the market. Green and Lee (2005), estimated that between 2000 and 2005 the number of diagnosed cases have more than doubled in the U.S.

Recently, in the U.S., a growing number of people have chosen to experiment with GFD to help alleviate symptoms such as abdominal pain, cramping, and generalized fatigue. In some cases, people have adopted the diet in simple protest against the current western culture of overconsumption of highly processed, nutritionally poor foods as many fresh, wholesome foods like fruits and vegetables are naturally GF while scientifically a GFD is only considered necessary for people suffering from CD (Pietzak, 2012).

## 2. Gluten-Free snack and bakery products in the United States

Yeast leavened products, *i.e.*, wheat bread is probably one of the most important staple foods in the Western world diet. Since the discovery and better understanding of CD, GF snack, and bakery products market has emerged.

According to MINTEL (2012), the segment sales for gluten-free chips, pretzels, and snacks increased from \$225 million in 2009 to \$306 million in 2010 and to \$388 million in 2011 (Figure 4). Consumer desire for healthy and certified GF snacks has greatly helped segment growth.



**Figure 4: Total sale of cereal based gluten-free foods over the 2009-2011 period in the U.S. (MINTEL, 2012).**

The GF snack market is very fragmented: many small to mid size companies are involved in the segment with no dominant companies creating a stiff competition.

Bigger companies are starting to get involved in this segment of the GF market. Another trend for the segment is that products with exotic and unusual ingredients and gourmet products have been successful, showing that consumers are willing to pay a premium for exotic gluten-free chips, pretzels, and snacks. As far as gluten-free bread and baked goods segment, sales increased from \$56 million in 2009 to \$79 million in 2010 to \$119 million in 2011. The gluten-free segment has grown far faster than the traditional bread category, which had minimal growth during the reviewed period (Mintel, 2012). These evolutions can be observed in figure 4.

### **3. Gluten-free consumers**

#### **a) Celiac and gluten intolerant individuals**

The most susceptible consumers of GF products are CD and gluten intolerant individuals. Indeed, as CD individuals have to comply with a strict GFD, they represent the core of the US GF market. Gluten intolerance or sensitivity is thought to exist in a patient when removal of gluten from the diet results in significant symptomatic improvement (mainly gastro intestinal). Scientifically, these persons do not have CD. Presently the clinical diagnosis of gluten sensitivity can only be based on response to a complete GFD, as there no serological tests available for this condition. Even if its mechanisms are not well understood, it is widely believed that in contrast to CD, gluten sensitivity is not an immune mediated reaction (Pietzak, 2012). People suffering from IBS might also be considered as the base consumer of GF products as some studies have shown that improvement of their condition was achieved by adhering to a GFD (Biesiekierski *et al.*, 2011; Pietzak, 2012).

Gluten purity of gluten-free products is the essential value of the original gluten-free market. Numbers vary depending on sources, but it is estimated that about 1% of the American population is gluten intolerant/sensitive or have celiac disease (Pietzak, 2012; Mintel, 2012).

#### **b) Other consumers**

Not all persons who claim a gluten sensitivity or suffering from CD have a real medical diagnosis. Indeed as discussed in the paragraph covering CD diagnosis, the path to a CD diagnosis can be long, difficult, and frustrating. This is a reason why some patients will

self-identify as having CD on the basis of an at-home test, self-diagnosis, or a perception of feeling better after going gluten-free (Worosz and Wilson, 2012).

Also, unlike the products associated with other food allergens such as those free of peanuts, eggs, and shellfish, gluten-free products have also a type of consumer base that is on the rise: individuals who consider a gluten-free lifestyle ethical or healthy (Worosz and Wilson, 2012). These new consumers enter the market mostly because eating such products is a fad or the products in the market satisfy a different need or interest from the needs or interests of the original consumers. Consumers without CD have been found to be more likely to pick a gluten-free product when provided with information about the potential health benefits of consumption and when the product is found in a health food store (Worosz and Wilson, 2012).

## **C. Sorghum: characterization and utilization in food products**

### **1. Presentation**

Sorghum (*Sorghum bicolor* L. Moench), also known as milo, is a tropical grass belonging to the tribe of *Andropogoneae* and the family of *Poaceae* having a variety of uses including food for human consumption, feed grain for livestock and industrial applications such as ethanol production. It is grown primarily in semiarid areas of the world and especially on the African continent. It is believed to have been domesticated between 3,000 to 5,000 years ago in the north east of Africa corresponding today, to Ethiopia (Dendy 1995). Sorghum is a self-pollinating plant; it is more resistant to drought than corn making its culture an interesting and advantageous alternative in regions where other crops might suffer from hot and dry weather conditions (U.S. Grain Council, 2012a).

### **2. Production**

The U.S. Grain Council established that sorghum is the third most important cereal crop grown in the U.S. and the fifth most important cereal crop grown in the world behind wheat, rice, corn, and barley (U.S. Grain Council, 2012a). The United States is the world's largest producer of grain sorghum followed by India, Nigeria, and Mexico. It is a leading cereal grain produced in Africa and is an important food source in India. Leading exporters are the United States, Australia, and Argentina (U.S. Grains Council, 2012b).

Sorghum is of particular importance in Kansas as it is the first state in terms of sorghum production. According to the 2010 data from the Food and Agriculture Organization



Statistical databases (FAO STAT, 2010), the world's production for sorghum was about 55,654,523 tonnes. This production was spread on 40,508,600 hectares area harvested.

Sorghum is grown in the United States, Australia, and other developed nations essentially for animal feed. However, in Africa and Asia the grain is used both for human nutrition and animal feed. It is estimated that more than 300 million people from developing countries essentially rely on sorghum as source of energy (Dicko *et al.*, 2006).

In most Western African countries, sorghum accounts for roughly 50% of the total cereal crop land area. Therefore, true food security is hard to achieve in those countries without a significant improvement of the production, use, and marketing of this major staple cereal. The yield is 1000-3000 kg/ha, while in the other countries (Argentina, China, and USA) it is 3,000 to 4,000 kg/ha. The low production in West Africa is essentially due to biotic such as insects, fungal diseases, or weeds and abiotic stresses such as drought, logging, photoperiod or soil quality (Dicko *et al.*, 2006; FAO STAT, 2010).

### **3. Structure**

#### **a) Kernel**

Sorghum kernels thresh easily free of hulls or glumes during harvesting. The kernel is usually spherical with a weight ranging from 20 to 30 milligrams. The test weight for sorghum ranges from 746 to 772 kg/m<sup>3</sup>. The sorghum caryopsis consists of three distinctive anatomical components: the pericarp (outer layer), the endosperm (storage tissue) and the germ (embryo). The relative proportion of the components varies depending upon variety and environment (Dendy, 1995).

#### **b) Pericarp**

In most sorghum varieties, the pericarp is the thick outer layer consisting of three layers: the epicarp, the mesocarp and the endocarp (see figure 5). Pericarp thickness ranges from 8 to 160 µm and varies within an individual kernel: The sections below the style and hilum are the thickest and the sides of the kernel are thinnest. The outermost layer or epicarp is usually covered with a thin protective layer of wax. The epicarp is two to three layers thick and is made of rectangular cells often containing pigmented material. Unlike other crops kernels, sorghum second layer (mesocarp) may contain starch granules. Sorghum with thick pericarp, contains three to four mesocarp cell layers filled with small starch granules. The inner pericarp tissue (endocarp), is composed of cross and tube cells (Dendy, 1995).

### **c) Inner integument**

The pigmented inner integument also called *testa* layer or seed coat, may or may not be present in mature sorghum caryopsis based on genetic factors. The pigmentation is due to the presence of condensed tannins in the testa layer. These molecules affect greatly the agronomic profile of the crop but also the end-use of the grain: high concentration of tannins gives a great pest resistance (especially against birds) but also lowers greatly protein digestibility (Dendy, 1995; Hoseney, 2010).

### **d) Endosperm**

As most of the cereal grains, sorghum endosperm is essentially a storage tissue. This storage organ is an assembly of the aleurone layer, peripheral, floury, and corneous regions. The aleurone cells are the outer cover of the endosperm consisting of a single layer of rectangular cells adjacent to the testa or tube cells (Waniska, 2000). These cells display a thick cell wall, large amounts of proteins (protein bodies, enzymes), ash (phytin bodies), and oil (sphaerosomes).

The peripheral area is composed of many layers of dense cells containing more protein and smaller starch granules than the corneous area. Both the peripheral and corneous areas appear translucent, or vitreous, and they affect processing and nutrient digestibility. Waxy sorghums contain larger starch granules and less protein in the peripheral endosperm than regular sorghums (Waniska, 2000).

The corneous and floury endosperm cells are composed of starch granules, protein matrix, protein bodies, and cell walls rich in cellulose,  $\beta$ -glucans, and hemicellulose. Starch granules and protein bodies are embedded in the continuous, protein matrix in the peripheral and corneous areas (Waniska 2000; Hoseney, 2010). The starch granules are polygonal and often contain dents from the protein bodies. Their size varies from 4  $\mu\text{m}$  to 25  $\mu\text{m}$ , the average being 15  $\mu\text{m}$ . Granules present in the corneous endosperm are smaller and angular whereas those in the floury endosperm are larger and spherical.

The opaque, floury endosperm is located near the center of the caryopsis. It has a discontinuous protein phase, air voids, and loosely packaged, round and lenticular shaped starch granules (Dendy, 1995; Hoseney, 2010). The presence of air voids diffract incoming light, giving the floury endosperm an opaque or chalky appearance.

#### e) Germ

The germ consists of two major parts: the embryonic axis and scutellum. The embryonic axis contains the new plant and is divided into a radicle and plumulae. Upon germination and development, the radicle forms primary roots whereas the plumulae forms leaves and stems. The scutellum is the single cotyledon and contains reserve nutrients, i.e., moderate amounts of oil, protein, enzymes, and minerals, and serves as the bridge or connection between the endosperm and germ (Dendy, 1995; Waniska 2000).

### 4. Composition

Sorghum proximate composition varies significantly due to genetic and environment factors. The protein content is usually the most variable and can range from 7 to 16% of the whole kernel. Sorghum is similar in composition to corn (*Zea mays*) but sorghum usually contains slightly more protein and has less oil than yellow dent corn (Dendy 1995).

The pericarp is rich in fiber, whereas the germ has a high crude protein, fat and ash content. Regarding the endosperm, it contains essentially starch and protein with little amounts of fat and fiber (Table 1).

**Table 1: Chemical composition of whole sorghum<sup>a</sup>.**

<b>Kernel fraction</b>	<b>% of kernel weight</b>	<b>Protein<sup>b</sup> (%)</b>	<b>Ash<sup>b</sup> (%)</b>	<b>Fat<sup>b</sup> (%)</b>	<b>Starch<sup>b</sup> (%)</b>	<b>Niacin<sup>b</sup> (mg/100g)</b>	<b>Riboflavin<sup>b</sup> (mg/100g)</b>	<b>Pyridoxin<sup>b</sup> (mg/100g)</b>
<b>Whole grain</b>	100	12.3	1.67	3.6	73.8	4.5	0.13	0.47
<b>Endosperm</b>	82.3	10.5 (80.9)	0.37 (20.6)	0.60 (13.2)	82.5 (94.4)	4.4 (76)	0.09 (50)	0.40 (76)
<b>Germ</b>	9.8	18.9 (14.9)	10.4 (68.6)	28.1 (76.2)	13.4 (1.8)	8.1 (17)	0.39 (28)	0.72 (16)
<b>Pericarp</b>	7.9	6.7 (4.0)	2.0 (10.8)	4.9 (10.6)	34.6 (3.8)	4.4 (7)	0.40 (22)	0.44 (8)

<sup>a</sup>Data adapted from Dendy, (1995).

<sup>b</sup>Values in parentheses correspond to percentage of whole kernel value.

### **a) Carbohydrates**

The carbohydrates of sorghum are composed of starch, soluble sugars, pentosans, cellulose and hemicelluloses. Starch is the most abundant chemical component while soluble sugars and crude fiber are present in very modest quantities.

Starch accounts for 50 to 75% of the sorghum caryopsis total weight. Starch molecules are arranged in highly organized granules in which amylose (linear polymer made of glucose units joined by  $\alpha$ -1,4 glycosidic bonds) and amylopectin molecules (highly branched polymer made of glucose units joined by  $\alpha$ -1,4 and  $\alpha$ -1,6 glycosidic bonds) are bound together by hydrogen bonding. This arrangement makes starches pseudo crystals with a crystalline and an amorphous area (Dendy, 1995).

In sorghum endosperm (similarly to maize), starch granules and protein bodies are in very close association with each other. The largely polygonal, tightly packed starch granules are surrounded with numerous, largely spherical protein bodies embedded in a protein matrix (Duodu *et al.*, 2003). While starch granules present in the corneous endosperm are smaller and angular, those in the floursy endosperm are larger and round. Regular endosperm sorghum types contain from 20 to 30% amylose and, hence, 70 to 80% amylose. The gelatinization temperature, which is critical in baking procedures, ranges from 70 to 80°C for sorghum starch. The water binding capacity of sorghum starch is lower than that of regular yellow corn (Dendy, 1995; Hosene, 2010). Soluble sugars can represent a significant part of sorghum carbohydrates and especially in sugary cultivars. In such varieties, sucrose is the major soluble sugar in the dry grain.

### **b) Protein**

As already mentioned, protein content and composition can vary greatly due to different factors such as sorghum genotype, water availability, soil fertility, temperatures, and environmental conditions during grain development.

Approximately 81, 15 and 4% of the sorghum protein is located in the endosperm, germ and pericarp, respectively (Table 1). The germ tends to be rich in albumins and globulins while the endosperm contains the prolamins (also called kafirins in the case of sorghum) and glutelins. Kafirins are mainly located within the spherical protein bodies and increase with increased level of proteins in the kernel (Dendy, 1995). They are the major storage protein of sorghum and represent from 72 to 82% of the total protein content. They

have been classified into three groups of prolamins, called  $\alpha$ ,  $\beta$  and  $\gamma$ -kafirins based on molecular weight and solubility (Belton *et al.*, 2006).

Depending on whether it is floury or vitreous, sorghum endosperm contains about 66% to 84%  $\alpha$ -kafirin, 8% to 13%  $\beta$ -kafirin, and 9% to 21%  $\gamma$ -kafirin and low levels of a poorly characterized  $\delta$ -kafirins. The  $\alpha$ -kafirins are divided into two groups of polypeptides with molecular weights of 23,000 and 25,000 daltons. These proteins are rich in non-polar amino acids and are found primarily as monomers and oligomers. These proteins do not crosslink extensively and form mainly intramolecular disulfide bonds. The  $\beta$ -kafirins have a molecular weight of approximately 18,000 daltons, are rich in the sulfur-containing amino acids methionine and cysteine, and are found in monomeric and polymeric forms. The  $\gamma$ -kafirins weigh approximately 20,000 daltons and are rich in the amino acids proline, cysteine, and histidine. These subunits are found as oligomers and polymers. Both  $\beta$ -kafirins and  $\gamma$ -kafirins form intermolecular and intramolecular disulfide bonds and are highly crosslinked (Belton *et al.*, 2006, De Mesa-Stonestreet *et al.*, 2010).

In pigmented sorghum cultivars, polyphenols (*e.g.* tannins) can form complexes with kafirins that are resistant to digestion. Cooking enhances the interaction of kafirins with these compounds, further reducing protein digestibility (De Mesa-Stonestreet *et al.*, 2010).

### **c) Fibers**

The term fiber has been controversial for a long time. Fibers are now defined as the endogenous components of plant materials (*i.e.*, cereal grains) that are resistant to digestion by enzymes in the monogastric stomach and upper gastrointestinal tract (Smith and Tucker, 2011).

The major individual components of grain fibers are cellulose, hemicellulose, lignin, pectins, and gums which are mainly located in the pericarp and endosperm cell walls. These dietary fibers are in general classified according to their solubility in water. Insoluble fiber components are present primarily in the pericarp where they have essential structural and protective functions. In sorghum, most of the dietary fiber is insoluble, representing about 86% of the total fiber (Dendy, 1995; Hosney 2010).

### **d) Lipids**

Lipids are relatively minor constituents in cereal grains; most of them are located in the scutellum of the sorghum caryopsis. The crude fat content of sorghum is 3%, which is higher than that of wheat percent (2.7%) and rice percent (2.3%) but lower than corn percent

(4.1%) (Hoseney, 2010). Nonpolar lipids (i.e. triglycerides, diglycerides) are the main lipids present in sorghum. The typical composition of sorghum oil is very similar to that of maize and is dominated by the unsaturated linoleic, oleic and palmitic acids (Dendy, 1995).

#### **e) Minerals, vitamins and enzymes**

Sorghum is a good source of minerals as its pericarp, aleurone, and germ are rich sources of ash. The mineral found in greatest amount is phosphates; however, its availability can be greatly affected by the presence of phytates (Hoseney, 2010). Sorghum has been reported to be an important source of B vitamins (Dendy, 1995). Unlike barley, the sorghum aleurone layer does not produce endosperm-degrading enzymes such as  $\alpha$ -amylase, protease, pentosanases or endo- $\beta$ -glucanases. Sorghum  $\alpha$ -amylase are secreted by the scutellum during germination.

### **5. Utilization**

Sorghum is a staple food grain in many semi-arid and tropic areas of the world, notably in Sub-Saharan Africa because of its good adaptation to hard environments and its fairly good yield of production (Dicko *et al.*, 2006). More than 35% of sorghum is grown specifically for human consumption. The rest is used mainly for animal feed, alcoholic, and non-alcoholic beverages production, and industrial products (Awika and Rooney, 2004).

Sorghum is used in a wide variety of foods. White sorghums are processed into flour and other products, including expanded snacks, cookies, and ethnic foods, and are gaining popularity in countries such as Japan. In the U.S., white sorghum products are used to a small extent and essentially to substitute for wheat in products for celiac individuals or people intolerant to wheat gluten (Awika and Rooney, 2004; De Mesa-Stonestreet *et al.*, 2010). In developing areas of the world, sorghum is the base of many staple foods including tortillas, porridges, boiled grain dishes, couscous, fermented foods, and traditional beers (Dicko *et al.*, 2006).

What makes sorghum interesting in western countries is that it is safe for celiac patients (Ciacci *et al.*, 2007). Sorghum flour is an attractive alternative to wheat flour for the celiac market because of its neutral flavor and the use of hybrids with a white pericarp devoid of pigmented components. These white grained sorghum lines produce flour that is similar to wheat flour in appearance and do not impart an unusual color to the flour. Good-quality sorghums are generally available with a nutritional feeding value that is equivalent to that of corn. Furthermore, sorghum utilization helps address food security issues because it is a

drought resistant crop that easily withstands harsh cultivating conditions in impoverished regions of Asia and Africa (De Mesa-Stonestreet *et al.*, 2010).

## **IV. Gluten-free technology in bakery products**

The formulation and production of gluten-free cereal-based products presents a tremendous challenge to food companies. Limited available literature on gluten-free bakery products reflects both the complexity of the involved technology and the lack of awareness of gluten-free product demand from celiac patients, gluten intolerant individuals or even health conscious consumers. The absence of gluten yields poor quality GF foods with low specific volumes, crumbly and dry texture, strong off flavors and short shelf lives.

In recent years, scientists have started to study different ways to overcome the absence of gluten proteins including the use of starches, dairy products, gums and hydrocolloids, enzymes, and other alternatives in order to improve the structure, texture, mouthfeel, shelf-life, and overall acceptability of gluten-free products ( Gallagher *et al.*, 2004; Houben *et al.*, 2012). It is important to note that GF foods should be palatable, nutritious convenient to manufacture and finally possess similar characteristics to their gluten-containing equivalents (Abdel-Aal, 2009).

### **A. Main ingredients**

#### **1. Flour**

Commercial GF bakery products available in the market are often starch-based as high fiber GF formulation, are much more technologically challenging, due to the change in water distribution and fiber interference with the structure formation of the product (Poutanen, 2009). This attribute makes them nutritionally poor due to the lack in fiber, vitamins, and other nutrient content. This results in a worsening effect on their nutritional diet. For this reason it is crucial to use GF flours as bulk ingredient in GF bakery foods and snacks (Arendt and Renzetti, 2009). In recent years several cereals and pseudocereals have been studied and evaluated for usage in GF products (*e.g.* rice flour, corn flour, sorghum flour, tapioca flour, arrow root flour, millet, potato flour, buckwheat flour, soy flour, and amaranth flour).

