EFFECTS OF VARIATIONS IN HIGH MOLECULAR WEIGHT GLUTENIN ALLELE COMPOSITION AND RESISTANT STARCH ON WHEAT FLOUR TORTILLA QUALITY

A Thesis

by

TOM ODHIAMBO JONDIKO

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2010

Major Subject: Food Science and Technology

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Approved by:

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December 2010

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ABSTRACT

Effects of Variations in High Molecular Weight Glutenin Allele Composition and Resistant Starch on Wheat Flour Tortilla Quality. (December 2010)

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Tortilla sales are projected to exceed 9.5 billion by 2014. However, currently no wheat cultivars have been identified that possess the intrinsic quality attributes needed for the production of optimum quality tortillas. Tortillas made with refined wheat flour low in dietary fiber (DF) are popular in the United States due to their sensory properties. This study explored the use of wheat lines (WL) possessing variations in high molecular weight glutenin allele sub-units (HMW-GS) for production of tortillas and also investigated the use of corn based resistant starches (RS), type II (RS2) and wheat based RS type IV (RS4) to increase DF in tortillas.

Tortillas were made with 0-15% RS and 100% whole white wheat (WW). Flour protein profiles, dough, and tortilla properties were evaluated to determine the effects of the allelic variations and RS substitution on tortilla quality. Sensory properties of tortillas with RS were determined. Variations in HMW-GS composition significantly affected the protein quality and tortilla properties. Flour from WL possessing allelic combinations (2*, 17+18, 7, 2+12), (1, 17+18, 5+10), (2*, 17, 2+12) and (1, 2*, 17+18, 2+12) had 12.8–13.3% protein. These WL had extensible doughs and produced large diameter tortillas with superior (≥ 3.0) flexibility after 16 days compared to control. However, WL with (17+18 and 5+10) and (2*, 17+7, 5) produced extensible doughs, large, but less flexible, tortillas compared to control. WL with (2*,17+18,5+10) and (1,2*,7+9,5+10) produced smaller diameter tortillas, but with superior flexibility compared to control.

RS2, WW, and cross-linked-pre-gelatinized RS4 (FiberRite) produced hard, less-extensible doughs and thinner tortillas compared to control, due to high water absorption. Cross-linked RS4 (Fibersym) dough and tortillas were comparable to control. 15% of RS2 and RS4 increase DF in control to 6 and 14% respectively, compare to control (2.8% DF). WW tortillas were less acceptable than control in appearance, flavor and texture, while tortillas with 15% Fibersym had higher overall acceptability than control. RS2 negatively affected dough machinability and tortilla shelf stability. However, 15% RS4 improved the DF in refined flour tortillas to meet FDA's "good source of fiber claim," without negatively affecting dough/tortilla quality.

DEDICATION

This thesis is dedicated to my parents, brothers and sisters for their encouragement and understanding. More specifically to my late mother Rachel Jondiko.

ACKNOWLEDGEMENTS

I am thankful to my committee co-chairs, Dr. Joseph Awika and Dr. Lloyd Rooney, for their immense guidance, support, patience and advice. I would like to thank Dr. Dirk Hays providing direction and encouragement for writing my thesis and Dr. Amir Ibrahim for his support and advice with data analysis and interpretation. I would also like to acknowledge Dr. Novie Alviola for her advice and critique during my studies and research. I acknowledge Dr. Geera Bhimalengeswarrapa for providing positive critique and guidance with my thesis. I am most grateful to Dr. Mike Tilley of Kansas State University for the help with protein analysis.

I am grateful to the companies that generously provided the flour and other ingredients for my experiments. I greatly appreciate the scholarships from the Intercollegiate Faculty of Food Science and Technology, and AACC International.

I thank the Cereal Quality Lab staff and fellow students for their assistance in processing the tortillas and insightful discussions of my research results.

Finally, thanks to my father, Prof. Job I. Jondiko, for his encouragement and to my brothers and sisters for their patience and love.

Above all, thanks to God for the opportunity to pursue my dream of attending graduate school in the USA and the blessings to successfully complete my master’s degree.

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CHAPTER I

INTRODUCTION

Tortillas are currently the most popular bread consumed in the United States as fajitas, burritos, wraps and soft tacos. Tortilla sales stood at 5.2 billion in 2002 (Anton 2008) and was projected to exceed $7.0 billion in 2006 (Alviola 2007). “According to the Tortilla Industry Association, tortilla sales were poised to surpass that of sandwich bread in 2009” (Food Procuct Design 2009). The Hispanic Food and Beverages in the U.S.: Market and Consumer Trends in Latino Cuisine, 4th Edition projects that the sales will exceed 9.5 billion in 2014 (Packaged Facts 2010). This growing popularity is attributed to its convenience as a flexible wrap for holding a wide variety of meals ranging from rice, meats, cheeses and sauces consumed at once. The wraps do not easily leak or get soggy with the food, due to sealing of tortilla surfaces as a result of hot-press method of production (McDonough et al. 1996). In the USA, consumers prefer refined wheat flour tortillas that are flexible, opaque, large in diameter and have long shelf life (Bello et al. 1991; Cepeda et al. 2000). Good quality tortillas must resist cracking, crumbling and breaking during preparation and consumption (Waniska 1999). Most tortillas are not consumed on the day of production, but over several weeks. Hence, the challenge is to produce shelf stable tortillas that retain flexibility over time.

This thesis follows the style of Cereal Chemistry.

Tortilla shelf stability and diameter are controlled predominantly by glutenin and gliadin more than other endosperm sub-fractions (Pascut et al. 2004; Waniska et al. 2004; Waniska et al. 2002). Wheat varieties with the right glutenin and gliadin alleles have been developed to provide the ideal protein requirements for bread baking. These varieties are blended and used for tortilla making.

Bread flour has strong gluten that gives small diameter tortillas with good flexibility over storage. Good quality tortilla flour should have extensible gluten that will provide rapid extension during pressing to form larger diameter tortillas that retain air bubbles and flexibility during storage (Waniska et al. 2004).

Tortilla producers use food additives such as reducing agents, fats and enzymes to increase gluten extensibility in dough during production of wheat flour tortilla. However, besides reducing the profitability of tortilla production, the use of these additives at high levels adversely affects sensory attributes.

There is no wheat variety that has been produced to provide uniform optimum quality tortillas. Mondal and others (2008) reported that wheat varieties possessing high molecular weight glutenin gene subunit (HMW-GS) 17 + 18 on Glu-B1 loci and have gene deletions in Glu-A1 and Glu-D1 loci give large diameter tortillas with poor flexibility (Mondal et al. 2008). There is need to increase the understanding of the roles of wheat glutenin in tortillas and utilize the information in developing identity protected wheat varieties for tortilla production.

