

AN EVALUATION OF THE ROLE OF EGGS AND DATEM ON THE QUALITY OF  
GLUTEN-FREE SORGHUM BREAD

by

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B.S., Ecole d'Ingénieurs de Purpan, 2009

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Food Science

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

2012

Approved by:

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# Abstract

Due to an increase in awareness of celiac disease, the gluten-free market continues to expand. However, gluten-free breads are still characterized by a poor structure and overall mediocre quality.

This research was aimed at determining the impact of egg addition as well as an antistaling agent (DATEM) on the quality of a batter-based gluten-free sorghum bread. Gluten-free bread loaves containing 20, 25, or 30% eggs (as is) on a flour basis were evaluated against a control (no egg). The impact of the antistaling agent, DATEM at 0.5% was also studied for each of these formulations.

Quality factors evaluated included water activity, color, specific volume, and cell size. Texture profile analysis was performed to evaluate staling rate based on changes in crumb hardness values and a trained panel evaluated staling attributes by descriptive analysis. Finally, a consumer acceptance test on sorghum bread with and without eggs was also conducted.

Results showed that sorghum breads with eggs had higher specific volumes than control (increase from 0.06 cm<sup>3</sup>/g to 0.11 cm<sup>3</sup>/g), while DATEM had a negative effect on the volume of gluten-free bread (decrease of 0.73 cm<sup>3</sup>/g). Eggs also improved cell structure and produced significantly darker crust ( $p < 0.05$ ). Additionally, the addition of eggs reduced bread hardness (from 54 g force to 142 g force on fresh bread) and slowed the rate of staling over the 12 day storage period studied. Descriptive analysis results confirmed the findings of the texture analysis, showing control bread significantly harder ( $p < 0.05$ ) than egg-containing bread at days 0 and 4. The consumer test indicated a significant preference ( $p < 0.05$ ) for sorghum bread with eggs over the control. The overall acceptability score for this bread was above 6 on a 1 to 9 hedonic scale. The score was closer to 7 when the bread was rated by consumers with celiac disease.

This research proved that the addition of eggs to a gluten-free sorghum bread formulation resulted in delayed staling and better overall quality and acceptability of the product.

Keywords: gluten-free bread, DATEM, eggs, staling, quality

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# Acknowledgements

My greatest thank you goes to Dr. Fadi Aramouni, for giving me the opportunity to do such an interesting research. I thank him for his continuous help, support and advice all along my research. And also, I really want to thank him for his friendship and everything he did to help me during my life in the United States!

I also want to thank Gwénaëlle Jard, for her guidance and for helping me deal with Purpan University's requirements.

Sincere appreciation is also due to Dr. Scott Bean and Dr. Kelly Getty, for serving as my committee members.

I would like to express my gratitude to Brennan Smith for all his precious help and advice, his knowledge and patience were essential to the success of this study. To Dr. Koushik Adhikary, your advice and insight on sensory analysis were much appreciated. Thanks also to Sarah Falke and all the K-State students who have helped me with everything.

On a more personal aspect, I would like to deeply thank Ashley Pruett for her daily support, her incredible friendship and all the moments we shared.

A special thought to Rémi Abbal, for being always there to listen and comfort me.

And at last but certainly not least, I want to thank my beloved parents Marie-Claude and Alain Bize without whom I wouldn't have had this opportunity, thank you so much for your love and support.

I couldn't have achieved what I did without you all. Thank you, from all my heart.

# INTRODUCTION

Awareness of celiac disease has increased among western populations during the past few years. This has led to an increasing demand for gluten-free products. However, many gluten-free baked goods, including breads, are characterized by a poor quality and lack of good structure, hence the need to improve the quality of gluten-free bread.

Staling of gluten-free bread is recognized to be a major issue as it happens relatively quickly. Some studies have focused on finding a way to delay staling in gluten-free breads by using diverse processes and additives known as antistaling agents. Even though the addition of egg in gluten-free bread formulation has been investigated for its ability to improve the bread quality, no research was found that really focused on the possible role of eggs in retarding staling. Although sorghum is the fifth largest crop produced worldwide and has been shown to be safe for people with celiac disease due to its naturally gluten-free nature, the literature is relatively rare compared to that of corn and rice in exploring sorghum for production of gluten-free foods.

Researchers with the Food Science Institute (FSI) at Kansas State University (KSU) and the United States Department of Agriculture Center for Grain and Animal Health Research (USDA CGAHR) have been focusing their work on sorghum grain products for the last few years. Several research projects have been conducted over this particular grain in order to improve its utilization in the food industry, since sorghum is an important crop for the state of Kansas.

This study, conducted in collaboration between the FSI at KSU and the USDA CGAHR, was a follow-up on previous research aiming at improving the quality of sorghum baked goods, and especially bread. Our research objective was to determine the effect of eggs on the overall bread quality and the specific impact of eggs and diacetyl tartaric ester of monoglycerides (DATEM) on the staling of sorghum gluten-free bread.

This report will include a literature review covering the different aspects of celiac disease, sorghum grain, gluten-free products, staling and ways to retard it. Then the materials and methods used in the study will be explained. The outcomes of the different tests will be presented and discussed. And finally, this study shortcomings and the recommended future work will be mentioned before the conclusion.

# PART I: LITERATURE REVIEW

## I. Celiac Disease

### A. Introduction

Wheat is one of the eight major food allergens that cause more than 90 percent of allergic reactions from food in the United-States (U.S.) (U.S. Food and Drug Administration, 2004). One characteristic of food allergies, including wheat allergy, is that people can sometimes grow out of it. This is not the case with celiac disease. Indeed, although wheat protein is responsible for celiac disease, celiac disease is not a food allergy; it is an autoimmune disease. More specifically, it is an inflammatory disorder of the upper small intestine triggered by the ingestion of gluten found in wheat, rye, barley, and possibly oat products (Wieser and Koehler, 2008).

The prevalence of celiac disease has extensively been studied in different countries for both at-risk and not-at-risk populations. Fasano et al. (2003) defined at-risk population as first and second-degree relatives of patients with biopsy-proven celiac disease, and children and adults with symptoms frequently associated with celiac disease. As a result, it has been estimated that the average prevalence of celiac disease is as high as 1: 266 worldwide and 1: 100 in the U.S. Moreover, in 2001, estimates placed the number of persons with celiac disease in the U.S. at roughly 3 million (Fasano and Catassi, 2001). In at-risk groups, the prevalence of celiac disease seems to be up to 20 percent in first-degree relatives, 2 percent in second-degree relatives, 3 to 6 percent in type 1 diabetic patients, 10 to 15 percent in symptomatic iron-deficiency anemia, and 1 to 3 percent in osteoporosis. These findings suggest that in at-risk populations, celiac disease occurs frequently not only in patients with gastrointestinal symptoms, but also in first- and second-degree relatives and patients with numerous common disorders even in the absence of gastrointestinal symptoms (Fasano et al., 2003; Dubé et al., 2005).

## **B. Mode of action**

When individuals with celiac disease eat gluten, it induces an inflammatory response in the small intestine. More specifically, the villi, which are tiny hair-like projections in the small intestine that absorb nutrients from food, are damaged. This is due to an autoimmune reaction to gluten. As the damage progresses, damaged villi will not effectively absorb basic nutrients: proteins, carbohydrates, fats, vitamins, minerals and, in some cases, water and bile salts. If celiac disease is left untreated, damage to the small bowel can become chronic and life threatening, causing an increased risk of associated disorders, both nutritional and immune related (Alaedini and Green, 2005; Wieser and Koehler, 2008; Celiac Disease Foundation, 2011).

## **C. Symptoms**

### ➤ *General:*

Celiac disease can appear at any time in a person's life. In adults, the disease can be triggered for the first time after surgery, viral infection, severe emotional stress, pregnancy or childbirth. Celiac disease is a multi-system, multi-symptom disorder (Celiac Disease Foundation, 2011). The clinical presentation of celiac disease varies greatly and ranges from asymptomatic to severe malnutrition. Although all symptoms are not gastrointestinal, these gastrointestinal symptoms are frequent and can often mimic other bowel disorders. The most common manifestations of celiac disease include abdominal pain, increased frequency of bowel movements, unexplained weight loss with large appetite or weight gain, bone disease, and weakness. Furthermore, celiac disease symptoms are broad; thus, classic features of celiac disease also include chronic diarrhea and sometimes malabsorption. There are also some atypical forms of the disease which are characterized by the following manifestations: gastrointestinal symptoms may be absent or less pronounced; instead, extra-intestinal features, such as anemia, osteoporosis, short stature, infertility and neurologic problems are more prominent. This list is not exhaustive but states most of the symptoms associated with celiac disease (Alaedini and Green, 2005; Celiac Disease Foundation, 2011).

### ➤ *Specific case of dermatitis herpetiformis:*

Dermatitis herpetiformis is the skin manifestation of celiac disease. It has been reported that 10 percent of celiac disease patients have dermatitis herpetiformis. This disease is characterized by blistering, intensely itchy skin. The rash has a symmetrical distribution and is

most frequently found on the face, elbows, knees and buttocks. Dermatitis herpetiformis patients can have intestinal damage without obvious gastrointestinal symptoms; however, more than 85 percent of dermatitis herpetiformis patients will have small-bowel sensitivity to gluten resulting in bloating, abdominal pain, diarrhea, and sometimes malnutrition (Celiac Disease Foundation, 2011).

## **D. Diagnosis**

Although celiac disease is hereditary, genetic testing does not diagnose it. Instead, noninvasive blood tests measuring specific antibodies to gluten are the initial step in screening for celiac disease. The recommended blood tests are the following:

Anti-tissue transglutaminase antibody (tTG – IgA and IgG), which is the most sensitive test available and is commonly used whether or not symptoms are present; anti-endomysial antibody (EMA-IgA), which is a highly specific marker for celiac disease; anti-deaminated gliadin peptide tests (DGP – IgA and IgG), which are used when tTG or EMA is negative and in cases where a patient is IgA deficient; total serum IgA, used to check levels to exclude selective IgA deficiency that results in a false negative test; and finally, anti-gliadin antibody (AgA – IgG and IgA) which is not considered sensitive or specific enough for adults, but is used for children under 2 because tTG and EMA antibodies may be absent (Wieser and Koehler, 2008).

These blood tests are useful for celiac disease diagnosis and monitoring of the response to therapy. However, antibody test results can only suggest the presence of celiac disease but cannot confirm it. A *positive* small bowel biopsy is required to confirm the diagnosis and assess the degree of damage to the villi in the intestinal lining. Finally, in trying to diagnose celiac disease, duodenal biopsy and tissue histology remain the gold standard, but are more invasive procedures (Wieser and Koehler, 2008; Celiac Disease Foundation, 2011).

Dermatitis herpetiformis is diagnosed by a biopsy of a skin lesion and staining for IgA in the tissues (Celiac Disease Foundation, 2011).

## **E. Treatment**

There is no cure for celiac disease. The only treatment is a lifelong adherence to a gluten-free diet. When gluten is removed from the diet, the small intestine will start to heal, overall health will improve, and nutrients from food will start to be absorbed again. Medication is not normally required but some nutritional supplements are sometimes given at the beginning to

correct any deficiencies. Every dermatitis herpetiformis patient also needs to follow a gluten-free diet (Celiac Disease Foundation, 2011).

A gluten-free diet requires the elimination of grains such as wheat, barley, and rye. The case of oats is a little bit more complex. Indeed, some studies have shown that oats may be acceptable for patients with celiac disease; however, their consumption is not recommended for U.S. consumers due to concerns about potential cross contamination from other grains of concern (Alaedini and Green, 2005; Kupper, 2005).

Traditionally, rice, corn, and potatoes were substitutes for gluten-containing grains. Nowadays, additional nutrient-dense grains, seeds, legumes, and nut flours are included in the gluten-free diet to increase variety and enhance palatability and nutritional quality. These grains and seeds include sorghum, amaranth, millet, buckwheat, quinoa, flax, Indian rice grass, and teff (Kupper, 2005).

## **II. Sorghum**

### **A. Introduction**

Sorghum, which scientific name is *Sorghum bicolor* (L.) Moench, is native to the tropical areas in Africa. The oldest cultivation record for this crop dates back to B.C. 3000 in Egypt. It seems that sorghum came to the Americas via trade routes in the 1700's. From a botanical point of view, sorghum belongs to the tribe *andropogonae* of the grass family *Poaceae*. Sugar cane is also a member of this tribe and is therefore a close relative of sorghum. Sorghum is known under a variety of names throughout the world: great millet and guinea corn in West Africa, kafir corn in South Africa, dura in Sudan, mtama in eastern Africa, jowar in India, kaoliang in China, and milo in Spain (U.S. Grain Council, 2011a).

According to the Food and Agriculture Organization (FAO) (2011), a branch of the United Nations, sorghum is one of the most important staple foods for millions of people in the semi-arid tropics of Asia and Africa. This is due to the fact that sorghum is one of the most drought tolerant cereal crops currently under cultivation and has the ability to grow in harsh environments, where other crops do not grow or yield poorly. This crop is still the principal source of energy, protein, vitamins, and minerals for millions of the poorest people in these regions (Taylor and Dewar, 2001).

*Sorghum bicolor* is divided into four classifications of sorghum by intended purposes: grain, sweet, broom, and grass. Grain sorghum is mainly used as human food and is often used as

raw material for alcoholic beverages, sweets and glucose. Sweet sorghum is used as a material for sweetener syrup. Broom sorghum is used as a material to make brooms. And finally, grass sorghum is grown for green feed and forage use (U.S. Grain Council, 2011a).

Grain sorghum, of interest to the food industry, is also subdivided into four classes. These classes are based on tannin content and color by the grain standards stipulated by the USDA Federal Grain Inspection Service (FGIS). While the classes can be visually identified, properties of the types are determined by tannin content. These classes are: sorghum, tannin sorghum, white sorghum, and mix sorghum. Sorghum class is categorized by low tannin content due to the absence of a pigmented subcoat and contains less than 98 percent white sorghum and not more than 3 percent tannin sorghum. Tannin sorghum is sorghum high in tannin content due to pigmented subcoat and contains not more than 10 percent non-tannin sorghum. White sorghum has low tannin content due to the absence of a pigmented subcoat and contains not more than 2 percent of other classes. Finally, mix sorghum is sorghum which does not meet the requirements for any of the other classes (U.S. Grain Council, 2011a).

## **B. Production**

According to the 2009 data from the Food and Agriculture Organization Statistical databases (FAOSTAT), the world's production for sorghum was 56,098,260 tonnes. This production was spread on a 39,969,624 hectares area harvested.

Furthermore, according to the U.S. Grain Council (2011b), grain sorghum is the third most important cereal crop grown in the United States and the fifth most important cereal crop grown in the world behind wheat, rice, corn, and barley. As a continent, Africa is the largest producer of sorghum but the United States is the world's largest producer country of grain sorghum followed by Nigeria and India. Sorghum is a leading cereal grain produced in Africa and is an important food source in India. The United States exports 42 percent of its production which makes it the world leading exporter followed by Argentina and Australia.

In North America, sorghum is cultivated in parts of the central and southern plains of the United States where rainfall is low and variable. Kansas (which accounts for almost half the production of the United States), Texas, Nebraska and Arkansas are the major producing states, accounting for about 80 percent of total production in the United States (U.S. Grain Council, 2011b).

Sorghum is an interesting crop as it is treated as an annual, although it is a perennial grass and it can be harvested many times a year in the tropics (U.S. Grain Council, 2011a). Sorghum is grown with limited water sources and usually without application of any fertilizers or other



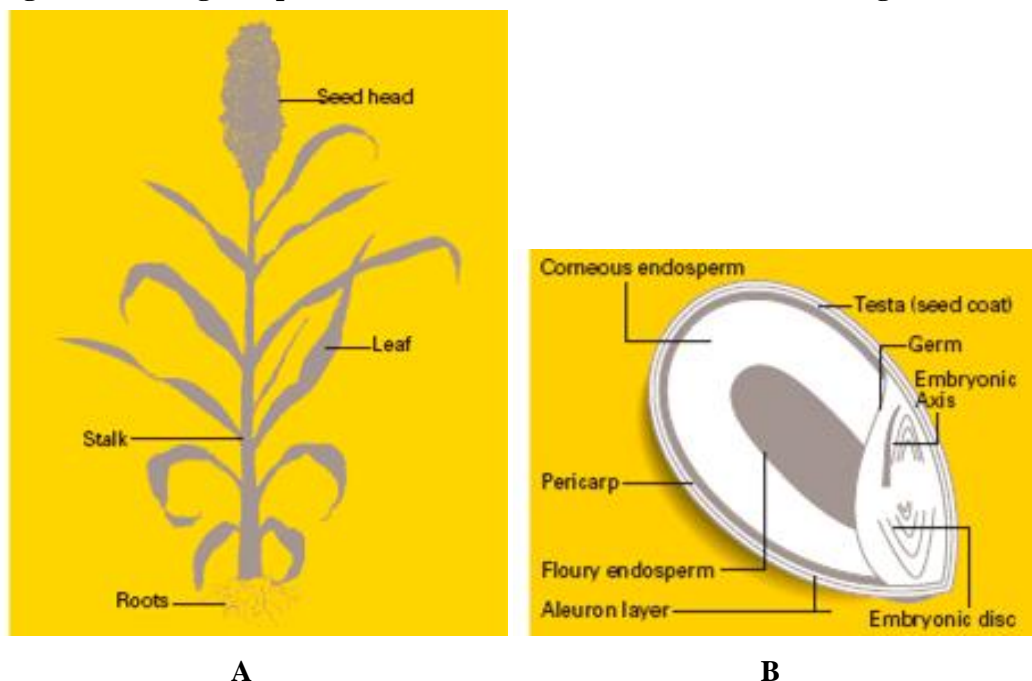
inputs, which makes it more adapted to hostile environment and poor countries. These particularities are also convenient to a multitude of small-holder farmers in many countries. Moreover, varieties of sorghum which contains a pigmented testa layer, and thus tannins, are resistant to birds, weathering, mold, and grain sprouting (Rooney and Serna-Saldivar, 2000).

## C. Structure

### 1) General

Sorghum is a self-pollinating plant. The height of the plant can be from about 60 cm to as high as 460 cm. The long, wide leaves grow from the stalk. The kernel is small and round. A seed head of about 25 cm to 36 cm is seen on the top of the stalk of a mature sorghum plant. Sorghum seed consists of three major anatomic sections: pericarp (outer layer), endosperm (storage organ), and the germ (*Figure 1*) (U.S. Grain Council, 2011a).

**Figure 1: A: sorghum plant structure. B: the different sections of a sorghum kernel**



Source: U.S. Grains Council (2011a).