However, studies have shown that differences among cereal flours and even among varieties of the same cereal can be important in terms of protein, starch, fiber, ash, moisture, nitrogen free extract, lipids, starch gelatinization temperatures, amylose to amylopectin ratio, functionality of proteins. These characteristics significantly affect technological properties

such as bread-making performance. Rice, corn, and sorghum flour have attracted more attention and demonstrated that acceptable GF products could be produced from these grains (Arendt and Renzetti, 2009; Abdel-Aal, 2009). In order to achieve good results, each bread system has to be optimized for use with specific flours.

## **2. Starches**

Starches are commonly used to impart appearance and textural properties to food products such as thickening, gelling, texturizing, moisture retention, stabilizing, anti-staling and adhesion properties. In GF food systems, starch is incorporated into the formula to provide one or more of these functionalities. The type of food being produced and other ingredients contained in formulation are also critical to determine starch properties (Abdel-Aal, 2009).

Every plant and cereal has its own unique starch granule that varies in size, shape, and chemical and physical properties. Starch is composed of two types of glucose polymers, the linear amylose and the highly branched amylopectin as well as other minor components (proteins and lipids). Both amylose and amylopectin molecules are  $\alpha$ -glucans ( $\alpha$ -branched glucose polymers) that differ essentially in chain configuration (linear and highly branched, respectively) and molecular weight. They are associated with hydrogen binding into crystal and form a crystalline structure, insoluble in cold water (Houben *et al.*, 2012). The structural differences between amylose and amylopectin, result in significant differences in starch properties and functionality and especially pasting, retrogradation, and gelling properties. The two polymers are present in varying amounts depending on the starch source (Abdel-Aal, 2009; Hoseney, 2010).

Different starches from naturally gluten-free sources such as corn, potato, cassava, tapioca and rice have been used by food technologists in gluten-free formulations (Arendt *et al.*, 2009). Starch functions in GF baking systems in many ways. First, it has the ability to absorb large amounts of water. When heated in combination with water, starch will gelatinize. Every starch has its own gelatinization temperature. Gelatinization within a bread system causes the starch granules to become partially soluble and irreversibly swell, maintaining a granular appearance, further cooking will lead to disruption and total loss of integrity of the granule. During the gelatinization process, amylose leaches out of the granule and solubilizes into the food matrix; amylose and amylopectin are able to interact via hydrogen bonding forming a composite continuous network (composed of swollen amylopectin, filling an



interpenetrating amylose gel matrix) that envelops and sticks the gelatinized starch granules together upon cooling (Arendt *et al.*, 2008; Hosney, 2010, Houben *et al.*, 2012). When baking the dough, the crumb structure is set as result of starch gelatinization which is found to be highly correlated to the expansion volume of the dough and eventually, after cooling specific volume and final crumb texture. Hence, gelatinization properties of starch such as gelatinization transition temperatures and pasting viscosity are essential in determining texture and appearance of GF bakery products (Abdel-Aal, 2009).

Due to the lack of gluten network in GF products, GF doughs are less viscous than wheat flour based dough and thus, are generally handled and processed as batters rather than regular bread dough (similar process to cake batter). Baked products from batters are limited in consistency and shape. Indeed, batter based foods can only take the shape of the pans they are baked in, whereas dough can be molded or formed into different shapes resulting in numerous different types of products (Smith *et al.*, 2012)

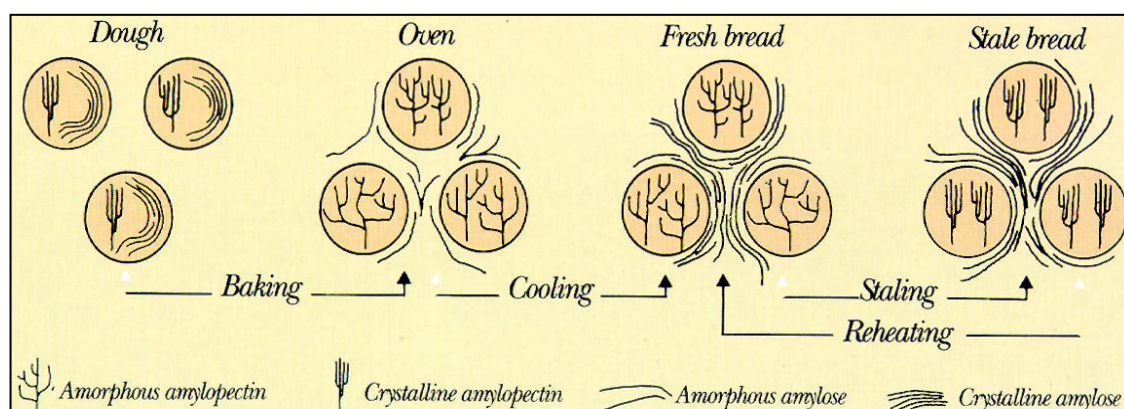
Starches forming strong gels can be used to improve consistency and GF batters. In combination with these gelling-forming starches, air cell stabilizers such as gums need to be used to provide gas holding capacity to the batter due to their gas occlusion and stabilizing properties. This results in a cohesive dough system with good air cell stabilization.

The retrogradation or crystallization process of gelatinized starch contributes to a phenomenon called staling in parallel with water migration. These two cause firmer crumb structure due to an increase in order between polymers of amylose and amylopectin. It also yields a leathery crust, with less elasticity of the crumb, and loss of flavor (Arendt *et al.*, 2008).

### **3. Hydrocolloids**

The viscoelastic characteristics of gluten containing dough are largely responsible for gas cell formation during the proofing and baking stages of bakery products. The absence of the gluten network (described previously) determines the properties of the gluten-free dough which is more fluid than wheat dough. Thus, polymeric substances that are able to imitate the viscoelastic properties of gluten to improve the structure (mainly through stabilization mechanisms), gas retention and occlusion ability are necessary to produce acceptable gluten-free leavened products (Abdel-Aal, 2009; Arendt *et al.*, 2009; Houben *et al.*, 2012). It has been reported that hydrocolloids have two key effects on starch structure in GF breads. Hydrocolloids can coat starch granules causing a decrease in starch swelling and leaching of

amylose to cause an overall increase in crumb firmness (which is generally not desirable). The other consequence of hydrocolloid addition is the reduction of retrogradation or crystallizing of amylose and amylopectin that may cause crumb structure softening, also called staling (Arendt *et al.* 2008). The staling mechanism in bread and its relationship to the amylose and amylopectin molecules is schematized in Figure 6.



**Figure 5: Starch retrogradation mechanism in bread (Schiraldi and Fessas, 2001).**

There are various different types of gums and hydrocolloids all of which have differing functional characteristics and can contribute differently depending on the food system. Hydrocolloids and gums used in food applications come essentially from hydrophilic polymers of vegetable, animal and microbial sources (Arendt *et al.* 2008). These are cellulose derivatives (carboxy-methyl-cellulose and hydroxy-propyl-methyl-cellulose), guar and locust bean gum, non-starch polysaccharides (*e.g.*  $\beta$ -glucans) or xanthan gum.

Cellulose derivatives hydrocolloids such as hydroxy-propyl-methyl-cellulose (HPMC) and carboxy-methyl-cellulose (CMC) have been widely studied in GF bread production. Arendt *et al.* (2009) mentioned that HPMC has tremendous positive effects on GF bread systems. HPMC (that have similar properties to CMC) helps to increase GF bread specific volume and improves gas retention and water absorbing characteristics generally conferred by gluten. This is due to the fact that HPMC gives some stability to the interface dough system during mixing and proofing and confers additional strength to the formed gas cells during the whole baking process and eventually increasing gas retention and specific volume (Arendt *et al.*, 2009).

Xanthan gum helps in strengthening by enhancing elasticity and resistance to deformation of the dough and produces a farinograph more similar to that of wheat dough. Xanthan acts by improving the strain hardening properties of the dough (Arendt *et al.*, 2009; Sabanis and Tzia, 2010). Abdel-Aal (2009) studied the effect of several hydrocolloids on GF bakery products, mentioning that xanthan had the most pronounced effect on viscoelastic properties of the dough among commonly used hydrocolloids. The crumb structure analysis revealed that xanthan increases elasticity, porosity, and cell expansion and thus, improving the overall quality of GF breads.

#### **4. Other ingredients**

The final ingredient that is necessary for bakery products is water. Water hydrates flour components as well as hydrocolloids. Water is also considered a universal solvent. When placed in contact with water, some bread ingredients like salt and sugar readily dissolve to form ionic solutions. Salt and sugar not only change the flavor of products, but they can also affect hydration properties of other ingredients, texture, water activity, and yeast activity. Leavening agents such as yeast and chemical based leavening agents are critical for achieving well leavened bakery products. Their ability to produce carbon dioxide coupled with a food system able to prevent the escape of the gas produces foam and when baked a leavened product can be produced (Hoseney, 2010).

### **B. Partial baking technology**

#### **1. Description**

Bread and similar baked products have a limited shelf life. Physicochemical changes (e.g. staling, firming) and microbiological spoilage (e.g. yeast, mold, and bacterial growth) shorten the shelf life of these bakery products (Karaoglu and Kotancilar, 2006). Because consumers demand fresh baked goods that do not stale within a short time frame, the economic losses resulting from bread staling are important. For this reason, considerable attention has been paid to bread staling (Schiraldi and Fessas, 2001, Ronda *et al.*, 2005).

Bread staling has been extensively investigated because of its importance in determining product acceptability and shelf life and great effort has been put to retard the staling process by changing the product formulation, processing technique or packaging, and storage conditions (Schiraldi and Fessas, 2001). Because crumb firming is related to starch retrogradation during bread storage (especially amylopectin), studies on bread staling have

focused on starch modifications and starch–gluten interactions in bread crumb. Other research aiming at prolonging freshness showed that the staling can be retarded by adding certain additives (called antistaling agents) such as fats, emulsifiers, proteins or enzymes (Schiraldi and Fessas, 2001; Karaoglu and Kotancilar, 2006).

A different approach for increasing the shelf life of bread is to modify the baking method. Partial baking (or par-baking) is a method of bread manufacturing involving two stages of baking with an intermediate freezing step (Vulicevic *et al.*, 2004; Karaoglu and Kotancilar, 2006). According to Vulicevic *et al.* (2004) the par-baking process follows an ordered protocol. Proofed dough pieces are baked under the defined oven conditions into partially baked products with the minimum crust coloration and the maximum moisture retention. The products are then cooled to ambient temperature, and, rapidly frozen by conventional blast freezing, before being packaged and stored until the final re-baking at the point of sale.

## **2. Gluten-free applications**

Par-baked products are convenient foods meant to be baked before consumption and have sufficient moisture for the development of desirable quality characteristics (Karaoglu and Kotancilar, 2006). Little research has been done on GF par-baking applications while this technology seems to have a great market potential since this process provides an opportunity to supply fresh baked bread safe to Celiac individuals with a simple baking stage at retail locations. Bize (2012) mentioned that staling (the major cause leading to poor consumer acceptance) occurred at a faster rate in GF than conventional bakery products. Especially a sorghum-based GF bread displayed a relatively short shelf life due to excessive staling. The scientist mentioned that par-baking might be an interesting alternative to extend gluten-free bread shelf-life.

Recently, Sciarini *et al.* (2012), studied the application of par-baking to GF bread production. Using an optimized GF bread formula they assessed the impact of a partial-baking process on the quality of gluten-free breads as compared to a conventional full-baking process, and studied the effect of different hydrocolloids (CMC and xanthan gum) on the process. According to Sciarini *et al.* (2012), par-baking process is a suitable process to obtain desirable gluten-free breads. This “interrupted baking” process decreased the overall final bread quality but the negative effects could be diminished by hydrocolloid addition.

Hydrocolloids improved specific volume and decreased crumb firmness as well as amylopectin retrogradation (Sciarini *et al.*, 2012).

## **C. Eggs and carob function in gluten-free formulation**

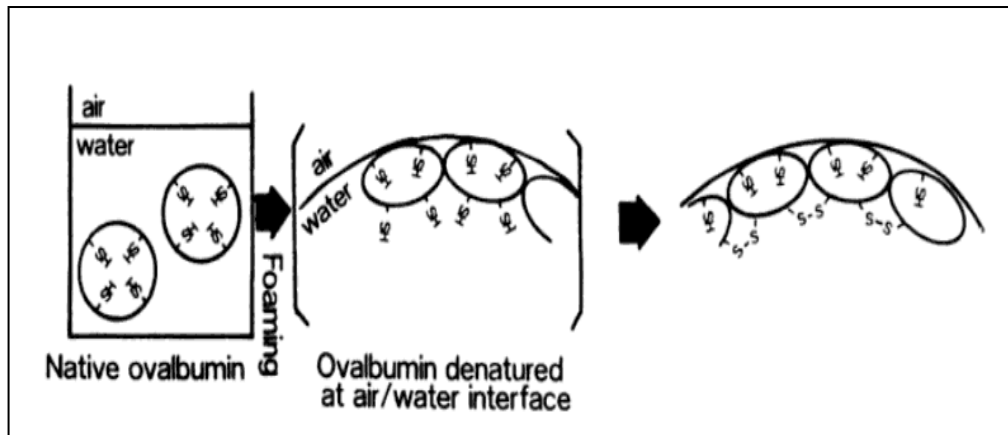
### **1. Eggs**

Eggs have been known for a long time as natural highly functional and nutritive ingredient. Even if eggs are rich in water (about 75%), they are a rich source of high quality proteins as well as an important source of unsaturated fatty acids, iron, phosphorus, trace minerals and the fat soluble vitamins A, E, K and B (Mine, 2002). Eggs can add many positive attributes to food products such as: foaming, emulsification, leavening, smoothness, and flavor (Jones, 2007). Yet, its main functional properties (e.g. emulsifying ability and gel structure formation upon heating in bakery products) have not attracted the attention of too many food scientists.

During the preparation of certain bakery products, such as cakes or variety breads, yolk constituents may have to function as emulsifiers and foaming agents, as well as network structure formers. Gluten-free bread batter is a mixed colloidal system of an emulsion, a foam and a suspension that, during baking, is transformed into a solid foam, the bread, exhibiting unique textural characteristics (Kiosseoglou, 2003); therefore, GF breads could benefit from the inclusion of eggs in formulations.

#### **a) Foaming ability**

Eggs play a major role in foaming of bakery products. Egg proteins are mostly responsible for this foaming ability (Yiu, 2002). These proteins are generally considered amphiphilic: they bear both hydrophilic and hydrophobic chemical groups on their surface. During the whipping process, air is incorporated in the dough; denaturated egg proteins concentrate and adsorb at the interface of the bubbles lamella lowering the surface tension and increasing the number of new interfaces and number of air bubbles (Figure 7).



**Figure 6: Network formation with Ovalbumin molecules surface-denaturated at air/water interface by foaming (Damodaran (1997)).**

The formation of stable foam requires great lamella intensity. Indeed, when heated, the air in the cell expands and the proteins at the interface of the lamella will either stretch or break leading to poorer volumes and specific densities. The capacity of protein to form and stabilize foams depends on various factors: the source and type of protein as well as its degree of denaturation, pH, and temperature of the food system, size and flexibility of proteins, whipping time and method, presence of ingredients affecting foams (*e.g.* ionic molecules, carbohydrates, fats and oils).

Egg white proteins, especially ovalbumins, ovomucins and globulins, are believed to have the highest foaming capacity in comparison to their yolk proteins counterparts (Mine, 2002; Yiu 2002). This ability to form a foam is very interesting for GF food applications since the foam produced by the release of carbon dioxide during the proofing step needs to be stabilized throughout the process.

### **b) Coagulation properties**

Coagulation is the term used to describe the change from a fluid to a solid or semi-solid state based on protein heat denaturation. It is well known that the heat induced gelation of protein molecules includes an initial uncoiling and unfolding step followed by an association reaction such as hydrogen bonding, hydrophobic interactions and disulfide bonding (similarly to disulfide bonding between two sulfhydryl residues in gluten proteins), which leads to the formation of the three dimensional gel structure. Ovalbumin proteins, which are the most abundant protein in egg white have a prevalent role during the gelation process (Mine, 2002). Thermal coagulation of egg proteins takes place from 62°C and above

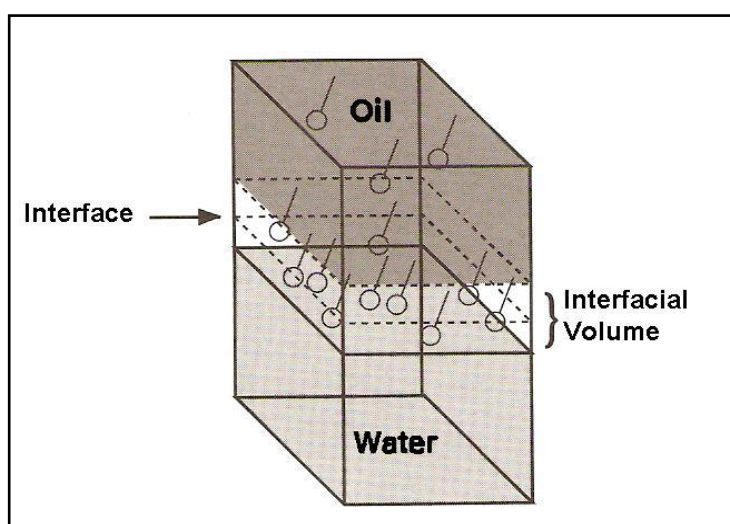
in egg white whereas, the egg yolk coagulates at 65°C. It is important to note that the gelling properties of the yolk proteins are associated with the lipoprotein molecules (Yiu, 2002).

Because of the coagulation properties in a food system, egg binds materials together and contributes greatly to thickening. The structural integrity and strength of some bakery products have been reported to be attributable to the coagulation properties of egg protein (Yiu 2002, Bize 2012).

### c) Role in emulsification

#### i. *Emulsion: definition*

Emulsifiers, or surfactants are amphiphilic molecules: they contain both an hydrophilic part and a lipophilic part. They have the ability to reduce the surface tension between two non-miscible phases (generally a lipid phase and an aqueous phase) and hence promote and stabilize the dispersion. The lipophilic part of emulsifiers is generally consisting of a long chain fatty acid. On the other side, the hydrophilic section of food emulsifiers can be non-ionic (eg: glycerol), anionic or amphoretic which are carrying both positive and negative charges (Stauffer, 1999). The amphiphilic character of emulsifiers allows them to concentrate and adsorb at the interfacial region (Figure 8). The hydrophilic phase is in the polar phase, and the lipophilic section is in the non-polar phase. As the surfactant molecules migrate, free energy (including surface tension) of the system is lowered. When the concentration of emulsifier is increased, it will form into micelles within the substance in which it is soluble (Morrison and Ross, 2002).



**Figure 7: Concentration of an oil soluble emulsifier in the water-oil interface (Stauffer, 1999).**

## ii. *Emulsion formation*

When making an emulsion, the amount of interface in a food system is increased. Input of mechanical energy is generally applied to subdivide and distort the droplets of the discontinuous phase in a final average droplet diameter of 1 to 100 micrometer is reached. The process is repeated until the shear forces are no longer greater than interfacial tension forces (Stauffer, 1999). An emulsifier's ability to decrease interfacial tension allows smaller droplets to be created with lower shear forces (McClements and Weiss, 2005).

The two major types of food emulsions are oil-in-water and water-in-oil emulsions. Oil-in-water emulsions are where oil is dispersed as micelles in a continuous water phase. This type of emulsion is the most common in food applications. Some examples of oil-in-water emulsions are non-dairy creamers, cake batters, and mayonnaise. Water-in-oil emulsions are where micelles of water are dispersed in oil (eg: margarines and butter) (Stauffer, 1999).

Emulsions created between immiscible substances are thermodynamically unstable. The instability due to the hydrophobic interactions causes dispersed droplets to combine or coalesce when they come in contact with each other (McClements and Weiss, 2005). If the emulsion is to remain stable, coalescence of the dispersed phase needs to be prevented. This phenomenon can be avoided through electrical repulsive force stabilization. Repulsive forces can range from building stronger, thicker, or more organized interfacial regions to creating an electrical charge on the surface of the dispersed substance. Increasing the viscosity of the continuous phase (eg: use of hydrocolloids) can also help stabilize emulsions as the mobility of the dispersed droplets is reduced so that they do not come into contact with each other (Stauffer, 1999; Morisson and Ross, 2002).