Despite the rapid growth in tortilla consumption, Majority of tortillas consumed in the USA are produced from refined wheat flour tortillas. Refined wheat flour is low in nutritional properties and dietary fiber (DF) due to the removal of bran. Refined flour tortillas have poor nutrition profile and generate a high glycemic response after digestion, similar to white wheat bread (Saldana and Brown 1984). However, consumers prefer refined wheat tortillas mostly due to their sensory attributes compared to whole wheat tortillas. Hence, technology and ingredients are needed to improve the nutritional quality and provide desired functionality in refined wheat tortillas.

The market for wheat flour tortilla is growing in North America (Dally and Navarro 1999). Hence, there is a need to make tortillas healthier as a vehicle to promote healthy eating. Less than 10% of Americans consume the recommended daily intake (RDI) of fiber. Dietary fibers are food components that are resistant to digestion and absorption in the small intestine. There are two main categories of dietary fiber - soluble and insoluble. Consumption of soluble dietary fiber such as gums, hydrocolloids, most pectins, mucilages and some hemicelluloses can reduce cholesterol levels. Consumption of insoluble fibers such as cellulose, some hemicelluloses, lignin and enzyme-resistant starches, increases transit time in the gut, thus reducing the risk of colon cancer, diverticulitis, colitis and other gastrointestinal ailments (Englyst and Cummings 1985).

Low consumption of dietary fiber in the USA is linked to increased occurrence of diabetes Type II. Increased fiber in commercial tortilla could improve total dietary fiber intake. Like other dietary fibers sources, resistant starch are not absorbed in the small intestines (Englyst et al. 1993), but are partially or completely fermented in the large intestines (Englyst and Cummings 1985). Resistant starch (RS) slow digestion of carbohydrates and results in a sustained, low elevation of blood sugar, hence provide a low glycemic load. They also delay hunger by acting as a bulking agent (Sajilata et al. 2006). Resistant starch also acts as a prebiotic (Seetharaman et al. 1994). Resistant starch can be used as a source of dietary fiber in tortillas.

This study hypothesizes that unique wheat glutenin functionalities can be optimized genetically to produce good quality tortillas. Resistant starch can be used to improve the nutritional profile of wheat flour tortillas. The goal of the study was to improve the understanding of the effects of HMW-GS and resistant starches on quality of wheat flour tortillas.

RESEARCH OBJECTIVES

1) Evaluate the tortilla making quality of wheat lines possessing variations in HMW glutenin allele’s composition at homologous loci on A, B and D genomes that were planted in three locations in Texas.
2) Determine the effect of type II and type IV resistant starches on wheat flour tortillas processing and quality.

CHAPTER II

EFFECTS OF VARIATIONS IN HMW GLUTENIN ALLELE COMPOSITION ON WHEAT FLOUR TORTILLA QUALITY

INTRODUCTION

Protein requirements for tortilla

Tortilla quality requires flour with unique protein functionality that are distinctively different from those of bread (Wang and Flores 1999). Tortillas must maintain flexibility during preparation, consumption and over storage (Waniska 1999). Flour properties are the primary determinant of tortillas quality.

The unique properties of wheat flour are due to the presence of gluten proteins (Mondal 2006). Upon hydration gliadin and glutenin form a complex network called gluten. Gluten is described as a bimodal distribution of gliadin and glutenin proteins (Wrigley and Bekes 1999). The gluten plays a fundamental role in baking and is responsible for the functionality of flour for specific wheat based products. Gluten is visco-elastic and hence, capable of trapping and holding gas contributing to increased volume during baking. Wheat gliadins are responsible for viscosity while glutenins provide elasticity to dough (MacRitchie 1987).

The suitability of wheat flour for tortilla processing is determined by dough extensibility and how long tortillas can retain their flexibility/rollability over storage time (Pascut et al. 2004). Dough extensibility is essential to the production of large-diameter tortillas. Both diameter and shelf stability are controlled mainly by wheat glutenin and gliadin, over any other endosperm sub-fractions such as globulin or albumin or starch or lipids (Pascut et al. 2004; Waniska et al. 2004). Wheat breeders are developing wheat cultivars that meet the unique requirements for tortillas (Mondal 2006). Currently, the tortilla industry uses bread wheat flour and chemical ingredients to achieve the required functionality for tortilla production.

Without modification hard winter wheat cultivars developed for bread making produce poor quality tortillas (Serna-Saldivar et al. 2004). This is because protein functionality requirements for wheat flour tortilla differ from that required for good quality bread. The desirable protein network (gluten) for good quality tortilla production is extensible and mellow. Bread dough requires a strong, resilient gluten network to retain air bubbles during fermentation and baking. Bread becomes firm and stales after five days of storage, while tortillas retain their flexibility and rollability over several weeks depending on the flour properties, formulation and method of processing (Seetharaman et al. 2002; Waniska et al. 2004). Hence, there is a need to modify the glutenins and gliadin composition to in new wheat cultivars to produce the unique requirements for tortillas.

In the hot-press procedure for tortilla production, proteins and starch in the flour are exposed to high temperatures for a short time (~ 40 seconds) compared to ~ 25 minutes for bread baking. Complete starch gelatinization occurs in both tortillas and bread. However, the longer exposure to heat in bread baking causes extensive starch dispersion, formation of amylose crystals, and firming of bread (Hug-Iten et al. 2003). A rigid retrograded starch gel surrounding the gluten (protein network) masks the functionality of the proteins in bread. In tortillas the starch receives less heat. And thus disperse less around the gluten matrix. This allows for retention of gluten functionality as exhibited by the extended flexibility and rollability over longer storage time. Wheat flour for tortilla production should provide rapid extensibility during hot-pressing and flexibility after baking.

Glutenin contributes to the strength and elasticity of dough. End use quality variations are governed by the glutenin-to-gliadin ratio and molecular weight distribution in glutenins which can be genetically determined (Cinco-Moroyoqui and MacRitchie 2008). Molecular weight distribution is dependent on variations in the high molecular weight glutenin allelic composition (Gupta and MacRitchie 1994 ; Payne et al. 1987), availability of chain terminators (Masci et al. 1998) and the ratio of low molecular weight/high molecular weight glutenins (Gupta et al. 1993). These can be affected by genetic and environmental factors (Cinco-Moroyoqui and MacRitchie 2008).