### 2) Kernel structure

The sorghum kernel varies in color from white through shades of red and brown to pale yellow to deep purple-brown. The most common colors are white, bronze and brown. Kernels are generally spherical but vary in size and shape. According to Rooney and Clark (1968), the

sorghum kernel is a flattened sphere around 4 mm long, 3.5 mm wide, and 2.5 mm thick. The 1000-kernel weight has a very wide range of values, from 3 g to 80 g, but in the majority of varieties it is between 25 g and 30 g. The grain is partially covered with glumes. Sorghum grain that has a testa contains tannin in varying proportions depending on the variety (FAO, 2011).

➤ *Pericarp*

The pericarp that is expressed from the ovary wall is made of three segments: epicarp, mesocarp and endocarp. The epicarp is the outermost layer and usually covered with a thin waxy film. It is further divided into epidermis and hypodermis. The mesocarp, the middle part, is the thickest layer of the sorghum pericarp, but its thickness varies widely among genotypes. Mold resistance in sorghum is associated with thin mesocarp. Sorghum is the only food grade crop that is reported to contain starch in this anatomical section. The endocarp, the innermost sublayer of the pericarp, consists of cross cells and a layer of tube cells which transport moisture into the kernel (FAO, 2011 and U.S. Grain Council, 2011a).

➤ *Seed-coat or testa*

Just underneath the endocarp is the testa layer or seed-coat. The presence of pigment and the color are a genetic character. In some genotypes there is a partial testa, while in others it is not apparent or is absent (FAO, 2011).

➤ *Endosperm*

The largest component of the cereal kernel is the endosperm, which is a major storage tissue. It is composed of an aleurone layer and peripheral corneous and floury zones. The aleurone contains proteins, ash, oil, minerals, B-complex vitamins and some hydrolyzing enzymes (Chandrashekar and Kirleis, 1988).

Grain texture, or endosperm hardness, is one of the most important determinants of the processing and food quality of sorghum and millets. For instance, for preparation of bread, fermented or unfermented, the flour of soft-endosperm sorghum is highly preferred (Rooney et al., 1986).

### ➤ Germ

The germ is comprised of two major parts, the embryonic axis and embryonic disc, also called scutellum. This scutellum is a storage tissue rich in lipids, protein, enzymes and minerals. Additionally, protein of the germ contains high levels of excellent quality lysine and tryptophan (U.S. Grain Council, 2011a).

## D. Composition

Like other cereal grains, the primary component of sorghum is starch. Sorghum has a similar chemical composition to corn but is often reported to have a slightly lower protein and starch digestibility (Rooney and Waniska, 2000).

Much of the work on sorghum composition was done earlier in the mid 20<sup>th</sup> century. According to Hubbard et al. (1950), sorghum bran is low in protein, and ash but rich in fiber components. The germ fraction in sorghum is rich in ash, protein and oil but very poor in starch. Over 68 percent of the total mineral matter and 75 percent of the oil of the whole kernel is located in the germ fraction. Its contribution to the kernel protein is only 15 percent. Sorghum germ is also rich in B-complex vitamins. Endosperm, the largest part of the kernel, is relatively poor in mineral matter, ash, and oil content. It is, however, a major contributor to the kernel's protein (80 percent), starch (94 percent) and B-complex vitamins (50 to 75 percent) (*Table 1*).

**Table 1: Sorghum nutrient content of whole kernel and its fractions on a dry basis<sup>a</sup>**

Kernel fraction (Sorghum)	% of kernel weight	Protein <sup>b</sup> (%)	Ash (%)	Oil (%)	Starch (%)	Niacin (mg/100g)	Riboflavin (mg/100g)	Pyridoxin (mg/100g)
<b>Whole kernel</b>	100	12.3	1.67	3.6	73.8	4.5	0.13	0.47
<b>Endosperm</b>	82.3	12.3	0.37	0.60	82.5	4.4	0.09	0.40
		(80)	(20)	(13)	(94)	(76)	(50)	(76)
<b>Germ</b>	9.8	18.9	10.4	28.1	13.4	8.1	0.39	0.72
		(15)	(69)	(76)	(20)	(17)	(28)	(16)
<b>Bran</b> (Pericarp + Testa)	7.9	6.7	2.0	4.9	34.6	4.4	0.40	0.44
		(43)	(11)	(11)	(4)	(7)	(22)	(8)

<sup>a</sup> Values in parentheses represent percentage of whole kernel value.

<sup>b</sup> N × 6.25

Source: Hubbard et al. (1950)

### ➤ *Carbohydrates*

Starch is the major storage form of carbohydrate in sorghum. According to Rooney and Clark (1968), about 70 to 80 percent of the sorghum starch is amylopectin and the remaining 20 to 30 percent is amylose. Like mentioned before, sorghum has a similar composition to corn. Therefore, their starches share some properties. However there are some minor differences in the rheological characteristics, among which swelling capacity and paste viscosity (Rooney and Clark, 1968). Another difference between sorghum starch and corn starch is their gelatinization temperature, with sorghum's being higher. Because of this, sorghum requires a longer cooking time as well as more thermal energy during processing to reach its starch gelatinization temperature (Rooney and Waniska, 2000). Lower starch digestibility in some varieties of sorghum is mainly due to the presence of tannins in the grain (Keregero and Mtebe, 1994).

### ➤ *Protein*

The second major component of sorghum grains is protein. The majority of these proteins are located in the kernel endosperm, divided between the protein bodies and the endosperm's protein matrix. About half of sorghum proteins are made of alcohol soluble proteins called prolamin. This prolamin fraction of sorghum is referred to as kafirin. Kafirin is also known to be high in glutamic acid and aspartic acid. Kafirins are made up of three fractions:  $\alpha$ -,  $\beta$ -, and  $\gamma$ -kafirin, with  $\alpha$ -kafirin being the prevalent form and is found in the innermost regions of the protein body (Rooney and Serna-Saldivar, 2000).

The other proteins of sorghum are in the form of glutelins, albumins and globulins that make up enzymes, cell material, and other proteins needed for seed structure and plant development. Like most other cereal grains, sorghum's limiting amino acid is lysine (Rooney and Serna-Saldivar, 2000; Taylor and Dewar, 2001).

### ➤ *Lipids*

The crude fat content of sorghum is 3 percent, which is higher than that of wheat percent and rice percent but lower than corn percent. The germ and aleurone layers are the main contributors to the lipid fraction. The germ itself provides about 80 percent of the total fat (Rooney and Serna-Saldivar, 2000). The main fatty acids in sorghum oil are: linoleic, oleic, and palmitic (Rooney and Clark, 1968).

➤ *Vitamins*

In general, sorghum is a rich source of B-complex vitamins. Some yellow-endosperm varieties of sorghum contain  $\beta$ -carotene which can be converted to vitamin A by the human body. Detectable amounts of other fat-soluble vitamins, namely D, E and K, have also been found in sorghum grain (FAO, 2011).

➤ *Fiber*

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

## **E. Application**

As the fifth most important cereal crop grown in the world, sorghum does have many diverse applications. However, only two of them, animal feed and food will be covered in this section. Sorghum is an important animal feed used in countries like the U.S., Mexico, South America and Australia (FAO, 2011). Good-quality sorghums are available with a nutritional feeding value that is equivalent to that of corn. Additionally, sorghum can be processed to further improve its feed value and techniques such as grinding, crushing, steaming, steam flaking, popping and extruding have all been used to enhance the grain for feeding. The products are then fed to beef, dairy cattle, laying hens, poultry, pigs, and are also used in pet foods. Grain use for animal feed has thus been a dynamic element in the stimulation of global sorghum consumption. The demand for sorghum for feed purposes has been the main driving force in raising global production and international trade since the early 1960's. The demand is heavily concentrated in the developed countries, where animal feed accounts for most of total use. However, the U.S. is re-evaluating sorghum purpose especially due to the development of food grade white sorghum and its interesting characteristics for the food industry (FAO, 2011; U.S. Grain Council, 2011b).

In many parts of the world sorghum has traditionally been used in food products and various food items. It is estimated that about 40 percent of the worldwide sorghum production is used for human food consumption (Rooney and Waniska, 2000).

Sorghum is consumed in the world in various ways. Boiled sorghums are one of the simplest uses and small, corneous grains are normally desired for this type of food product. The whole grain may be ground into flour or decorticated before grinding to produce either a fine particle product or flour, which is then used in various traditional foods. Traditional food preparations range from stiff and thin porridge, leavened and unleavened bread, boiled sorghum, baked and steamed products, snack foods, alcoholic and non-alcoholic beverages (Keregero and Mtebe,1994; U.S. Grain Council, 2011b).

Sorghum has unique properties that give it considerable potential in foods and beverages. Some sorghum varieties are rich in antioxidants and all sorghum varieties are gluten-free, which make it a suitable alternative for celiac patients. Furthermore, sorghum absorbs other flavors well. The development of white sorghum lines has enabled white, bland-tasting flour to be produced from sorghum grain. This flour is then useful in food products because it does not impart unusual colors or strong flavors (Taylor et al., 2006; Asif et al., 2010).

### **III. Gluten-free**

#### **A. Definition and Labeling**

##### **1) Definition**

The International Codex Alimentarius Commission (2007) defines gluten as a protein fraction from wheat, rye, barley, oats or their crossbred varieties and derivatives, to which some persons are intolerant and that is insoluble in water and 0.5M NaCl. The prolamins are a fraction from gluten that can be extracted by 40 to 70 percent of ethanol.

According to the International Codex Alimentarius Commission (2007), gluten-free products are defined as dietary food:

a) “consisting of or made only from one or more ingredients which do not contain wheat (this is, 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 (this is, 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.”

They also specify that oats can be tolerated by most but not all people with celiac disease. Therefore, the use of oats not contaminated with gluten permitted in gluten-free foods for the dietary management of celiac disease may be determined at national level.

## **2) Labeling**

In 2007, the U.S. regulatory agency Food and Drug Administration (FDA) published a proposed rule for the definition of gluten and the labeling of gluten-free products. The rule recommends that a “gluten-free” statement can be made on any products that do not contain wheat, rye and barley and their crossbred derivatives. In addition, derivatives of these ingredients, for instance wheat starch, would not be allowed in gluten-free foods, unless they are processed to remove the gluten to levels of less than 20 mg/Kg of food.

The new European Union regulations (2009) states that only foods that contain less than 20 mg gluten/Kg can be labeled as gluten-free and foods containing less than 100 mg/Kg can be claimed as “very low gluten”.

Thus, the proposed rules of both the United States and the European Union conform to the standard set by the Codex Alimentarius Commission.

## **B. Gluten-free market**

### **1) From a niche category to a mainstream food group**

Heller (2009) explained that in the past, food manufacturers would avoid the gluten-free segment because there was a relatively low demand and especially because of the technical and cost challenges involved in the manufacturing of such products.

However, the market for gluten-free foods has exploded in recent years and today’s consumer can find more gluten-free products on supermarket shelves than ever before. According to Heller (2009), the growth of the category is due to three major factors:

- An increase in the number of people diagnosed with celiac disease, and corresponding increased consumer demand.
- More developed and responsive food industry eager to tap into any new niche that represents a market opportunity.
- Developments in science and technology that allow for the production of tasty and marketable gluten-free foods.

Thus, for these reasons, gluten-free products have started penetrating the market all around the world. In fact, it would be appropriate to say that today the category has made its way into the mainstream food supply.

More than that, because the gluten-free market is evolving rapidly, gluten-free products are now present in major supermarkets. In its report (2011) analyzing the U.S. market in detail, Packaged Facts, the publishing division of MarketResearch.com, says that more mass market retailers are selling gluten-free products, probably causing the gluten-free market share for health and natural food stores to fall from 30 percent in 2008 to 16 percent in 2010. Packaged Facts reported that just under half of gluten-free shoppers buy from Walmart, and 44 percent buy from supermarket chains.

## **2) Who wants gluten-free?**

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

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

## **3) Market growth**

“The growth of the gluten-free market is incredible”, Mintel Global New Products Database, which tracks new product development trends around the world, stated in 2010 report. In this report, Mintel states that the most common conditions currently impacting the market are



celiac disease and lactose intolerance. Moreover, it was declared that in the United States, gluten-free is thought to be the dominant player in the “free-from” marketplace.

Furthermore, Mintel (2010) also explained that the popularity of gluten-free products was particularly boosted in 2003 and 2004, paralleling the low-carb diet boom. Hence, according to the report, some consumers seeking low-carb or low glycemic index products go “one step further” and seek out gluten-free products.

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

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

### **C. Nutritional aspect of gluten-free foods**

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

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

Finally, it is important to specify that gluten-free cereal foods are generally not enriched and are frequently made with refined flour and/or starch. As a result, they may not provide the same nutritional value as wheat-based foods, especially if we consider that wheat-based foods are often whole grain or enriched. This is why recommendations have been made that persons with celiac disease should be advised to consume more nutrient dense gluten-free cereal foods in the form of whole grains or enriched products and that manufacturers should produce more of these kinds of products (Thompson, 2009).

Concerning the glycemic index of gluten-free foods, a controversy exists in the literature. Some research have pointed out that gluten-free breads have a higher glycemic index than regular wheat breads (Schober, 2009). However they did not look at specific grains to determine whether or not some gluten-free grains have lower or higher glycemic indexes. More recently, a study investigated this issue by determining glycemic index of different gluten-free grains (sorghum, rice, and corn) compared to wheat's (personal communication: Pruett, 2012).

## **D. Gluten-free breads**

### **1) Introduction**

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

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

Studies on the rheological properties of gluten-free doughs as well as reports addressing the possible relation between those properties and the quality attributes of the end product are limited. However like specified by Lazaridou and Biliaderis (2009), it was demonstrated that doughs produced from gluten-free formulations do not have the cohesive and elastic characteristics obtained from wheat flour, because of the absence of gluten. Gluten-free

doughs are more fluid than wheat doughs and, due to the lack of gluten network, are closer to cake batter in viscosity and rheological behavior. Accordingly, many researchers use the term batter to characterize gluten-free doughs used for breadmaking. Therefore, these batter-type doughs have to be handled like cake batters rather than typical bread doughs. Obviously, this makes hand kneading no longer appropriate.

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

## **2) Starch-based breads**

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

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

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

### **3) Sorghum breads**

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

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

Furthermore, the kind of sorghum flour used plays an important role in the end product. In their research, Schober et al. (2005), showed that clear differences were found between various sorghum hybrids in their potential to produce gluten-free leavened bread. In their results they explained that crumb structure differed most characteristically, whereas volume and height did not show significant differences among the samples evaluated. Kernel hardness and damaged starch seem to be key elements in these differences. The flour starch damage will depend on the milling technique and on the sorghum grain. Thus, flour with low starch damage might more likely require adding pregelatinized starch or a hydrocolloid to promote water binding in the batter than flour with high starch damage. This confirms the results of Rooney et al. (1986) which specified that endosperm hardness plays a role in the food quality. Like mentioned in previous part about sorghum, these authors demonstrated that the flour of soft-endosperm sorghum is highly preferred for preparation of bread. These findings were also verified by Fernholz (2008). In this study, it was shown that sorghum hybrids can differ in kernel and flour properties; and that smaller particle size and higher damaged starch flour produced a better end product. Frederick (2009) also established that sorghum flour

composition and particle size have an effect on the quality of gluten-free bread. This study also validated the impact of starch damage on bread performance.

Additionally, Marston (2009) revealed that treating sorghum flour with ozone and heat affect the quality of gluten-free bread. When sorghum flour was ozonated, the bread produced was characterized by an extremely poor structure. However, heat treatment showed positive effects on the quality of gluten-free bread. When sorghum flour was heated to 125°C prior to use, bread volume, was improved probably due to the oxidation of sulfhydryl units. This volume improvement also led to an amelioration of both the crumb structure and the texture.

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

## **IV. Staling**

Consumers demand for fresh baked goods presents a difficult task for the baking industry. Indeed, consumers want baked goods that do not stale too fast while still conserving the taste and texture expected from such products. This is the reason why formulation and processing technologies designed to control the staling rate have been studied for over a century and half. Nevertheless, bread remains a processed food with one of the shortest shelf lives (Chinachoti and Vodovotz, 2001).

### **A. Definition**

Bechtel et al. (1953) defined staling as “a term which indicates decreasing consumer acceptance of bakery products caused by changes in crumb other than those resulting from the action of spoilage organisms”. It is still used by many researchers as the general definition.

Staling is a process that begins long before storage, it starts during cooling. Moreover, like the definition specifies, consumer acceptance decreases when staling occurs due to some organoleptic differences in the texture, taste, and aroma of bread (Pateras, 2007).

Bread staling falls into two categories: crust staling also known as crust softening, and crumb staling also known as crumb firming. The most widely used indicator of staling is

measurement of the increase in crumb firmness, which is the attribute most commonly recognized by consumers. Furthermore, crumb staling presents a much greater concern to the consumer than crust staling. This is why crumb staling has been studied more even though it is less understood due to its complexity (Gray and Bemiller, 2003).

## **B. Mechanisms**

The roles of starch, water, gluten, lipids, and other components of bread are continuously studied with advanced analytical methodologies and sophisticated multidisciplinary approaches in order to better understand staling mechanisms (Chinachoti and Vodovotz, 2001).

### **1) Moisture redistribution**

Moisture content and moisture transfer among bread components is believed by many to be a significant factor contributing to bread staling. This is the reason why it has been widely studied (Gray and Bemiller, 2003).

Consequently, crust staling is generally thought to be caused by moisture transfer from the crumb to the crust. This moisture redistribution results in a soft, leathery texture. Piazza and Masi (1995) proved that as baked bread begins to cool, a moisture gradient forms in the loaf. Moreover, moisture migration occurs from the crumb to the crust due to differences in vapor pressures between the crumb and the external region of the loaf. As a result, crust which has initially a low moisture rate (around 12 percent) will soak up moisture from the higher moisturized crumb (45 percent). Eventually, Bechtel et al. (1953) demonstrated, the moisture content in the center of the loaf will decrease while the one in the external region will increase.

Baik and Chinachoti (2000) measured moisture and water activity and then confirmed that there is a moisture migration from the crumb to the crust during storage. However, further experiments revealed that even when the bread is stored without its crust, it did not prevent firming.

This moisture redistribution is also responsible for crumb staling. Water is a major component of bread and it logically plays a crucial role in the firming process, either by enhancing the molecular mobility of polymer chains or by acting as a coordinator agent between them. There were many studies about the possible role of water in crumb staling. However, many of them are contradictory and at the end, the mechanisms involved are still not fully understood.

This is partially due to the fact that crumb has a very complex structure and the interactions between its diverse components are not all known (Pateras, 2007).