## iii. *Hydrophilic/lipophilic balance*

As mentioned above, food emulsifiers consist of both a hydrophilic and a lipophilic portion. The equilibrium between these two parts conditions the functionality of the emulsifier at interfacial areas and thus, its function in food systems. This balance is called the hydrophilic/lipophilic balance (HLB). From the HLB value, various properties of emulsifiers can be assumed. For example, emulsifiers with HLB values below 6 indicate good water-in-oil emulsifiers, between 7 and 9 indicate good wetting agents, and above 10 indicate good oil-in-water emulsifiers. Furthermore, the HLB value indicates whether the emulsifier is water or oil



soluble. Emulsifiers with low HLB values are soluble in oil while high values are soluble in water (Stauffer, 1999).

#### iv. *Egg role in emulsification*

Egg yolk is a homogeneously emulsified fluid. It contains approximately 50% of suspended solids where proteins and lipids account for respectively 15.7 to 16.6% and 32 to 35% of its weight. Egg yolk is well known to be an excellent emulsifying agent in the production of various food products. The main molecules responsible for these emulsifying properties are essentially phospholipids (PLs) and lipoproteins (phospholipid-protein complex). Egg yolk phospholipids and lipoprotein HLB values vary greatly (from 0 to 72) allowing these molecules to emulsify various types of emulsions (Shahidi, 2005). When diluted into water, egg yolk can be separated into plasma and granule by centrifugation. It has been mentioned that yolk plasma and granules have similar emulsifying activities with granule having the best stabilization properties (Mine, 2002).

The granule consists essentially of high-density lipoproteins (HDL); it has been successfully used to stabilize oil-in-water emulsions. Furthermore, low-density lipoproteins (LDL), another yolk component thought to be essential in the emulsifying and heat stability properties of egg yolk, interact very efficiently at an oil-in-water interface. It has been also noted that in emulsions prepared with egg yolk, the contribution of proteins and lipoproteins to emulsifying activity is significantly greater than that of PLs. The relative adsorption of the proteins is based on their concentration, HLB value, ability to uncoil, and the packing configuration at the interface (Mine, 2002). In the case of bakery products, a complex aerated emulsion of a shortening and oils in an aqueous phase is formed. Lakshminarayan *et al.* (2006), estimated that emulsifiers have several roles in the baking industry. Among them, emulsifiers help optimize the distribution of oil in water dispersions and stabilize the resultant emulsion. They also improve the distribution of air, stabilizing the foam and the product internal characteristics. Finally, emulsifiers have been proven to enhance bakery products shelf life. Due to these characteristics, emulsifiers are now used for the development of reduced fat products but also to implement the use of liquid oils with low levels of trans fatty acids.

During the baking step, the batter is heated and the liquid emulsion phase is transformed, viscosity increases, air bubbles expand, moisture is lost and finally the internal and external cake structures are obtained. The stability of the bubbles and uniform expansion helps to increase the final product specific volume. Maintenance of emulsion stability and

batter viscosity during the baking process is positively influenced by the presence of emulsifying agents (Kiosseoglou, 2003; Lakshminarayan *et al.*, 2006).

## **1. Carob germ proteins**

### **a) Description**

The carob tree (*Ceratonia siliqua*) is a typical Mediterranean tree. In many countries, the fruit is used for preparing popular beverages and confectionery products. In Western countries, carob powder and gum have various usages. After deseeding carob pods, the pods are roasted and milled to obtain carob powder. Carob pods are characterized by high sugar content (more than 50%) mainly composed of sucrose. Carob powder is a natural sweetener with flavor and appearance similar to chocolate; therefore it is often used as cocoa substitute (Bengoechea *et al.*, 2008).

The seeds obtained can be further processed to obtain high value products. In order to separate the germ from the endosperm, the whole seed is milled so that the endosperm remains in large scale like pieces and the germ is turned into a fine powder. This can be achieved due to the differences in friability of the two fractions (the germ is much more brittle and reduces in size easily when compared to the endosperm). After separation the germ is further processed to be used for protein supplementation in both food and feed. The endosperm goes through another milling step to produce a fine powder that is sold under the trade name carob bean gum or locust bean gum. Carob bean gum has many uses as a food additive due to its textural and hydration properties. In food systems carob bean gum is recognized as a food thickener, stabilizer, and emulsifier (Smith, 2009).

Carob germ flour has been traditionally used as a protein additive in animal feeds and foods for human consumption because of its well balanced amino acid content (Smith, 2009; Smith *et al.*, 2012). Carob germ flour is a by-product of carob milling; it contains a high protein content (almost 50%) with a high level of lysine and arginine amino acids. Carob germ flour can find usage as dietetic human food or as a potential ingredient in cereal-derived foods for Celiac people (Feillet and Roulland, 1998; Bengoechea *et al.*, 2008; Smith *et al.*, 2012). The embryo accounts for 23 to 25% of the total seed weight. It is primarily composed of protein (almost half of the embryo's weight) and fiber with little amounts of water, lipid, minerals, polyphenols, and soluble carbohydrates. Bengoechea *et al.* (2008) reported the proximate analysis of defatted carob germ flour (Table 2)

**Table 2: Chemical characterization of defatted flour from carob germ meal (Bengoechea *et al.*, 2008).**

Flour component	Percent and standard deviation of flour
Protein content	48.2 $\pm$ 0.24
Lipids	2.26 $\pm$ 0.13
Moisture	5.76 $\pm$ 0.32
Ash	6.34 $\pm$ 0.15
Polyphenols	0.45 $\pm$ 0.01
Soluble carbohydrates	2.92 $\pm$ 0.03
Total fiber	24.3 $\pm$ 0.09

### **b) Caroubin proteins**

Caroubin is the water-insoluble protein isolated from carob bean embryo. It is a mixture composed of a large number of polymerized proteins of different size (Bengoechea *et al.*, 2008). Feillet and Roulland (1998) were first to study the unique wheat-like proteins caroubins. They achieved a protein separation in two caroubin fractions using extraction and centrifugation. The two fractions have nearly identical amino acid profiles and molecular weight distributions. However, they differed significantly in compressibility, elastic recovery, and viscoelastic index as determined by texture profile analysis (Feillet and Rouland 1998). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and size exclusion-high pressure liquid chromatography (SE-HPLC) experiments revealed that caroubin has an average molecular weight greater than that of gluten. SE-HPLC also demonstrated that wheat gluten has greater amounts of large polymeric proteins than caroubin (Feillet and Rouland 1998). When hydrated, the proteins form aggregates linked via non-covalent and disulfide bonding that have molecular weights between ~13 kDa and ~95 kDa with major bands appearing at 95.5, 55, 26.3, and 13.8 kDa (Bengoechea *et al.*, 2008).

The types of proteins present in carob germ flour were first determined by Plaut *et al.* (1953) based on the Osborne protein classification by solubility. They found that carob germ protein was composed of 14.5% albumins, 50% globulins, 3.4% prolamin, and 32.1% glutelins (Osborne, 1903; Plaut *et al.* 1953). Although carob germ proteins have similar properties to wheat, Osborne fractionation showed that the proteins are quite different. Wheat gluten typically contains 5% albumin, 10% globulin, 69% prolamin, and 16% glutelin

(Osborne 1903). As discussed previously, prolamins in wheat (Glutenins and Gliadins) are the major contributors to viscoelastic properties and carob germ flour contains small amounts of prolamins.

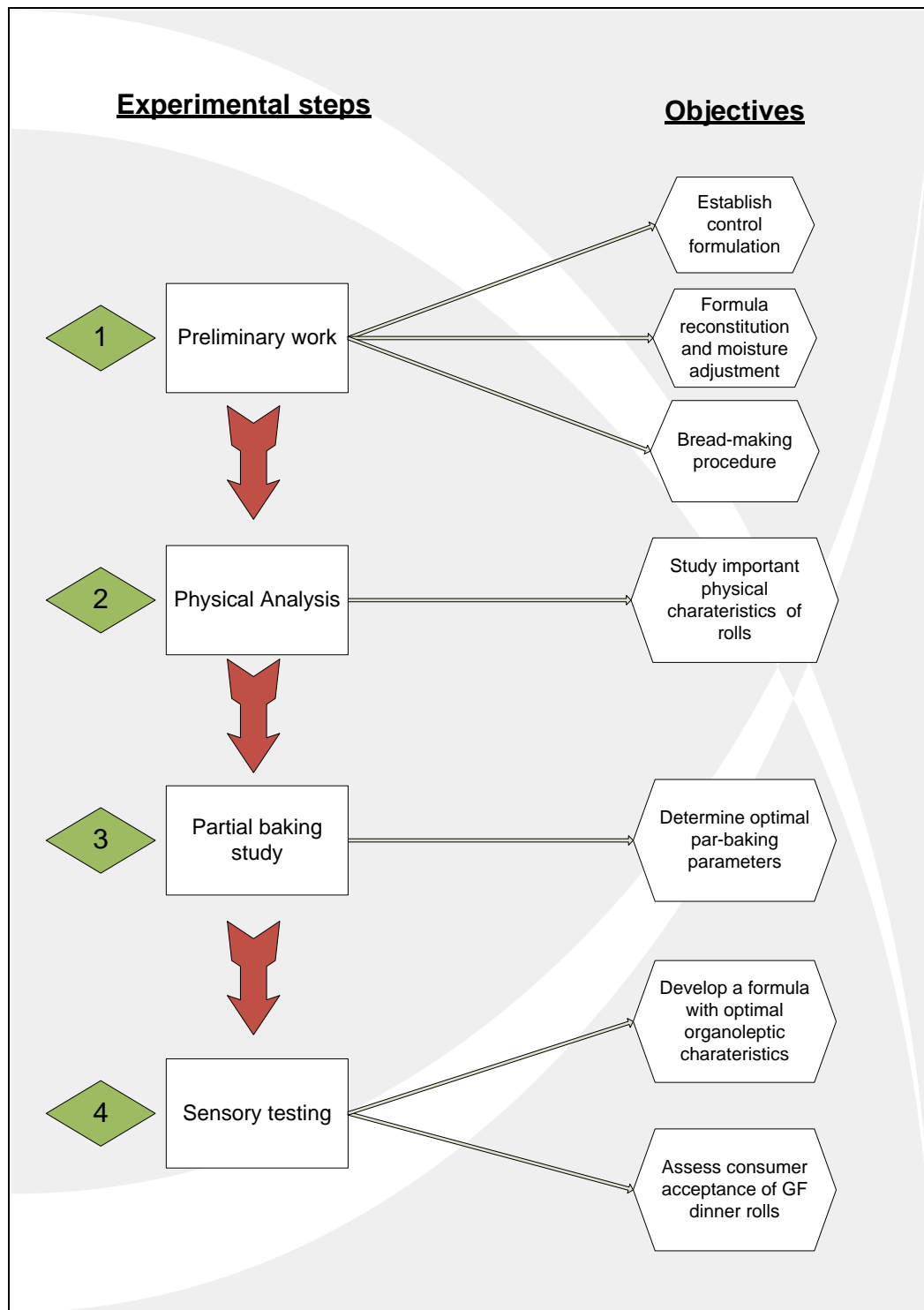
### **c) Carob germ flour and gluten-free technology**

Caroubin has been reported to possess similar rheological properties to gluten. The essential difference is that caroubin has a more ordered structure and presents minor changes in secondary structure when hydrated (Bengoechea *et al.*, 2008). Carob germ flour was first identified to have gluten-like properties in a 1935 U.S. patent. When used in a yeast leavened bread system containing approximately 30% carob germ flour and 70% gluten-free flour, a bread was produced with similar qualities to a European rye bread (Bienenstock *et al.*, 1935). Since this patent, little work has been done to characterize caroubin functional properties when compared to wheat. Until the discovery of Celiac disease and the development of cereal-based GF foods, there was very little data published on the functional properties of carob germ proteins when compared to that of wheat.

It has been stated in many publications that carob germ protein shows significant potential in gluten-free foods due to its viscoelastic nature and its acceptance as being safe for Celiac patients. Smith (2009) studied the characteristics and functionality of carob germ flour in starch-based bread. It was found that the baked dough made from carob germ protein and maize starch is capable of producing bread similar in appearance to a dense wheat bread. Carob also helped in delaying staling which is one of the biggest flaws of GF products (Smith, 2009). Smith *et al.* (2012), studied the effect of HPMC on bread made from carob germ flour-starch mixtures. A high quality GF bread was produced using carob germ flour and protein. Formulation was unique in that a true dough was formed. However, it can be argued that the bread presented a poor nutritional value with low fiber and mineral content essentially due to the use of refined starch as major ingredient.

## PART II: MATERIALS AND METHODS

The scientific approach followed during this research work is summarized in Figure 9.



**Figure 8: Scientific approach used to develop and improve par-baked gluten-free dinner rolls using carob germ flour and egg ingredients.**

# I. Preliminary work

## A. Formulation

Formulation of gluten-free dinner rolls was adapted from previous work on GF bread by Schober *et al.* (2007) as well as Bize (2012). The control sorghum bread studied by these researchers was batter-based. Therefore, modification of their formula was necessary to obtain a dough-like food system that would take a similar shape to traditional dinner rolls after hand-molding. Composite flour including 70% of sorghum flour and 30% of native potato starch was used in order to obtain acceptable crumb properties. The formula is expressed on flour basis: formulation is based on 100 parts of weight of flour rather than actual ingredient percentages (Table 3). Together, the sorghum flour and the native potato starch (and also carob germ flour when used), determined the flour weight basis. The optimal moisture level for corresponding formula (90% on a flour basis for the control) was then determined based on visual observation as well as texture analysis using a cell extrusion rig (TA-XT2, Stable Micro Systems, Godalming, United Kingdom). The force applied to extrude the control dough out of the extrusion cell was recorded in the computer software (Exponent 32, Stable Micro Systems, Godalming, United Kingdom) and compared to that of tested dough

**Table 3: Gluten-free dinner roll control formula.**

<b>Ingredients</b>	<b>Flour Basis (%)</b>	<b>Overall percentage (%)</b>
Sorghum flour	70	33.78
Native potato starch	30	14.48
<i>(Total flour)</i>	<i>(100)</i>	<i>(48.26)</i>
Sucrose	4	1.93
Butter	4	1.93
Non-fat dry milk	4	1.93
Xanthan powder	1.5	0.72
Salt	1.75	0.84
Water	90	43.43
Instant dry yeast	2	0.97
TOTAL	207.25	100

After determination of the base formula, it was modified to comply with the concept of dinner rolls: sweet and buttery. Butter and non-fat dry milk were used to reproduce regular dinner roll flavor attributes according to Wayne's (1985) recommendations.

In the first experimental formulas, HPMC was used to control the rheology and texture of the dough. However, the bread-making process yielded products with very poor textural and appearance characteristics. This was probably due to a loss of functionality after a strong fat-HPMC interaction. Further baking trials showed xanthan gum was a hydrocolloid compatible with a shortening containing food system.

Because the goal of this study was to improve the products using different type of egg ingredients as well as carob germ proteins, their percentage had to be determined. Several levels of carob germ flour were used in preliminary experimentations. Following these, it appeared that replacing 10% of the sorghum flour by carob germ flour yielded the best handling and processing characteristics of the dough. Because carob germ proteins are highly hygroscopic and act as a hydrocolloid, carob containing formulas required a water adjustment. According to the texture analyzer equipped with a forward extrusion cell (TA-93, Stable Micro Systems, Godalming, United Kingdom), in the control formula containing 10% of carob germ flour, the percentage of water had to be raised up to 105% on a flour basis. This composite flour consisted of sorghum flour, potato starch and, if required by formulation, carob germ flour.

Bize (2012) estimated in her work on sorghum batter-based gluten-free bread that fresh egg at a level of about 30% on a flour basis provided significant positive effects on the bread texture, appearance and flavor. That level was hence chosen to be used in this present research.

## **B. Reconstitution formulas**

The optimal level of fresh shell egg was determined to be of 30% on a flour basis (Bize 2012). As the base formula does not contain eggs, adjustments needed to be made to get an identical moisture content and dough consistency in the control formulation, as well as egg containing formulas. A formula containing 30% of fresh shell eggs on a flour basis was used as a reference for the calculations.

The moisture content of fresh shell eggs, commercial egg whites, and whole dry eggs were determined using an electronic moisture analyzer (MX-50 moisture analyzer, A&D

Company limited, Tokyo, Japan). This method indicated a moisture content of 76.73% for fresh shell eggs, 4.96% in dried whole eggs and 87.39% in the egg whites.

To calculate the amount of water to add in the formula containing 30% of fresh shell egg a water percentage calculation was used (Table 4). Then, to determine the water adjustments a reconstitution formulation (Table 5) was adapted from a previous study (Yiu 2002). The adapted formulations are presented in Table 6 and Table 7.

**Table 4: Water percentage calculation in formula containing 30% fresh shell egg.**

Percentage of water necessary in formula (%) = $x$
Water percentage in control (%) = $y$
Moisture brought by shell eggs (%) = $z$
$x = y - z$
Where $y = 90\%$ and
$z = 23\%$ (egg percentage in formula(%) $\times$ egg moisture content(%))
Thus $x = 67\%$

**Table 5: Reconstitution calculation for egg containing formula.**

	Fresh shell egg	Dried whole egg	Egg whites
Moisture $y$ (%)	76.73%	4.96%	87.39%
Solids (%)	23.27%	95.04%	12.61%
Fresh egg moisture (%) $\times$ total solids (%)= $m$		72.92%	9.68%
$1-0.7673= n$		0.2327	0.2327
$m/n = x$		313.38	41.58
$x-y = z$ (water in ml per 100g of egg sample)		308.42	-45.81
$z / (z + 100) * 100 = p$ (water percentage to be added per 100g of egg sample)		75.52%	-84.54%
Fresh egg (%) from formula $\times$ (100 - $p$ ) = egg ingredient (%) in baking formula		7.35%	55.36%
(Fresh egg (%) $\times p$ ) + fresh egg moisture (%)= water(%) in baking formula		89.65%	41.64%



**Table 6: Final gluten-free dinner rolls formulas without carob germ flour.**

	<b>Formulation 1</b>	<b>Formulation 2</b>	<b>Formulation 3</b>	<b>Formulation 4</b>
<b>Ingredients</b>	<b>Control (%)</b>	<b>Fresh shell egg (%)</b>	<b>Dried whole egg (%)</b>	<b>Egg product (%)</b>
Egg ingredient	0%	30%	7.35%	55.36%
Water	90%	67.00% <sup>a</sup>	89.65%	41.64%
Sorghum flour	70%	70%	70%	70%
Potato Starch	30%	30%	30%	30%
Sucrose	4%	4%	4%	4%
Butter	4%	4%	4%	4%
Non-fat dry milk	4%	4%	4%	4%
Xanthan	2%	2%	2%	2%
Salt	2%	2%	2%	2%
Yeast	2%	2%	2%	2%

<sup>a</sup> Amount of water in reference shell egg formula (Formulation 2) was calculated using the equation from Table 4.

**Table 7: Final gluten-free dinner rolls formulas with carob germ flour.**

	<b>Formulation 5</b>	<b>Formulation 6</b>	<b>Formulation 7</b>	<b>Formulation 8</b>
<b>Ingredients</b>	<b>Control (%)</b>	<b>Fresh shell egg (%)</b>	<b>Dried whole egg (%)</b>	<b>Egg product (%)</b>
Egg ingredient	0%	30%	7.35%	55.36%
Water	105%	82.00%	104.65%	56.64%
Sorghum flour	60%	60%	60%	60%
Potato Starch	30%	30%	30%	30%
Carob germ flour	10%	10%	10%	10%
Sucrose	4%	4%	4%	4%
Butter	4%	4%	4%	4%
Non-fat dry milk	4%	4%	4%	4%
Xanthan gum	2%	2%	2%	2%
Salt	2%	2%	2%	2%
Yeast	2%	2%	2%	2%

## **C. Processing**

Establishing the optimal particle size for used sorghum flour was an important step of the processing determination. It appeared that sorghum milled and screened through a 0.5 mm screen on an experimental cyclone mill (UDY corp., Fort Collins, Colorado, USA) was the optimal in terms of hydration and end-product texture. Proofing parameter needed to be determined in order to get desirable volume and textural characteristics. Proofing is the final dough-rise step before baking during which fermentation happens allowing the leavening of the dough. Such parameters included proofing time and temperatures which were optimal for a duration of 90 minutes at a temperature of 37°C respectively.