Synthesis of glutenin and gliadin proteins

There are nine and six major genetic loci that control the synthesis of glutenin and gliadin proteins that are responsible for flour quality of hexaploid and tetraploid wheat's respectively. In hexaploid wheat there are three loci of glutenin Glu-1 (Glu-A1, Glu-B1, Glu-D1) located on the long arm of 1A, 1B and 1D, respectively, coding for high molecular weight glutenin subunits (HMW-GS) and three complex Gli-1/Glu-3 loci (Gli-A1/ Glu-A3, Gli-B1/ Gli-B3, Gli-D1/GliD3) on the short arms of 1A, 1B and 1D (Mondal et al. 2009; Mondal et al. 2008). These loci contain allelic variations. The most significant of these alleles occur at Glu-D1, where high molecular weight glutenin sub-units (HMW-GS) can occur as allelic pairs of genes encoding HMW-GSs designated as

5+10 or 2+12 (MacRitchie and Lafiandra 2001). Deletion of some HMW-GS has been shown to give good diameter and rollability in tortillas (Mondal 2006). Wheat with deletions in the Gli 1 loci exhibited greater dough strength (MacRitchie and Lafiandra 2001). Variation in the HMW-GS composition also alters tortilla properties (Mondal et al. 2009; Mondal et al. 2008). Wheat flour without these proteins gave large diameter tortillas, but with a compromise on shelf stability (Mondal 2006). HMW-GS 5, in particular, has been shown to play a role on tortilla shelf life when combined with HMW-GS 10 (Mondal et al. 2008). Deletion of the HMW-GS 17+18 does not negatively affect tortilla properties. Tortillas made with flour that do not contain these proteins have larger diameter and good shelf stability (Mondal et al. 2008). The growing popularity and diversity of wheat based tortilla products has created a bigger task for wheat breeders and food scientists to develop quality wheat that can provide uniform optimum tortilla quality. Hence, this study utilized wheat lines possessing variations HMW glutenin alleles to increase our understanding of the role played by varying alleles present at the homologous loci Glu1 on the genomes A, B and D in the quality of wheat flour tortillas.

MATERIALS AND METHODS

Wheat lines

Each of the 15 wheat lines with variations in high molecular weight glutenin composition (Table I) were planted in two fields; Texas Agricultural Experiment Station at McGregor, Texas in 2008 and both at the Texas Agrilife Research Station at College Station and at McGregor, Texas, in 2009. The wheat was harvested, milled and processed into tortillas. Lines with similar allele composition were grouped together (Table I). Commercial tortilla flour (untreated, bleached, enriched: ADM Milling Company., Overland Park, KS) was used as control.

Table I

Wheat lines with different HMW glutenin allele composition

Group	**Entry**	**Wheatlines**	**Pedigree**	**GluA1**	**GluB1**	**GluD1**
1	1	GABO		2*	17+18,7	2+12
2	2	Ogallala		2*	20x + 20y	5+10
2	11	TX04CS00237	FM3/OGALLALA	2*	20	5+10
5	5	TX04CS00233	FM3/5009	1	17+18	5+10
5	14	TX04CS00240	FM3/OGALLALA	1	17+18	5+10
5	18	TX04CS00245	FM6/5009	1	17+18	5+10
5	9	TX04CS00235	FM6/JAGGER	1	17+18	5+10
6	6	TX04CS00229	FM1/JAGGER	2*	17+18	5+10
7	7	TX04CS00230	FM1/JAGGER	-	17+18	5+10
8	8	TX04CS00232	FM3/5009	2*	17	2+12
10	10	TX04CS00236	FM3/OGALLALA	2*	7+9	2+12
10	19	TX04CS00249	GLID2/5009	2*	7+9	2+12
13	13	TX04CS00239	FM3/OGALLALA	1,2*	17+18	2+12
15	15	TX04CS00241	FM6/5009	1,2*	7+9	5+10
16	16	XT04CS00231	NTX(FM6/Ogallala) STX (FM2A/OGALLALA)	2*	17,7	5
20	20	Control	Tortilla flour (ADM Inc.)		Unknown	

Seed and flour evaluation

Protein analysis

Lab on chip capillary electrophoresis was performed to determine the high molecular weight glutenin (HMW–GS) composition. Lab on chip capillary electrophoresis can be used to identify the protein composition of the deletion lines (Uthayakumaran et al. 2003). The protein analysis was conducted by Dr. Mike Tilley, USDA-ARS, Manhattan, Kansas. A 10mg sample of flour samples was extracted with 0.5 ml 1% SDS solution containing 1% dithiothreitol (D-TT) by vortex –mixing (5 sec) and shaking for 3 min at 65°C. After centrifugation extracts were ready for loading. Ten extract 4 µL each were applied with Agilent sample buffer for Analysis in the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). The software provided results both as quantitative profiles and as simulated gel patterns.

Polymeric protein analysis

The analysis of the percentage of insoluble polymeric proteins was conducted at Kansas State University, Kansas, Manhattan. A 0.01 g flour sample was suspended in 1.0 ml of 0.5% (w/v) SDS buffer. The suspension was then stirred for 5 min at 20, 000 rpm and centrifuged for 20 min at 15900 rpm to obtain a supernatant (extractable protein). The residue was then sonicated for 30 sec in 0.5% (w/v) SDS buffer (1ml) to solubilize the remaining protein (unextractable protein). Both the extracts were filtered through 0.45 µm filters. The percentages of extractable and unextractable polymeric protein were calculated as [peak 1 area (extractable)/peak 1 area (total)] x 100 and [peak

1 area (unextractable)/peak 1 area (total)] x 100 respectively. Peak 1 (total) refers to the sum of peak 1(extractable) and peak 1 (unextractable) (Mondal 2006).

Single kernel hardness test (SKHT)

The single kernel hardness tester (Perten Single Kernel Characterization System SKCS 4100, Perten Instruments, Springfield, IL), was used to evaluate kernel hardness, diameter, weight and moisture content (Mondal et al. 2008).

Milling

Based on the moisture content from SKHT, the clean grains were tempered (24hours/34rpm) to a moisture content of 14% to improve the flour yield during milling. The amount of tempering water was determined using the following formula:

$$\left[\left(\frac{100 - \text{Moisture Content}}{100}\right) - 1\right] * \text{Weight of grain}$$

The grains were placed in plastic bottles with water added and shaken overnight for the proper distribution and tempering of water (Mondal 2006). The tempered grains were milled using a quad junior mill (Brabender GmbH & Company KG, C. W. Barbender Instruments, Incorporation, South Hackensack, NJ) to obtain refined flour.

Near-infrared reflectance spectrophotometer (NIR)

Near-infrared reflectance spectrophotometer (Perten PDA 7000 Dual Array with Grams Software) was used to determine protein and moisture content of the milled flour. Tortilla flour (ADM Milling Company, Overland Park, Kansas) was used as a control. Three replicates of each sample were analyzed (AACC 2000)

Mixograph

A mixograph (National Manufacturing Co., Lincoln, NE) was used to estimate dough mixing properties; mixing time, and tolerance. Ten grams of flour was used (14%mb) (AACC 2000).