Schiraldi and Fessas (2001) explained that crumb is composed of aqueous interphases separating interpenetrated gels. According to them, this water is rather mobile and can enhance the crumb-to-crust migration of moisture. This local drying makes the walls of the crumb alveoli more rigid.

However, water redistribution is not the only factor responsible for bread staling. Studies have proved that even with preventing this moisture migration, bread still stales (Pateras, 2007).

## **2) Role of starch**

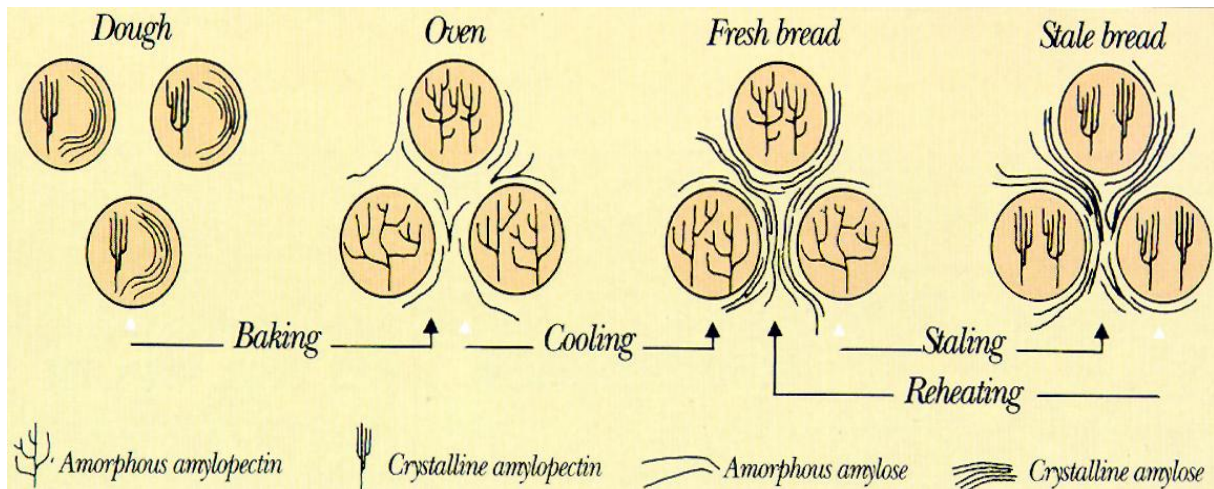
The changes occurring in starch and particularly in starch polymers during baking process and storage are critical in giving the bread its structure, texture and quality. Bread is mainly composed of flour and water, with flour at about 75 percent starch. Therefore, starch is the main component in a loaf of bread, approximately 50 percent, while water is 40 percent and proteins are 7 percent (Pateras, 2007).

Zobel and Kulp (1996) proposed a model in which the firmness in stale crumb originated from the formation of tight junctions between polysaccharides chains. These junctions are mainly the result of inter-chain hydrogen bonds. This process can eventually progress toward the formation of crystalline structures. More than that, many other studies also focused on starch retrogradation to explain staling.

Starch retrogradation is the aggregation of polysaccharides chains which may form a crystal phase within and outside the contours of the native starch granules. This process only concerns 15 to 20 percent of amylose and amylopectin of gelatinized bread starch. The rest remains amorphous (Hug-Iten et al., 2003).

Staling is indeed linked to the re-crystallisation of amylopectin which occurs with time. This is characterized by a change from the completely amorphous state (fresh bread) to a partially crystalline state (stale bread). The process of starch retrogradation makes gelatinized starch molecules re-associate to form back a double helix crystalline structure via hydrogen-bonding (Pateras, 2007). (*Figure 2*)

**Figure 2: Starch retrogradation process in bread**



Source: Figure provided by Grinsted (2011)

The relationship between crumb firming and starch retrogradation has been questioned by many researchers while others have proved the implication of starch retrogradation in bread staling.

The retrogradation of amylose and amylopectin, the two major components of starch, have been deliberated. What emerged from these studies is that even though right after baking amylose associates in bread and therefore affects initial firmness, it does not participate more in crumb firming. The branched amylopectin molecules of starch within the swollen granule however play an important role in this firming by its changes in physical orientation. At first, the branched chains of amylopectin are unfolded and spread out within the limits of available water. With time, these chains will gradually aggregate, aligning with one another by various types of intra-molecular bonding. This will lead to an increase in the internal structure of the swollen starch granules rigidity. For that reason, it will cause crumb hardening (Pateras, 2007).

Gray and Bemiller (2003) reached the conclusion that amylopectin retrogradation is part of the staling process, but is not the only responsible mechanism for the observed changes in texture.

Finally, in spite of the contradictory outcomes of the different research, most agree that there is at least a correlation between amylopectin retrogradation/crystallization and staling, even though the two events may not be part of the same process.



### 3) Role of other crumb components

#### ➤ *Protein (gluten)*

Many studies have focused on the possible role of gluten in staling mechanisms. Now, it is generally believed that starch-gluten interactions are somehow involved in the firming process (Martin et al., 1991).

One of the existing hypotheses states that bread firming is a result of hydrogen bonding between gelatinized starch granules and the gluten network. It could also involve hydrogen bonding between retrograded starch molecules and the gluten network with retrogradation occurring either before or after association of amylopectin and/or amylose molecules with the protein network. Finally, it was affirmed that the primary effect of protein in reducing staling is its action on diluting starch (Pateras, 2007).

Several studies focusing on the role of gluten in staling have pointed toward the concept that gluten serves as a moisture reservoir from which water is transferred to retrograding starch molecules. It has also been proposed that gluten-starch cross-links are responsible for staling. However, these interactions between starch and gluten cannot explain by themselves bread staling (Gray and Bemiller, 2003).

Since there is much more starch than protein in bread, the staling process is probably more due to starch retrogradation (Pateras, 2007).

#### ➤ *Nonstarch polysaccharides*

Arabinoxylans and arabinogalactans are the “pentosans” of wheat flour. Arabinoxylans are divided into two classes: “water-soluble” and “water-insoluble”. They have been more studied than arabinogalactans because they are present in greater concentrations and are believed to play a more important role in both the preparation and the shelf-life of bakery products (Biliaderis et al., 1995).

Their influence on bread-making and on bread properties, including bread staling, is not clear, although the subject has been studied for many years (Jankiewicz and Michniewicz, 1987; Biliaderis et al., 1995; Cleemput et al., 1997). In fact, controversy exists in the literature, as some studies have demonstrated that both water-soluble and -insoluble pentosans affect staling rate, while other concluded that pentosans do not take part in staling.

### ➤ *Native lipids*

Rogers et al. (1988) advanced the concept that native lipids may play a role in bread staling. Their study showed that shortening was quite effective in retarding the rate of bread firming by acting on the native flour lipids. Besides, they identified that the influence of flour lipids on firming rate is concentration-dependant. At low levels, total free lipids enhanced firming and at higher levels the lipids delayed firming. However, this pattern is a mirror image of the effect of total free lipids on loaf volume.

## **C. External factors affecting staling**

### **1) Storage temperature**

An interesting peculiarity of bread is that the rate of staling has a negative temperature coefficient. Therefore, the rate of bread staling is accelerated at lower storage temperatures. Indeed, Slade and Levine (1987) correlated bread staling with starch recrystallization at storage temperatures of -1, 10, and 21°C, and found that the role of starch crystallization in staling was diminished at higher temperatures (32 and 43°C). Then, in their study on starch characteristics, they also came to the conclusion that 4°C (refrigerator temperature) is the single optimum temperature between the glass transition temperature and the melting temperature of crystalline amylopectin which is about 60°C. This balances nucleation and crystallization and the melting temperature involved implicates amylopectin as the polymer crystallizing. This proves that low storage temperature has an accelerating effect on bread staling.

### **2) Processing factors**

Different studies on effects of technological factors, which include manufacturing methods, formulas, and operational steps, on both loaf characteristics and bread staling, have been performed (Kulp and Ponte, 1981).

Giovanelli et al. (1997) showed that baking temperature significantly affects bread staling. Thus, bread baked at lower temperatures stales at a slower rate in terms of both crumb hardening and starch retrogradation. Higher baking temperatures led to increased protein denaturation and starch granule disruption. Consequently, Giovanelli et al. (1997) suggested baking under slight vacuum to achieve crumb cooking at temperatures lower than 100°C, and possibly enhance the shelf-life of bread.

In addition, Axford et al. (1968) found that both the rate and extent of staling decreased as the loaf volume increased in bread stored at the same temperature. They showed that breads made with the same dough ingredients, but by different processes (and stored at the same temperature), underwent staling at different rates because of differences in loaf volume.

Finally, Maleki et al. (1980) demonstrated that breads baked from a lower protein flour seemed to stale faster than breads from higher protein flours. Moreover, they showed that decreased baking times produced breads with higher moisture contents. This higher moisture content resulted in softer bread. However, they also specified that these results only concern the three first days as there was no significant difference after three days of storage.

## **D. Staling in gluten-free breads**

### **1) Introduction**

Although different gluten-free breads have been developed, only a few published studies are available on the staling profile of gluten-free breads (Sciarini et al., 2010). However, the literature indicates that in gluten-free bread, staling seems to be mainly due to starch retrogradation. Indeed, because there is no possible interaction between starch and gluten in gluten-free foods, studies have tried to determine role of starch in gluten-free breads staling. Hence, it has been reported that interactions between starch and gluten are not essential for the crumb firmness to increase. Starch retrogradation alone can be sufficient to cause bread firming (Morgan et al., 1997).

Osella et al. (2005) demonstrated that in gluten-free bread, staling is mainly linked to starch retrogradation as well as to moisture redistribution. However, staling in gluten-free bread occurs faster than in wheat bread, giving credibility to the hypothesis that gluten present in wheat bread slows down the movement of water by forming an extensible protein network, thus keeping the crumb structure together. The end-product of this phenomenon is therefore gluten-free bread being more prone to staling (Gallagher et al., 2003b; Schober, 2009; Sciarini et al., 2010).

### **2) Role of non-gluten proteins**

Different research has been done on the role of proteins in bread staling. Some concluded that crumb firmness is not significantly correlated to flour protein type or concentration. Gerrard et al. (2001) conducted a study aiming at determining the role of non-gluten proteins on the staling of bread. Addition of milk, soya, and meat proteins to starch breads produced loaves of

comparable appearance to conventional bread, although they were characterized by an unusual odor. Besides, bread of equivalent specific loaf volume staled at the same rate irrespective of protein concentration, or type of protein. This adds further credence to the hypothesis that the staling process is not critically dependent on the particular properties of gluten proteins. However, their studies suggested that other properties of bread, such as specific loaf volume, may be altered by specifically changing the protein component in the flour.

Gallagher et al. (2003b) working with dairy ingredients indicated how the incorporation of dairy ingredients is long established in the baking industry. Moreover, dairy proteins are highly functional ingredients, and can be readily incorporated into many food products. They may be used in bread for both nutritional and functional benefits including flavor and texture enhancement, as well as storage improvement. This study's outcome proved that the addition of dairy powders in gluten-free bread formulations resulted in improved volume, appearance, and sensory aspects of the loaves. Besides, the addition of milk protein isolate and a novel rice starch to a gluten-free bread formulation resulted in loaves with an increased volume and better appearance and acceptability than the control. These loaves were also characterized by a softer crust and better crumb characteristics. However, eventually, loaves from both formulations staled at a similar rate.

## **E. Conclusion**

Retrogradation of starch molecules remains the most widely accepted factor contributing to bread staling. However, it is important to remember that there is also good evidence that there is no cause-and-effect relationship between retrogradation and staling. There is at least a correlation between starch retrogradation (particularly amylopectin retrogradation) and staling, even though the two events may not be part of the same process. As specified, amylopectin retrogradation is believed to play the major role, and amylose is now also thought to be involved. The common belief now is that amylopectin retrogradation is part of the staling process, but is not the only responsible mechanism for the observed changes in texture. Moisture content and moisture transfer among bread components is also believed by many to be a significant factor contributing to bread staling. Gluten has also been studied extensively and it appears that gluten may be involved in staling by its interactions with starch. Despite all this research, it is still unclear what other bread components and processes contribute to the overall staling process (Gray and Bemiller, 2003).

Overall, considerable improvements have been done to extend baked goods shelf-life by analyzing the staling process. However, bread staling is incontestably a very complex process that is still intensively studied but yet not well understood in its entirety. As a result, it is still responsible for huge economic losses to both the baking industry and the consumer.

## **V. Ways to retard staling**

### **A. Anti-staling additives**

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

#### **1) Emulsifiers**

##### *➤ Emulsifiers in general*

Emulsifiers, also referred to as food surfactants or surface active agents, are considered as optional additives and are used as dough and bread improvers. Indeed, emulsifiers are commonly included in bakery products' formulations to enhance the structure by increasing dough strength or crumb softness. The main characteristic of emulsifiers is their amphiphilic nature, which allows molecules to migrate to interfaces between two physical phases lowering surface tension and forming dispersions (Nunes et al., 2009).

Whether surfactants actually decrease the rate of firming or produce softer breads that then stale at the same rate as the control, has been debated. Kulp and Ponte (1981) concluded that a surfactant's ability to retard firming is more important than an initial softening of crumb in freshly baked bread.

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

Finally, Knightly (1988) confirmed this theory by showing that the anti-staling abilities of emulsifiers come mainly from their interaction with starch: they inhibit the process of amylopectin re-crystallization. Knightly (1988) explained that the formation of an emulsifier

and amylose complex would contribute to a decrease in the initial firmness of the crumb, while the formation of a complex with amylopectin would result in a distinct reduction in the rate of firming during storage. Because emulsifiers mainly interact with the amylopectin part of starch, their action as anti-staling agents is thus about reducing the firming rate of the crumb.

Application of emulsifiers in gluten-free products is still a non-widely studied point of interest. Nevertheless, some studies have been conducted on this subject.

Nunes et al. (2009) studied the effect of the addition of several emulsifiers on gluten-free breads. The emulsifiers tested were lecithin, diacetyl tartaric ester of monoglycerides (DATEM), distilled monoglycerides and sodium stearoyl lactylate (SSL). This study demonstrated that addition of distilled monoglycerides and SSL at significantly high levels reduce the staling rate of the crumb. Yet, retrogradation of starch over a five-day period did not seem to be affected by the addition of emulsifiers.

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

#### ➤ *Specific case of DATEM*

Typically, mono- and diacetyl tartaric acid esters of mono- and diglycerides are used as dough conditioners for all baked products, particularly yeast-leavened products (Gaupp and Adams, 2004).

Diverse authors have studied the effects of DATEM as anti-staling agents. Krog et al. (1989) established that DATEM surfactants were as effective anti-staling agents as SSL over five days of storage but less effective in reducing retrogradation of amylopectin. DATEM impact on crumb firming reduction was also proved. It was suggested that the anti-firming properties of DATEM may be due to changes in cell wall thickness and elasticity.

It was further reported that optimal reduction in firmness increase over extended periods of storage can be achieved when DATEM is used in combination with monoglycerides (Gray and Bemiller, 2003).

Recently, in their review on DATEM, Gaupp and Adams (2004) explained that the most important effects induced by the addition of DATEM are the stabilization of a soft crumb which will have as consequence a delay in starch retrogradation as well as an improvement of dough performance during manufacturing (tolerance towards raw material quality, mechanical resistance, sticking to manufacturing equipment, mixing and fermentation tolerance).

To sum up, the application of DATEM has a lot of advantages as a bread improving ingredient. Also, the economics of its use is considered even more important than its organoleptic effects (Gaupp and Adams, 2004).

## 2) Enzymes

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

### ➤ *α-amylases*

The most useful enzymatic approach to staling rate reduction has been the use of  $\alpha$ -amylases, which catalyze a small amount of hydrolysis of the starch. Several studies about the role of  $\alpha$ -amylases have been conducted (Miller et al., 1953; Martin and Hosene, 1991; Morgan et al., 1997; León et al., 2002). As a result, it has been shown that the addition of these enzymes to the dough retard crumb firming. It has been demonstrated that  $\alpha$ -amylases have most certainly an indirect effect. The anti-staling effect of this enzyme is thus due to in-situ formation of starch dextrans and/or maltodextrans. Indeed, the role of amylases is to partially hydrolyze starch during baking into a mixture of smaller dextrans (shorter-chains). Like the  $\alpha$ -amylases, enzymes cannot access intact starch granules, they mainly hydrolyze damaged starch. This is done by the enzyme attacking  $\alpha$ -(1,4) linkages along starch chains. This action is stopped at  $\alpha$ -(1,6) branch points of amylopectin. So, the addition of  $\alpha$ -amylases enzymes results in the formation of increased amounts of dextrans. These dextrans are characterized by a particular low degree of polymerization (DP 3–9) and are presumably responsible for the anti-firming effect. So, because of their lower molecular weight, branched dextrans have a decreased ability to retrograde or interfere with retrogradation in any manner, and so they can reduce the extent of firming (Martin and Hosene, 1991; León et al., 2002; Gray and Bemiller, 2003; Pateras, 2007).

### ➤ *Transglutaminase*

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

Renzetti et al. (2008) confirmed these results by demonstrating that transglutaminase can be successfully applied to gluten-free flours to improve their breadmaking potentials by promoting network formation. However, as Moore et al. (2006) did, they emphasized the fact that the protein source is a key element determining the impact of the enzyme.

## **3) Hydrocolloids**

### ➤ *General*

Hydrocolloids are water soluble polysaccharides with a range of functional properties that make them very useful in food technology. They are indeed widely used as additives in the food industry especially for their action in modifying the rheology and texture of aqueous suspensions. However, a different approach is the use of hydrocolloids as anti-staling agents (Guarda et al., 2004).

Hydrocolloids' action as anti-staling agents is linked to moisture content loss that occurs during staling. Certainly, all hydrocolloids interact with water, reducing its diffusion and presence (Anton and Artfield, 2008).

Different studies about diverse hydrocolloids demonstrated that breads containing hydrocolloids showed lower loss of moisture content, hence higher water retention in the crumb. So, hydrocolloid addition reduces the dehydration rate of crumb samples during storage. It was also demonstrated that out of all the hydrocolloids tested (xanthan gum,



carboxymethylcellulose (CMC), guar gum, carrageenan, locust bean gum alginates, and hydroxypropylmethylcellulose (HPMC)), HPMC at a level of 0.5% (flour basis) was the one with an improvement effect on all the parameters tested which were: specific volume index, width/height ratio, and crumb hardness. Moreover, breads with HPMC showed good sensory properties for visual appearance, aroma, flavor, crunchiness, and overall acceptability, and produced a better effect as anti-staling agent (Guardia et al., 2004; Barcenas and Rosell, 2005; Lazaridou et al., 2007).