## **II. Processing conditions**

### **A. Baking materials**

The following ingredients were used during the experimentation:

- White sorghum flour (Fontanelle 1000 variety, harvested in 2008 on Earl Roemer's farm, Lane county, Kansas, USA)
- Native potato starch (Bob's Red Mill, Milwaukie, Oregon, USA)
- Carob germ flour if required (Danisco USA Inc., Kansas City, Missouri, USA)
- Iodized salt (Great Value, Wal-mart Stores, Inc., Bentonville, Arkansas, USA)
- Granulated sugar (Extra Fine, Great Value, Wal-mart Stores Inc., Bentonville, Arkansas, USA)
- Xanthan powder (Ticaxan® Xanthan powder, Tic Gums Inc., Belcamp, Maryland, USA)
- Instant dry yeast (Fleishmann's Yeast, Chesterfield, Missouri, USA)
- Non-fat dry milk ( Kroger Co., Cincinnati, Ohio, USA)
- Sweet cream unsalted butter (Land O Lakes Inc., Arden Hills, Minnesota, USA).  
Ingredients: pasteurized cream, natural flavorings.
- Fresh shell (Grade A large eggs, Kansas State University Tom Avery poultry farm, Manhattan, Kansas, USA)
- Egg whites (Egg Beaters, ConAgra Foods inc., Omaha, Nebraska, USA). Ingredients: egg whites (99%), contains less than 1%: natural flavor, natural color (including beta-

carotene), spices, salt, onion powder, vegetable gums (xanthan gum, guar gum), maltodextrins.

- Dried whole pasteurized eggs (DEB EL Food Products LLC, Elizabeth, New Jersey, USA).

## **B. Manufacture**

### **1. Baking procedure**

Dry ingredients including sorghum flour, potato starch, xanthan gum, non-fat dry milk and finally, carob germ flour if applicable were blended in a separate bowl using a hand spatula. In a different mixing bowl, sugar was dissolved into water kept at room temperature (21°C); butter, egg ingredient if necessary were then added and instant dry yeast was poured over this aqueous solution in order to re-hydrate for 150 seconds. When re-hydration was completed, the dry mix was added in the mixing bowl. The bowl was placed in the receptacle of a stand mixer (Kitchen Aid 300 Watts model, Whirlpool, Benton Charter Township, Michigan, USA) equipped with a flat beater. The blend was first blended for 30 seconds at the first mixer speed out of 10 speeds, and then scraped down from the sides of the bowl. Mixing was continued for 150 seconds at the second speed. After complete mixing and agglomeration, 100 grams of dough was manually rolled and placed into greased square baking pans with dimensions of 7.6 x 7.6 x 3.2cm. The rolls were put in a proofing chamber (Metro C5 1 Series, InterMetro Industries Corporation, Wilkes-Barre, Pennsylvania, USA) at 37°C and 95% humidity for 90 minutes until the dough was at 1 cm above the top of the pan. The pans were then transferred into a double deck bread oven (Artisan Stone Deck oven 1T2, Doyon Equipment Inc., St-Come Linière, Québec, Canada) to bake at 210°C for 18 minutes if the intended product was fully baked dinner rolls or less (0, 8, 10, and 12 minutes) to obtain par-baked rolls. Upon final baking completion, bread rolls were removed from the pans and kept at room temperature (21°C) for one hour to let moisture and temperature equilibrate throughout the product before further testing could be conducted. While testing the various GF bakery products' properties, the room temperature was of 21°C and the relative humidity of 60%.

### **2. Par-baking experiment**

Rolls were par-baked for respectively 0, 8, 10, 12 and 18 minutes at 210°C. Par-baked bread rolls were then blast frozen at -28°C upon completion of the one hour cooling down

period and subsequently stored in a regular home freezer at -20°C. Later on, rolls were fully thawed and reheated for 10 minutes in the double deck bread oven to complete baking. They were subsequently cooled at room temperature for one hour before measurements were taken. Par-baking testing was only performed on the control (Formulation 1) and 30% fresh egg +10% carob germ flour (Formulation6) (Table 6 and 7).

### **III. Design of experiment**

To best determine the rolls physical properties, a randomized complete block design (RCBD) was used. According to Shieh and Jan (2004), RCBD designs are particularly useful to compare treatment means when there is a possible extraneous source of variability that the researcher needs to control. In such designs, treatments are randomly assigned to experimental units within a block (representing the eventual source of variability), with each treatment appearing exactly once in every block. Both randomization and blocking in RCBD are statistical tools that help reduce bias and adverse effects of random variability in experimentation (Ott and Longnecker, 2004).

In the physical analysis experiment of fully baked dinner rolls, there were a total of eight treatments chosen to be tested (Table 6 and 7) in three replicates (one replicate produced and analyzed every day during a three day experiment) with six sub-samples (loaves of bread). The blocking effect was the day the experimentation was performed.

The par-baking study was comprised of 10 treatments that were similarly tested in triplicates with six sub-samples and a “day” blocking effect. The treatments consisted of 2 formulations (control or Formulation 1 versus Formulation 6 that contained 30% fresh shell eggs and 10% carob germ flour on a flour basis) and 5 par-baking times (0, 8, 10, 12 and 18 minutes). Physical analysis data (except crumb analysis data) were analyzed using SAS software by using the analysis of variance (ANOVA) technique and a restricted maximum likelihood (REML) approach (Software Release 9.2, SAS Institute Inc., Cary, North Carolina, USA). Crumb data was analyzed using the same software using SAS and the ANOVA technique with a GLIMMIX procedure.

Significant differences between the treatment means were then determined at a statistical level of significance at  $\alpha \leq 0.05$ . When treatment effects were found significantly different, the least square means with Tukey-Kramer adjustments and groupings were used to differentiate treatment means.

During the sensory evaluation of a large consumer panel, only three formulations were evaluated:

- Control without eggs and carob germ flour.
- Treatment with 30% fresh shell eggs and 10% carob germ flour (flour basis).
- Treatment with 30% fresh shell eggs, 10% carob germ flour, 15% clover honey (Ficher's pure clover honey, Ficher Honey Co., North Little Rock, AR, USA) and 15% roasted sunflower seeds on a flour basis (Nature's harvest, Amport foods, Minneapolis, MN, USA).

Basic statistical analysis (mean, standard deviation and relative standard deviation) as well as ANOVA with grouping of the means were run on collected data. The statistical difference between the means was determined using a classic Student T-Test at a level of significance  $\alpha \leq 0.05$ .

## **IV. Physical analyses**

### **A. Specific volume**

After moisture and temperature equilibrium were reached, rolls were weighed (g) and their respective loaf volumes (cm<sup>3</sup>) were determined by rapeseed displacement according to the standard American Association of Cereal Chemists (AACC) international approved method 10-05.01. Specific volume (cm<sup>3</sup>/g) was deducted by dividing volume by weight of rolls.

### **B. Texture Profile Analysis**

Texture Profile Analysis (TPA) of the rolls crumb was performed on each slice using a texture analyzer equipment (TA-XT2, Stable Micro Systems, Godalming, United Kingdom) equipped with a 25mm diameter and 35mm tall acrylic cylindrical probe (model TA-11, 1 inch) along with a 30kg load measurement electronic cell. A two cycle crumb compression test with a 5 second wait time between the first and second compression cycles was performed. The analysis was carried out at constant test speed of 2.0 mm/s and a pre and post-testing speed of 10mm/s on a distance of 10.0mm. Finally, the trigger force was 10.0 g (minimal force necessary to start the data acquisition). The dinner roll samples were previously sliced in the middle using a custom made cutting case and a regular bread knife to obtain uniform slices of 2.5cm thickness. Main studied parameters generated using the texture

exponent software included hardness, springiness, cohesiveness, and chewiness. These are good indicators of crumb quality (Kilcast, 2004). A similar analysis was performed on both directly fully baked and par-baked dinner rolls.

### **C. Crumb structure**

The 25 millimeters bread roll slices were used to determine the overall quality of the crumb structure using a C-Cell Instrument (Calibre Control International Ltd., Appleton, Warrington, United Kingdom). The C-Cell apparatus uses high resolution optics to obtain high definition images. These images are then analyzed using a proper software (CC.200: version 2, Calibre Control International Ltd., Appleton, Warrington, United Kingdom) to provide data such as the number of cells, cell diameter and cell wall thickness.

### **D. Color analysis**

Color attributes of rolls crust and crumb were assessed using a Hunter L.a.b. colorimeter (Model MiniScan EZ, Hunter Associates Laboratory, Inc., Reston, Virginia, USA). Readings were displayed as L\*, a\* and b\* color parameters according to the CIELAB international system of color measurement. The L\* value indicates the measure of lightness and ranges from 0 (black) to 100 (white), a\* is a measure of greenness (from -100 or green to 100 or magenta) and, finally, b\* value ranges from -100 (blueness) to 100 (yellowness). The colorimeter was calibrated with a reference white tile ( $L^*=94.06$ ,  $a^*=-0.73$ ,  $b^*=2.26$ ) and a reference black glass ( $L=0.61$ ,  $a=0.27$ ,  $b=-0.35$ ).

## **V. Sensory analysis**

To assess the acceptance and quality of the products, a consumer study was carried out in the Call Hall building of Kansas State University. Consumers were asked to evaluate three different types of GF dinner rolls. The bread rolls formulations tested consisted of a control, the treatment presenting the best physical characteristics (Formulation 6), and finally Formulation 6 with added honey and sunflower seed presenting the most pleasant organoleptic properties (these two ingredients helped cover the musty flavor of sorghum and the beany aroma of CGF). These desired organoleptic characteristics were determined during three informal sensory testing sessions featuring three food scientists from the KSU Food Science Institute. Rolls were par-baked for 12 minutes, cut in half slices of 1 cm in thickness,

blast frozen and stored at -20°C. Before presenting roll samples to panelists, slices were heated at 232°C for 3 minutes in a conventional convection oven (General Electric Co., Fairfield, CT, USA) and kept at 77°C for no more than ten minutes and served at a temperature of about 50°C.

A total of 137 untrained panelists volunteered to participate in this study including 9 individuals suffering from celiac disease or gluten allergy/sensitivity. Every participant started by signing an informed consent statement form informing about the purpose and guidelines of the study before participating (*Appendix B*). Then, they completed the numbered pre-screening form containing information about their age, gender, highest education completed, if they suffer from any gluten-related illness (celiac disease or gluten allergy), the frequency of their gluten-free bakery products consumption, and, finally, any known food allergies other than celiac disease or gluten allergy they might have (*Appendix C*).

The three samples were presented in a random sequence as half slices of about 1 cm in thickness in coded dishes. At the time the three samples were distributed, numbered ballots (matching with the pre-screening form number) bearing the identical three-digit codes were handed to the panelists.

The volunteers were asked to test each sample in the specified order they were served to eliminate possible bias and complete the ballots following the instructions. Out of 137 panelists, 70 persons tasted the rolls with approximately 1.5 grams of unsalted butter (Sweet cream unsalted butter, Land O Lakes Inc., Arden Hills, Minnesota, USA) while the 67 others consumed them plain. Analyses were performed under normal lighting conditions. The panelists cleansed their palates between samples with tap water.

Each ballot contained a 9-point hedonic scale matching with the five tested attributes (overall acceptability, appearance, flavor, color, and texture). These 9-point hedonic scales displayed the degree of liking corresponding to the specific attributes (9 being “like extremely”, 5 being “neither like nor dislike”, and 1 being “dislike extremely”). Rolls were considered acceptable if their mean scores for overall acceptance were above 5 (“neither like nor dislike”).

Another scale was used to assess the likelihood of buying the product if it was marketed. On this hedonic scale, 9 corresponded to “definitely yes”, 5 was “maybe” and 1 was “definitely not”. When panelists were finished with tasting and rating, they had the opportunity to write additional comments to detail more explicitly their grading as well as any improvement suggestions (*Appendix D*).

## PART III: RESULTS AND DISCUSSION

### I. Physical analyses of fully baked rolls

#### A. Specific volume

Specific volume (SV) of leavened baked goods is a very important parameter to assess quality. Gluten-free products have been found to generally have low SVs; hence, increasing this parameter is critical to improve the overall quality of the leavened goods (Lazaridou, 2007; Sabanis and Tzia, 2010). According to Sciarini *et al.* (2010a), GF batter-based breads commonly yield products having a SV ranging around  $2 \text{ cm}^3.\text{g}^{-1}$  which can seem fairly low when compared to their wheat-based counterparts (presenting SVs often around  $4\text{-}7 \text{ cm}^3.\text{g}^{-1}$ ). It is nevertheless important to note that generally, shortening containing bakery products often present worse volume characteristics (Wayne, 1985).

There was an increase ( $p<0.05$ ) in rolls volume between the control and all the other treatments (table 8 and graph 8), meaning that both egg ingredients and carob germ flour improved bread roll quality. The type of egg ingredients used clearly had a significant impact on SVs. Whereas the two formulations containing fresh shell eggs displayed the highest SVs ( $1.96 \text{ cm}^3.\text{g}^{-1}$ ), the ones containing egg white-based product and whole dried eggs had a significantly ( $p<0.05$ ) lower SVs (Table 8). In the last two stated formulations (containing egg white-based product and whole dried eggs), adding carob germ flour did not influence the rolls SVs ( $p>0.05$ ). This observation implies that the type of egg ingredient had a greater impact on the product volume characteristics than the presence of carob germ flour. However, it is crucial to note that for the control formulation, adding only carob germ flour at a level of 10% (on a flour basis), significantly raised its SV ( $p<0.05$ ). Overall, from these numbers, it appears that egg ingredients and carob germ proteins played a positive role on gluten-free sorghum breads.

The differences observed between the egg ingredients can be explained by various reasons. Egg whites helped improve SV values but did not produce the best volume characteristics. Egg whites are essentially composed of water and proteins. Egg proteins help produce stable foams during the mixing step. This protein foam is further stabilized during baking as protein coagulation occurs giving a more cohesive crumb structure (Mine, 2002).



Moreover, sulfur containing egg white proteins, especially the main egg white protein, ovalbumin, further stabilizes the formed gel by polymerization via the thiol disulfide exchange (Houben *et al.*, 2012). These proteins may also be causing some oven spring which is the sudden increase in volume during the first minutes of baking resulting from the increased rate of yeast fermentation as well as the production and expansion of gases that are trapped by a protein matrix. Oven spring supports a second rise in final loaf volume during the baking stage.

Formulations containing fresh shell eggs had the best SV values. Besides benefiting from white proteins foaming and coagulation abilities, these formulations were favored by the emulsion properties of yolk lipo-proteins and phospholipid. Emulsion stability in rolls seemed particularly important as it improves the dispersion and stabilization of gas bubbles, the product internal characteristics and stabilizes the foam.

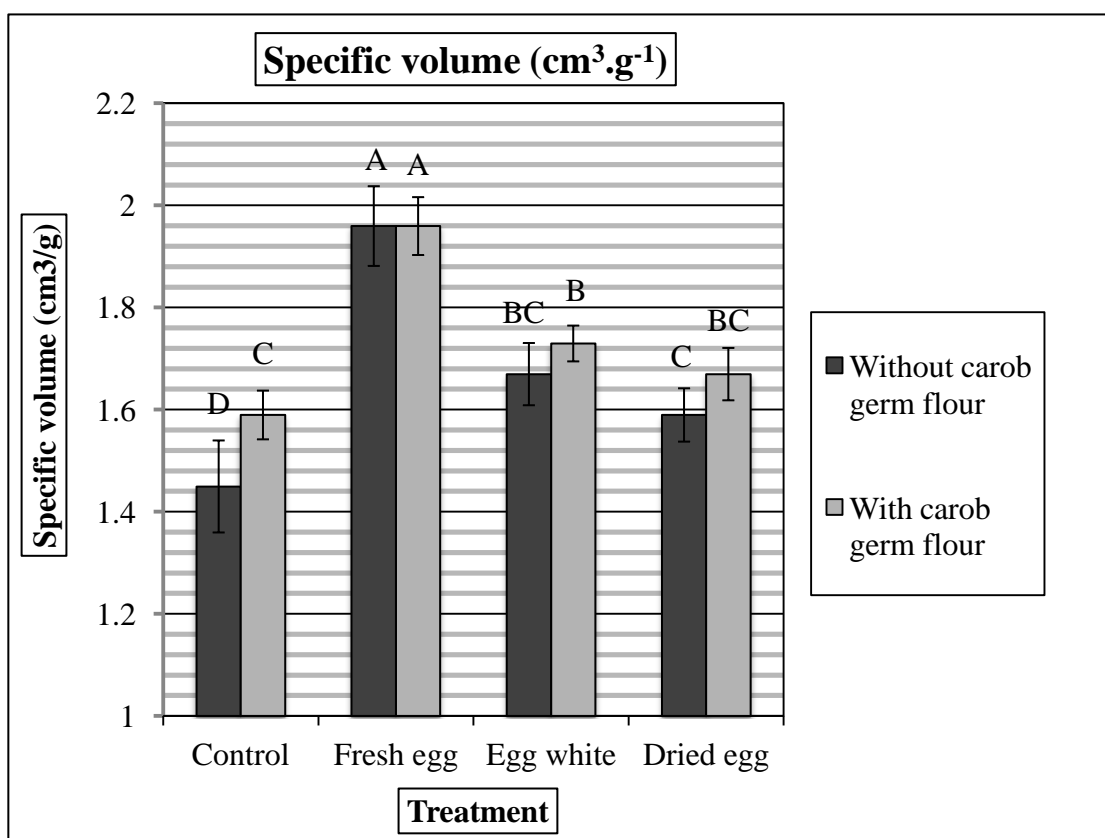
Finally, whole dried eggs (which should function similarly to fresh shell eggs), when added to the dinner roll formulation, imparted little volume improvement compared to the fresh egg containing formulations. Because, this type of product is obtained by drying whole fresh eggs during a thermal processing, it is reasonable to think that dried egg powder loses some of its functional properties during the transformation processing.

Although carob germ proteins were not found to have such a great effect as egg ingredients, the control formulation with CGF had a significantly greater SV than the control. This can be simply explained by the gluten-like properties of caroubins described by Smith (2009). During proofing, carbon dioxide is released from the yeast fermentation and released into the food matrix. Because caroubins are able to form a tri-dimensional network, they permit the system to entrap formed gases and allow rising of the dough.

**Table 8: Specific volume means and standard deviations of fully baked gluten-free dinner rolls made with various egg ingredients, with or without carob germ flour, n=18 ( $\text{cm}^3.\text{g}^{-1}$ ).**

Specific volume ( $\text{cm}^3.\text{g}^{-1}$ )				
	Control	Fresh egg	Egg white	Dried egg
Without carob germ flour	1.45 $\pm$ 0.09 <sup>D</sup>	1.96 $\pm$ 0.08 <sup>A</sup>	1.67 $\pm$ 0.06 <sup>BC</sup>	1.59 $\pm$ 0.05 <sup>C</sup>
With carob germ flour	1.59 $\pm$ 0.05 <sup>C</sup>	1.96 $\pm$ 0.06 <sup>A</sup>	1.73 $\pm$ 0.04 <sup>B</sup>	1.67 $\pm$ 0.05 <sup>BC</sup>

<sup>A-D</sup> Values with a common uppercase letter are not significantly different ( $p>0.05$ ).



**Figure 9: Specific volume means and standard deviations of fully baked gluten-free dinner rolls made with various egg ingredients, with or without carob germ flour, n=18. <sup>A-D</sup> Values with a common uppercase letter are not significantly different ( $p>0.05$ ).**

## B. Texture Profile Analysis

Several crumb characteristics including hardness, springiness, cohesiveness, and chewiness were studied during the Texture Profile Analysis (TPA) of the rolls. According to Kilcast (2004), these parameters can be defined as follows:

- Hardness or firmness: Force required to compress a food between the molars. In a TPA experiment hardness corresponds to the maximum force applied.
- Springiness or elasticity: The extent to which a compressed food returns to its original size when the load is removed. It is basically the height that the food recovers between the end of the first bite and the second bite. Springiness values range from 0 to 1.
- Cohesiveness: The strength of the internal bonds making up the food. Cohesiveness is the ratio of the positive force area during the second compression to that during the first compression and hence is a uniteless value.
- Chewiness: The energy necessary to chew and disintegrate a solid food so that it is ready for swallowing.

It is widely recognized that the hardness (here, the measure of maximum force required to compress crumb by a specific length at a specific rate and expressed as gram of force) is the most important parameter to look at when assessing crumb quality. Soft texture is generally the sign of better quality bread since it is strongly correlated with consumers' perception of bread freshness. Higher springiness and cohesiveness values as well as low chewiness values are also good indicators of bakery products quality (Kilcast, 2004; Sabanis and Tzia, 2010; Sciarini *et al.*, 2010a)

### 1. Hardness

Carob germ flour as well as the type of egg ingredient (except for fresh shell egg), affected crumb firmness of GF dinner roll significantly ( $p < 0.05$ ) as it can be observed in Table 9 and Figure 9. The presence of carob germ flour had a great positive effect on crumb hardness. Rolls with 10% carob germ flour all had lower hardness values ( $p < 0.05$ ) than rolls without CGF. Fresh eggs did not have a significant impact on crumb hardness ( $p > 0.05$ ), but egg white and whole dried eggs increased crumb hardness ( $p < 0.05$ ). Control with carob germ flour and the treatment containing 30% fresh egg and 10% carob germ flour presented the best crumb firmness with 1404 and 1468 grams of force respectively while the treatment containing egg whites had the less soft crumb with 3058 grams of force (Figure 9).