Tortilla formulation

The tortilla formulation included 500 g flour from each of the wheat lines. 500g of white wheat flour (ADM, Inc.) was used as control. Each 500g batch included: 30 g of shortening (Sysco Corporation, Houston, TX), 7.5 g salt (Morton International, Inc., Chicago, IL), 3 g sodium bicarbonate (Arm and Hammer, Church and Dwight Company, Inc., Princeton, NJ), 2.9 g sodium aluminum sulfate (Budenheim USA Inc., Plainview, NY), 2.5 g sodium steroyl lactylate (Caravan Ingredients, Lenexa, KS), 2.5 g sodium propionate (Niacet Corp., Niagara Falls, NY), 2 g potassium sorbate (B. C. Williams, Dallas, TX), 1.65 g encapsulated fumaric acid (Balchem Corp., New Hempton, NY) and distilled water. Dough was prepared by mixing dry ingredients in a mixer (model A-200, Hobart Corp, Troy, OH) with a paddle at slow speed (speed 1) for 2 minutes. Shortening was then added to the dry ingredients and was mixed at slow speed (speed 1) for 3 minutes. Amount of water added to the dry ingredients was based on an adjusted value from the mixograph water absorption; this was mixed using a hook at low speed for 1 minute. The dough was mixed at medium speed (speed 2) for the time equal to each flours mixograph peak time.

The dough was then subjectively evaluated for smoothness, softness, extensibility and force to extend. The dough was rested for 5 minutes at 32º C and 65-

70% relative humidity in a proofing chamber (Model 57638, National Manufacturing Co., Lincoln, NE).

At the end of proofing the dough’s were pressed on a stainless steel rounding plate and rated for press rating, then divided and rounded into 36 dough balls (Duchess Divider/Rounder, Bakery Equipment and Service Co., San Antonio, TX). The dough balls were then rested for 10 minutes at 32º C and 65-70% relative humidity in the proofing chamber.

Evaluation of dough properties

Subjective dough evaluation

The dough properties were evaluated subjectively (Seetharaman et al. 2002; Waniska 1999) on a 5 point scale as described in Table II for smoothness, softness, extensibility and force to extend after dough formation (Mondal et al. 2009; Mondal et al. 2008). Press rating was evaluated before dough dividing and rounding as described by Alviola et al. (2008). These properties were used to determine dough machinability (Alviola et al. 2008).

Smoothness refers to the appearance and texture of the dough surface; it was used as an indicator of dough cohesiveness. Softness is the viscosity or firmness of the dough when pressed with fingers. Force to extend refers to the elasticity of the dough when pulled apart. It was obtained by pulling the dough at the same point where softness is ranked. Extensibility refers to the length the dough extends when pulled apart. It was obtained by pulling the dough. Press rating refers to the force required to press the dough on the stainless steel round plate before dividing and rounding.

Table II

Dough subjective evaluation scale

Rating	Smoothness	Softness	Force to Extend	Extensibility	Press Rating
1	very smooth	very soft	less force	breaks immediately	less force
2	**Smooth***	**soft**	**slight force**	some extension	**slight force**
3	slightly smooth	slightly hard	some force	**extension**	some force
4	rough	hard	more force,	more extension	more force
5	very rough	very hard	extreme force	extends readily	extreme force

*** BOLD** values = desired dough properties.

Temperature

Immediately after mixing the doughs were placed on a plastic tray and the temperature measured using a thermometer.

Objective dough evaluation

Dough compression force

Dough texture was measured using dough compression test (Barros 2009; Bejosano et al. 2005), two dough balls of approximately equal weight and size were subjected to 70% compression using a 10 centimeter diameter probe on a texture analyzer (Model TA-XT2, Micro Systems, Scarsdale, NY). Maximum dough compression force was recorded and averaged for each of the treatments.

Stress Relaxation

Stress relaxation was measured by compressing two dough balls on a texture analyzer (TA.XT2i Texture Analyzer,Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK) after10 min resting time. A cylindrical probe with a diameter of 10 cm was attached to the texture analyzer arm and was calibrated to a distance of 35 mm from the texture analyzer platform. When the cylindrical probe was compressing the dough ball, force at 25 seconds, 100 seconds, maximum force and relaxation time were collected.

Modified dough extensibility test

Dough Preparation

Dough was prepared using 100 grams of flour from each wheat line. 2 grams of salt was added to the flour and mixed in the Hobart mixer (Model N-50, Hobart Manufacturing Company Corp, Troy, OH) for 1 minute at speed 1, with a paddle at slow speed (speed 1) for 1 minute. Warm water (~ 35º C) was added to the dry ingredients. The amount of water used was based on an adjusted value from the mixograph water absorption values The mixture was then mixed with a paddle at slow speed (speed 1) for 2½ minutes to hydrate the flour after which the dough was mixed at medium speed (speed 2) for a time equal to each wheat line’s mixograph peak time. The dough was rested for 25 minutes at 32º C and 65-70% relative humidity in a proofing chamber (Model 57638, National Manufacturing Co., Lincoln, NE).

Extensibility test

Dough extensibility test was measured using Kieffer dough extensibility rig, as described by Barros (2009). Immediately after the dough balls were rested for 10 minutes in the proofing chamber (Model 57638, National Manufacturing Co., Lincoln, NE). A 20 g of dough was weighed from one dough ball and rolled into a cylindrical shape with minimal manipulation. A dough press with a grooved base and a top form was used to prepare the samples; Paraffin oil was placed along the grooved base to aid in the removal of dough strips and prevents sample adhesion. The cylindrical shaped dough sample was placed on the grooved base with its length perpendicular to the groove direction. The top form was then placed on the grooved base. The dough press was placed in the clamp and screwed down. Excess dough extruding from the sides was removed using a spatula. This process sliced the sample into uniform dough strips. The dough clamp was placed in a plastic bag and left to relax for 40 min at room temperature (Approx 25º C). After which, the plastic bag was opened, and the clamp was released and the dough press removed. Dough strips were removed using a thin spatula and placed across the grooved region of the sample plate. The extensibility probe (hook) was lowered to the surface of the spring loaded clamp. The lever of the spring loaded clamp was lowered and the sample plate was inserted into the rig. The handle was slowly released then the test was conducted as per the settings by Barros (2009).

Tortilla processing

Tortillas were prepared according to the standard hot-press method (Bello et al. 1991). Dough balls were hot-pressed (400°F, 1150 psi, 1.4 sec), baked (380-390°F, 30 sec) on a three-tier gas fired oven (Model 0P01004-02, Lawrence Equipment, El Monte, CA), then cooled for $1^1/_2$ minutes on a 3-tier conveyor (Superior Food Machinery Inc., Pico rivera, CA). Immediately after cooling each tortilla was placed on a table for 2 minutes then packaged in 1 mil polyethylene bags and stored at 22^{o}C for subjective and objective evaluation as described by Alviola et al. (2008).