#### ➤ *Hydrocolloids in gluten-free breads*

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

## **B. Physical methods to reduce bread staling**

### **1) Packaging**

Packaging methods have been studied extensively to improve bread shelf-life (Pateras, 2007). In bread packaging, the most common kind of material used is low density polyethylene. This form of packaging is known to help retard moisture loss which is a big issue occurring during bread storage. Additionally, other packaging methods are widely used. It is the case of modified atmosphere packaging, known as MAP, a method referring to vacuum packaging, gas packaging or active packaging. Regarding gluten-free breads, it is common to find product packaged in a double wrap in order to improve the moisture barrier. This moisture barrier was demonstrated to be an important aspect of packaging as it helps prevent microorganism spoilage while gas barrier acts more towards, preventing oxidation (Galić et al., 2009).

Thus, packaging has been shown to improve bread shelf-life by inhibiting bread microbiological spoilage (mainly mold) as well as chemical spoilage (rancidity) (Pateras, 2007). Nevertheless, it has been demonstrated that bread staling cannot really be countered by

a specific type of packaging. So, no matter how well-developed packaging technologies are now, it seems that packaging can do little to delay staling (Pateras, 2007; Galić et al., 2009).

## **2) Storage temperature**

As mentioned previously, storage temperature does have an effect on staling rate. For this reason, in order to retard bread staling, it is important to control the temperature to which the bread is stored. To delay bread staling, bread must be stored at temperatures close to room temperatures (Slade and Levine, 1987).

In a study conducted by Edelman and Cathcart (1949), effect of storage at 10°C and room temperature (authors approximated it at 28°C) on the degree and rate of increase in crumb firmness during a 96 hour staling period was evaluated. Firmness of bread crumb was found to increase when bread was stored at 10°C. Therefore, this study confirmed the increased firmness effect of low temperatures.

## **3) Freezing**

Deep freezing of bread is a solution to delay the process of staling as well as inhibiting microbiological activity in the bread. However, this cannot be done by simply storing loaves in a standard freezer. Indeed, such storage will for sure stop the process of starch re-crystallization. Nevertheless, this technique does not procure optimal results as after thawing, staling will resume. Moreover, in these conditions, after thawing, staling will not only resume but also proceed at a faster rate than in unfrozen loaves. This phenomenon is due to the fact that during the freezing and thawing process the bread passes through the temperature range at which it stales fastest (Pateras, 2007).

This question has been studied and it has been concluded that for maximum storage stability, the product must be stored at a temperature that is below its glass-transition temperature (Levine and Slade, 1990). When products are stored below their glass-transition temperature, they reach the glassy state form. Once in this state, the polymers, such as starch, are immobilized and reactions and movement of solutes, such as sugar, and plasticizers, such as water, are limited.

Furthermore, the results of the study performed by Wang and Jane (1994) indicated that the lower the difference between the freezer temperature and the glass-transition temperature of the sample, the greater the storage stability of foods will be.

## **C. Role of eggs**

### **1) Influence of eggs in bread staling**

When trying to improve characteristics and staling of gluten-free breads, most studies have focused on the addition of a dairy ingredient to achieve the desired quality (Gallagher et al., 2003b). Nevertheless, it is well-known that some important functions of egg ingredients are surface activity, emulsifying properties of egg white and yolk, and heat coagulation of egg white that helps in the setting of the bread or cake structure (Cauvain, 2007). Because of these properties, eggs have also been thought to add some improvement in gluten-free breads. Thus even if less studied than dairy ingredients, some researchers have focused on the impact of eggs in gluten-free formulations.

Because egg proteins have strong cohesive viscoelastic films, they are essential for foam stability. Consequently, egg albumen improves gas retention properties when used in gluten-free bread (Kato et al., 1990). This was demonstrated in the study by Moore et al. (2004) who added 30 percent eggs to their gluten-free formulation. Confocal laser-scanning microscopy pictures of batters showed some similarities to a wheat dough which was taken as a reference. A continuous area of proteins was visible in the gluten-free batter. With the formulation with eggs, a protein network similar in appearance to the gluten one was visible. However, it is important to specify that in this study by Moore et al. (2004), eggs were not the only protein source: skim milk powder was also used in their formula.

The ability of egg proteins to create a protein viscoelastic network similar to gluten is more evident in another study by Moore et al. (2006). In this study, the authors compared gluten-free breads with various protein sources (skim milk powder, soya flour, and egg powder). Batters obtained with the egg powder were characterized by a more homogeneous dispersion of proteins and starch granules compared to a soya flour batter and with a less significant degree to the skim milk powder batter. Moreover, the bread supplemented with egg powder showed a significant higher volume than the two others (milk and soya). The limitation of these results is that the different protein sources were implemented with various levels of transglutaminase. Thus, the breads obtained were not the result of the effect of eggs only.

Despite these limitations, the overall results reported so far suggest that egg proteins are a valuable tool for replacement of gluten in gluten-free formulations. They have indeed shown to bring some improvements in the textural characteristics of gluten-free breads. Additionally, among all protein sources tested, egg proteins are among sources more likely to mimic the viscoelastic properties of gluten and promote the formation of a protein network in gluten-free

bread (Arendt et al., 2009). The other protein source that would be able to replace gluten network is the carob bean germ protein. Indeed, Smith et al. (2010) demonstrated that despite major biochemical differences between caroubin and gluten, this carob germ protein has the ability to form a protein network.

Among all these positive effects of eggs on gluten-free breads, the impact of eggs on staling has also been considered. From the eggs properties, we can expect that egg addition to gluten-free bread would help in the stabilization of the gas cell due to its surface activity as well as the setting of the crumb due to the heat coagulation of the egg white (Schober, 2009).

Moore et al. (2004) and Ahlborn et al. (2005) reached the same conclusion that staling was delayed in gluten-free rice breads which formulations included eggs. Both studies found that these breads with eggs had web-like structures resembling gluten in rice gluten-free breads crumbs. These were not found in rice gluten-free bread with other kind of proteins. Both studies hypothesized that these protein matrices were the factor counteracting staling, for example by simply masking some of the changes originating from starch retrogradation.

In conclusion, by recreating a protein network in gluten-free breads, eggs improve the quality and structure of such breads as well as they produce breads with delayed staling compare to egg-free gluten-free breads.

## **2) Commercial egg products**

Several egg products are currently commercialized. The most common ones are fresh eggs; whole liquid eggs and liquid egg white (both pasteurized and possibly frozen); dried/powdered whole and white eggs. In large-scale operations usually pasteurized eggs are used for sanitary and convenience reasons.

# **VI. Study's context**

This study was conducted in collaboration between the Food Science Institute (FSI) at Kansas State University (KSU) and the United States Department of Agriculture Center for Grain and Animal Health Research (USDA CGAHR). The cooperation between these two establishments on gluten-free projects originally came from a push from the Kansas Sorghum Commission in 2005, which was looking for new uses of food grade sorghum grain. Their

first joint project was to develop and commercialize gluten-free sorghum Belgium waffles but since then, they have expended their focus to gluten-free products in general. The team is composed of three permanent researchers at the USDA CGAHR, four professors associated with the KSU's FSI and several master's and Ph.D. students.

As specified, the team focus is mainly oriented to developing new and adequate gluten-free products. This general theme is divided into four distinct projects:

- Research on food grade sorghum production
- Research on development of sorghum-based products
- Research on the functionality of Kafirin proteins (from sorghum)
- Research on the functionality of carob bean germ proteins (Caroubin), also gluten-free.

Several research projects have been performed and published on these three different subjects and some more are currently being conducted.

The main financial support for these projects comes from the United Sorghum Board (USB) which is a national organization for sorghum growers. They provide funding for the Center for Sorghum Improvement locally based at Kansas State University. There is an annual call for proposals based on the agenda set by the USB. Grants are funded on a competitive basis. Additional supports can come from other commodity groups such as the American Egg Board and government agencies such as the USDA.

The present study is part of the project on sorghum grain products and is a follow-up to previous studies working on the subject. Three previous studies have already evaluated sorghum flour characteristics and different treatments that would improve the end product of sorghum flour use. The first study by Fernholz (2008) focused on sorghum variety, and characterized four sorghum hybrids in terms of their grain properties and flour. The second study by Frederick (2009) examined the effects of sorghum flour composition and particle size on functionality in gluten-free batter bread. And finally, the study by Marston (2009) examined the effects of heat and ozone treatments on sorghum flour functionality in gluten-free bread and cake.

## **VII. Objectives of the study**

Celiac disease is becoming more and more known to both consumers and the food industry. People suffering from this disease require a gluten-free diet. Thus a lot of effort is currently put into developing gluten-free products.

Thus after researching the flour characteristics impacts on gluten-free sorghum bread, the next step was to focus on improving the quality and shelf-life of a gluten-free sorghum bread.

Based on literature review, there is obviously a lot of room to improve gluten-free breads and investigate the role of eggs. Gluten is responsible for the formation of a viscoelastic network which procures the bread structure. Eggs may be able to compensate for this protein matrix which is absent in gluten-free bread.

Therefore, the objectives of this study were to investigate the effect of eggs on the staling rate of gluten-free sorghum bread, as well as the effect of an antistaling agent: DATEM. The impact on overall quality for end use product was also studied. This was investigated by using different kinds of tests:

- Physical: texture profile analysis to evaluate staling rate, water activity, color, specific volume and cell size;
- Chemical: proximate analysis to determine moisture, fat, protein, fiber and ash content of bread;
- Sensory: descriptive analysis with a trained panel to estimate texture and staling flavor; and a consumer test to assess the preference and difference of breads with and without egg.

The hypothesis regarding this study was that egg protein may help delaying staling by creating a network similar to gluten and that egg functionality may also help alleviate some of the common poor characteristics of gluten-free bread. Concerning DATEM, the hypothesis was that the presence of this antistaling agent would also help delaying staling.

# PART II: MATERIALS AND METHODS

## I. Preliminary work

Initially, numerous formulations and processing parameters were tested in order to find the optimal ones. Different mixing times and speeds were evaluated as well as oven temperatures. From a formulation point of view, different ingredient amounts were evaluated. HPMC levels, water and sugar amounts were optimized, based mostly on loaf volume and informal taste testing. Levels of eggs that would be tested in the experiment were also determined, starting at 5% eggs level (flour basis) and going up to 30%. Finally, 20%, 25%, and 30% were the levels retained. Different forms of egg products were also tested: fresh eggs, white and whole liquid eggs, white and whole egg powder, and high gel strength egg powder. No major differences were found between the use of whole liquid eggs and whole fresh eggs. Eventually whole fresh eggs were retained since they were easier to acquire from Kansas State University's Call Hall Dairy Bar.

## II. Breadmaking

Table 2: Sorghum bread formulation

Ingredients	% Flour Basis
White sorghum flour	70
Potato Starch	30
<i>Total flour</i>	<i>100</i>
Salt	1.75
Sugar	1
HPMC	1.5
DATEM	Variable (0 or 0.5)
Water	100
Instant Dry yeast	2
Eggs	Variable (0, 20, 25 or 30)

The base formulation was adapted from previous work by Schober et al. (2007) and is shown in Table 2. This formulation is expressed on flour basis as it is usually done in the baking industry in the U.S. Indeed, everything is based on 100 parts of weight of flour rather than actual ingredient percentages.

The ingredients used were: white sorghum flour (Twin Valley Mills, LLC., Ruskin, Nebraska, USA), unmodified potato starch (Bob's Red Mill, Milwaukie, Oregon, 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), hydroxypropyl methylcellulose (HPMC) (Methocel K4M, E 464, Dow Chemical Co., Midland, Michigan, USA), Instant dry yeast (Fleishmann's Yeast, Chesterfield, Missouri, USA), Diacetyl tartaric acid esters of monoglycerides (DATEM) (PANODAN DATEM, Danisco, Copenhagen, Denmark), and Eggs (Grade A large eggs, Kansas State University Tom Avery poultry farm, Manhattan, Kansas, USA). The sorghum flour and the potato starch together determined the flour weight basis.

The dry yeast was allowed to pre-hydrate for 5 minutes in water at room temperature for activation. The dry ingredients were separately and thoroughly mixed manually with a spatula and then eggs were added to this mixture, if needed in the formulation. This was then added to the yeast and water. The batter was mixed with a 300 W Kitchen Aid mixer (Ultra Power, St Joseph, Michigan, USA) equipped with a flat beater attachment for 90 seconds at the lowest speed, and then scraped down. Mixing was carried on for another 3 minutes at the second speed (out of 10 speeds). Then, 250 g of batter was divided into each of 4 greased baking pans (15x9x5.5cm). The pans were put in a proofing chamber (Metro C5 1 Series, InterMetro Industries Corporation, Wilkes-Barre, Pennsylvania, USA) at 32 °C and 95% humidity until the batter was at 1 cm to the top of the pan (this took about 30 minutes). The pans were then put in a double deck-type oven (Artisan Stone Deck oven 1T2, Doyon Equipment Inc., St-Come Linière, Québec, Canada) at 190.5 °C for 30 minutes. After baking, loaves were turned out of the pans and allowed to cool for 2 hours at room temperature.

### **III. Physical Analyses**

After the 2 hours cooling-time, physical analyses were performed on the eight different loaves (one for each formulation).



## **A. Loaf Specific Volume**

Loaves were weighed and their volumes determined by the rapeseed displacement method (AACC method 10-05). The specific volume for each loaf was calculated by dividing the sample volume (cm<sup>3</sup>) by the sample weight (g). Specific volume was expressed in cubic centimeters per gram (cm<sup>3</sup>/g).

## **B. Texture Profile Analysis**

Breads were sliced with a bread knife inside a constructed bread slicer box with slots 25 mm apart. Three 25 mm thick slices were obtained.

Texture Profile Analysis (TPA) of the crumb was performed on three slices from each loaf using a texture analyzer (TA-XT2, Stable Micro Systems, Godalming, United Kingdom) equipped with a 38 mm Perspex cylinder probe along with a 30 kg load cell. TPA was carried out with a constant speed of 2.0 mm/s (applying to the pre-test speed, test speed, and post-test speed) for a distance of 10.0 mm, corresponding to 40% compression of the 25 mm slices. There was a 5 second wait time between the first and second compression cycles; the trigger force was 20.0 g.

This analysis was also performed on other loaves from the same treatment, at days 0, 4, 8, and 12 after baking. These loaves were individually packaged in a MylarFoil bags (Impak Corporation, Los Angeles, California, USA) on day 0 after cooling-time. These are metallized polyester film ('Mylar') laminated with aluminum. An oxygen absorber (Impak Corporation, Los Angeles, California, USA) with an active ingredient of powdered iron oxide was also put in the package with the loaf of bread. These oxygen absorbers are completely safe, not toxic and do not remove the fresh smell and taste of the product. They bring the oxygen level down reliably to .01% or less (Impak Corporation). The Mylar foil was then heat-sealed with a vacuum sealer (Rival Seal a meal, Sunbeam Products, Inc., Boca Raton, Florida, USA). This packaging method provides light, moisture and oxygen barriers to ensure product integrity. All this was done in order to prevent spoilage organisms (especially mold) growth. Indeed, according to Pateras (2007) and Galić et al. (2009), a modified atmosphere packaging, in that case without oxygen, is favorable to inhibit mold spoilage.

## **C. Crumb Structure**

On day 0, one of the 25 mm slices was used to determine the overall quality of the crumb structure using a C-Cell Instrument (Calibre Control International Ltd., Appleton, Warrington,

United Kingdom). C-Cell uses high resolution optics to obtain high definition images (Calibre Control International Ltd.).

## **D. Crumb and Crust Color**

Both crumb and crust colors were measured for each formulation. A HunterLab MiniScan (Model EZ/ 4500L, Hunter Associates Laboratory Inc., Reston, Virginia, USA), was used that was calibrated following the standard procedure given by Hunter Associates Laboratory Inc., using a black glass port and a white tile. The outputs of this measurement are “L\*”, “a\*”, and “b\*” values. “L\*” indicates lightness and “a\*” and “b\*” are the chromaticity (saturation) coordinates. “L\*” values range from 0 = black to 100 = white. Red and green colors are indicated by the “a\*” value with +a = red and -a = green. The “b\*” value indicates yellow (+b) and blue (-b) colors.

## **E. Water activity**

Water activity of both the crumb and the crust was measured using an Aqualab Series 3 water activity meter (Decagon Devices, Inc., Pullman, Washington, USA). Water activity ( $a_w$ ) is a measured value and is defined as follows:

$$a_w = p / p_o = ERH / 100$$

With:

p = partial pressure of water vapor above the food (solid or liquid)

p<sub>o</sub> = partial pressure of water

ERH = equilibrium relative humidity (%) relative humidity above a food where there is no difference in moisture between the food system and the environment.

Water activity determines the amount of water available for chemical and microbial growth.

Water activity values range from 0 to 1 (pure water).

## **IV. Proximate Analysis**

Samples were frozen on day 0 after cooling-time and then proximate analysis was performed a few days later at the department of Animal Sciences and Industry Analytical Chemistry Laboratory (Weber Hall, KSU).

## **A. Moisture Content**

The moisture contents of the breads were measured using the Association of Official Analytical Chemists (AOAC) approved method 930.15 (AOAC, 2005). The procedure determines the dry matter of the sample by oven drying. A 2 g sample is placed in an aluminum dish which is in turn placed in a forced air draft oven set to 135 °C. The dish is removed after exactly two hours, covered and placed in a desiccator. When cool, the dish is weighed and the loss in weight is moisture.

## **B. Protein Content**

The crude protein contents of the breads were measured using AOAC approved method 990.03: Nitrogen Determination by Combustion (AOAC, 2005). Nitrogen in the sample is freed by combustion at high temperatures in pure oxygen, and then measured by thermal conductivity. This value is then converted to the equivalent protein by a numerical factor of 6.25.

## **C. Fat Content**

The fat contents of the breads were measured using AOAC approved method 920.39 (AOAC, 2005). This method determines crude fat in the samples by ether extraction with subsequent solvent evaporation. The fat content is reported as a percentage of the original sample weight.

## **D. Fiber Content**

The crude fiber contents of the breads were measured using the Ankom Method (Arkon Technology). The Ankom Crude Fiber solvent solubilizes non-fiber components of the bread, and then the sample is filtered, rinsed, and dried to determine the crude fiber content. Crude fiber is reported as a percentage of the original sample weight.

## **E. Ash Content**

The ash contents of the breads were measured using AOAC approved method 942.05 (AOAC, 2005). Two grams of each sample was weighed into a porcelain crucible and placed in a temperature controlled furnace preheated to 600°C. The sample was held at this temperature for 2 hours. The crucible was then transferred directly to a desiccator, cooled, and then weighed. Ash content is reported as a percentage of the whole sample.