According to Sciarini *et al.* (2010a), there is a negative correlation between crumb hardness and specific volume (product with lower specific volumes present higher crumb firmness values. This was not always the case in our study. For example, control with 10% carob germ flour had a much lower crumb hardness (1404 grams) as well as a higher SV (1.59 cm<sup>3</sup>.g<sup>-1</sup>) than the control (2074 grams and 1.45 cm<sup>3</sup>.g<sup>-1</sup> respectively). On the contrary, the treatment containing egg whites had a much firmer crumb structure (3058 grams) but a higher SV (1.67 cm<sup>3</sup>.g<sup>-1</sup>) than the control. Bize (2012) determined that adding fresh eggs to sorghum batter-based breads should decrease bread crumb firmness. According to our results, this assessment cannot be extrapolated to the present GF bread roll formulations.

The main conclusion from this crumb hardness study is that caroubins, thanks to their gluten-like properties, significantly affected crumb hardness and helped produce a much more acceptable GF raised bakery product.

## **2. Other TPA parameters**

Because there was no significant differences in springiness (values close to 1) among all the treatments ( $p>0.05$ ), freshly baked dinner rolls presented optimal characteristics for this parameter (Table 9). Marco and Rosell (2008) found springiness values that ranged from 0.77 to 0.94 when studying the protein enrichment of rice based gluten-free breads. Low springiness value is indicative of brittleness and this reflects the tendency of the bread to crumble when it is sliced. Hence, the elasticity characteristics of the rolls can be considered as desirable.

Cohesiveness characterizes the extent to which a material can be deformed before it ruptures, reflecting the internal cohesion of the material. Bread with high cohesiveness is desirable because it forms a bolus rather than disintegrates during mastication, whereas low cohesiveness indicates increased susceptibility of the bread to fracture or crumble. Cohesiveness values were similarly comparable for most of the tested products. The only significant improvement was found when adding CGF to the formulation containing 30% fresh shell eggs ( $p<0.05$ ) and cohesiveness increased from 0.49 to 0.57 (Figure 10). These results differ slightly from what we might have expected. The logical results would have been that egg ingredients and caroubins would impart more subsequent structural strength to the bread rolls translated by higher cohesiveness values for the corresponding treatments. In a study conducted by Matos and Rosell (2012), 11 commercialized GF breads were analyzed

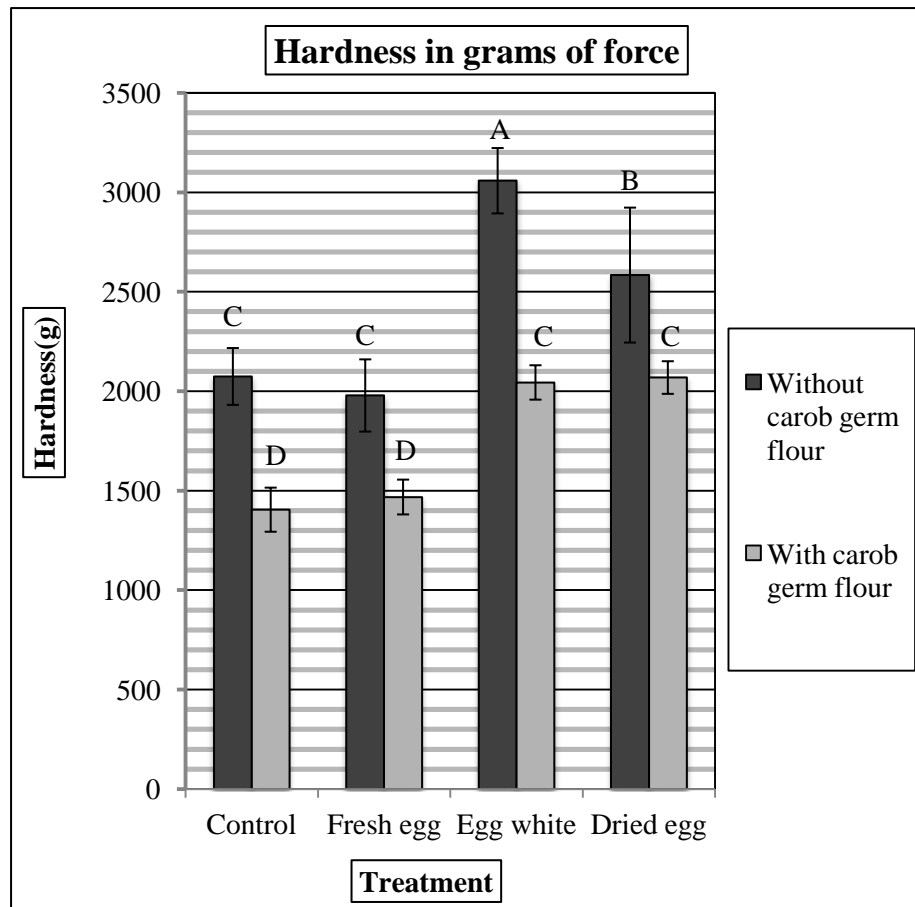
and found to have cohesiveness values ranging from 0.20 to 0.44. The values found for the rolls were consequently of better quality regarding this parameter.

Finally, chewiness values were augmented ( $p < 0.05$ ) compared to control when egg whites and dried eggs were added but did not change when CGF was added (except for the formulation having egg whites). This implies that these two formulations would require more energy to be masticated. It can be inferred that these types of egg products, because of their coagulation properties impart strong resilience potency to that type of food matrix.

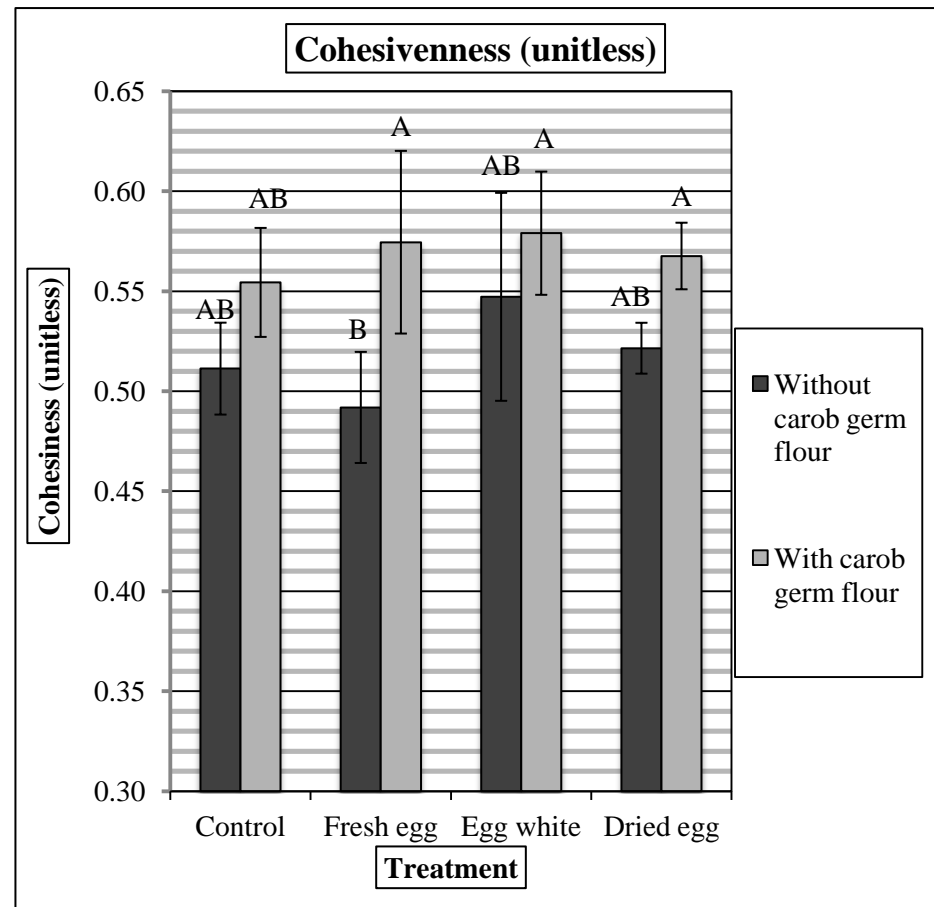
**Table 9: Crumb texture profile means and average deviations of fully baked gluten-free dinner rolls made with various egg ingredients, with or without carob germ flour, n=18.**

	Hardness (g)				Springiness (unitless)			
	Control	Fresh egg	Egg white	Dried egg	Control	Fresh egg	Egg white	Dried egg
<b>No carob germ flour</b>	2074 ± 142 <sup>C</sup>	1978 ± 181 <sup>C</sup>	3058 ± 164 <sup>A</sup>	2583 ± 339 <sup>B</sup>	0.97 ± 0.03 <sup>A</sup>	0.96 ± 0.02 <sup>A</sup>	0.99 ± 0.01 <sup>A</sup>	0.95 ± 0.03 <sup>A</sup>
<b>With carob germ flour</b>	1404 ± 110 <sup>D</sup>	1468 ± 87 <sup>D</sup>	2044 ± 86 <sup>C</sup>	2068 ± 82 <sup>C</sup>	0.97 ± 0.01 <sup>A</sup>	0.97 ± 0.02 <sup>A</sup>	1.00 ± 0.02 <sup>A</sup>	0.95 ± 0.01 <sup>A</sup>
	Cohesiveness (unitless)				Chewiness (g)			
	Control	Fresh egg	Egg white	Dried egg	Control	Fresh egg	Egg white	Dried egg
<b>No carob germ flour</b>	0.51 ± 0.02 <sup>AB</sup>	0.49 ± 0.03 <sup>B</sup>	0.55 ± 0.05 <sup>AB</sup>	0.52 ± 0.01 <sup>AB</sup>	1002 ± 80 <sup>BCD</sup>	936 ± 157 <sup>BCD</sup>	1687 ± 237 <sup>A</sup>	1274 ± 164 <sup>AB</sup>
<b>With carob germ flour</b>	0.55 ± 0.03 <sup>AB</sup>	0.57 ± 0.05 <sup>A</sup>	0.58 ± 0.03 <sup>A</sup>	0.57 ± 0.02 <sup>A</sup>	753 ± 42 <sup>D</sup>	803 ± 63 <sup>CD</sup>	1201 ± 164 <sup>BC</sup>	1126 ± 55 <sup>BCD</sup>

<sup>A-D</sup> Values with a common uppercase letter within the same parameter are not significantly different (p>0.05).



**Figure 11: Crumb hardness means and standard deviations of fully baked gluten-free dinner rolls made with various egg ingredients, with or without carob germ flour, n=18. <sup>A-D</sup> Values with a common uppercase letter are not significantly different (p>0.05).**



**Figure 10: Crumb cohesiveness means and standard deviations of fully baked gluten-free dinner rolls made with various egg ingredients, with or without carob germ flour, n=18. <sup>A-B</sup> Values with a common uppercase letter are not significantly different (p>0.05).**

## C. Crumb cell analysis

Figure 13 presents the C-Cell images of the eight different dinner roll formulations. These digital pictures were analyzed using C-cell software (CC.200: version 2, Calibre Control International Ltd., Appleton, Warrington, United Kingdom) giving three crucial indications on the rolls crumb structure (Table 10) (number of cells per slice, average cell diameter (mm) and cell wall thickness (mm)).

In bakery products, the extent to which cells are formed is a function of the protein-starch interaction. Formation of the dough viscoelastic network occurs due to this interaction. Air cells, are created during mixing and CO<sub>2</sub> which is produced as a by-product of yeast fermentation, diffuses into these air cells, which causes them to expand (Hoseney, 2010). In leavened goods, a fine crumb characterized by numerous small gas cells is the sign of good quality. However, due to the lack of gluten proteins, GF bakery products often display very poor crumb and cell characteristics, including a coarse and dense structure linked to the presence of a small number of very large cells as well as high cell-wall thickness (Sciarini *et al.*, 2010b). This observation directly results from the incapability to incorporate gases during the mixing stage and to retain the carbon dioxide formed during proofing, owing to the lack of a three-dimensional visco-elastic network.

Results indicated that adding egg ingredients to the control increased ( $p < 0.05$ ) the number of cells observable in slices (Table 10 and Figure 11). Further substantial augmentation of number of cells ( $p < 0.05$ ) was observed when adding 10% CGF (flour basis) with the samples containing egg ingredients and CGF having the highest number of visible cells. This indicates that egg proteins and caroubins are good gluten substitutes to create a proper network in bread crumb. These observations aligns with research by Moore *et al.* (2006) who reported that higher number of cells could be obtained when adding egg powder. There were no real differences in number of cells as a function of the type of egg ingredient ( $p > 0.05$ ).

The second crumb cell parameter that is of importance in determining GF products' crumb quality is the average cellular size. While fresh eggs did not have a considerable effect on average alveoli size ( $p > 0.05$ ) egg whites and dried eggs reduced significantly the average cell size ( $p < 0.05$ ). When carob germ flour was added to the control and 30% fresh egg formula it reduced ( $p < 0.05$ ) the cell diameter.



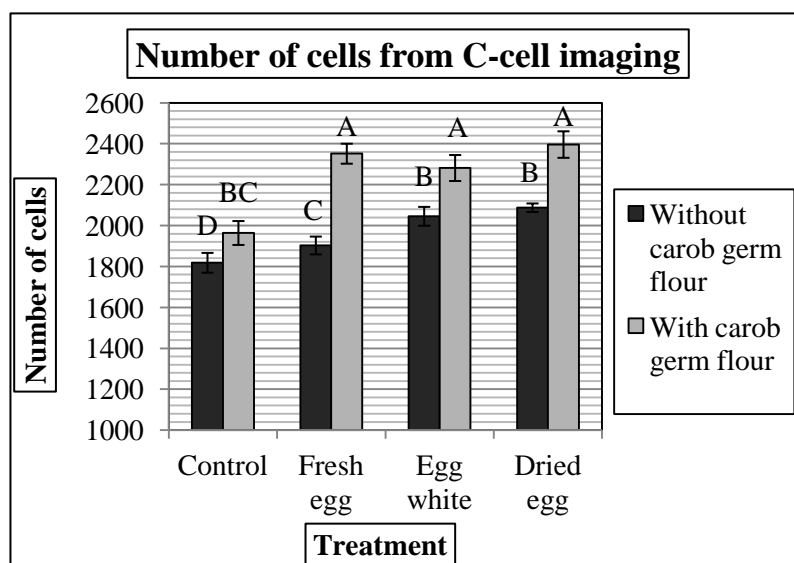
Cell wall thickness values did not seem to follow a very specific trend. It seemed that the whole dried eggs formulation yielded the rolls with thinnest cell walls. In every case adding CGF reduced slightly alveoli wall thickness but not significantly ( $p>0.05$ ).

Close examination of slice pictures (Figure 13) indicate that formulations having both egg ingredients and CGF have overall better crumb properties. Changes in volume can also be identified with the two treatments containing fresh eggs having an apparent larger surface area (and hence a higher specific volume). Egg proteins and caroubins containing rolls seemed to result in a more continuous food matrix compared to control with an improved crumb structure with less noticeable bigger cells. According to these results, egg ingredients and CGF addition were very beneficial to the crumb structure as well as to the cell characteristics.

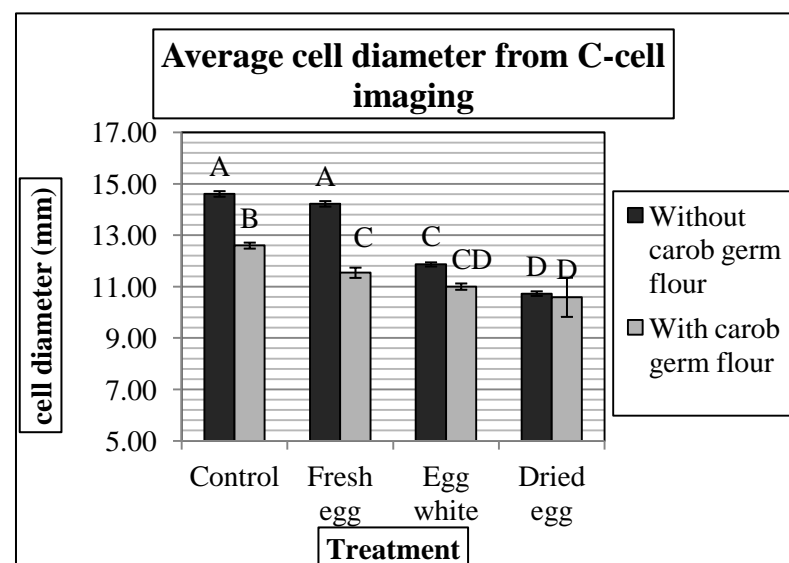
**Table 10: Cell profile means and standard deviations from C-Cell analysis of fully baked gluten-free dinner rolls made with various egg ingredients, with or without carob germ flour, n=18.**

	Number of cells		Cell diameter (mm)		Wall thickness (mm)	
	No carob	With carob	No carob	With carob	No carob	With carob
No egg	1818 ± 48 <sup>D</sup>	1964 ± 58 <sup>BC</sup>	14.61 ± 0.11 <sup>A</sup>	12.60 ± 0.12 <sup>B</sup>	3.32 ± 0.01 <sup>A</sup>	3.23 ± 0.11 <sup>A</sup>
Fresh egg	1903 ± 43 <sup>C</sup>	2352 ± 48 <sup>A</sup>	14.22 ± 0.11 <sup>A</sup>	11.54 ± 0.20 <sup>C</sup>	3.44 ± 0.05 <sup>A</sup>	3.15 ± 0.02 <sup>AB</sup>
Egg white	2045 ± 46 <sup>B</sup>	2282 ± 63 <sup>A</sup>	11.87 ± 0.09 <sup>C</sup>	11.00 ± 0.12 <sup>CD</sup>	3.28 ± 0.07 <sup>A</sup>	3.10 ± 0.12 <sup>B</sup>
Dried egg	2087 ± 20 <sup>B</sup>	2396 ± 64 <sup>A</sup>	10.73 ± 0.09 <sup>D</sup>	10.59 ± 0.76 <sup>D</sup>	3.08 ± 0.01 <sup>B</sup>	2.99 ± 0.12 <sup>B</sup>

<sup>A-D</sup> Values with a common uppercase letter within the same parameter are not significantly different (p>0.05).

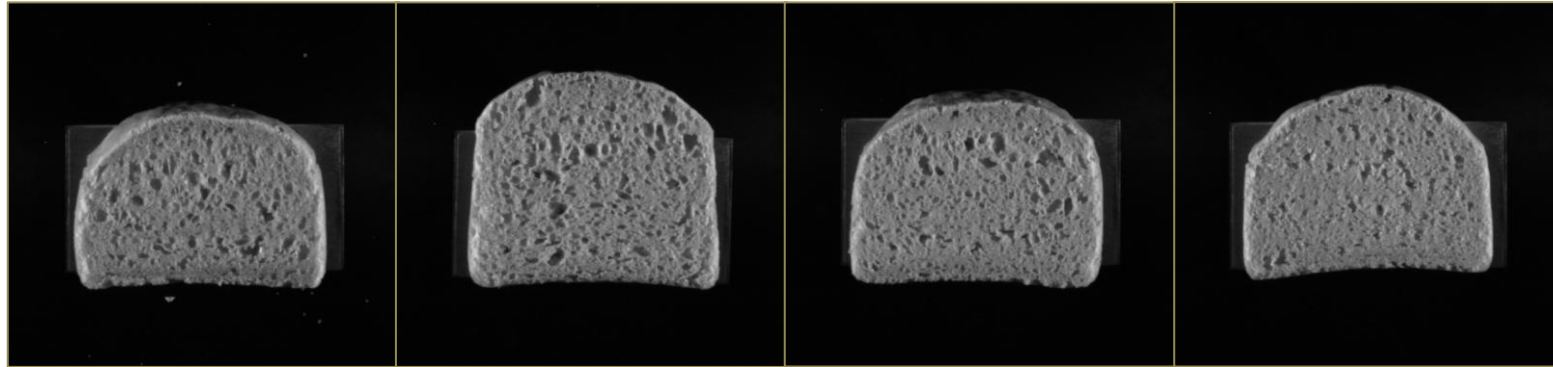


**Figure 13: Cell number means and standard deviations from C-Cell analysis of fully baked gluten-free dinner rolls made with various egg ingredients, with or without carob germ flour, n=18.** <sup>A-D</sup> Values with a common uppercase letter are not significantly different (p>0.05).