Evaluation of tortilla physical properties

Ten tortillas were selected randomly and weight, diameter, height, opacity, and moisture were measured on the first day after processing (Bello et al 1991). Tortilla flexibility/ rollability and extensibility were measured at 4, 8, 12 and 16 days after production as described by Alviola and Waniska (2008).

Moisture

Tortilla moisture content was determined using a two-stage procedure in a hot-air oven (AACC 2000). Pre-weighed tortillas were dried for 96 hours after production in ambient conditions followed by a one hour drying at 100º C in an oven (model 16, Precision Scientific Co. PS, Chicago, IL). Moisture was calculated as a percentage of weight loss from the drying process.

Weight

Ten randomly selected tortillas (Friend et al. 1995) were weighed using an analytical scale (Ohaus, Houston TX). The values were recorded and averaged to obtain the weight of one tortilla.

Diameter

Diameter of ten tortillas was measured by using a ruler at two points across the tortilla. These values were recorded and used to obtain the average diameter of one tortilla (Alviola et al. 2008).

Height/ Thickness

The average height/ thickness of a one tortillas were obtained by measuring the height of a stack of ten tortillas using a digital caliper (Chicago Brand 12” Electronic Digital Caliper, Chicago, IL).

Opacity

Opacity (%) was evaluated subjectively on a 100 point scale for ten tortillas from each wheat line and control. A highly opaque tortilla was given a 100% rating and completely translucent tortillas were rated as 0%. The values were recorded and used to get the average opacity.

Color

Color values L* (whiteness-gray), ± a* (red-green) and b* (yellow-blue) were measured at two points on each side of two randomly selected tortillas from each treatment using a Minolta Color Meter (Chroma Meter CR-310, Munilta, Tokyo, Japan).

Specific Volume

Tortilla specific volume was determined as follows:$\mathrm{Specific\ volume} = (\mathrm{Height} * \pi r^2)/\mathrm{weight}$). Where; height = height of a single tortilla (cm); weight = weight of a single tortilla (g), r=average radius of a tortilla (cm).

Rollability/ Flexibility

Tortilla shelf stability was evaluated subjectively by a rollability test (Friend et al. 1995), which is a 5 point measure of the cracking and breakage of a tortilla. Two randomly selected tortillas from each wheat line were evaluated. Each tortilla was wrapped around a 1.0cm diameter wooden dowel and were allocated a rollability/flexibility score (RS) (Alviola and Waniska 2008; Cepeda et al. 2000; Mondal et al. 2009; Mondal et al. 2008) on continuous scale for rollability as follows: 5 = no cracking; 4 = signs of cracking, but no breaking; 3= cracking and breaking beginning on the surface; 2 = cracking and breaking imminent on both sides; and 1 = unrollable, breaks easily. A rollability/flexibility score below 3 (many cracks and breaks on tortilla surface) was indicative of undesirable shelf stability during storage. Shelf stability/flexibility was measured for the tortillas at days 4, 8, 12 and 16 of storage.

Tortilla texture-2D extensibility

Tortilla textural changes during storage were measured at day 0, 4, 8, 12 and 16 using the two-dimensional extensibility tests (Barros 2009; Bejosano et al. 2005) on the texture analyzer (model TA-XT2i, Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Survey, UK) (Suhendro et al. 1999). The extensibility test was conducted using the return to start option, at a trigger force of 0.05 N. Pre and post test speed was 10.0 mm/s. The test speed was 1.0mm/s. The modulus of deformation (N/mm), force (N), distance (mm) and work to rupture (N.mm) were recorded for data analysis (Barros 2009).

Data analysis

Microsoft office excel 2007 (Microsoft Corporation, Redmond, WA) was used to derive means, standard deviations and plots. Statistical was done using SPSS version 16.0 (SPSS Inc. Chicago, Il) and SAS version 9.2 (SAS Institute, Cary, NC). Analysis of variance (ANOVA) and determination of least significant difference (LSD) were performed at $\alpha = 0.05$ significance level to determine differences among the samples and treatments.

RESULTS AND DISCUSSION

Flour protein properties

The flour protein content (%) measured using the NIR ranged between (12.2 and 13.7 % as is) and was significantly affected by the variations in allelic composition ($p < 0.05$). Flour from line with (2* ,17+7, 5) at A, B and D loci had the highest protein content whereas flour from lines with (2* 7+9, 2+12) had the lowest protein content (Table III).

The ratio of glutenin/gliadin content of the flours varied between 0.5 and 0.6 (Table III). Flour from lines with (2*, 17+7, 5) had the lowest glutenin:gliadin ratio whereas the highest ratio was from lines with (2*, 7+9, 2+12).(Table III).

The HMW- GS to LMW – GS ratio was significantly ($p < 0.05$) affected by the variations in allelic composition and varied between 0.5 and 0.3 (Table III). Lines with (2*, 17+7, 5) and (2*, 7+9, 2+12) had the lowest HMW/LMW GS ratios whereas the highest ratio was exhibited by lines with (2*, 17, 2+12). This is attributed to the variations on the Glu B and Glu D1 loci.

Variation in the HMW allelic composition significantly ($p < 0.05$) affected the percentage of insoluble polymeric proteins (% IPP). The lowest % IPP was exhibited by lines with (2*, 17+7, 5). This agrees with findings by Mondal et al. (2008) that deletions

Table III

Effects of different HMW glutenin allele's composition on the flour protein profile[1]

Group	**HMW-GS Allele composition**			**Entries**[3]	**Ratios**			**%IPP**	**% Protein**
	Glu A1	**GluB1**	**GluD 1**		**Gliadin/Glutenin**	**Glutenin/Gliadin**	**HMW-GS/LMW-GS**		
1	2*	17+18, 7	2+12	1	0.5 ± 0.1 abc	1.9 ± 0.2 abc	0.4 3 ± 0.0 ab	**45.8 ± 2 a**	13.3± 0.2 ab
2	2*	20	5+10	2,11	0.5 ± 0.0 bc	2.0 ± 0.2 ab	0.31 ± 0.1 c	39.6 ± 6 a-d	13.2± 0.5 ab
5	1	17+18	5+10	5,9,14,18	0.6 ± 0.1 abc	1.7 ± 0.2 bc	0.40 ± 0.1 abc	44.2 ± 6 ab	12.8± 0.5 bcd
6	2*	17+18	5+10	6	0.5 ± 0.0 abc	1.9 ± 0.1 abc	0.40 ± 0.1 abc	43.9 ± 3 ab	13.1± 0.4 abc
7	-	17+18	5+10	7	0.6 ± 0.1 ab	1.7 ± 0.3 bc	0.37 ± 0.1 abc	34.9 ± 5 cd	13.1± 0.4 abc
8	2*	17	2+12	8	0.5 ± 0.1 abc	1.9 ± 0.2 abc	0.46 ± 0.1 a	38.5 ± 3 bcd	13.3± 0.5 ab
10	2*	7+9	2+12	10,19	**0.6** ± 0.1 a	**1.7** ± 0.2 c	0.30 ± 0.0 c	41.3 ± 5 abc	**12.2± 0.5 d**
13	1,2*	17+18	2+12	13	0.6 ± 0.0 abc	1.8 ± 0.1 abc	0.33 ± 0.1 bc	40.1 ± 4 a-d	13.3± 0.5 ab
15	1,2*	7+9	5+10	15	0.5 ± 0.0 abc	1.9 ± 0.0 abc	0.43 ± 0.0 ab	45.2 ± 2 ab	12.5± 0.6 cd
16	2*	17+7	5	16	**0.5** ± 0.0 c	**2.0** ± 0.1 a	0.31 ± 0.0 c	**33.4 ± 4 d**	**13.7± 0.4 a**
20	Tortilla flour (Control)								12.5± 0.1 cd
LSD					0.1	0.3	0.11	7.3	0.7