## V. Sensory Analysis

### A. Descriptive Analysis

Descriptive analysis was performed to determine the sensory characteristics of two different breads upon aging: sorghum bread without DATEM and no egg (control) and the same bread with 25% eggs. This choice was determined by the outcomes of physical and chemical analyses. The formulation for these breads was the exact same used for the previous tests.

Six graduate and undergraduate food science students (3 females and 3 males in the age range of 18-27 years) were chosen for the panel. The panel training was performed over six sessions of 1.5 hour each. This training followed both the ballot training method and the consensus method (Lawless and Heymann, 2010).

The first two sessions focused on texture attributes for bread. The ballot method was used for this part. Panelists were given a list of attributes characterizing bread. Some references for each attribute were also provided. This was adapted from Gámbaro (2002) and Bejosano et al. (2005) (*Appendix A*).

The next three sessions consisted of following the consensus training method: panelists determined what flavor attributes would be best to describe “staling”. The third session was divided into two parts; first, each panelist had to work individually and come up with a list of attributes characterizing stale bread, then, all panelists discussed the attributes found and a consensus was made in order to retain the four main and more significant attributes. These attributes were associated to those found in grain sorghum (Brannan et al., 2001) and bread (Callejo, 2011; Elía, 2011). References for each attribute were then determined. The fourth and fifth sessions were used to apply these attributes and references to different sorts of bread. By consensus, panelists decided if the attributes were still relevant for the new kinds of bread. Then references were scored on a scale from 0 to 15. (*Appendix A*).

Finally, the sixth session was used as a review session where all references for both texture and flavor attributes were available. Then, a practice evaluation was performed by the panelists. With all the descriptive terms, their definitions and references, they had to evaluate different bread samples. One slice from each of the breads was evaluated at room temperature. They worked individually and quietly. Each sample was given with a random three-digit code to preserve panelist unawareness of what was tested. Distilled water and unsalted saltine crackers were provided for the panelists to cleanse their palate in between each sample. A group discussion followed for the panelists to comment on their findings.

After the training was done, two more sessions were held to evaluate control and test breads. Two bread samples were evaluated on the first session and two more on the second one. This was done in order to avoid panelist fatigue. Samples were evaluated following the same method used in the sixth session for the practice evaluation. On the first session, panelists evaluated sorghum bread control and sorghum bread with 25% eggs both four days old. On the second one, the same kinds of bread were evaluated on day 0.

## **B. Consumer Response Evaluation**

A consumer study was held at Kansas State University in Call Hall. Consumers had to evaluate two different breads: the first one was sorghum bread without DATEM and 0% egg (control); the other one was the same bread but with 25% egg. The bread formulations were the same used for the descriptive analysis. However, there was a change in flour supplier due to a recent deviation in flour quality from the previous supplier; therefore we used sorghum flour from Authentic Foods (Gardena, California, USA).

A total of 103 untrained panelists volunteered to participate in this study. Among them, 13 were consumers suffering from celiac disease or gluten allergy. Each panelist was asked to sign an informed consent statement form to notify them about the purpose and guidelines of the study before participating (*Appendix B*). The panelists were also given a pre-screening form to obtain information about age, gender, education completed, if they suffer from celiac disease or gluten allergy, frequency of gluten-free breads consumption, and any known food allergies other than celiac disease or gluten allergy (*Appendix C*). If the panelist claimed to have a food allergy other than celiac disease or gluten allergy, then they were asked not to participate in the study.

The two samples were randomly coded with a three-digit number and presented to the consumers on a white paper plate. Both samples were given to the panelists at the same time along with ballots having corresponding three-digit codes. The panelists were asked to test each sample in the specified order they were served to them to eliminate bias. Unsalted saltine crackers (only for consumers without celiac disease or gluten allergy) and distilled water were provided for cleansing their palate between samples.

Each ballot contained a 9-point hedonic scale for each attribute. The 9-point scale displayed the degree of liking with 9 being like extremely, 5 being neither like nor dislike, and 1 being dislike extremely. The attributes evaluated were overall acceptability, appearance, flavor, color, and texture. Another scale was used to assess the willingness to buy the product. For this specific test, the 9-point hedonic scale was divided as follows: 9 was definitely yes, 5 was

maybe and 1 was definitely not. Finally, consumers were also given the opportunity to write additional comments on the bottom of the ballot (*Appendix D*). On these ballots, consumers were also asked to report their panelist number, provided on the pre-screening form, in order to link their findings with their eating habits and especially to be able to separately analyze consumers with celiac disease or gluten allergy and consumer without any food allergy. Confidentiality was still assured, as it was not possible to link any panelist number to their name.

## **VI. Statistical design**

The physical and chemical analyses utilized a split-plot design with a completely randomized main plot.

A split-plot experiment has a main plot effect with larger experimental units, and is then subdivided into smaller experimental units to which a secondary treatment factor is applied. A split-plot design allows for experiments with a factor requiring a larger amount of experimental material to be paired with a second factor that requires a relatively smaller experimental unit (Kuehl, 2000).

For our study, the main-plot effect was the combination of eggs and antistaling agent levels which means the whole plot experimental unit was the batch of batter corresponding to a particular formulation. And the split plot experimental unit was the loaf of bread to which was applied a storage time factor.

Eight different formulations were tested for all physical and proximate analyses. For sensory studies, only two formulations were evaluated, one control without eggs and one with 25% eggs (flour basis). None of these two formulations had antistaling agent (DATEM).

Both physical and chemical tests were performed in triplicates. However, the sensory tests, both descriptive analysis and consumer response evaluation, were only conducted once due to lack of time and resources.

All data were analyzed using SAS, Software Release 9.2 (SAS, Institute Inc., Cary, North Carolina, USA). When treatment effects were found significantly different, the least square means with Tukey-Kramer groupings were used to differentiate treatment means. A level of significance was reported at  $\alpha \leq 0.05$ .

# PART III: RESULTS AND DISCUSSION

## I. Physical Analyses

### A. Loaf Specific Volume

There were differences ( $p < 0.05$ ) in loaf volumes due to the presence or absence of DATEM (Figure 3 and Table 3). This antistaling agent decreased loaf volume of gluten-free sorghum bread for 0% and 25% egg levels. Volumes differences due to DATEM were  $0.73 \text{ cm}^3/\text{g}$  and  $0.17 \text{ cm}^3/\text{g}$  for the 0% and 25% egg level, respectively.

Although DATEM has been shown to increase loaf volume in regular gluten breads (Gaupp and Adams, 2004), Nunes et al. (2009) showed that it did not have such a positive impact on loaf volume for gluten-free breads. This was similar to our study, where results showed that DATEM had a negative impact on loaf volume. This might be due to the functionality of this kind of emulsifier that usually improves dough strength by its ability to form liquid films of lamellar structure in the inter-phase between the gluten strands and the starch (Stampfli and Nersten, 1995). The absence of gluten might thus interfere with DATEM functionality.

According to Sciarini et al. (2010), gluten-free breads have a smaller specific volume, with an average of  $2.32 \text{ cm}^3/\text{g}$ , than wheat bread (average specific volume of  $4.41 \text{ cm}^3/\text{g}$ ).

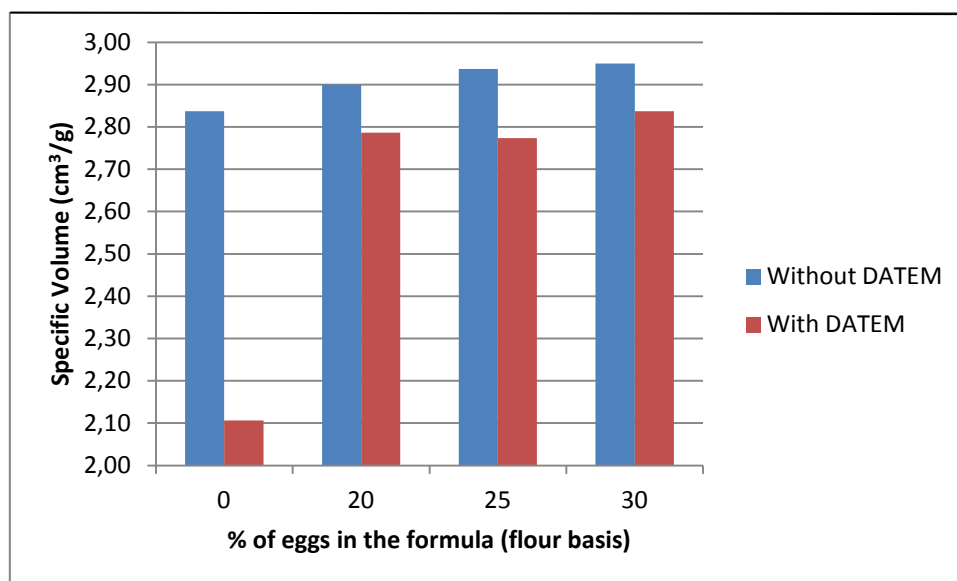
Our basic sorghum bread (without eggs) had a higher specific volume ( $2.84 \text{ cm}^3/\text{g}$ ) compared to the average gluten-free breads reported by Sciarini et al. (2010). The breads containing eggs in their formulation showed even higher volumes than the control one (respectively 2.90, 2.94, and  $2.95 \text{ cm}^3/\text{g}$  for the 3 egg levels). However, these differences were not found to be significant ( $p > 0.05$ ) for formulations without DATEM (Table 3).

Specific volumes were higher ( $p < 0.05$ ) (from  $0.66 \text{ cm}^3/\text{g}$  to  $0.73 \text{ cm}^3/\text{g}$ ) when eggs were added to a formulation containing DATEM. Eggs seemed to have a counter effect on the antistaling negative action. When eggs were added to the formulation with DATEM, volume of the end product almost reached volumes of breads without DATEM (Figure 3 and Table 3). This is obvious when looking at the bread containing DATEM but no egg, which had the lowest specific volume ( $2.11 \text{ cm}^3/\text{g}$ ).

Overall, whether the difference was significant or not, it seems obvious that eggs played a positive role on gluten-free sorghum breads. This can be due to egg proteins functionality. Egg white proteins in particular may be causing oven spring, which is the sudden increase in the volume during the first 10-12 minutes of baking, resulting from the increased rate of fermentation and expansion of gases. This oven spring is thus promoting a second rise in final loaf volume during baking (Crockett et al., 2011). Moreover, egg whites are well-known for their capacity to promote foaming. This foam may also have a role on this increased bread volume (Mine, 1995).



**Figure 3: Specific volumes (cm<sup>3</sup>/g) of sorghum breads according to percent eggs in the formula (flour basis) and the presence or absence of antistaling agent (DATEM)<sup>1</sup>**



n=3

<sup>1</sup> DATEM = diacetyl tartaric ester of monoglycerides

**Table 3: Comparison of specific volume (cm<sup>3</sup>/g) for sorghum breads with four different egg levels and the presence or absence of antistaling agent (DATEM)<sup>1</sup>**

Specific Volume (cm <sup>3</sup> /g)		
Egg level (%)	Without antistaling agent	With antistaling agent
0	2.84±0.03 <sup>Aa</sup>	2.11±0.04 <sup>Ba</sup>
20	2.90±0.06 <sup>Aa</sup>	2.79±0.07 <sup>Ab</sup>
25	2.94±0.06 <sup>Aa</sup>	2.77±0.07 <sup>Bb</sup>
30	2.95±0.01 <sup>Aa</sup>	2.84±0.08 <sup>Ab</sup>

<sup>a,b</sup> For each column, mean values with the same lowercase superscript are not significantly different (p>0.05).

<sup>A,B</sup> For each row, mean values with the same uppercase superscript are not significantly different (p>0.05).

<sup>1</sup> DATEM = diacetyl tartaric ester of monoglycerides

## B. Texture Profile Analysis

The hardness of the crumb was given by the texture profile analysis. Hardness is the measure of maximum force required to compress crumb by a specific length at a specific rate and is expressed as gram of force (g force).

Crumb hardness is major quality factor in baked goods, as it is strongly associated with consumers' perception of bread freshness (Ahlborn et al., 2005). Indeed, in white pan bread, most consumers prefer a soft, resilient, and short crumb as they relate these attributes to product freshness (Cogswell, 2008).

The presence of eggs did have a positive effect on crumb hardness (*Table 4*). Loaves with eggs had a significantly lower hardness values ( $p < 0.05$ ) than the control loaves (without eggs) at each of the four days studied (Days 0, 4, 8, and 12).

For formulations without DATEM, the lowest hardness was found for the loaf with 30% eggs at day 0 (517.6 g force) and the highest for the loaf without egg at day 12 (2440.4 g force). It was the same for formulations with the presence of the antistaling agent, the lowest hardness was found for the loaf with 30% eggs at day 0 (565.3 g force) and the highest for the loaf without egg at day 12 (4361.2 g force).

The positive effect of eggs was even more pronounced on loaves with DATEM. For these, there was also a significant difference ( $p < 0.05$ ) between the three different egg levels (20, 25, and 30%) at day 4 and day 12. For the breads without DATEM, even though overall the more eggs the lower the hardness values were, these differences were not found to be significant ( $p > 0.05$ ) for any of the four days observed.

The addition of eggs to the formulation helped reduce the differences between the hardness values for formulations with and without DATEM (*Table 4*). For the controls, at any moment over the 12 day storage period the two different formulations were significantly different ( $p < 0.05$ ): control with DATEM was significantly harder than the one without DATEM. However, when eggs were also added to the bread formulation, these differences, although still present, were lower and not significant anymore ( $p > 0.05$ ). The only significant difference was found at day 12 for an egg level of 20%.

Studies by Sabanis et al. (2009) and Ciarini et al. (2010) proved a negative correlation can be observed between crumb hardness and specific volume, meaning that bread with lower specific volumes had denser and more tightly-packed crumb structures resulting in higher

crumb hardness values. This was also the case in our study. Loaves with less egg were characterized by a smaller specific volume as well as by a significantly harder crumb.

Furthermore, this negative correlation existing between loaf specific volume and crumb hardness may explain our outcome for formulations containing DATEM. Loaves with this particular antistaling agent were characterized by a smaller specific volume due to the apparent negative effect of DATEM on this attribute. Therefore, due to the negative correlation mentioned, it is then normal that we found loaves with DATEM having a harder crumb.

Focusing on the differences over time for one specific formulation (*Table 4*), we can note that overall, for formulations without DATEM, the only values not different ( $p>0.05$ ) from one another were the ones at day 8 and day 12. Nevertheless, there was an exception for the loaf with 25% eggs. For formulations with DATEM, it was the opposite. The values over time were found to be different ( $p<0.05$ ) from one another except for 30% eggs where day 8 and day 12 were not significantly different. This might be due to the overall lower hardness for the loaf with 30% eggs.

As done in the study by Barcenas and Rosell (2005), the hardening rate was measured as the slope of the hardness time curves. Thus, if we consider the linear equations (*Figures 4 and 5*), we can see that not only egg addition on gluten-free bread formulation had a positive effect in reducing hardness at any time; but it also reduced the rate at which hardness increased. Indeed, in *Figures 4 and 5* we can see on the linear equations that the slopes for loaves containing eggs were smaller than the one for the control. This is true for both loaves without DATEM with a slope of 146.23 g/day for the control (*Figure 4*); and loaves with DATEM with a slope of 237.15 g/day for the control (*Figure 5*). However, this positive impact of egg is more pronounced on loaves with DATEM. For these formulations, we can see that as egg content increased in the formulation, the lower the slope value was.

While for the loaves without DATEM, there is an important difference between the control and the loaves with eggs, although this difference is not as prominent as the one for formulations with DATEM. Moreover, there is not much of a difference between the three egg levels. In addition, when more eggs were added, the slope does not get necessarily lower. Thus, regardless the presence or absence of the antistaling agent, it was demonstrated that the addition of eggs to a sorghum based gluten-free bread formulation reduced the rate of increase for the crumb hardness.

In conclusion, we can say that eggs had a positive effect in reducing gluten-free sorghum bread hardness over time. This lower hardness was found all over the 12 days period, with a more marked difference at the end of the storage period. Additionally, when eggs were added to the formulation of gluten-free sorghum bread, staling was delayed as the hardness increase was slower.