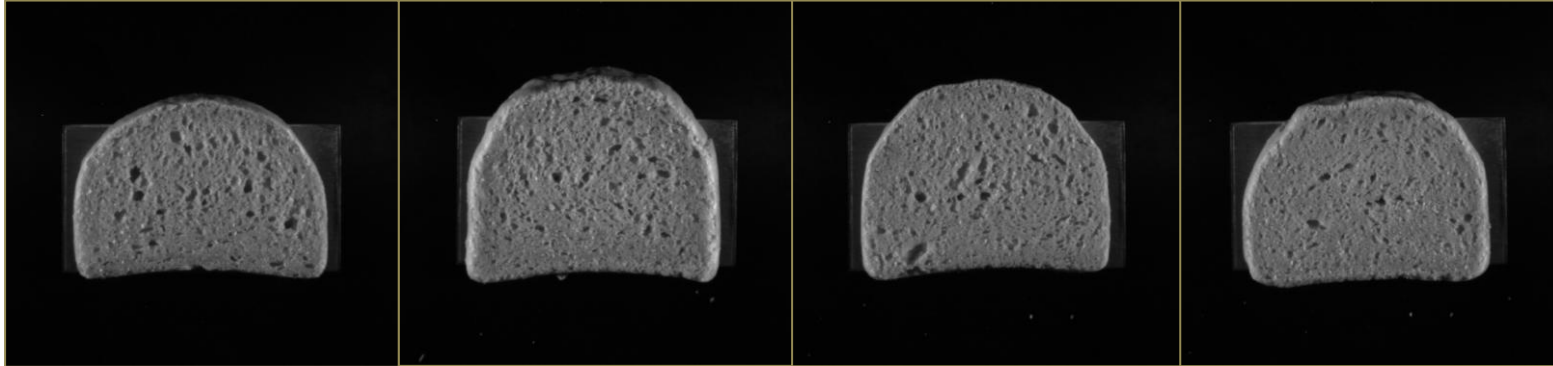


**Figure 12: Average cell elongation means and standard deviations from C-Cell analysis of fully baked gluten-free dinner rolls made with various egg ingredients, with or without carob germ flour, n=18.** <sup>A-D</sup> Values with a common uppercase letter are not significantly different (p>0.05).

**Top pictures: Formulations without carob germ flour. From left to right: Control (no egg ingredient and no carob germ flour), 30% Fresh egg, 55.36%, Egg whites, 7.35% whole dried eggs.**



**Bottom pictures: Formulations with carob germ flour. From left to right: 10% carob germ flour, 10% carob germ flour and 30% Fresh egg; 10% carob germ flour and 55.36% Egg whites, 10% carob germ flour and 7.35% whole dried eggs.**



**Figure 14: C-Cell digital images of the eight gluten-free formulations of sorghum rolls made with various egg ingredients, with or without carob germ flour (% on flour basis).**

## **D. Crumb and crust color parameters**

### **1. Crumb**

There was a difference ( $p < 0.05$ ) in crumb lightness when egg ingredients were added to the control formulation (Table 11). Rolls with eggs were characterized by higher  $L^*$  values which means that the crumb color was lighter. When 10% of CGF was added all the treatments had their lightness decreased ( $p < 0.05$ ). According to Matos and Rosell (2012), the darkening of the crumb color is desirable as GF bakery products generally tend to have much lighter color than regular wheat bread and because darker breads are often associated with wholegrain and wholesomeness by consumers. Concerning this parameter, samples containing CGF presented the most desirable values.

Egg ingredients inclusion (except dried eggs) imparted change ( $p < 0.05$ ) in  $a^*$  values. All samples showed low positive  $a^*$  values indicating a hue on the red axis. Whereas, fresh eggs significantly decreased the value, egg whites significantly increased it. CGF had a significant impact on the samples' redness as  $a^*$  values decreased when 10% were added. This indicated that with this kind of ingredient, the crumb had less of a red color. However, the reduction in  $a^*$  values were not sufficient to have hue on the green axis and have a greenish color. Regarding the rolls crumb, there was no particular pattern in the results according to the type of egg ingredient or the presence of CGF (Table 11). Because  $b^*$  values were all positive there was a hue on the yellow axis. The control formulation presented the lowest value ( $b^* = 22.53$ ) while the formulation containing egg whites had the highest value ( $b^* = 29.45$ ). Carob germ flour did not seem to really affect this parameter.

### **2. Crust**

Differences ( $p < 0.05$ ) in  $L^*$  values were observed during the crust color analysis. The control and the formulation with only egg powder displayed the lightest color ( $L^* = 75.89$  and  $L^* = 74.32$ , respectively). Similarly to the crumb  $L^*$  values, 10% of CGF dramatically increased ( $p < 0.05$ ) the darkness of the crusts. The darkest crust was the formulation containing CGF and 30% fresh eggs ( $L^* = 59.61$ ). The fact that samples containing CGF and egg ingredients (except dried eggs) displayed darker crust is essentially linked to the increase in production of aromatic and colored compounds during baking due to the Maillard reaction.

The Maillard reaction is the condensation of a reducing sugar with an amino acid in presence of water during a thermal treatment that results in the formation of melanoidins

which are brown-colored compounds and pyrazines compounds responsible for the characteristic flavors and aromas (Capuano *et al.*, 2008).

In the Maillard reaction, egg ingredients and CGF can increase the amount of substrate (*i.e.* reducing sugars and amino acids from proteins) in the food system. Because egg contains naturally some glucose which is a reducing monosaccharide, it will readily react with amino-containing compounds such as proteins and any free amino acid, especially lysine, and induce more browning of the crust and production of desirable aromatic molecules. Because glucose is removed from eggs using glucose oxidase before the drying procedure, it is logical that the treatment containing dried eggs has a lighter crust value (Sisak *et al.*, 2006).

There was a difference ( $p < 0.05$ ) in  $a^*$  values with the type of egg ingredient and the presence of CGF. While control had the lowest  $a^*$  value ( $a^* = 2.05$ ), the formulation containing CGF and 30% fresh eggs had the highest value ( $a^* = 59.61$ ). Rolls with egg ingredients and CGF had a more red color. This is the opposite of what happened for the crumb. Higher  $a^*$  values (more red) might be explained by the formation of red-brown pigments during the Maillard reaction.

Finally, there was also a significant difference ( $p < 0.05$ ) in crust  $b^*$  values between the control and the different treatments which had much higher  $b^*$  values reflecting a yellow-orange hue. This is due to the natural yellow pigments contained in egg yolk as well as CGF coupled with some Maillard pigment compounds.

**Table 11: Crumb and crust color means and standard deviations of fully baked GF dinner rolls made with various egg ingredients, with or without carob germ flour, n=18.**

Treatment	L*		a*		b*	
	Crumb	Crust	Crumb	Crust	Crumb	Crust
1	66.83 ± 0.84 <sup>CD</sup>	75.89 ± 0.54 <sup>A</sup>	3.03 ± 0.11 <sup>B</sup>	2.05 ± 0.10 <sup>G</sup>	22.53 ± 0.37 <sup>E</sup>	19.81 ± 0.31 <sup>E</sup>
2	68.85 ± 0.86 <sup>B</sup>	71.22 ± 0.92 <sup>B</sup>	2.62 ± 0.08 <sup>DE</sup>	5.48 ± 0.90 <sup>D</sup>	22.40 ± 0.26 <sup>E</sup>	27.24 ± 1.38 <sup>C</sup>
3	68.40 ± 1.65 <sup>CB</sup>	69.00 ± 1.89 <sup>BC</sup>	3.84 ± 0.15 <sup>A</sup>	5.32 ± 0.56 <sup>D</sup>	29.45 ± 0.38 <sup>A</sup>	31.68 ± 0.83 <sup>B</sup>
4	70.57 ± 0.39 <sup>A</sup>	74.32 ± 0.55 <sup>A</sup>	2.90 ± 0.10 <sup>BC</sup>	3.56 ± 0.41 <sup>F</sup>	23.45 ± 0.36 <sup>D</sup>	25.10 ± 0.92 <sup>D</sup>
5	63.84 ± 0.93 <sup>E</sup>	68.46 ± 0.76 <sup>C</sup>	2.50 ± 0.13 <sup>E</sup>	4.57 ± 0.90 <sup>E</sup>	22.90 ± 0.38 <sup>DE</sup>	26.22 ± 1.00 <sup>CD</sup>
6	64.97 ± 0.49 <sup>E</sup>	59.61 ± 1.73 <sup>F</sup>	2.16 ± 0.11 <sup>F</sup>	11.87 ± 1.29 <sup>A</sup>	23.43 ± 0.27 <sup>D</sup>	32.65 ± 1.00 <sup>B</sup>
7	64.35 ± 0.65 <sup>E</sup>	62.48 ± 1.18 <sup>E</sup>	2.73 ± 0.11 <sup>CD</sup>	9.69 ± 1.00 <sup>B</sup>	26.85 ± 0.33 <sup>B</sup>	34.86 ± 0.59 <sup>A</sup>
8	66.63 ± 0.51 <sup>D</sup>	65.63 ± 0.94 <sup>D</sup>	2.53 ± 0.11 <sup>DE</sup>	7.71 ± 0.77 <sup>C</sup>	24.22 ± 0.31 <sup>C</sup>	31.28 ± 1.13 <sup>B</sup>

<sup>A-G</sup> Values with a common uppercase letter within the same parameter (same column) are not significantly different (p>0.05).

## II. Partial baking experiment

### A. Par-baked rolls Texture Profile Analysis

Par-baking time and formulation had a significant impact on rolls crumb texture profile (Table 12). When dough was directly frozen after proofing and re-heated for 10 minutes at 410°C, it yielded the hardest, most cohesive and chewy rolls with no fracturability point. Formulation 6 with 10% CGF and 30% fresh eggs yet presented the best TPA profile when compared to control Formulation (Formulation 1).

An increase in par-baking time resulted in an ( $p < 0.05$ ) increase of crumb hardness (Table 12). Compared to the fully baked replicates, formulations containing fresh eggs and CGF had a much lower hardness value than their control counterparts (Figure 15). This is in accordance with the findings of Sciarini *et al.* (2012), who found that hydrocolloid addition decreases crumb hardness of par-baked GF breads. No obvious trend was observed in terms of crumb springiness. According to Marco and Rosell (2008) the springiness of the rolls was acceptable since most of the values were close to 0.90.

Cohesiveness is a very important value when it comes to par-baked GF bakery products. Recently, Sciarini *et al.* (2012), studied the impact of hydrocolloid addition to par-baked GF breads. They indicated that few of the par-baked products had decent cohesiveness values meaning that after baking completion breads tend to be crumbly and disintegrate easily. Hydrocolloids helped to improve this problematic characteristic. Frozen dough samples had the highest cohesiveness values. Par-baking had a negative impact on crumb cohesiveness ( $p < 0.05$ ). There did not seem to be any trend in changes of cohesiveness in function of par-baking time. However, it is important to note that treatments containing fresh eggs and CGF had higher cohesiveness values than control equivalents (Figure 16) and that the sample par-baked for 12 minutes had the highest cohesiveness value ( $c=0.47$ ) which is close to the fully baked rolls or directly frozen dough but still significantly inferior ( $p < 0.05$ ).

Chewiness values seemed to be directly linked to cohesiveness values: when the crumb was cohesive it also had a more chewy texture indicating that more energy is required to disintegrate it. As a result Control frozen dough had the highest chewiness value ( $g=1642$ ) followed by the frozen dough containing fresh eggs and CGF ( $g=1072$ ) and the sample baked for 12 minutes containing fresh eggs and CGF ( $g=615$ ). Concerning their chewiness value, other replicates did not seem to follow a strong pattern as a function of par-baking time or presence of eggs and carob.

Finally the last parameter that was of importance was the fracturability. In fully baked rolls this parameter was not studied as no fracturability points were observed. It was a different case here. Because, similarly to chewiness, fracturability is directly linked to the propensity to fracture, it appeared that the samples that did not have a fracturability point were the same that had the highest cohesiveness values: control frozen dough, CGF and egg containing frozen dough, 12 minutes par-baked dinner roll containing eggs and carob. Concerning other treatments, eggs and CGF reduced significantly the fracturability force ( $p < 0.05$ ) while rising par-baking time increased it significantly.

According to Sciarini *et al.* (2012) poorer par-baked bread crumb characteristics are essentially linked to the amylopectin re-crystallization (figure 5). After the final baking step, crumb hardness is reduced as the high temperature melts the re-crystallized amylopectin. When par-baking time is increased higher amounts of water are lost and more entanglements are formed between starch and macromolecules such as proteins. Furthermore, during cold storage, more of these interactions are formed leading to an incomplete and heterogeneous refreshing of bread after reheating. This was the case with most of our par-baked samples. However the sample containing fresh eggs and CGF presented desirable TPA parameters similar to the fully baked replicates (table 9).

## **B. Specific volume**

Specific volumes (table 13) were significantly lower in frozen dough samples ( $p < 0.05$ ). Par-baking time did not affect the SV but formulation clearly did: adding carob and fresh eggs significantly raised the SV values ( $p < 0.05$ ) for every tested par-baking time (figure 16). All formulations containing eggs and CGF had high SV values close to the fully baked samples (ranging from  $1.86$  to  $1.97 \text{ cm}^3 \cdot \text{g}^{-1}$ ). Hence egg proteins and caroubins continue to function as gluten replacers enhancing dough air holding capacity leading to better bread roll volume.

Domingues *et al.* (2005), described in a patent a new method of preparing an un-proofed dinner roll dough composition capable of being frozen and baked without thawing to create a yeast-leavened baked dough product. They claimed that their invention yielded yeast-leavened baked rolls with a specific volume of at least 2.5 cubic centimeters per gram.

## **C. Color analysis**

As one could expect, increasing the par-baking time induced darker crumb and crust indicated by lower  $L^*$  values (table 14). Once again, adding fresh eggs and CGF reduced  $L^*$



values through more Maillard reaction pigmented compound production as well as the addition of naturally occurring pigments.

In the control formulation, there did not appear to be any significant changes in terms of redness ( $a^*$  value) of the crumb nor the crust. Formulation having eggs and CGF had the highest crust  $a^*$  value (most red color) when the par-baking time was 18 minutes. Most other values (in both crumb and crust) were not significantly different ( $p>0.05$ ).

$b^*$  values were significantly ( $p<0.05$ ) higher in formulation 6 crumb and crust. This means that the crumb and crust had a more yellow color than the control.

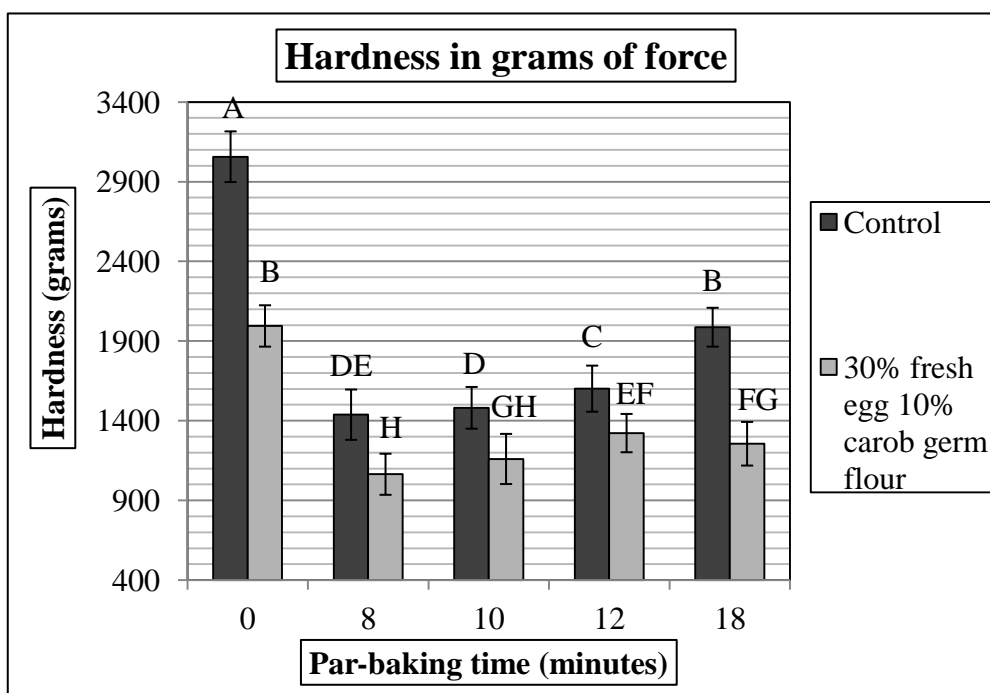
## **D. Overall quality**

It is clear that the treatment containing fresh eggs and CGF par-baked for 12 minutes was the best among all the replicates with close characteristics to directly fully baked rolls. In the light of these physical analyses, interrupted-baking process is hence a suitable process to obtain acceptable GF dinner rolls. Storage of the bread rolls at  $-20^{\circ}\text{C}$  makes them adequate for home consumption following a reheating step and allows avoiding the fast staling rate of such fully-baked GF product.

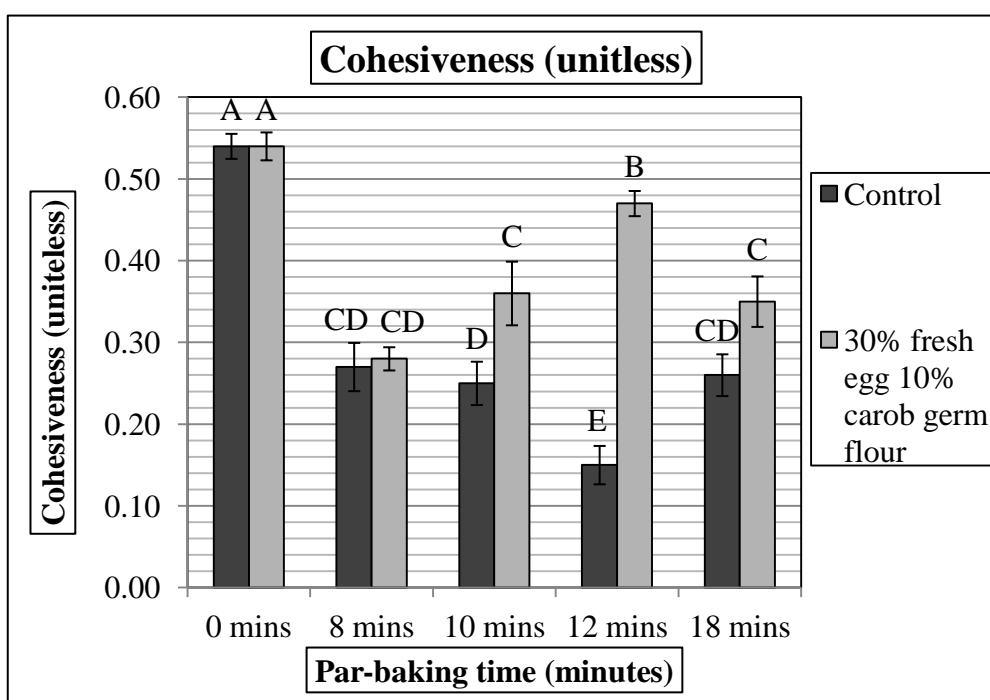
**Table 12: Crumb hardness, cohesiveness, fracturability, springiness, and gumminess means and standard deviations of partially baked control and fresh egg and carob germ flour gluten-free dinner rolls, par-baked for 0, 8, 10, 12 and 18 min, blast frozen at -28°C, reheated for 10 min at 210°C, n=18.**

<b>Hardness (g)</b>					
Par-baking time	0 mins	8 mins	10 mins	12 mins	18 mins
Control	3057 ± 59 <sup>A</sup>	1438 ± 158 <sup>DE</sup>	1481 ± 131 <sup>D</sup>	1601 ± 145 <sup>C</sup>	1987 ± 122 <sup>B</sup>
30% fresh egg 10% carob germ flour	1995 ± 30 <sup>B</sup>	1064 ± 129 <sup>H</sup>	1160 ± 157 <sup>H</sup>	1323 ± 120 <sup>EF</sup>	1256 ± 137 <sup>FG</sup>
<b>Cohesiveness (unitless)</b>					
Par-baking time	0 mins	8 mins	10 mins	12 mins	18 mins
Control	0.54 ± 0.015 <sup>A</sup>	0.27 ± 0.030 <sup>CD</sup>	0.25 ± 0.027 <sup>D</sup>	0.15 ± 0.023 <sup>E</sup>	0.26 ± 0.026 <sup>CD</sup>
30% fresh egg 10% carob germ flour	0.54 ± 0.017 <sup>A</sup>	0.28 ± 0.014 <sup>CD</sup>	0.36 ± 0.039 <sup>C</sup>	0.47 ± 0.015 <sup>B</sup>	0.35 ± 0.031 <sup>C</sup>
<b>Fracturability (g)</b>					
Par-baking time	0 mins	8 mins	10 mins	12 mins	18 mins
Control	0 <sup>F</sup>	1332 ± 58 <sup>C</sup>	1528 ± 86 <sup>C</sup>	1724 ± 40 <sup>B</sup>	2034 ± 25 <sup>A</sup>
30% fresh egg 10% carob germ flour	0 <sup>F</sup>	944 ± 22 <sup>E</sup>	1037 ± 50 <sup>ED</sup>	0 <sup>F</sup>	1121 ± 34 <sup>D</sup>
<b>Springiness (unitless)</b>					
Par-baking time	0 mins	8 mins	10 mins	12 mins	18 mins
Control	0.88 ± 0.007 <sup>CBD</sup>	0.88 ± 0.011 <sup>CBD</sup>	0.93 ± 0.006 <sup>A</sup>	0.74 ± 0.025 <sup>F</sup>	0.90 ± 0.006 <sup>B</sup>
30% fresh egg 10% carob germ flour	0.93 ± 0.003 <sup>A</sup>	0.86 ± 0.009 <sup>DE</sup>	0.85 ± 0.005 <sup>E</sup>	0.89 ± 0.005 <sup>BC</sup>	0.88 ± 0.004 <sup>CD</sup>
<b>Gumminess (g)</b>					
Par-baking time	0 mins	8 mins	10 mins	12 mins	18 mins
Control	1642 ± 59 <sup>A</sup>	382 ± 53 <sup>DEF</sup>	374 ± 47 <sup>EF</sup>	235 ± 31 <sup>G</sup>	525 ± 52 <sup>CD</sup>
30% fresh egg 10% carob germ flour	1072 ± 36 <sup>B</sup>	294 ± 22 <sup>FG</sup>	415 ± 64 <sup>DEF</sup>	615 ± 25 <sup>C</sup>	442 ± 52 <sup>DE</sup>

<sup>A-G</sup> Values with a common uppercase letter within the same parameter are not significantly different (p>0.05).