[1] Average from two trials of lines planted in three locations, Values followed by the same letter in the same column are not significantly different ($p < 0.05$).
[2] Least significant difference ($\rho < 0.05$).
[3] Wheat lines with similar HMW glutenin allele composition

at Glu D1 and Glu B1 results in decreased % IPP. Presence of 2*, 17+18,7 and 2+12 on A, B & D loci resulted in significantly higher % IPP (Table III). The variations in protein properties resulted into varied flour functionality as evidence in differences in dough properties.

Subjective dough properties

Dough smoothness, softness, extensibility, force to extend and press rating were significantly ($p < 0.05$) affected by the variations in HMW glutenin allele composition. Overall, dough softness was negatively correlated with overall tortilla diameter (-0.86 at $p < 0.05$). Doughs from wheat lines possessing (2*, 20, 5+10) were rougher than control dough. All other lines produced doughs that were similar in smoothness rating to control dough (Table IV). Doughs from wheat lines with (2*, 20, 5+10) and (2*, 17, 2+12) were soft and extensible compared to control dough, this indicates that the interactive effect of the presence of (20, 5+10) and (17, 2+12) on Glu B1 and Glu D1 respectively, contribute to weaker dough strength. However, presence of both 1 and 2* on Glu A combined with 7+9 and 5+10 on GluB1 and GluD1 respectively, produced strong gluten and resulted in the least extensible doughs (Table IV). Dough from lines with (2*, 17+18, 7, 2+12) required the highest force to extend ($p < 0.05$). All the dough’s were easy to press on the stainless steel plate for dividing and rounding (Table IV).

Table IV

Effects of different HMW glutenin allele's composition on subjective dough properties[1]

Group	HMW Glu Allele composition			Entry	Subjective dough properties[2]				
	GluA1	GluB1	GluD1		Smoothness	Softness	Extensibility	Force to extend	Press rating
1	2*	17+18,7	2+12	1	2.0 abc (1.5 -3.0)[4]	2.1 a-d (1.5 - 2.5)	3.2 abc (3 -3.5)	**3.0 a** (2.0 - 3.5)	2.1 abc (1.5 - 3.0)
2	2*	20	5+10	2,11	**1.7 c** (1.0 - 2.0)	**1.54 e** (1.0 - 2.0)	**3.5 a** (2.5 -4.0)	2.3 cd (1.5 - 3.5)	1.8 c (1.5 - 2.5)
5	1	17+18	5+10	5,9,14,18	2.0 abc (1.0 -2.5)	2.0 bcd (1.5 - 2.5)	2.9 b-e (2.0 - 3.5)	2.6 abc (1.5 - 3.5)	2.0 bc (1.5 - 3.0)
6	2*	17+18	5+10	6	1.9 abc (1.5 - 2.5)	2.2 abc (2.0 - 2.5)	2.8 cde (2.5 - 3.0)	2.8 abc (2.0 - 3.5)	2.0 bc (1.5 - 2.5)
7	-	17+18	5+10	7	2.1 ab (1.5 - 2.5)	2.2 abc (2.0 - 2.5)	2.7 cde (2.5 - 3.0)	3.0 ab (2.5 - 3.5)	2.0 bc (1.5 - 2.5)
8	2*	17	2+12	8	1.8 bc (1.5 - 2.5)	1.8 de (1.5 - 2.5)	**3.6 a** (3.0 - 4.0)	2.6 abc (2.0 - 3.0)	1.8 c (1.5 - 2.0)
10	2*	7+9	2+12	10,19	2.3 a (1.5 - 3.0)	2.4 ab (1.5 - 3.0)	2.5 de (1.5 - 3.5)	2.9 ab (2.0 - 4.0)	2.5 ab (2.0 - 3.5)
13	1,2*	17+18	2+12	13	1.9 abc (1.5 - 2.5)	2.1 a-d (1.5 - 2.5)	3.1 a-d (2.0 - 4.0)	2.4 bcd (2.0 - 3.0)	1.8 c (1.5 - 2.0)
15	1,2*	7+9	5+10	15	2.3 a (2.0 - 2.5)	2.4 a (2.0 - 2.5)	**2.5 e** (2.0 - 3.5)	2.8 abc (2.0 - 3.5)	2.4 ab (2.0 - 2.5)
16	2*	17,7	5	16	1.8 bc (1.5 - 2.5)	2.0 cd (1.5 - 2.0)	**3.4 ab** (2.5 - 4.0)	**1.9 d** (1.5 - 2.0)	2.4 ab (1.5 - 3.0)
20	Refined wheat flour (Control)			20	2.1 ab (2.0 - 2.5)	2.2 abc (2.0 - 2.5)	2.8 cde (2.5 - 3.5)	2.7 abc (2.0 - 3.0)	2.5 a (2.0 - 3.0)
	LSD[3]				0.40	0.36	0.55	0.58	0.5

[1] Values followed by the same letter in the same column are not significantly different ($\rho < 0.05$)

[2] 5 - point Subjective dough evaluation scales:- Smoothness: (1 = rough and 5 = smooth), Softness : (1 = very soft, 5 firm), Extensibility: (1 = not extensible and 5 = very extensible), force to extend: (1 = less force and 5 = much force), Press rating: (1 = easy to press and 5 = much force to press).

[3] Least significant difference ($\rho < 0.05$). [4] Range of lines with similar HMW Glu composition

Objective dough properties

Dough compression force

Dough compression force (N) measured using the TAXT2i was significantly affected by the variations in HMW glutenin alleles ($P < 0.05$). The compression force ranged between 82 - 127 N (Table V). Dough made using wheat lines possessing (2*, 20, 5+10), (2*, 17, 2+12), and (2*, 17, 7, 5) required significantly lower force to compress compared to control; this agrees with the results from subjective tests. Presence of 5+10 at the Glu D1 loci is believed to contribute to dough strength (Payne 1987). However, the interactive effect of 20 at GluB1 and 5+10 resulted in significant loss of dough strength. These doughs were easy to press into a round disc producing large diameter tortillas compared to control tortillas.