**Table 4: Comparison of hardness (g force) of sorghum breads with four different egg levels and presence or absence of antistaling agent (DATEM)<sup>1</sup> over a 12 day storage period**

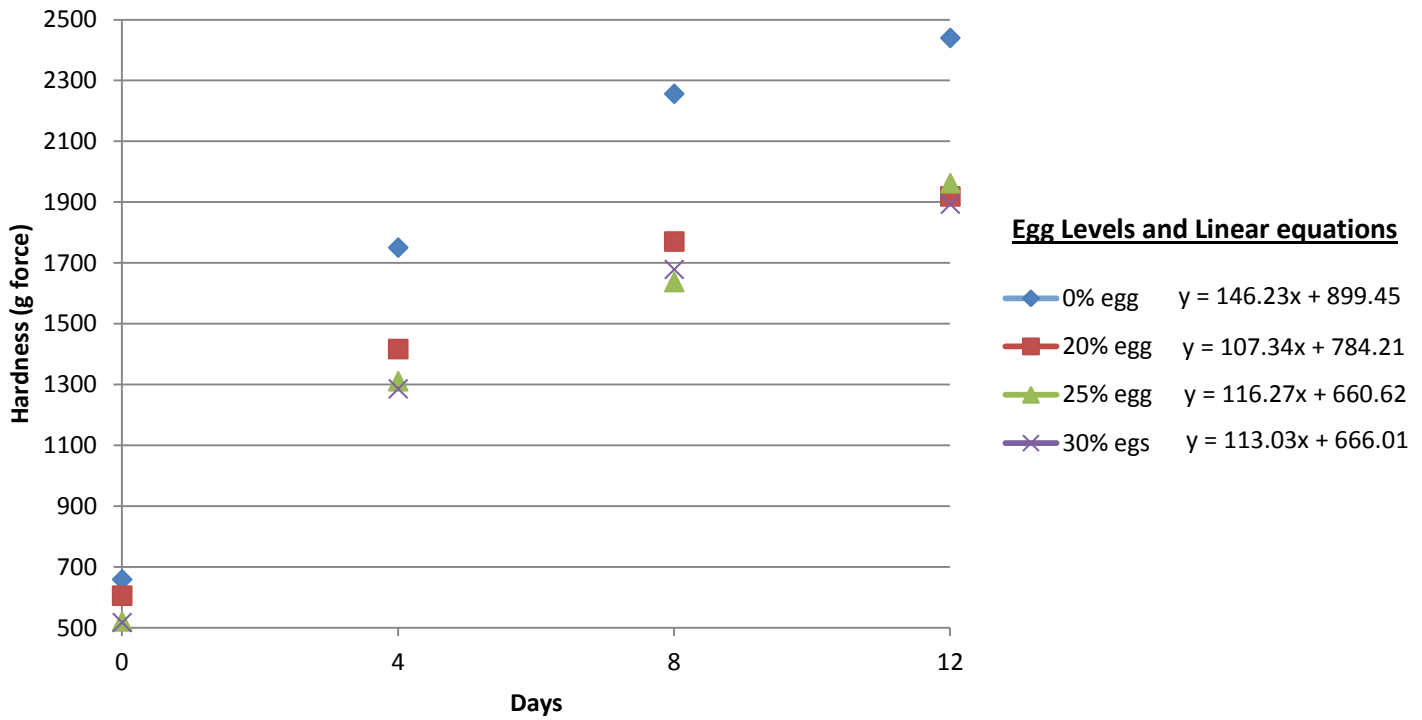
Hardness (g force)								
Egg level (%)	Without antistaling agent				With antistaling agent			
	Day 0	Day 4	Day 8	Day 12	Day 0	Day 4	Day 8	Day 12
<b>0</b>	659.3±73.0 <sup>Aa</sup>	1750.9±136.7 <sup>Ba</sup>	2256.7±103.9 <sup>Ca</sup>	2440.4±47.9 <sup>Ca</sup>	1307.3±34.5 <sup>Da</sup>	3407.8±130.5 <sup>Ea</sup>	3732.1±163.3 <sup>Fa</sup>	4361.2±133.5 <sup>Ga</sup>
<b>20</b>	605.9±20.2 <sup>Ab</sup>	1417.1±107.3 <sup>Bb</sup>	1770.8±90.1 <sup>Cb</sup>	1919.2±151.6 <sup>Cb</sup>	716.4±51.8 <sup>Ab</sup>	1536.6±70.4 <sup>Bb</sup>	1867.7±105.6 <sup>Cb</sup>	2273.1±237.9 <sup>Db</sup>
<b>25</b>	521.3±9.9 <sup>Ab</sup>	1311.2±30.6 <sup>Bb</sup>	1637.7±49.3 <sup>CEb</sup>	1962.7±16.9 <sup>DFb</sup>	675.2±54.7 <sup>Ab</sup>	1491.8±51.5 <sup>BCbc</sup>	1831.9±99.1 <sup>EDb</sup>	2165.2±145.3 <sup>Fb</sup>
<b>30</b>	517.6±7.1 <sup>Ab</sup>	1286.3±55.3 <sup>Bb</sup>	1679.1±99.5 <sup>Cb</sup>	1893.7±9.3 <sup>Cb</sup>	565.3±19.5 <sup>Ab</sup>	1315.5±31.4 <sup>Bc</sup>	1769.4±104.1 <sup>Cb</sup>	1863.1±51.6 <sup>Cc</sup>

<sup>a,b,c</sup> For each column, mean values with the same lowercase superscript are not significantly different (p>0.05).

<sup>A-G</sup> For each row, mean values with the same uppercase superscript are not significantly different (p>0.05).

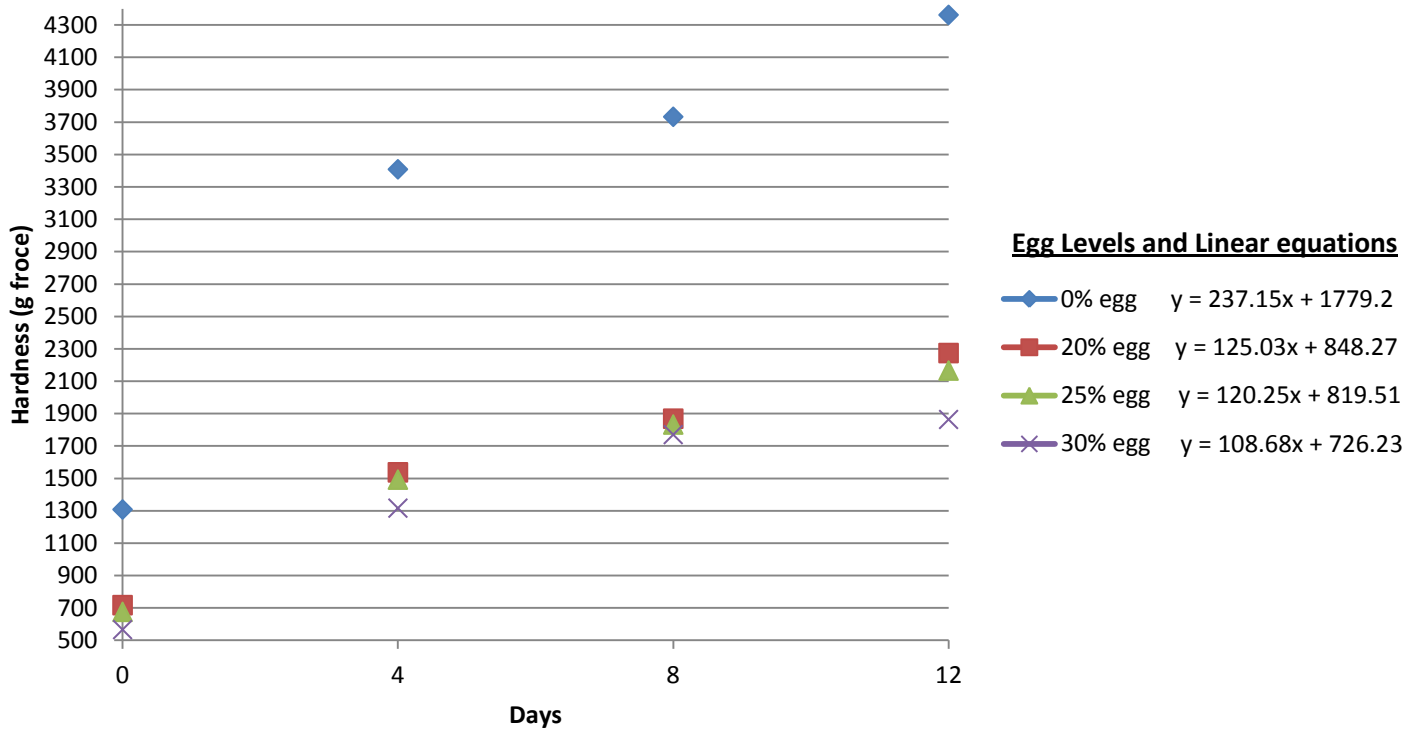
<sup>1</sup> DATEM = diacetyl tartaric ester of monoglycerides

**Figure 4: Changes in hardness (g force) of sorghum breads with four different egg levels without DATEM<sup>1</sup> over time (days)**



<sup>1</sup> DATEM = diacetyl tartaric ester of monoglycerides

**Figure 5: Changes in hardness (g force) of sorghum breads with four different egg levels with DATEM<sup>1</sup> over time (days)**



## C. Crumb structure

Figure 6 presents the C-Cell images of the eight different formulations. In wheat breads, the extent to which cells are formed is a function of the protein-starch interactions (especially from gluten). These interactions are responsible for the formation of the viscoelastic network of the dough. Air cells, also called alveoli, are created during mixing. Then, carbon dioxide, which is produced as a by-product of yeast fermentation, diffuses into these air cells, which causes them to expand (Gan et al., 1995).

Good quality wheat breads are characterized by small gas cells (Hayman et al., 1998). Smaller cells are also preferred in gluten-free bread products, since they have been found to produce loaves with higher specific volumes (Gallagher et al., 2003).

The top row in Figure 6 shows pictures of breads without any antistaling agent. The addition of eggs did improve the crumb structure. Cell size is indeed smaller. This desirable attribute is usually hard to find in gluten-free breads.

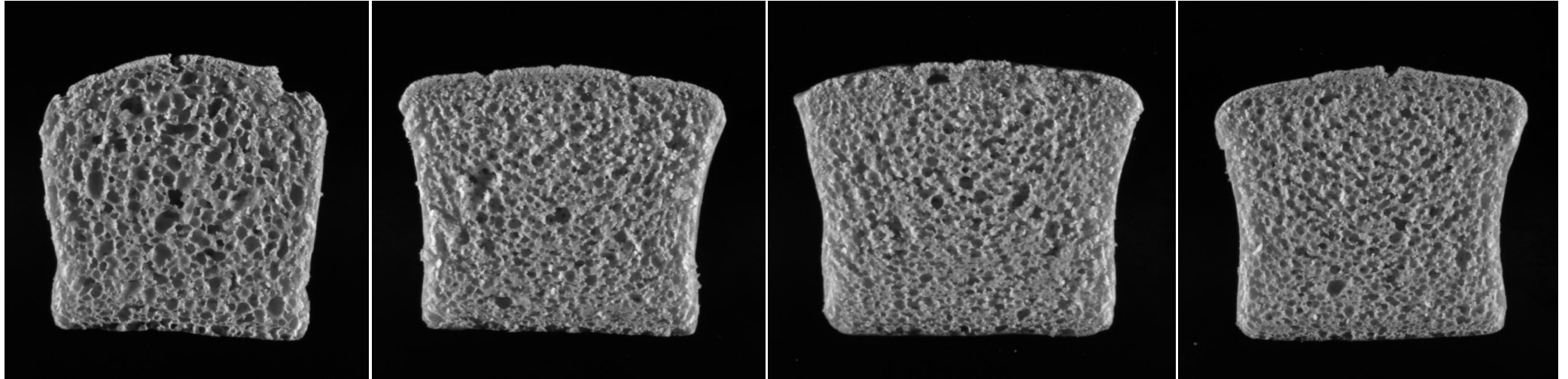
Finally, it is well-known that because of the absence of gluten, it is hard to form a web-like structure in gluten-free breads. This issue is even accentuated when larger cells are present as they are synonym with gas cell coalescence (Ahlborn et al., 2005). Our results indicated that egg proteins helped decrease the cell size and, as suggested by Arendt et al. (2009), they would be a good gluten replacement to create a proper network in bread crumb.

On the bottom row, pictures of loaves with DATEM show an obvious negative effect of DATEM on the crumb structure. For the slice of bread without egg but with DATEM, the crumb is really packed and the bread seems to have collapsed, indicating a very weak structure. This phenomenon is worse at the bottom part of the slice. Eggs seem to have a beneficial effect on this poor crumb structure. With the increase in eggs level, we can see the crumb arrangement not as dense. Though, the bottom part of the slices still presents a packed crumb although this has also been significantly improved by the addition of eggs.

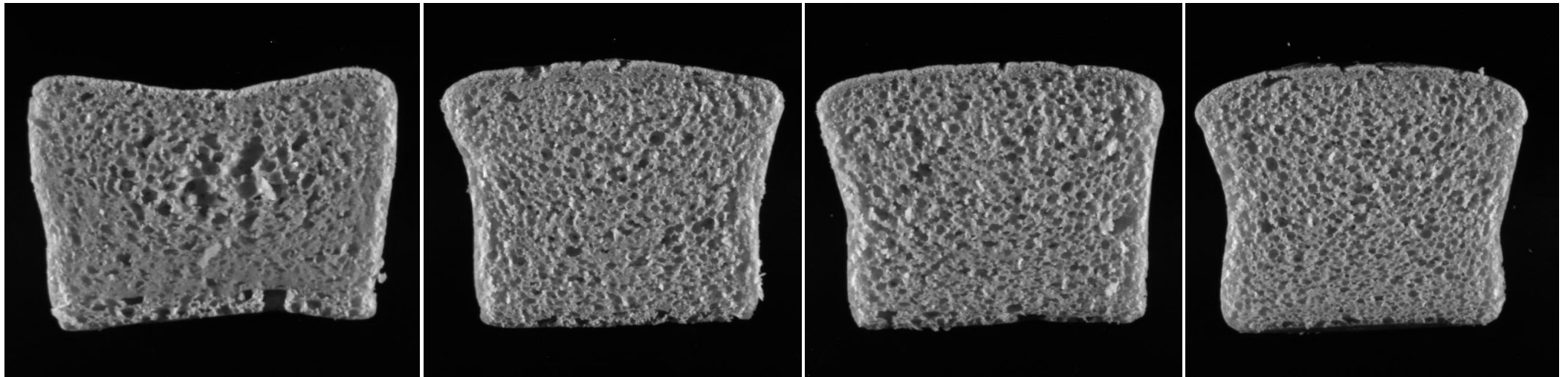
Furthermore, it is possible to see that breads with DATEM seemed flatter than breads without DATEM. This was a little bit counter-balanced by the addition of eggs.

In conclusion we can note that egg addition showed to be beneficial for the crumb structure as well as for the cell size. When eggs were added to the formulation, the end product presented more of the desirable attributes that are usually looked for in bread.

Figure 6: C-Cell images for eight different formulations of sorghum breads



Top pictures: Formulations without DATEM<sup>1</sup>. From left to right: 0% eggs, 20% eggs, 25% eggs, and 30% eggs.



Bottom pictures: Formulations with DATEM<sup>1</sup>. From left to right: 0% eggs, 20% eggs, 25% eggs, and 30% eggs.

<sup>1</sup> DATEM = diacetyl tartaric ester of monoglycerides



## D. Crumb and Crust Color

### 1) Crumb

#### ➤ *L\* values*

There was a significant difference ( $p < 0.05$ ) in crumb lightness when eggs were added to the formulation (*Table 5*). This was the case for breads with and without DATEM. Breads with eggs were characterized by higher  $L^*$  values which means that the crumb was lighter.

Although the presence of eggs made a difference on the lightness of the crumb, there was not any significant difference ( $p > 0.05$ ) between the three egg levels, regardless of the presence or absence of DATEM.

A significant difference in crumb color between loaves with and without DATEM was found for the 25% egg level only.

Krupa-Kozak et al. (2011) demonstrated that consumers have a preference for breads with lighter crumb. As a consequence, loaves with eggs present an advantage over the ones without any eggs.

#### ➤ *a\* values*

There was a significant difference in  $a^*$  values ( $p < 0.05$ ) among the different eggs level with and without the antistaling agent (*Table 5*). The more eggs in the formulation the lower the  $a^*$  values were. This indicated that with the increase in egg level, the crumb had less of a red color.

There were no differences ( $p > 0.05$ ) between loaves due to the presence of DATEM at the same egg level.

#### ➤ *b\* values*

For the crumb's  $b^*$  values, there was no particular pattern in the results according to egg level or the presence of DATEM (*Table 5*). The only significant difference with the presence of DATEM was for the 25% egg level. Once again, loaf without antistaling agent and with 25% eggs was characterized by a single lower value ( $b^* = 21.05$ ), thus by a less yellow color. There were no significant differences ( $p > 0.05$ ) for loaves without DATEM, but there were differences ( $p < 0.05$ ) for loaves with this antistaling agent. Only loaf with 20% eggs was significantly different from the control. However, it was not significantly different from the two other breads with eggs (at a level of 25 and 30%). It is possible that these results, for

formulations with antistaling agent, are not really representative because no clear pattern did emerge from the  $b^*$  value outcomes.

## 2) Crust

### ➤ $L^*$ values

There were significant differences ( $p < 0.05$ ) in  $L^*$  values for loaves without DATEM between breads without eggs and the control one (*Table 6*). There were also significant differences between the three egg levels: the more eggs the lower the  $L^*$  values. For breads containing DATEM, 25% and 30% egg level breads were significantly different ( $p < 0.05$ ) from the control but 20% eggs bread was not ( $p > 0.05$ ). Moreover, loaf is also not significantly different from the other 2 loaves with eggs. This means that the decrease in  $L^*$  values with the addition of eggs was less pronounced with the presence of DATEM. As a result, the crust was found to be darker with the presence of eggs. This is the opposite than for the crumb.

Regarding differences between the presence or absence of DATEM for a specific egg level, the only significant difference ( $p < 0.05$ ) was found for the control loaves. This might be due to the low  $L^*$  value ( $L^* = 73.68$ ) of the control loaf with DATEM. Except for these, there was no major difference between loaves although breads with the antistaling agent were always characterized by lower  $L^*$  values.

The fact that the crust becomes darker (lower  $L^*$  values) the more eggs are present can be easily explained. According to Breeding and Beyer (2000) eggs are composed of less than 1 g of carbohydrates in the form of glucose. Even if this amount seems insignificant, it is the appropriate amount to induce the Maillard reaction. This Maillard reaction is the condensation of a sugar with an amino acid that results in the formation of brownish-colored products. Thus the more eggs added to a formulation, the more glucose is added and as a consequence, a darker product is obtained.

It is general knowledge that most of the gluten-free breads are characterized by a lighter crust than regular breads. Hence, wheat breads are generally characterized by an  $L^*$  value of about 38 (Gallagher et al., 2003). This peculiarity of gluten-free breads to be quite light in color is one of the attributes that make them less attractive for the consumers like demonstrated by Krupa-Kozak et al. (2011).

Therefore we can see that even if our sorghum breads did not reach the regular wheat bread crust color, they were darker with eggs than without. Thus, they would be more attractive to consumers. This adds a component to the benefits of eggs in gluten-free breads formulations.

➤ *a\* values*

No matter the presence or absence of DATEM, there was a significant difference ( $p < 0.05$ ) in  $a^*$  values with the level of egg. The more egg there was in the formulation, the higher the  $a^*$  values were. This means that with the increase of eggs percent, the loaves have a more red color. Once again this is the opposite than what happens for the crumb. DATEM did not have an influence whatsoever on  $a^*$  values of the crust loaves (*Table 6*). As it was the case for the crust  $L^*$  values, these higher  $a^*$  values (more red) can be explained by the Maillard reaction which produces red-brown pigments.

➤ *b\* values*

For  $b^*$  values (*Table 6*), there was also a significant difference ( $p < 0.05$ ) with the level of egg without distinction of the presence or not of DATEM. The more egg there was in the formulation, the higher the  $b^*$  values were. This implies that the more we add eggs into the formulation, the more the bread's crust tends to have a yellow color. As for the  $a^*$  values, there was no significant difference ( $p > 0.05$ ) between loaves with and without the presence of DATEM at a similar egg level.

The higher yellow color found with the increase in egg amount can be explained by the natural yellow-orange pigments present in egg yolk (Breeding and Beyer, 2000).