**Figure 15 :** Hardness means and standard deviations of partially baked control and fresh egg and carob germ flour gluten-free dinner rolls, par-baked for 0, 8, 10, 12 and 18 min, blast frozen at  $-28^{\circ}\text{C}$ , reheated for 10 min at  $210^{\circ}\text{C}$ ,  $n=18$ . <sup>A-H</sup> Values with a common uppercase letter are not significantly different ( $p>0.05$ ).

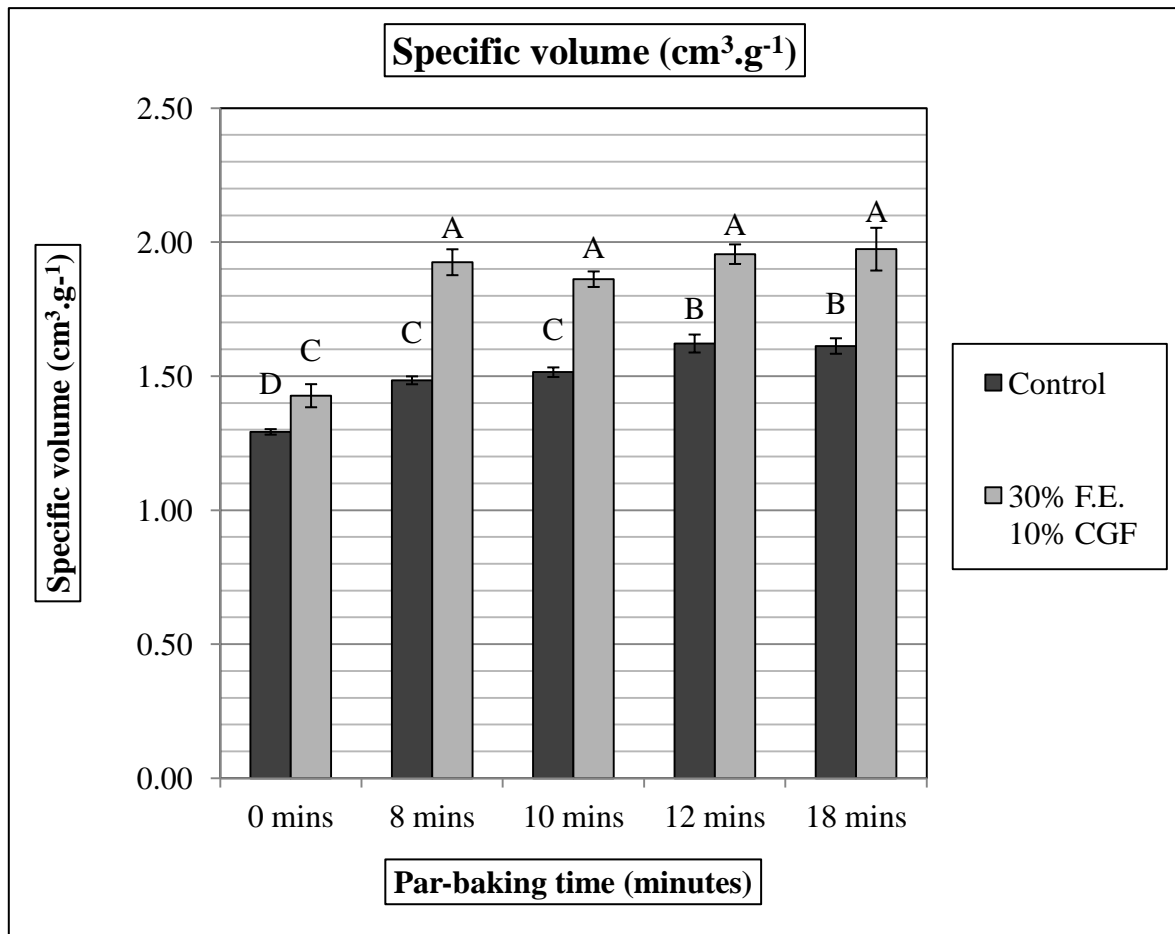


**Figure 16:** Cohesiveness means and standard deviations of partially baked control and fresh egg and carob germ flour gluten-free dinner rolls, par-baked for 0, 8, 10, 12 and 18 min, blast frozen at  $-28^{\circ}\text{C}$ , reheated for 10 min at  $210^{\circ}\text{C}$ ,  $n=18$ . <sup>A-E</sup> Values with a common uppercase letter are not significantly different ( $p>0.05$ ).

**Table 13: Specific volume means and standard deviations of partially baked control and fresh egg and carob germ flour gluten-free dinner rolls, par-baked for 0, 8, 10, 12 and 18 min, blast frozen at -28°C, reheated for 10 min at 210°C, n=18, (cm<sup>3</sup>.g<sup>-1</sup>).**

	Specific volume (cm <sup>3</sup> .g <sup>-1</sup> )				
	0 min	8 min	10 min	12 min	18 min
Control	1.29 ± 0.01 <sup>D</sup>	1.48 ± 0.02 <sup>C</sup>	1.52 ± 0.02 <sup>C</sup>	1.62 ± 0.03 <sup>B</sup>	1.61 ± 0.03 <sup>B</sup>
30% F.E. 10% CGF	1.43 ± 0.04 <sup>C</sup>	1.93 ± 0.05 <sup>A</sup>	1.86 ± 0.29 <sup>A</sup>	1.96 ± 0.37 <sup>A</sup>	1.97 ± 0.08 <sup>A</sup>

<sup>A-D</sup> Values with a common uppercase letter are not significantly different (p>0.05).



**Figure 17: Specific volume means and standard deviations of partially baked control and fresh egg (F.E.) and carob germ flour (CGF) gluten-free dinner rolls, par-baked for 0, 8, 10, 12 and 18 min, blast frozen at -28°C, reheated for 10 min at 210°C, n=18.**  
<sup>A-D</sup> Values with a common uppercase letter are not significantly different (p>0.05).

**Table 14: Crumb and crust color means and standard deviations of partially baked control and fresh egg (F.E.) and carob germ flour (CGF) gluten-free dinner rolls, par-baked for 0, 8, 10, 12 and 18 min, blast frozen at -28°C, reheated for 10 min at 210°C, n=18.**

Treatment	Par baking time	L*		a*		b*	
		Crumb	Crust	Crumb	Crust	Crumb	Crust
<b>Control</b>	0 min	62.62 ± 0.79 <sup>D</sup>	75.54 ± 0.22 <sup>A</sup>	3.54 ± 0.23 <sup>A</sup>	2.03 ± 0.12 <sup>E</sup>	23.23 ± 0.78 <sup>AB</sup>	19.98 ± 0.32 <sup>C</sup>
	8 min	72.08 ± 0.61 <sup>A</sup>	75.09 ± 0.22 <sup>A</sup>	2.55 ± 0.34 <sup>B</sup>	2.47 ± 0.09 <sup>D</sup>	20.30 ± 0.37 <sup>C</sup>	18.74 ± 0.32 <sup>D</sup>
	10 min	71.37 ± 0.13 <sup>AB</sup>	74.05 ± 0.81 <sup>AB</sup>	2.28 ± 0.22 <sup>BC</sup>	2.46 ± 0.28 <sup>DE</sup>	20.23 ± 0.27 <sup>C</sup>	17.78 ± 0.33 <sup>D</sup>
	12 min	71.54 ± 0.73 <sup>AB</sup>	73.52 ± 0.43 <sup>B</sup>	2.54 ± 0.29 <sup>B</sup>	2.29 ± 0.07 <sup>DE</sup>	19.82 ± 0.59 <sup>C</sup>	19.19 ± 0.63 <sup>CD</sup>
	18 min	69.75 ± 0.53 <sup>BC</sup>	72.83 ± 0.24 <sup>B</sup>	2.61 ± 0.26 <sup>B</sup>	2.40 ± 0.20 <sup>D</sup>	19.76 ± 0.32 <sup>C</sup>	18.96 ± 0.34 <sup>D</sup>
<b>30% F.E. 10% CGF</b>	0 min	61.39 ± 0.96 <sup>D</sup>	64.26 ± 0.10 <sup>D</sup>	2.40 ± 0.28 <sup>B</sup>	3.71 ± 0.13 <sup>B</sup>	24.09 ± 0.18 <sup>A</sup>	26.91 ± 0.38 <sup>B</sup>
	8 min	68.32 ± 0.56 <sup>C</sup>	68.06 ± 0.25 <sup>C</sup>	1.81 ± 0.08 <sup>CD</sup>	3.48 ± 0.17 <sup>BC</sup>	22.55 ± 0.33 <sup>B</sup>	27.93 ± 0.15 <sup>A</sup>
	10 min	68.99 ± 0.34 <sup>C</sup>	67.95 ± 0.54 <sup>C</sup>	1.77 ± 0.08 <sup>D</sup>	3.24 ± 0.08 <sup>C</sup>	22.74 ± 0.47 <sup>B</sup>	27.14 ± 0.91 <sup>AB</sup>
	12 min	68.81 ± 0.67 <sup>C</sup>	67.66 ± 0.34 <sup>C</sup>	1.82 ± 0.07 <sup>CD</sup>	3.86 ± 0.14 <sup>B</sup>	22.12 ± 0.64 <sup>B</sup>	28.30 ± 0.20 <sup>A</sup>
	18 min	68.66 ± 0.99 <sup>C</sup>	63.24 ± 0.46 <sup>E</sup>	1.66 ± 0.07 <sup>D</sup>	7.09 ± 0.81 <sup>A</sup>	21.15 ± 0.70 <sup>BC</sup>	28.97 ± 0.51 <sup>A</sup>

<sup>A-D</sup> Values with a common uppercase letter within a same parameter (same column) are not significantly different (p>0.05).

### **III. Consumer response evaluation**

Out of 137 volunteers in the consumer sensory testing, 85 were females and 52 were males. The age of the panelists ranged from 18 to 90 years with 55.5% of the panelists belonging to the 18-25 age group. The general population can be divided into two distinct subgroups: 9 persons suffering from celiac disease and 128 “normal” persons without this disease.

Out of the 128 panelists without celiac disease, 78 were female while 50 were males. The age of these non-celiac panelists ranged from 18 to 80 years with 57.8% of panelists in the 18-25 age group. These consumers had widespread GF product consumption habits. About 44% of them claimed to never consume gluten-free bakery products. Of the 66% remaining individuals, 20% indicated that they consumed GF products once a year, 20% once a month and, finally, 26% of them claimed to eat GF items at least once every two weeks. Even without having celiac disease, more than two thirds of the “healthy” population indicated that they consume GF products. It corroborates the GF market analysis which indicated that some consumers eat GF products for the supposed wholesomeness of that type of food. Within the 9 panelists having celiac disease, 7 were females and 2 were male. The age of these celiac panelists ranged from 18 to 90. Logically, the GF product consumption frequency for these celiac individuals ranged from once a week for 33% of the celiac panelists to every day for 67% of the celiac panelists.

Table 15 presents the average scores from the consumer study for the general population comprising both celiac and non-celiac panelists representing 137 persons. These response scores revealed that differences were found for most sensory parameters ( $p < 0.05$ ). For the six attributes, the improved dinner roll formulation (containing 30% fresh eggs, 10% CGF, 15% honey and 15% roasted sunflower seeds) was found to be significantly better; improved roll scores were higher than control by 1 and up to 2 points for each attribute. Rolls containing only fresh eggs and CGF had a higher ( $p < 0.05$ ) overall acceptability and willingness to buy, as well as a better texture. This observation aligns with the observations made during the crumb physical analysis: caroubins as well as egg proteins and lipoproteins greatly improve the crumb matrix resulting in higher acceptability and desirability of the rolls to the consumers.

It is important to note that the improved roll had an overall very high acceptability considering that tested products were gluten-free (7.54 out of 9). It was the same for the willingness to buy score (6.87 out of 9). As a comparison, Bize (2012) found that acceptability of batter-based sorghum bread was 4.50 for the control and 6.43 for the bread with eggs. In the same research, willingness to buy the product was 3.42 for the control, while sorghum bread with eggs scored 5.40. Generally, for a product to be launched on the market, an average of 7 or more for overall acceptability is used by many food companies (Lawless and Heymann, 1999). Consequently, the improved dinner rolls could be produced to be sold successfully in commodity places, catering stores and restaurants. High variation was observed for most of the parameters. According to Lawless and Heymann (1999), difference in perception of sensory parameters is often an issue with untrained panelists testing.

When comparing scores from panelists suffering from celiac disease to the scores of healthy panelists (Table 16), it is noticeable that the two categories perceive sensory parameters in a different manner. Overall acceptability and appearance of the control and improved rolls was significantly greater for consumers with celiac disease ( $p < 0.05$ ). Flavor and texture scores for the control were significantly higher for celiac consumers. Color was perceived as more acceptable by celiac people for the control and the roll with carob and fresh egg. Finally, because celiac panelists tasting the products were all regular buyers of GF bakery products, they indicated that their willingness to buy all the rolls was high (above 6 for all samples) and significantly ( $p < 0.05$ ) higher to that of non-celiac consumers. For panelists not suffering from celiac disease, the overall acceptability score was of 5.40 for the control roll, 5.98 for the roll with fresh eggs and carob and 7.50 for the improved roll. For celiac individuals, scores for this attribute were 6.22 for the control, 6.56 for the roll with fresh eggs and carob and, finally, 8.11 for the improved roll. A conclusion of this comparison is that the 9 celiac patients had a highest acceptability for most of the tested sensory attributes as well as a greater willingness to buy. For example the control replicate was considered acceptable while non-celiac panelist would barely accept it.

During the sensory testing, 70 panelists tasted the roll slices with 1.5 grams of unsalted butter spread onto it whereas, the other 67 panelists consumed them plain. The idea came from the recommendations of Bize (2012) to test GF bakery products with butter, fruit preserve, peanut butter or any other kind of condiment to improve perception of the tasted products.

From the obtained results, (Table 17), that hypothesis cannot be fully validated. Although butter did improve ( $p < 0.05$ ) the overall acceptability and willingness to buy the slice containing fresh eggs and CGF, other attributes were not significantly affected. Nevertheless it can be noticed that when butter was consumed with the slices, scores slightly increased for most of the parameters (but not significantly).

This sensory testing revealed that the improved par-baked dinner rolls with an optimized texture (due to addition of eggs and CGF), taste (added clover honey and roasted sunflower seeds) was highly accepted by most panelists (including those not suffering from celiac disease). In their comments consumers often stated that they preferred the last treatment because of the wholesome look brought by the seeds as well as the pleasant darker coloration of the crust which was due to the honey (good source of glucose and fructose which are substrates for the Maillard reaction). Hence, this product could reasonably be thought to be ready to be launched on the GF market as is.



**Table 15: General population response means and standard deviations to control, fresh egg and carob germ flour, and improved gluten-free dinner roll slices, par-baked for 12 min, blast frozen at -28°C, reheated for 3 min at 232°C, served at 50°C (on a 9-point hedonic scale).**

General population						
	Overall acceptability	Appearance	Flavor	Color	Texture	Willingness to buy
Control	5.45 ± 1.63 <sup>A</sup>	6.03 ± 1.67 <sup>A</sup>	5.39 ± 1.81 <sup>A</sup>	5.68 ± 1.82 <sup>A</sup>	5.69 ± 1.82 <sup>A</sup>	4.63 ± 2.08 <sup>A</sup>
Carob and Fresh Egg	6.02 ± 1.66 <sup>B</sup>	6.55 ± 1.40 <sup>A</sup>	5.76 ± 1.83 <sup>A</sup>	6.46 ± 1.42 <sup>B</sup>	6.12 ± 1.77 <sup>B</sup>	5.18 ± 1.99 <sup>B</sup>
Improved	7.54 ± 1.13 <sup>C</sup>	7.26 ± 1.24 <sup>B</sup>	7.45 ± 1.45 <sup>B</sup>	7.08 ± 1.37 <sup>C</sup>	7.28 ± 1.43 <sup>C</sup>	6.87 ± 1.75 <sup>C</sup>

<sup>A-D</sup> Values with a common uppercase letter within the same parameter (same column) are not significantly different (p>0.05).

**Table 16: Comparison of celiac and non-celiac population response means and standard deviations to control, fresh egg and carob germ flour, and improved gluten-free dinner roll slices, par-baked for 12 min, blast frozen at -28°C, reheated for 3 min at 232°C, served at 50°C (on a 9-point hedonic scale).**

	Consumers without celiac disease			Consumers with celiac disease		
	Control	Carob and Fresh Egg	Improved	Control	Carob and Fresh Egg	Improved
Overall acceptability	5.40 ± 1.52 <sup>D</sup>	5.98 ± 1.63 <sup>CD</sup>	7.50 ± 1.14 <sup>B</sup>	6.22 ± 2.77 <sup>C</sup>	6.56 ± 2.07 <sup>C</sup>	8.11 ± 0.93 <sup>A</sup>
Appearance	5.98 ± 1.68 <sup>D</sup>	6.52 ± 1.41 <sup>BC</sup>	7.21 ± 1.24 <sup>B</sup>	6.67 ± 1.50 <sup>BC</sup>	7.00 ± 1.32 <sup>B</sup>	8.00 ± 1.00 <sup>A</sup>
Flavor	5.32 ± 1.74 <sup>C</sup>	5.77 ± 1.82 <sup>C</sup>	7.42 ± 1.48 <sup>A</sup>	6.44 ± 2.55 <sup>B</sup>	5.56 ± 2.01 <sup>C</sup>	7.78 ± 0.97 <sup>A</sup>
Color	5.59 ± 1.77 <sup>C</sup>	6.40 ± 1.42 <sup>B</sup>	7.04 ± 1.38 <sup>AB</sup>	7.00 ± 2.06 <sup>AB</sup>	7.22 ± 1.39 <sup>A</sup>	7.67 ± 1.00 <sup>A</sup>
Texture	5.64 ± 1.79 <sup>C</sup>	6.09 ± 1.75 <sup>B</sup>	7.24 ± 1.43 <sup>A</sup>	6.44 ± 2.24 <sup>B</sup>	6.56 ± 2.13 <sup>B</sup>	7.89 ± 1.27 <sup>A</sup>
Willingness to buy	4.51 ± 1.98 <sup>E</sup>	5.12 ± 1.96 <sup>D</sup>	6.81 ± 1.76 <sup>B</sup>	6.33 ± 2.78 <sup>BC</sup>	6.11 ± 2.32 <sup>C</sup>	7.67 ± 1.41 <sup>A</sup>

<sup>A-D</sup> Values with a common uppercase letter within the same parameter (same row) are not significantly different (p>0.05).