Though studies show that 2+12 contributes to lack of dough strength (Payne 1987), the interactive effect of the presence of 7+9 and 2+12 on Glu B1 and Glu D1 respectively, resulted in a strong dough as exhibited by doughs from lines with 2*, 7+9, 2+12 that required the highest force to compress (Table V).

Dough stress relaxation

The force after 100 seconds of compression was significantly ($P < 0.05$) affected by the variations in allelic composition. All the lines exhibited low force after 100 seconds of compression compared to control (Table V). Presence of 1, 17+18 and 5+10 at Glu A, GluB and Glu D respectively resulted in high force after 100 seconds, whereas dough from wheat lines with (2*, 17+7, 5) exhibited low force.

Table V

Effects of different HMW glutenin allele's composition on the objective dough properties[1]

Group	GluA1	GluB1	GluD1	Entry	n	Compression force (N)	Force at 100 seconds (N)	Resistance to extension (N)	Extensibility (mm)	Work to extend (N.mm)
1	2*	17+18,7	2+12	1	6	103 cd (72 - 136)	5.76 b-e (5.4 - 6.0)	0.31 e (0.2 - 0.7)	103 ab (49 - 149)	20 ab (12 - 30)
2	2*	20	5+10	2,11	12	**82 f** **(67 - 95)**[2]	5.15 cde (4.0 - 6.8)	**0.21 f** **(0.1 - 0.4)**	**107 a** **(51 - 140)**	15 de (8 - 24)
5	1	17+18	5+10	5,9,14,18	24	103 cd (60 - 122)	**7.27 ab** **(4.5 - 11.1)**	0.38 cd (0.1 - 1.4)	72 d (25 - 117)	18 c (9 - 32)
6	2*	17+18	5+10	6	6	98 cde (84 - 109)	6.43 b-e 95.5 - 7.5)	0.33 de (0.3 - 0.4)	93 c (67 - 117)	21 ab (13 - 26)
7	-	17+18	5+10	7	6	108 cd (96 - 126)	5.52 cde (5.0 - 6.2)	0.20 f (0.1 - 0.2)	92 c (75 - 113)	**13 f** **(11 - 17)**
8	2*	17	2+12	8	6	**94 def** **(82- 103)**	5.05 de (2.4 - 7.0)	**0.2 f** **(0.1 - 0.3)**	105 ab (60 - 130)	14 ef (10 - 18)
10	2*	7+9	2+12	10,19	12	**127 a** **(88 - 151)**	6.64 bcd (4.1 - 11.1)	**0.49 b** **(0.2 - 1.0)**	**72 d** **(29 - 122)**	19 b (10 - 34)
13	1,2*	17+18	2+12	13	6	106 cd (101 - 114)	4.90 ef (4.2 - 5.4)	0.24 f (0.2 - 0.5)	98 bc (54 - 119)	17 cd (13 - 23)
15	1,2*	7+9	5+10	15	6	125 ab (115 - 140)	6.81 bc (5.9 - 8.3)	0.41 c (0.3 - 0.8)	74 d (16 - 106)	21 a (16 - 25)
16	2*	17,7	5	16	6	**87 ef** **(75 - 95)**	**3.37 f** **(3.1 - 3.9)**		**>150** [3]	
20		Tortilla flour		Control	6	110 bc (90 - 126)	8.81 a (7.7 - 10.7)	0.71 a (0.3 - 1.1)	28 e (19 - 53)	13 f (7 - 23)
LSD						15	1.68	0.05	8.4	1.6

[1] Average from two trials of lines planted in three locations, Values followed by the same letter in the same column are not significantly different ($\rho < 0.05$).

[2] Range for lines with similar HMW Glutenin allele composition. [3] Least significant difference ($\rho < 0.05$). n = number of repetitions from lines with similar allele composition.

[3] Too extensible and could not be evaluated using TAXT2i

The latter can be attributed to the lack of 10 on Glu D because, presence of 5+10 is associated with dough strength. This line had the highest ration of gliadin/glutenins hence, produced very extensible dough that could not be evaluated using the TAXT2i.

Dough extensibility

Dough extensibility test was carried out using the TAXT2 to objectively determine three dough parameters; dough extensibility is a measure of how far in millimeters the dough extended before it ruptures. Resistance to extension is a measure of the amount of force (N) needed to cause the dough strip to rupture, and work to extend was calculated as the area under the extensibility curve (N.mm).

Variations in the allelic composition significantly affected dough resistance to extension, extensibility, and work to extend at $\alpha = 0.05$ with 29.2, 20.4, and 17.3 co-efficient of variations, respectively. Resistance to extension was negatively correlated with tortilla diameter. Dough from all the wheat lines had significantly lower resistance to extension compared to control ($p < 0.05$) (Table V). Doughs from lines with (2*, 7+9, 2+12) exhibited highest resistance to extension, this confirms the subjective and dough compression results that 7+9 at GluB1 can be associated with increased strength of dough with 2+12 on the GluD1. However, lines with (2*, 17, 2+12) produced the least resistant doughs (Table V), this indicates that the presence of 17 at GluB1 did not improve the dough strength, the dough from this line was easy to press and produced larger diameter tortillas compared to control tortillas. On the other hand, doughs from lines with (2*, 20x+20y, 5+10) had high resistance to extension due to the presence of 5+10 which is believed to provide dough strength.

The mean dough extensibility (mm) values varied between (27 – 107 mm). Dough from all the lines were significantly ($P < 0.05$) more extensible compared to control dough (Table V). Dough from lines with (2*, 17, 7, 5) were very extensible and could not be objectively evaluated using the TAXT2i. Lines with (2*, 20, 5+10) produced highly extensible dough and produced larger tortillas compared to control. The least extensible dough was from lines with (2*, 7+9, 2+12) this confirms that the presence of 7+9 on GluB1 improves dough strength (Table V).

Work to extend dough made using the wheat lines was generally higher than control and averaged between (12.6 – 21.1 N.mm) (Table V). Lines with (1, 2*, 7+9, 5+10) required the highest work to extend and produced tortillas with small diameter due to the interactive effect of the presence of 7+9 at GluB2 and 5+10 at GluD1 which is associated with strong dough. Absence of HMW alleles at GluA in combination with 17+18 and 5+10 at GluB1 and GluD1 respectively resulted in reduced work to extend and hence, dough from lines with (17+18 and 5+10) required the least work to extend. (Table V). Dough extensibility is essential for production of large diameter tortillas. Dough that required high force to extend (resistance to extension) is very elastic and shrinks back after pressing thereby producing small diameter tortillas (Wang and Flores 1999). Tortillas require gluten (protein) network that is extensible with minimal shrink. The variations in flour and dough properties resulted in significant variations in tortilla quality as reported below.