**Table 5: Comparison of crumb color of sorghum breads with four different egg levels and the presence or absence of antistaling agent (DATEM) <sup>1</sup>**

Crumb color						
Egg level (%)	L		a		b	
	Without antistaling agent	With antistaling agent	Without antistaling agent	With antistaling agent	Without antistaling agent	With antistaling agent
0	62.55±0.55 <sup>Aa</sup>	63.80±0.16 <sup>Aa</sup>	2.65±0.17 <sup>Aa</sup>	2.65±0.07 <sup>Aa</sup>	21.41±0.27 <sup>Aa</sup>	21.35±0.50 <sup>Aa</sup>
20	65.36±1.07 <sup>Ab</sup>	66.21±0.27 <sup>Ab</sup>	2.35±0.06 <sup>Ab</sup>	2.39±0.09 <sup>Ab</sup>	21.51±0.37 <sup>Aa</sup>	21.96±0.17 <sup>Ab</sup>
25	64.89±0.88 <sup>Ab</sup>	66.73±0.82 <sup>Bb</sup>	2.13±0.05 <sup>Ac</sup>	2.28±0.08 <sup>Ab</sup>	21.05±0.32 <sup>Aa</sup>	21.76±0.26 <sup>Bab</sup>
30	65.45±0.78 <sup>Ab</sup>	65.87±0.09 <sup>Ab</sup>	2.07±0.09 <sup>Ac</sup>	2.14±0.04 <sup>Ac</sup>	21.18±0.18 <sup>Aa</sup>	21.58±0.17 <sup>Aab</sup>

<sup>a,b,c</sup> For each column, mean values with the same lowercase superscript are not significantly different (p>0.05).

<sup>A,B</sup> For each row, mean values with the same uppercase superscript are not significantly different (p>0.05).

<sup>1</sup> DATEM = diacetyl tartaric ester of monoglycerides

**Table 6: Comparison of crust color of sorghum breads with four different egg levels and the presence or absence of antistaling agent (DATEM) <sup>1</sup>**

Crust color						
Egg level (%)	L		a		b	
	Without antistaling agent	With antistaling agent	Without antistaling agent	With antistaling agent	Without antistaling agent	With antistaling agent
0	77.32±1.67 <sup>Aa</sup>	73.68±1.84 <sup>Ba</sup>	1.83±0.35 <sup>Aa</sup>	1.84±0.19 <sup>Aa</sup>	21.15±1.03 <sup>Aa</sup>	20.18±0.64 <sup>Aa</sup>
20	73.07±1.10 <sup>Ab</sup>	71.02±0.43 <sup>Aab</sup>	2.96±0.19 <sup>Ab</sup>	2.97±0.10 <sup>Ab</sup>	25.35±0.83 <sup>Ab</sup>	24.82±0.43 <sup>Ab</sup>
25	70.96±0.95 <sup>Abc</sup>	69.99±1.07 <sup>Ab</sup>	3.38±0.16 <sup>Abc</sup>	3.44±0.24 <sup>Abc</sup>	26.12±0.37 <sup>Abc</sup>	25.83±0.65 <sup>Abc</sup>
30	68.89±1.14 <sup>Ac</sup>	68.56±0.59 <sup>Ab</sup>	3.82±0.15 <sup>Ac</sup>	3.68±0.19 <sup>Ac</sup>	26.64±0.07 <sup>Ac</sup>	26.54±0.35 <sup>Ac</sup>

<sup>a,b,c</sup> For each column, mean values with the same lowercase superscript are not significantly different (p>0.05).

<sup>A,B</sup> For each row, mean values with the same uppercase superscript are not significantly different (p>0.05).

<sup>1</sup> DATEM = diacetyl tartaric ester of monoglycerides

## E. Water activity

There were no significant differences in water activity ( $p>0.05$ ) between eggs level and presence or not of DATEM for both the crumb and crust (*Table 7*).

Water activity values for the crumb varied from 0.986 for the bread with DATEM and with 30% eggs to 0.992 for the breads without the antistaling agent and either 20 or 30% eggs. For the crust, water activity values varied from 0.884 for the bread with DATEM and with 30% eggs to 0.912 for the bread without the antistaling agent and 25% eggs.

The only coherent pattern lies in the water activity of the crust for breads with DATEM. Although the differences were not significant, breads were characterized by lower crust water activity when eggs were added. But, because this pattern is only showing in the crust of breads with antistaling agent, it is not possible to draw general conclusions from this outcome. Therefore, the addition of eggs with or without the presence of DATEM had no influence on the water activity of both the crumb and the crust of sorghum bread. The addition of DATEM made no difference either on water activity.

**Table 7: Comparison of water activity of sorghum breads with four different egg levels and the presence or absence of antistaling agent (DATEM)<sup>1</sup>**

Water Activity				
Egg level (%)	Crumb		Crust	
	Without antistaling agent	With antistaling agent	Without antistaling agent	With antistaling agent
0	0.989±0.010 <sup>Aa</sup>	0.990±0.004 <sup>Aa</sup>	0.907±0.040 <sup>Aa</sup>	0.908±0.030 <sup>Aa</sup>
20	0.992±0.007 <sup>Aa</sup>	0.987±0.010 <sup>Aa</sup>	0.894±0.020 <sup>Aa</sup>	0.896±0.010 <sup>Aa</sup>
25	0.991±0.006 <sup>Aa</sup>	0.991±0.010 <sup>Aa</sup>	0.912±0.001 <sup>Aa</sup>	0.893±0.020 <sup>Aa</sup>
30	0.992±0.004 <sup>Aa</sup>	0.986±0.010 <sup>Aa</sup>	0.902±0.010 <sup>Aa</sup>	0.884±0.010 <sup>Aa</sup>

<sup>a</sup> For each column, mean values with the same lowercase superscript are not significantly different ( $p>0.05$ ).

<sup>A</sup> For each row, mean values with the same uppercase superscript are not significantly different ( $p>0.05$ ).

<sup>1</sup> DATEM = diacetyl tartaric ester of monoglycerides

## **II. Proximate Analysis**

### **A. Moisture Content**

There was a significant increase in moisture with eggs ( $p < 0.05$ ) (*Table 8*). However, there was no differences ( $p > 0.05$ ) among the three egg levels. On the contrary, for formulations containing the antistaling agent, there were no significant differences ( $p > 0.05$ ) among the four loaves. This deviation from the outcome of breads without DATEM might be due to a control loaf with higher moisture (35.15%) compared to the one without the antistaling agent (33.75%). There was no significant difference ( $p > 0.05$ ) between loaves with and without DATEM for a specific egg level.

### **B. Protein Content**

As expected, the addition of eggs to the formulation significantly increased ( $p < 0.05$ ) the protein content regardless the presence or absence of the antistaling agent (*Table 8*) because eggs are high in protein.

However, there was no difference ( $p > 0.05$ ) among the three egg levels. This indicates that even if there was a 5 to 10% increase in eggs level, the protein content did not necessarily increase. Maybe a more important increase in eggs is needed in order to see a difference on the protein content.

Lastly, there was also no difference ( $p > 0.05$ ) between loaves with and without DATEM.

### **C. Fat Content**

There were significantly higher ( $p < 0.05$ ) fat values when eggs were added to the formulation (*Table 8*). The more eggs the higher the values were. This was even more marked on formulations without DATEM. For these, significant differences ( $p < 0.05$ ) were also found between the different egg levels, while for formulations with DATEM, the three egg levels were not significantly different ( $p > 0.05$ ) from one another.

This increase in fat content as eggs are added to the formulation also makes sense as the egg yolk is composed by 69% of lipids (on a dry basis) (Breeding and Beyer, 2000).

There was no differences ( $p > 0.05$ ) between breads containing the same amount of egg with or without DATEM.

## **D. Fiber Content**

There were no differences ( $p>0.05$ ) in fiber content with the variation in egg level or with the presence or not of DATEM (*Table 9*). Thus, we can conclude that these two ingredients do not influence the bread fiber content.

## **E. Ash Content**

The increase in egg level in sorghum bread formulations did not impact ( $p>0.05$ ) the ash content of the bread which remained pretty similar (*Table 9*).

Only breads with 0% and 30% egg levels presented a significant difference ( $p<0.05$ ) according to the presence or absence of DATEM. It does not seem that DATEM had a real influence on ash content.

**Table 8: Comparison of moisture, protein and fat contents of sorghum breads with four different egg levels and the presence or absence of antistaling agent (DATEM) <sup>1</sup>**

Proximate (1)						
Egg level (%)	% Moisture		% Crude Protein*		% Crude Fat*	
	Without antistaling agent	With antistaling agent	Without antistaling agent	With antistaling agent	Without antistaling agent	With antistaling agent
0	33.75±0.31 <sup>Aa</sup>	35.15±0.41 <sup>Aa</sup>	5.50±0.06 <sup>Aa</sup>	5.84±0.67 <sup>Aa</sup>	2.32±0.09 <sup>Aa</sup>	2.84±0.78 <sup>Aa</sup>
20	36.29±1.87 <sup>Ab</sup>	35.78±0.59 <sup>Aa</sup>	7.24±0.64 <sup>Ab</sup>	7.40±0.28 <sup>Ab</sup>	4.35±0.57 <sup>Ab</sup>	5.01±0.29 <sup>Ab</sup>
25	35.81±0.29 <sup>Ab</sup>	36.27±0.72 <sup>Aa</sup>	7.89±0.11 <sup>Ab</sup>	7.47±0.77 <sup>Ab</sup>	5.04±0.21 <sup>Abc</sup>	5.46±0.29 <sup>Ab</sup>
30	36.34±0.28 <sup>Ab</sup>	36.38±0.41 <sup>Aa</sup>	7.70±1.41 <sup>Ab</sup>	7.41±1.36 <sup>Ab</sup>	5.70±0.62 <sup>Ac</sup>	5.74±0.11 <sup>Ab</sup>

<sup>a,b,c</sup> For each column, mean values with the same lowercase superscript are not significantly different (p>0.05).

<sup>A,B</sup> For each row, mean values with the same uppercase superscript are not significantly different (p>0.05).

<sup>1</sup> DATEM = diacetyl tartaric ester of monoglycerides

\*Express as dry basis

**Table 9: Comparison of fiber and ash contents of sorghum breads with four different egg levels and the presence or absence of antistaling agent (DATEM) <sup>1</sup>**

Proximate (2)				
Egg level (%)	% Crude Fiber*		% Ash*	
	Without antistaling agent	With antistaling agent	Without antistaling agent	With antistaling agent
0	0.48±0.03 <sup>Aa</sup>	0.15±0.10 <sup>Aa</sup>	3.03±0.03 <sup>Aa</sup>	3.25±0.03 <sup>Ba</sup>
20	0.57±0.13 <sup>Aa</sup>	0.50±0.07 <sup>Aa</sup>	3.11±0.06 <sup>Aa</sup>	3.21±0.09 <sup>Aa</sup>
25	0.57±0.09 <sup>Aa</sup>	0.59±0.09 <sup>Aa</sup>	3.13±0.03 <sup>Aa</sup>	3.21±0.07 <sup>Aa</sup>
30	0.63±0.06 <sup>Aa</sup>	0.54±0.04 <sup>Aa</sup>	3.10±0.05 <sup>Aa</sup>	3.28±0.01 <sup>Ba</sup>

<sup>a</sup> For each column, mean values with the same lowercase superscript are not significantly different (p>0.05).

<sup>A,B</sup> For each row, mean values with the same uppercase superscript are not significantly different (p>0.05).

<sup>1</sup> DATEM = diacetyl tartaric ester of monoglycerides

\*Express as dry basis



### **III. Sensory Analysis**

#### **A. Descriptive Analysis**

##### **1) Texture Attributes**

###### ➤ *Springiness*

Staling did impact the springiness of the bread with time (*Table 10*). When the bread was fresh, the springiness values were about three times higher than when the bread was 4 days old (mean scores of 10.00 and 10.67 for fresh breads against 3.17 and 3.67 for 4 days old breads).

However, for both fresh and 4 days old breads, even if breads with eggs presented a higher springiness, this difference was not found to be significant ( $p>0.05$ ). Thus, although eggs seem to help in increasing bread springiness, they do not significantly improve this attribute.

###### ➤ *Tooth packing*

There was no significant difference ( $p>0.05$ ) either with the egg factor or the time one on tooth packing (*Table 10*).

###### ➤ *Hardness*

As expected, bread got harder over time (*Table 10*). When comparing the same formulation at day 0 and day 4, the hardness after 4 days of storage was significantly higher ( $p<0.05$ ) than the one at day 0. This was true no matter which formulation.

Furthermore, for the two days studied, there was a significant difference ( $p<0.05$ ) depending on the presence or absence of egg. Breads with eggs were found to be considerably less hard. It is also interesting to note that a loaf of bread with 25% eggs (flour basis) after 4 days of storage (mean score of 11.00) was harder but not in a significant way ( $p>0.05$ ) than the fresh control loaf (mean score of 9.83). This demonstrates that bread without eggs is really harder than bread with eggs after 4 days of storage. Bread with eggs was not significantly different from fresh bread without eggs.

These outcomes produced by the semi-trained panel can therefore be correlated to the output furnished by the instrumental test as both indicated a 'softening' effect of eggs. Both tests showed the advantage of egg over gluten-free sorghum bread hardness.

## 2) Flavor Attributes

### ➤ *Sour*

Bread appears to get more sour over time (*Table 11*). This comes out to be normal as an increase in sourness is one of the multiple characteristics of stale bread.

Even though the values found were lower for breads with eggs, at both storage times, these differences were not significant ( $p>0.05$ ).

Still, in addition to the fact that the values for the formulations with eggs are lower, we can assume that eggs tend to attenuate the sourness of gluten-free sorghum bread because the loaf with eggs after 4 days of storage (mean score of 4.00) was not found to be significantly different from both fresh breads (mean scores of 2.67 for loaf with eggs and 3.17 for loaf without egg).

### ➤ *Rancid*

For the rancid attribute, there was no significant difference ( $p>0.05$ ) either over time nor with the egg level (*Table 11*).

Nonetheless, it might be interesting to point out that even if the differences are not significant; the rancidity value for the 4 days old bread with eggs (mean score of 4.33) is lower than the one for the 4 days old bread without egg (mean score of 6.33). This value is additionally quite close to the unique value of fresh bread (mean score of 4.00). Thus, once again, even if it is not significantly different, eggs seem to attenuate the increase in rancidity happening overtime.

### ➤ *Doughy*

For this attribute, it is important to specify that contrary to the other flavor attributes, a higher score would correspond to fresher bread.

There was a significant difference ( $p<0.05$ ) after 4 days of storage between the bread with egg and the one without (*Table 11*). This difference is quite impressive as it is more than double.

For the fresh breads, there was no significant difference ( $p>0.05$ ) between the two loaves but we can note that the bread without egg presented a higher value.

However, when comparing the differences overtime for one specific formulation, we can see that the bread with eggs tend to have a lower decrease in its value. Indeed the 4 days old bread with eggs is characterized by a doughy value (mean score of 4.50) not significantly different ( $p>0.05$ ) from the ones for fresh breads (average scores of 5.33 for the loaf with eggs and 5.50

for the loaf without egg). Consequently, even if eggs do not seem to have a positive effect on the doughy attribute on day 0, they seem to delay the loss of such desirable attribute overtime.

➤ *Musty*

Musty is a characteristic flavor of sorghum grain; as a result, this might explain why the values were quite high even for fresh breads.

There were no significantly different ( $p>0.05$ ) values for 4 days old breads (*Table 11*). Actually, they even were the exact same (average score of 10.83). There also was no significant difference between fresh breads. However, fresh bread with eggs presented a lower value (average score of 8.16).

Furthermore, fresh bread without eggs was characterized by an average musty score of 10.33 which is quite close to the one found for 4 days old breads (average score of 10.83). Thus, we might assume that eggs tend to palliate the musty flavor of gluten-free sorghum bread when fresh. Nevertheless, this difference was not significant and did not persist over time.

Some of the values from our descriptive analysis were characterized by quite high standard deviations. Day N’Kouka et al. (2004) explained that this is not unusual in a descriptive analysis panel. It is typically indicating the fact that panelists were using the scales differently for evaluation. This might be due to the fact that our panel was semi-trained; panelists were not professionally trained judges. Therefore, more training emphasizing group discussion on intensity of these attributes or use of multiple anchors could have been beneficial. No research was found in the literature about either eggs and/or gluten-free breads thus we were not able to cross-check our study with previous work.

**Table 10: Descriptive analysis of three texture attributes of sorghum bread without (control) and with (25% flour basis) eggs at day 0 (Fresh) and day 4**

<b>Texture</b>			
<b>Sample</b>	<b>Springiness<sup>1</sup></b>	<b>Tooth packing<sup>2</sup></b>	<b>Hardness<sup>3</sup></b>
<b>4-days control</b>	3.17±1.17 <sup>a</sup>	4.67±1.03 <sup>a</sup>	13.33±0.81 <sup>a</sup>
<b>4-days with eggs</b>	3.67±0.82 <sup>a</sup>	5.17±1.47 <sup>a</sup>	11.00±1.26 <sup>b</sup>
<b>Fresh control</b>	10.00±2.83 <sup>b</sup>	5.00±1.26 <sup>a</sup>	9.83±1.17 <sup>b</sup>
<b>Fresh with eggs</b>	10.67±1.03 <sup>b</sup>	3.67±1.21 <sup>a</sup>	6.83±1.72 <sup>c</sup>

<sup>a, b, c</sup> For each column, mean values with the same superscript are not significantly different ( $p>0.05$ ).

<sup>1</sup>Springiness: 1=not springy (undesirable); 15= very springy (desirable)

<sup>2</sup>Tooth packing: 1=not tooth packed (desirable); 15= very tooth packed (undesirable)

<sup>3</sup>Hardness: 1=not hard (desirable); 15= very hard (undesirable)

**Table 11: Descriptive analysis of four flavor attributes of sorghum bread without (control) and with (25% flour basis) eggs at day 0 (Fresh) and day 4**

<b>Flavor</b>				
<b>Sample</b>	<b>Sour<sup>1</sup></b>	<b>Rancid<sup>2</sup></b>	<b>Doughy<sup>3</sup></b>	<b>Musty<sup>4</sup></b>
<b>4-days control</b>	5.17±1.47 <sup>a</sup>	6.33±1.03 <sup>a</sup>	2.00±0.89 <sup>a</sup>	10.83±0.98 <sup>a</sup>
<b>4-days with eggs</b>	4.00±1.26 <sup>ab</sup>	4.33±2.50 <sup>a</sup>	4.50±1.22 <sup>b</sup>	10.83±0.75 <sup>a</sup>
<b>Fresh control</b>	3.17±1.60 <sup>b</sup>	4.00±2.00 <sup>a</sup>	5.50±1.05 <sup>b</sup>	10.33±1.03 <sup>ab</sup>
<b>Fresh with eggs</b>	2.67±0.81 <sup>b</sup>	4.00±1.26 <sup>a</sup>	5.33±1.03 <sup>b</sup>	8.16±2.93 <sup>b</sup>

<sup>a, b</sup> For each column, mean values with the same superscript are not significantly different ( $p>0.05$ ).