**Table 17: Comparison of response means and standard deviations to control, fresh egg and carob germ flour, and improved gluten-free dinner roll slices, par-baked for 12 min, blast frozen at -28°C, reheated for 3 min at 232°C, served without or with butter at 50°C (on a 9-point hedonic scale).**

	Rolls served without butter			Rolls served with butter		
	Control	Carob and Fresh Egg	Improved	Control	Carob and Fresh Egg	Improved
Overall acceptability	5.30 ± 1.56 <sup>C</sup>	5.76 ± 1.77 <sup>C</sup>	7.51 ± 1.22 <sup>A</sup>	5.60 ± 1.69 <sup>C</sup>	6.26 ± 1.52 <sup>B</sup>	7.58 ± 1.04 <sup>A</sup>
Appearance	5.99 ± 1.67 <sup>C</sup>	6.52 ± 1.49 <sup>B</sup>	7.27 ± 1.25 <sup>A</sup>	6.07 ± 1.68 <sup>BC</sup>	6.59 ± 1.33 <sup>B</sup>	7.27 ± 1.23 <sup>A</sup>
Flavor	5.28 ± 1.70 <sup>C</sup>	5.57 ± 1.79 <sup>BC</sup>	7.36 ± 1.40 <sup>A</sup>	5.50 ± 1.92 <sup>BC</sup>	5.91 ± 1.85 <sup>B</sup>	7.52 ± 1.50 <sup>A</sup>
Color	5.64 ± 1.98 <sup>C</sup>	6.45 ± 1.47 <sup>B</sup>	7.04 ± 1.45 <sup>A</sup>	5.71 ± 1.66 <sup>C</sup>	6.49 ± 1.39 <sup>B</sup>	7.11 ± 1.28 <sup>A</sup>
Texture	5.66 ± 1.88 <sup>C</sup>	5.87 ± 1.86 <sup>BC</sup>	7.30 ± 1.49 <sup>A</sup>	5.73 ± 1.78 <sup>C</sup>	6.38 ± 1.67 <sup>B</sup>	7.25 ± 1.38 <sup>A</sup>
Willingness to buy	4.42 ± 2.10 <sup>C</sup>	4.84 ± 2.06 <sup>C</sup>	6.88 ± 1.70 <sup>A</sup>	4.83 ± 2.06 <sup>C</sup>	5.49 ± 1.89 <sup>B</sup>	6.87 ± 1.79 <sup>A</sup>

<sup>A-D</sup> Values with a common uppercase letter within the same parameter (same row) are not significantly different (p>0.05).

## PART IV: RECOMMENDED FUTURE WORK

### I. Recommended future work

#### A. Study hindsight

Although promising results were obtained during our research, some aspects of the study would have benefited from some more extensive testing or a slightly different scientific approach. During the testing part of the research, the levels of egg ingredients and carob germ flour were arbitrarily fixed based on the results from previous research by KSU FSI researchers. This choice was essentially made due to a lack of time availability and personnel. A more rigorous approach would have been to test several levels (probably at least three) of egg ingredients and CGF in order to see if level of these kinds of ingredients can significantly affect GF dinner roll quality. Instead of 8 treatments we would have tested 24 different treatments. Because we used the days of experimentation as the blocking factor in our design of experiment, these 24 treatments would have had to be processed and tested on the same day requiring several experimenters.

The second limitation of our study was the relative low specific volume values obtained (up to  $1.97\text{cm}^3/\text{g}$ ). Besides the patent by Domingues *et al.* published in 2005, there is very little indication of what an acceptable specific volume for GF or conventional bread rolls would be. Hence, all our comparisons were based on bread products which generally weigh between 250 grams and one kilogram with specific volume ranging from about 4 to  $5\text{ cm}^3/\text{g}$ . It is important to note that although our roll specific volume values could not rival wheat breads common specific volume, the main difference lies in the weight of the products. Comparatively, rolls after baking weighed only about 80 grams. Because they present a smaller food matrix it seems plausible that the weight of the leavened goods determined its final specific volume.

It would be interesting to see if our breads would have a noticeable increased specific volume if they were proofed and baked in larger containers. This could be the base for future studies.

## **B. Market introduction study**

This research on GF dinner rolls focused mainly on the scientific aspect of food product development. Another interesting approach to the subject could have been more oriented towards the economic feasibility of market introduction. Since the best roll formulation from this work had good quality characteristics and was greatly accepted by the consumers, it would be reasonable to consider a market launch in the U.S.

With at least three out of four newly launched food products withdrawn from the food market within the first two years, the food product segment is characterized by a high rate of product failures (Menrad, 2003). Hence, a very cautious strategy should be employed to introduce the GF bread roll on the market.

In 2012, the Udi's Healthy Foods Company introduced a new frozen GF product named "classic French dinner rolls". This product sold in bags of 8 count for \$8 a bag would be the most direct competitor product. Therefore, for a market introduction to be successful, the market positioning is crucial and should be done in function of this competition.

A sound strategy would be to advertise the product in the GF premium or gourmet category, emphasizing on the rolls health benefits and its original appealing flavor. The use of sorghum as first ingredient should also be mentioned because it is often perceived by consumers as "healthy and whole grain" (Ciacci *et al.*, 2007). Finally, according to Menrad (2003), the GF product would have more chance to be successfully launched if it was to be distributed by a large food company already implanted in the GF market with established and well-known brands and with the necessary economical resources. Such a study would be very interesting and useful not only from this particular product stand point but also for all the small or medium-sized companies considering entering the GF food market with novel GF products.

## **C. Other recommended work**

### **1. Hot air impingement oven**

During the research, bread rolls were baked in a conventional oven using heated air with no air recirculation and with low heat transfer. This heat is essential to drive off the excess moisture, develop the crust, texture, color and flavor in the product. Impingement heating is directed hot air jets of high velocity that impinge orthogonally on a surface with enhanced heat transfer. According to Olsson *et al.* (2005), jet impingement is a rapid heating method that increases the rate of color development and shortens the total heating time. It was found that the jet impingement products required lower cooking temperatures and times, and as a result, tended to have higher final moisture contents and lower staling rates (Marcroft and Karwe, 1999). For these reasons it is sometimes used in food-serving establishments to rapidly re-heat frozen bakery goods of good quality.

Studying the use of impingement technology to re-heat par-baked GF products appears to be an interesting study subject. It would be especially interesting to determine the optimal baking parameters and assess any improvement in overall quality and consumer acceptability. The main drawbacks of such an experiment would be the acquisition, maintenance and operation costs of this oven and the amount of time necessary to conduct a statistically objective project.

A possible design of experiment for this study would be the following. After selecting the best GF roll formulation and par-baking time combination, three re-heating times would be tested in combination with four sets of air temperature and flow in an air jet impingement oven. The suggested design of experimentation is detailed in Table 18. To be sure to avoid any environment-induced variability, treatments should be tested in triplicates following a RCBD testing procedure. Tested parameters should be similar to those tested during this research.

**Table 18 : Possible design of experiment for a gluten-free product testing with an air jet impingement oven using two different air flows, two different air temperatures and three re-heating times.**

Air flow	Low air flow (LF)						High air flow (HF)					
Air Flow/Air temperature	LF/ Low air temperature (LT)			LF/ High air temperature (HT)			HF/ Low air temperature (LT)			HF/High air temperature (HT)		
Air Flow/Air temperature/ Re-heating time	LF/ LT/ S <sup>a</sup>	LF/ LT/ M <sup>b</sup>	LF/ LT/ L <sup>c</sup>	LF/ HT/ S	LF/ HT/ M	LF/ HT/ L	HF/ LT/ S	HF/ LT/ M	HF/ LT/ L	HF/ HT/ S	HF/ HT/ M	HF/ HT/ M
Treatment number	1	2	3	4	5	6	7	8	9	10	11	12

<sup>a</sup>S=Short

<sup>b</sup>M=Medium

<sup>c</sup>L=Long

## 2. Further study on use of carob germ proteins

In our research, CGF was used at a pre-determined level to form a three dimensional network replacing, at least partially, the gluten network. Similarly to the study by Smith *et al.* (2012), a research on the best CGF and hydration levels using a Response Surface Methodology (RSM) would be appropriate to determine optimal parameters while keeping the number of tested treatments reasonably low. As mentioned in the preliminary work of this research, good hydration of the CGF-containing flour is essential. Before starting the testing procedures the composite flours should be tested for moisture absorption percentage. This can be accurately measured using a farinograph (Brabender Instruments Inc., South Hackensack, NJ, USA) or a mixolab (Chopin technologies, Villeneuve-la-Garenne, France).

## 3. Frozen storage study

Sciarini *et al.* (2012) indicated that storage temperature and duration have a negative effect on GF bread quality. A study looking into different storage temperature and time combinations might indicate if the quality of GF dinner rolls would similarly be impacted. A possible design of experiment for this study would be the following. After selecting the best GF roll formulation and par-baking time combination, three storage temperatures would be

tested in combination with three storage durations. Re-heating parameters should be optimized and fixed. A possible design of experimentation is described in the table 19 below.

Once again, to be sure to avoid any environment-induced variability, treatments should be tested in triplicates following a RCBD testing procedure. Tested parameters should be similar to those tested during this research on par-baking optimization.

**Table 19: Possible design of experiment for a gluten-free product frozen storage testing based on three different storage temperatures and three different storage durations.**

Storage temperature	Low storage temperature (LT)			Medium storage temperature (MT)			High storage temperature (HT)		
Storage temperature/ storage time	LT/ storage duration 1	LT/ storage duration 2	LT/ storage duration 3	MT/ storage duration 1	MT/ storage duration 2	MT/ storage duration 3	HT/ storage duration 1	HT/ storage duration 2	HT/ storage duration 3
Treatment number	1	2	3	4	5	6	7	8	9

## PART V: CONCLUSION

This research principally aimed at studying the development and improvement of gluten-free dinner rolls using two functional ingredients (eggs and carob germ flour) and partial baking technology. During preliminary work a control formula was established upon which all analyses and comparisons were made.

Rapeseed displacement analysis revealed that fresh eggs improved ( $p < 0.05$ ) products specific volume due to foaming and gelling properties of its proteins and emulsification power of yolk lipoproteins and phospholipids. Specific volume up to  $1.96 \text{ cm}^3 \cdot \text{g}^{-1}$  was obtained using 30% of fresh eggs while egg whites and dried eggs were not as efficient.

TPA showed that there is a synergy effect between fresh eggs and carob proteins yielding more acceptable crumb characteristics. It was found that adding 30% of fresh eggs and 10% of carob germ flour, on a flour basis, to the control decreased ( $p < 0.05$ ) the hardness of the crumb down to 1404 grams of force and its chewiness to 830 grams while yielding cohesive and springy bread rolls that did not crumble apart. C-cell analysis supported the improvement of crumb quality induced by addition of CGF and fresh eggs. Indeed, it was observed that this same treatment was at the origin of rolls with the highest number of alveoli per slice (2352 in total), the smallest cell diameter (11.54 on average) and the thinnest cell walls (average of 3.15). These are all characteristics indicative of higher quality GF leavened baked breads.

Due to increased Maillard reaction, dinner rolls with 30% of fresh eggs and 10% carob germ flour (flour basis), had more appealing crumb and crust color. With a low  $L^*$  value of 59.61, this treatment was found to be the darkest of all treatments, which is desirable in GF products.

A par-baking experiment was then conducted on the control formulation and the best formulation from the fully baked roll analysis: control with 30% fresh eggs and 10% CGF. Five par-baking times were tested. According to this part of the study the formulation containing 30% fresh eggs and 10% CGF, par-baked for 12 minutes had very acceptable attributes: a low hardness value (1323 grams of force), a high springiness (0.89), a decent cohesiveness value (0.47) and a low chewiness value (615 grams). Furthermore, its specific



volume was identical to the fully-baked sample and it also had a dark appealing crust ( $L^*=67.66$ ).

Sensory consumer testing indicated that a high overall acceptability (7.54 out of 9) and willingness to buy (6.87 out of 9) were achieved with the improved formulations (control with 30% fresh eggs, 10% CGF, 15% honey and 15% roasted sunflower seeds on a flour basis). Furthermore, it was clear that panelists suffering from celiac disease perceived sensory attributes differently than non-celiac individuals; their acceptability was significantly higher for most tested attributes and willingness to buy than “healthy” panelists. Finally serving roll slices along with butter did not seem to change significantly the acceptance of the panelists.

This study allowed the production of good quality gluten-free dinner rolls with very acceptable physical attributes and desirable sensory attributes. It could have a significant impact on retail stores and catering establishments giving them the possibility to offer safe and convenient foods to celiac patients and health conscious individuals. In the light of these observations, it would be judicious to conduct some further research.

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# **Appendices**



## **Appendix A: Consent statement form**

### **Informed consent statement for consumer sensory analysis of gluten-free bakery products**

The purpose of this project is to determine consumer acceptance of two types of gluten free products. Testing is expected to take less than 10 minutes. All ingredients in these products are food grade and approved by FDA. If you have no food allergies, there are no known risks or discomforts associated with consumption of these products. Your data will be treated as research data and will in no way be associated with you other than for identification purposes, thereby assuring confidentiality of your performance and responses.

1. I (print name)\_\_\_\_\_, agree to participate as a panelist in a sensory consumer testing conducted by Dr. Fadi Aramouni and Marc Bianchi.

2. I understand that this study is part of a thesis project.

3. I understand that there will be a free ice cream certificate upon completion of the testing session.

4. I understand that I do not have to participate in this research and there will be no penalty if I choose not to participate.

5. I understand that I may withdraw from the research at any time.

6. If I have any questions concerning this study, I understand that I can contact Dr. Fadi Aramouni at 216 Call Hall (785-532-1668).

7. If I have any questions about my rights as a panelist or about the manner in which the study is conducted, I may contact the Committee on Research Involving Human Subjects, 103 Fairchild Hall, Kansas State University, Manhattan, KS 66506 (785-532-6195).

SIGNATURE:\_\_\_\_\_

DATE:\_\_\_\_\_

## **Appendix B: Screening form**

Panelist #: \_\_\_\_\_

### **Consumer pre-screening form for gluten-free products**

This study is perfectly anonymous, the panelist number specified on this sheet will be reported on your score sheet but it is only to link your results with your eating habits.

Please complete the information below:

**Age:**

- |                                |                                |                                |                                |                                |                                  |
|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|----------------------------------|
| <input type="checkbox"/> 18-25 | <input type="checkbox"/> 26-30 | <input type="checkbox"/> 31-35 | <input type="checkbox"/> 36-40 | <input type="checkbox"/> 41-45 | <input type="checkbox"/> 46-50   |
| <input type="checkbox"/> 51-55 | <input type="checkbox"/> 56-60 | <input type="checkbox"/> 61-70 | <input type="checkbox"/> 71-80 | <input type="checkbox"/> 81-90 | <input type="checkbox"/> Over 90 |

**Gender:**

- ☐ Male ☐ Female

**Education Completed:**

- |                                      |                                       |                               |                               |                                |
|--------------------------------------|---------------------------------------|-------------------------------|-------------------------------|--------------------------------|
| <input type="checkbox"/> High School | <input type="checkbox"/> Some College | <input type="checkbox"/> B.S. | <input type="checkbox"/> M.S. | <input type="checkbox"/> Ph.D. |
| <input type="checkbox"/> MD          | <input type="checkbox"/> Other        |                               |                               |                                |

**Do you have celiac disease or gluten allergy?**

- ☐ Yes ☐ No

**About how often do you eat gluten-free bakery products?**

- |                                       |   |   |
|---------------------------------------|---|---|
| <input type="checkbox"/> Every Day    | <input type="checkbox"/> At least once a Week | <input type="checkbox"/> Once every Two Weeks |
| <input type="checkbox"/> Once a Month | <input type="checkbox"/> Once a Year          | <input type="checkbox"/> Never                |

**Do you suffer from any food allergies other than celiac disease or gluten allergy?**

- ☐ Yes ☐ No

If you have any food allergies other than celiac disease or gluten allergy, you cannot participate in this study. Thank you for your willingness to help.

## **Appendix C: Ballot forms for consumer evaluation**

Panelist # \_\_\_\_\_

### **Instructions:**

You will be testing three samples of gluten free dinner rolls. Samples are presented in the order to be tasted. Make sure to use the ballot with the sample number that matches the number by the sample. Please be sure to answer the questions completely and honestly. Check the box that best describes your answer. Take a drink of water before you start and as needed throughout testing.

## **SAMPLE: 731**

*Please check only one box that represents your response (X)*

### **1. Please rate your overall acceptability of this sample**

Dislike		Neither				Like		
Extremely		Like nor Dislike				Extremely		
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
1	2	3	4	5	6	7	8	9

### **2. How much do you like or dislike the appearance of this sample?**

Dislike		Neither				Like		
Extremely		Like nor Dislike				Extremely		
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
1	2	3	4	5	6	7	8	9

### **3. How much do you like or dislike the flavor of this sample?**

Dislike		Neither				Like		
Extremely		Like nor Dislike				Extremely		
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
1	2	3	4	5	6	7	8	9

### **4. How much do you like or dislike the color of this sample?**

Dislike		Neither				Like		
Extremely		Like nor Dislike				Extremely		
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
1	2	3	4	5	6	7	8	9

### **5. How much do you like or dislike the texture of this sample?**

Dislike		Neither				Like		
Extremely		Like nor Dislike				Extremely		
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
1	2	3	4	5	6	7	8	9

### **6. Will you be likely to buy this product?**

Definitely		Maybe				Definitely		
Not						Yes		
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
1	2	3	4	5	6	7	8	9

**Additional**  
**Comments:** \_\_\_\_\_

Panelist#\_\_\_\_\_

## SAMPLE: 389

*Please check only one box that represents your response (X)*

### 1. Please rate your overall acceptability of this sample

Dislike		Neither					Like	
Extremely		Like nor Dislike					Extremely	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
1	2	3	4	5	6	7	8	9

### 2. How much do you like or dislike the appearance of this sample?

Dislike		Neither					Like	
Extremely		Like nor Dislike					Extremely	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
1	2	3	4	5	6	7	8	9

### 3. How much do you like or dislike the flavor of this sample?

Dislike		Neither					Like	
Extremely		Like nor Dislike					Extremely	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
1	2	3	4	5	6	7	8	9

### 4. How much do you like or dislike the color of this sample?

Dislike		Neither					Like	
Extremely		Like nor Dislike					Extremely	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
1	2	3	4	5	6	7	8	9

### 5. How much do you like or dislike the texture of this sample?

Dislike		Neither					Like	
Extremely		Like nor Dislike					Extremely	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
1	2	3	4	5	6	7	8	9

### 6. Will you be likely to buy this product?

Definitely		Maybe					Definitely	
Not							Yes	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
1	2	3	4	5	6	7	8	9

**Additional**  
**Comments:**\_\_\_\_\_

Panelist#\_\_\_\_\_

## SAMPLE: 622

*Please check only one box that represents your response (X)*

### 1. Please rate your overall acceptability of this sample

Dislike		Neither					Like	
Extremely		Like nor Dislike					Extremely	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
1	2	3	4	5	6	7	8	9

### 2. How much do you like or dislike the appearance of this sample?

Dislike		Neither					Like	
Extremely		Like nor Dislike					Extremely	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
1	2	3	4	5	6	7	8	9

### 3. How much do you like or dislike the flavor of this sample?

Dislike		Neither					Like	
Extremely		Like nor Dislike					Extremely	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
1	2	3	4	5	6	7	8	9

### 4. How much do you like or dislike the color of this sample?

Dislike		Neither					Like	
Extremely		Like nor Dislike					Extremely	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
1	2	3	4	5	6	7	8	9

### 5. How much do you like or dislike the texture of this sample?

Dislike		Neither					Like	
Extremely		Like nor Dislike					Extremely	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
1	2	3	4	5	6	7	8	9

### 6. Will you be likely to buy this product?

Definitely		Maybe					Definitely	
Not							Yes	
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
1	2	3	4	5	6	7	8	9

**Additional**  
**Comments:**\_\_\_\_\_