<sup>1</sup>Sour: 1=not sour (desirable); 15= very sour (undesirable)

<sup>2</sup>Rancid: 1=not rancid (desirable); 15= very rancid (undesirable)

<sup>3</sup>Doughy: 1=not doughy (undesirable); 15= very doughy (desirable)

<sup>4</sup>Musty: 1=not musty (desirable); 15= very musty (undesirable)

## **B. Consumer Response Evaluation**

Out of 103 panelists, 59 were female while 44 were male. The age of panelists ranged from 18 to 90 years with 66% of panelists in the 18-25 age group.

This general population can be divided into two distinct subgroups: people having celiac disease and people without the disease.

- Out of 90 panelists without celiac disease, 48 were female while 42 were male. The age of these non-celiac panelists ranged from 18 to 70 years with 70% of panelists in the 18-25 age group. Regarding gluten-free bread consumption, 68% claimed never consuming this kind of bread. Of the remaining 32%, 10% claimed to eat gluten-free bread at least once a week, while 34% claimed to eat this kind of bread once every two weeks to once a month. This demonstrates that even without having celiac disease, a third of this healthy population consumes gluten-free bread.

- Finally, out of 13 panelists having celiac disease, 11 were female while 2 were male. The age of these celiac panelists ranged from 18 to 90 years with 38% of panelists in the 18-25 age group. Regarding gluten-free bread eating frequency, it fluctuates between every day to once every two weeks.

Table 12 presents the average scores from the consumer study for the general population, this is the entire 103 panelists. These scores reveal that significant differences were found for each attributes ( $p < 0.05$ ). For the six attributes, sorghum bread with 25% (flour basis) eggs was found to be better; score was higher from almost 2 points for each attribute. Overall acceptability was 4.50 for the control against 6.43 for the bread with eggs. Furthermore, there was also an important and representative difference in the willingness to buy the product. The control scored 3.42 for this, while the bread with eggs scored 5.40 and was therefore above the middle point (5) on the 9-points hedonic scale.

From this study we can then draw the following conclusion: from a consumer point of view, more acceptable gluten-free bread was obtained when eggs were added to the formulation. Finally, we can state that with its higher scores than the control and its overall acceptability over 6, our gluten-free sorghum bread made with eggs has great potential in the gluten-free market. Indeed, for a product to enter into the market, an average of 7 for overall acceptability is typically used by many food companies. Thus, our product being scored close to 7, there are just few improvements to be done in order for the product to be ready for sale.

In their comments, many consumers found the sorghum bread without egg to be characterized by a strong bitter aftertaste. Overall, comments about sorghum bread with eggs were more positive but also more focused on its better taste. Even though some consumers picked up a bitter note, they also specified that it was a softer taste than for the bread without egg and that it was not as unpleasant.

For each of the six attributes, celiac disease patients scored the control lower and the bread with eggs higher (*Table 13*). The only exception was for the color attribute of the bread with eggs where celiac patients scored it lower (6.15) than non celiac suffering consumers (6.80). This attribute also presented a significant difference ( $p < 0.05$ ). The second attribute presenting a significant difference between the two subgroups was the willingness to buy the bread with eggs. This last point is going to be talked about in a following paragraph. Except for these, all scores between the two subgroups were not significantly different from one another for one given sample.

For consumers non suffering from celiac disease, the overall acceptability scored 4.52 for the control bread and 6.37 for the bread with eggs. For celiac disease patients, scores for this attribute were 4.31 for the control and 6.85 for the bread with eggs. It seemed that celiac disease patients had a higher acceptability of our sorghum bread with eggs than the non celiac consumers.

Finally, the willingness to buy from consumers without celiac disease was 3.46 for the control and 5.21 for the bread with eggs which is very similar to what we have for the general population. However, we can notice that these scores are a little bit different when we look at consumers having celiac disease. Indeed, they scored the control 3.15 but 6.69 the bread with eggs. This major increase in the score for bread with eggs is very encouraging as it is close to 7. A willingness to buy close to 7 on a 9-points hedonic scale from our main target market is a good and promising sign.

In conclusion, it is important to specify that the overall acceptability as well as the willingness to buy was higher for the formulation with eggs. Even if this bread might not be ready to commercialize as it did not reach an average of 7 on the hedonic scale; it is essential to state again that it was plain bread. Usually consumers will not eat bread plain. Most of the time in

the U.S., bread is eaten with butter, preserve, peanut butter or any other kind of condiment. Therefore, knowing that we attain a score over 6 with plain bread, there are good reasons to believe that served with the appropriate accompaniment, our gluten-free sorghum bread with eggs may be ready to commercialize.

**Table 12: Comparison of scores from the consumer study of sorghum bread without egg (control) and with 25% (flour basis) eggs for 6 attributes**

General Population											
Overall acceptability		Appearance		Flavor		Color		Texture		Willingness to buy	
Control	With eggs	Control	With eggs	Control	With eggs	Control	With eggs	Control	With eggs	Control	With eggs
4.50±1.70 <sup>a</sup>	6.43±1.25 <sup>b</sup>	5.41±1.67 <sup>a</sup>	6.92±1.33 <sup>b</sup>	4.19±1.88 <sup>a</sup>	6.07±1.63 <sup>b</sup>	5.93±1.53 <sup>a</sup>	6.72±1.27 <sup>b</sup>	4.21±1.87 <sup>a</sup>	6.47±1.65 <sup>b</sup>	3.42±2.01 <sup>a</sup>	5.40±2.04 <sup>b</sup>

<sup>a, b</sup> For each attribute, mean values with the same superscript are not significantly different (p>0.05).  
For all attributes, 1= dislike extremely; 9= Like extremely

**Table 13: Comparison of scores from consumers with and without celiac disease of sorghum bread without egg (control) and with 25% (flour basis) eggs for 6 attributes**

	Consumers <u>without</u> celiac disease		Consumers <u>with</u> celiac disease	
	Control	With eggs	Control	With eggs
<b>Overall acceptability</b>	4.52±1.64 <sup>a</sup>	6.37±1.29 <sup>b</sup>	4.31±2.14 <sup>a</sup>	6.85±0.80 <sup>b</sup>
<b>Appearance</b>	5.47±1.64 <sup>a</sup>	6.91±1.37 <sup>b</sup>	5.00±1.87 <sup>a</sup>	7.00±1.08 <sup>b</sup>
<b>Flavor</b>	4.26±1.89 <sup>a</sup>	5.97±1.61 <sup>b</sup>	3.77±1.88 <sup>a</sup>	6.77±1.69 <sup>b</sup>
<b>Color</b>	6.07±1.56 <sup>a</sup>	6.80±1.26 <sup>c</sup>	5.00±0.82 <sup>b</sup>	6.15±1.28 <sup>a</sup>
<b>Texture</b>	4.23±1.89 <sup>a</sup>	6.36±1.70 <sup>b</sup>	4.08±1.75 <sup>a</sup>	7.23±0.93 <sup>b</sup>
<b>Willingness to buy</b>	3.46±1.93 <sup>a</sup>	5.21±1.88 <sup>b</sup>	3.15±2.58 <sup>a</sup>	6.69±2.62 <sup>c</sup>

<sup>a, b, c</sup> For each row, mean values with the same superscript are not significantly different (p>0.05).  
For all attributes, 1= dislike extremely; 9= Like extremely



# **PART IV: SHORTCOMINGS OF THE STUDY AND RECOMMENDED FUTURE WORK**

## **I. Shortcomings of the Study**

In light of the results obtained it seems undeniable that the use of DATEM as antistaling agent was not a sound choice. It was established that this specific antistaling agent, although widely used in baked goods, had a negative impact on loaf volume and crumb hardness for gluten-free bread.

This pitfall with the use of DATEM limits the scope of the study. Indeed, it was not possible to properly evaluate the impact of an antistaling agent on delaying staling for gluten-free sorghum bread. Additionally, neither was it possible to assess the combined effect of eggs and an effective antistaling agent.

Another limitation of this study was encountered during descriptive sensory analysis specifically. Even though our panel was properly trained, their judgment was not as accurate as would be a highly trained panel. Consequently, the evaluation of these different breads by a professional panel might homogenize the scores and thus be able to lower some of the standard deviations in our results

Finally, this study showed promising results, eggs had a considerable positive effect in reducing crumb hardness as well as slowing down staling. Nevertheless, even with the addition up to 30% (flour basis) eggs to its formulation, gluten-free sorghum bread still staled too fast regarding consumer usual acceptance. This was highlighted by the descriptive analysis. Regarding bread hardness, which is the main quality factor defining freshness for consumers, after only 4 days post baking, and even with 25% (flour basis) eggs, breads were already described by our panel with scores above the medium point on the 15-point scale (mean score of 11.00). However, this issue can be countered commercially through freezing of gluten-free breads as it will be mentioned in the next part.

## **II. Recommended future work**

While this study's main question about determining egg impact on gluten-free sorghum bread has been addressed, many others still remain and there is still room for further progress in delaying staling as well as improving overall quality of gluten-free sorghum bread.

### **A. Increasing shelf-life**

One point of interest regarding delaying staling may be the freezing process. The effect of freezing on the quality and shelf-life of wheat breads, fully baked or partially baked, has been widely studied (Barcenas et al., 2003; Ronda et al., 2011). Nevertheless, there are only few studies on the effect of freezing on gluten-free bread quality. One of them, by Ronda and Roos (2011), demonstrated that frozen storage at  $-28^{\circ}\text{C}$ , the lowest achievable temperature in common home freezers, retained quality of gluten-free bread near to fresh bread one. They concluded that a frozen storage temperature of  $-20^{\circ}\text{C}$  seems to be satisfactory for high quality retention and lower bread staling rates subsequent to thawing of gluten-free bread.

Therefore, freezing seems to be an interesting alternative to extend gluten-free bread shelf-life. It would be valuable to study what could be the outcomes of freezing gluten-free sorghum bread with eggs. If the freezing process does not damage our gluten-free bread existing quality, this would be a way to make its commercialization easier as the addition of eggs already improved its quality to an acceptable level, all we need is a longer shelf-life.

Furthermore, further research can be undertaken to develop a new formulation, based on our gluten-free sorghum bread with eggs but with the addition of calcium propionate. This preservative is widely used in the baking industry mainly as mold inhibitor. The incorporation of such an ingredient to our formulation would allow us to optimize storage packaging in order for it to better correspond to what consumers are used to. With this calcium propionate, it would also be possible to realize a proper shelf-life study to determine with more accuracy when the bread will become unacceptable.

### **B. Improving overall quality**

Because this plain, unflavored bread scored pretty high and close to a 7 on a 9-points hedonic scale, it would be interesting to develop another kind of sorghum bread with eggs. Flavored bread may be better idea as it may score even higher. This may specifically help to cover the

bitterness and musty attribute peculiar to sorghum bread. Furthermore flavored breads are quite popular for American consumers.

Such bread was already developed at some point during this study. A variation of our sorghum bread with 25% eggs was produced with raisins, orange, nuts and spices. This bread was a finalist at the annual America's Best Raisin Bread Contest.

As part of this overall project and in order to improve gluten-free sorghum bread quality, an additional study is also going to be done on exploring the combined effects of particle size and heat treatment of sorghum flour on the quality of sorghum baked goods.

Finally, it is commonly known that the nutritional aspect of gluten-free breads is still open for improvement. Most of these wheat-free products are produced from purified starch and refined flours which results in breads low in fiber. Moreover, gluten-free functional foods as those containing high levels of anti-oxidants are also not widely available on the market.

Our gluten-free sorghum bread is also characterized by poor fiber content as demonstrated by our results (our highest value was 0.63% crude fiber, on a dry basis).

For that reason, research might focus on improving these deficiencies. Another study our team worked on already did so by investigating on the addition of tannin containing sorghum bran to gluten-free sorghum bread in order to improve its fiber content as well as its anti-oxidant capacity.

Efforts should keep going in that direction in order to improve our gluten-free sorghum bread with eggs formulation.

## SUMMARY AND CONCLUSION

The objectives of this study were to evaluate the role of egg addition and the presence of the antistaling agent DATEM on staling and overall quality of gluten-free sorghum bread.

Gluten-free breads are indeed still characterized by a prompt staling, poor structure and an overall mediocre quality. Although different studies have dealt with finding a way to delay staling in such breads, most of these studies have focused on the impact of additives or dairy products.

This study thus assessed the impact of egg addition on staling by analyzing the crumb hardness over a 12 day storage period and by using descriptive analysis for a trained panel to evaluate staling attributes. Additionally, water activity, color, specific volume, cell size, proximate analysis and a consumer study were performed in order to discriminate quality factors for gluten-free sorghum bread with and without egg.

For all physical and chemical tests, the addition of the antistaling agent DATEM at 0.5% was also studied.

Overall, this study confirmed our initial theory that the addition of eggs to a gluten-free sorghum bread formulation will have a positive effect on both delaying staling and improving bread quality and acceptance.

Breads with eggs presented higher specific volumes than control (from 0.06 cm<sup>3</sup>/g to 0.11 cm<sup>3</sup>/g). This improvement was even more significant when DATEM was added to the formulation (from 0.66 cm<sup>3</sup>/g to 0.73 cm<sup>3</sup>/g) as it was revealed that this antistaling agent tends to have a negative effect on gluten-free breads' volume (decrease of 0.73 cm<sup>3</sup>/g for the control). Furthermore, thanks to eggs proteins, loaves with eggs also presented improved cell structure. Crust color was found to be significantly darker when eggs were added to the formulation (L\* values were lower from 4 to 9 points). This is a quality improvement as it is known that consumers prefer darker breads and are usually reluctant to typical white gluten-free breads.

Regarding staling, it was established that the addition of eggs not only reduced bread hardness at each of the four days studied (day 0 and days 4, 8 and 12 post baking) but also slowed down the rate of staling over the 12 day storage period. Thus, the inclusion of eggs into

sorghum bread formulation had a desirable effect on bread staling. The antistaling agent DATEM however, did not impact staling on a positive way, although it was suppose to fulfill this function. This might mainly be due to DATEM negative effect on hardening the batter and then the bread. Thus regarding DATEM and staling, our initial hypothesis was not confirmed as it turned out DATEM had a negative impact on loaf volume, crumb hardness, and crumb structure, and did not help delaying staling.

Descriptive analysis results confirmed the findings of this texture analysis, showing control bread significantly harder than egg-containing bread at days 0 and 4.

Finally, the consumer test indicated a significant preference for sorghum bread with eggs over the control for 5 different attributes (overall acceptability, appearance, flavor, color and texture) as well as for willingness to buy the product. The overall acceptability score for the egg-containing bread was above 6 on a 1 to 9 hedonic scale. The score was closer to 7 when the bread was rated by consumers with celiac disease.

Adding eggs to bread is going to increase its cost as eggs are the most expensive ingredients in a baked product. However, this is not too much of an issue as from a general standpoint, gluten-free breads are more expensive than wheat breads. There is no specific data published regarding this. But from our own study at a local store, we were able to note that the price for a loaf of wheat bread ranges from U.S. \$0.98 to U.S. \$3.49, while gluten-free bread prices range from U.S. \$5.29 to U.S. \$5.99. Thus usually, people buying gluten-free products do so out of necessity or by choice for personal reasons, therefore their willingness to buy the product is not going to be dictated much by the price, and they are going to more interested in a better quality that is currently lacking in the product.

Therefore, this study demonstrated that a quantity of eggs up to 30% (flour basis) can be added to sorghum bread formulation to improve its overall quality, acceptance and to delay its staling.

Our product is then one step closer to be marketable as the addition of egg gives promising results. Even though the product is not quite ready yet and that further research can be done on keep improving the staling issue and/or the quality, our research successfully conferred decent gluten-free bread.

In conclusion, the results of this study are of good implications to sorghum and egg producers, food processors, and mostly, consumers requiring a gluten-free diet

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# Appendices

# Appendix A: Definitions of attributes and Reference Sheet for Descriptive Analysis

Adapted from: Brannan et al. (2001); Gámbaro (2002); Bejosano et al. (2005); Callejo (2011); and Elía (2011)

## TEXTURE (by the mouth)

Springiness: Degree to which the sample can be condensed and return to its original shape.

*Compress partially without breaking using front teeth.*

References: *Kraft Philadelphia original cream cheese = 2*

*Oscar Meyer wiener = 7*

*Kraft jet-puffed marshmallow = 14*

Tooth packing: Amount of sample packed in and between the teeth after swallowing.

References: *Nabisco Honey Maid graham cracker = 7*

*Great Value party peanuts = 10*

*Wonka Laffy Taffy candy = 15*

## TEXTURE (by the hand)

Hardness: The relative resistance to deformation.

*Press down evenly using two fingers.*

References: *Wet sponge = 3*

*Kraft jet-puffed marshmallow = 8*

*Great Value sharp cheddar cheese = 15*

## FLAVOR

Sour: A basic taste factor of which citric acid is typical; Flavor typical of sour milk; Fundamental taste sensation evoked by acids, for example, lactic acid

References: *Cream Cheese = 2*

*Plain yogurt = 8*

Rancid: Aromatic associated with decomposition of fats or oils.  
References: *Crisco all-vegetable shortening* = 2  
*Rancid oil* = 15

Doughy: A flavor associated with wet flour or dough.  
References: *Pita bread* = 2  
*Pillsbury Grands homestyle canned biscuit dough* = 15

Musty: Aromatic associated with a dust or earth from cereal grain.  
References: *Wheat flour* = 4  
*Sorghum flour* = 12



**Appendix B:**  
**Informed consent statement for consume sensory analysis of**  
**gluten-free breads**

The purpose of this project is to determine consumer acceptance of gluten free breads. 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 Magali Bize.
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 C:**

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

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:**

- 18-25       26-30       31-35       36-40       41-45       46-50  
 51-55       56-60       61-70       71-80       81-90       Over 90

**Gender:**

- Male  Female

**Education Completed:**

- High School       Some College       B.S.       M.S.       Ph.D.  
 MD       Other

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

- Yes  No

**About how often do you eat gluten-free breads?**

- Every Day       At least once a Week       Once every Two Weeks  
 Once a Month       Once a Year       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 D: Consumer ballots

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

Instructions:

You will be testing two samples of gluten free breads. 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 and a bite of cracker before you start and as needed throughout testing.

## **SAMPLE: 156**

*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 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 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 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 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 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 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:** \_\_\_\_\_

# SAMPLE: 487

*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 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 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 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 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 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 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:** \_\_\_\_\_