Tortilla properties

Tortillas were prepared from all the wheat lines and tortilla flour as control. Flour attributes have been shown to significantly affect tortilla quality (Waniska et al 2004). In order to better understand the effect of the glutenin gene variations, reducing agents were not used in the production of the tortillas.

Tortilla moisture content was significantly affected by HMW allele variations ($p < 0.05$) and ranged between 31.6 – 35.8%. Tortilla from the wheat line possessing 2*, 17+18, 5+10 had the highest moisture content but were similar to control ($p < 0.05$). Lines with 2*, 20, 5+10 produced tortillas with significantly low moisture content compared to control tortillas (Table VI). More studies should be carried to determine the role played by the presence of 17+18 and 20 on Glu-B1 on tortilla moisture content.

Variations in the HMW glutenin composition did not significantly affect tortilla weight and thickness. Tortillas from all the lines and control had similar thickness and weight (Table VI).

Tortilla opacity was significantly affected by the variations in HMW glutenin allele composition ($p < 0.05$) (CV = 9.3). Tortillas made from wheat line with 2*, 17+18, 5+10 were the least opaque at 74.7 %, this was due to the formation of strong and elastic gluten that shrunk back producing a dense tortilla with fairly small diameter that were similar to control but were less opaque due to escape of gas formed during pressing ($P < 0.05$) as can be seen in the figure on page 33. On the other hand wheat lines with deletion at GluA1 loci, 17+18 and 5+10 at the Glu B1 and D1 respectively produced the most opaque tortillas; these tortillas were more opaque than control tortillas.

Table VI

Effects of different HMW glutenin allele's composition on the physical properties of tortillas[1]

Group	HMW Glu Allele composition			Entry	Diameter	Moisture	Weight	Height	Opacity	Specific Volume	L-Value
	GluA1	GluB1	GluD1								
1	2*	17+18,7	2+12	1	167.7 bcd (160 - 176)[2]	34.6 ab (32.7 - 39.2)	40.2 b (38 - 42)	2.97 a (2.7 - 3.1)	80 a-d (72 - 85)	1.63 bc (1.5 - 1.8)	80.5 d (80 - 81)
2	2*	20	5+10	2,11	**173.7 ab** **(162 - 187)**	31.6 d (21.2 - 34.6)	39.2 b (37 -41)	2.96 a (2.5 - 3.4)	85 abc (74 - 99)	1.79 ab (1.5 - 2.1)	83.0 a (81 - 85)
5	1	17+18	5+10	5,9,14,18	167.6 bcd (152 - 181)	33.6 a-d (30.3 - 38.4)	40.5 b (39 -43)	2.97 a 92.5 - 3.4)	82 a-d (69 - 95)	1.62 bc (1.2 - 2.0)	81.9 abc (80 - 84)
6	2*	17+18	5+10	6	**163.1 d** (159 - 166)	35.8 a (32.9 - 43.8)	39.5 b (37 - 42)	2.91 a (2.5 - 3.1)	75 d (68 - 84)	1.54 cd (1.4 - 1.7)	82.3 abc 980 - 84)
7	-	17+18	5+10	7	**172.7 b** (168 - 178)	32.5 bcd (29.7 - 34.1)	40.1 b (38 - 42)	2.95 a (2.6 - 3.2)	88 a (78 - 100)	1.73 abc 91.5 - 1.9)	82.8 ab (81 - 84)
8	2*	17	2+12	8	170.9 bc (168 - 174)	31.9 cd (25.6 - 34.6)	39.7 b (37 - 42)	3.08 a (2.9 - 3.3)	84 abc (77- 91)	1.78 ab (1.6 - 1.9)	82.7 abc (81 - 84)
10	2*	7+9	2+12	10,19	166.1 bcd (150 - 180)	32.8 bcd (27.7 - 35.1)	40.5 b (38 - 43)	3.11 a 92.9 - 3.5)	79 bcd 959 - 97)	1.68 abc (1.3 - 2.1)	82.2 abc 980 - 85)
13	1,2*	17+18	2+12	13	171.9 b (165 - 176)	33.3 a-d (32.3 - 34.8)	39.3 b 938 - 42)	3.01 a (2.5 - 3.3)	81 abc 979 - 87)	1.78 ab (1.5 - 2.0)	82.7 abc (82 - 84)
15	1,2*	7+9	5+10	15	164.3 cd (158 - 168)	33.2 bcd (32.4 - 34.6)	39.7 b (38 - 42)	2.96 a (2.7 - 3.3)	77 cd 962 - 84)	1.58 bcd (1.4 - 1.9)	82.4 abc (81 - 84)
16	2*	17,7	5	16	**180.7 a** (172 - 185)	31.9 cd (28.5 - 33.4)	40.7 b (38 - 47)	2.98 a (2.8 - 3.2)	86 ab (78 - 95)	1.88 a (1.7 - 2.0)	81.6 abc (80 - 83)
20	Refined wheat flour (Control)			20	161.4 d (152 - 167)	34.3 abc (32.7 - 35.7)	42.5 b (40 - 46)	2.92 a 92.6 - 3.3)	78 cd (69 - 85)	1.40 d (1.4 - 1.5)	81.5 cd (80 - 82)
	LSD[3]				7.6	2.5	1.7	0.26	8	0.21	1.3

[1] Average from two trials of lines planted in three locations, Values followed by the same letter in the same column are not significantly different ($\rho < 0.05$).

[2] Range for lines with similar HMW Glutenin allele composition. [3] Least significant difference ($\rho < 0.05$).

This is attributed to the weakening of dough strength conferred by the interactive effect of deletion at GluA1 and lack of sub–unit 10 on GluD1.

Tortilla lightness (L –Value) was affected by the HMW glutenin variations. The lightest tortillas were produced from wheat lines possessing (2*, 20, 5+10), these tortilla had high opacity score (85%) and significantly high specific volume compared to control tortillas due to retention of gas formed during pressing and baking by the action of leavening agents. Lines with 2*, 17+18, 7, 2+12 produced tortillas with the lowest L-value (Table VI). L – values agreed with opacity scores. Lines that had high opacity scores also had high L-values. This high values are attributed to the retention of air bubbles produced from leavening agents. The lines produced tortillas with gluten matrix that formed well sealed surfaces during pressing that helped to retain the air bubbles.

The HMW glutenin allele variations significantly affected the tortilla specific volume. Presence of (2*, 17+7, 5) on the Glu A1, B1 and D1 respectively resulted in tortillas with the highest specific volume (Table VI) whereas tortillas produced from wheat lines possessing (2*, 17+18, 5+10) had the lowest specific volume.

Tortilla diameter

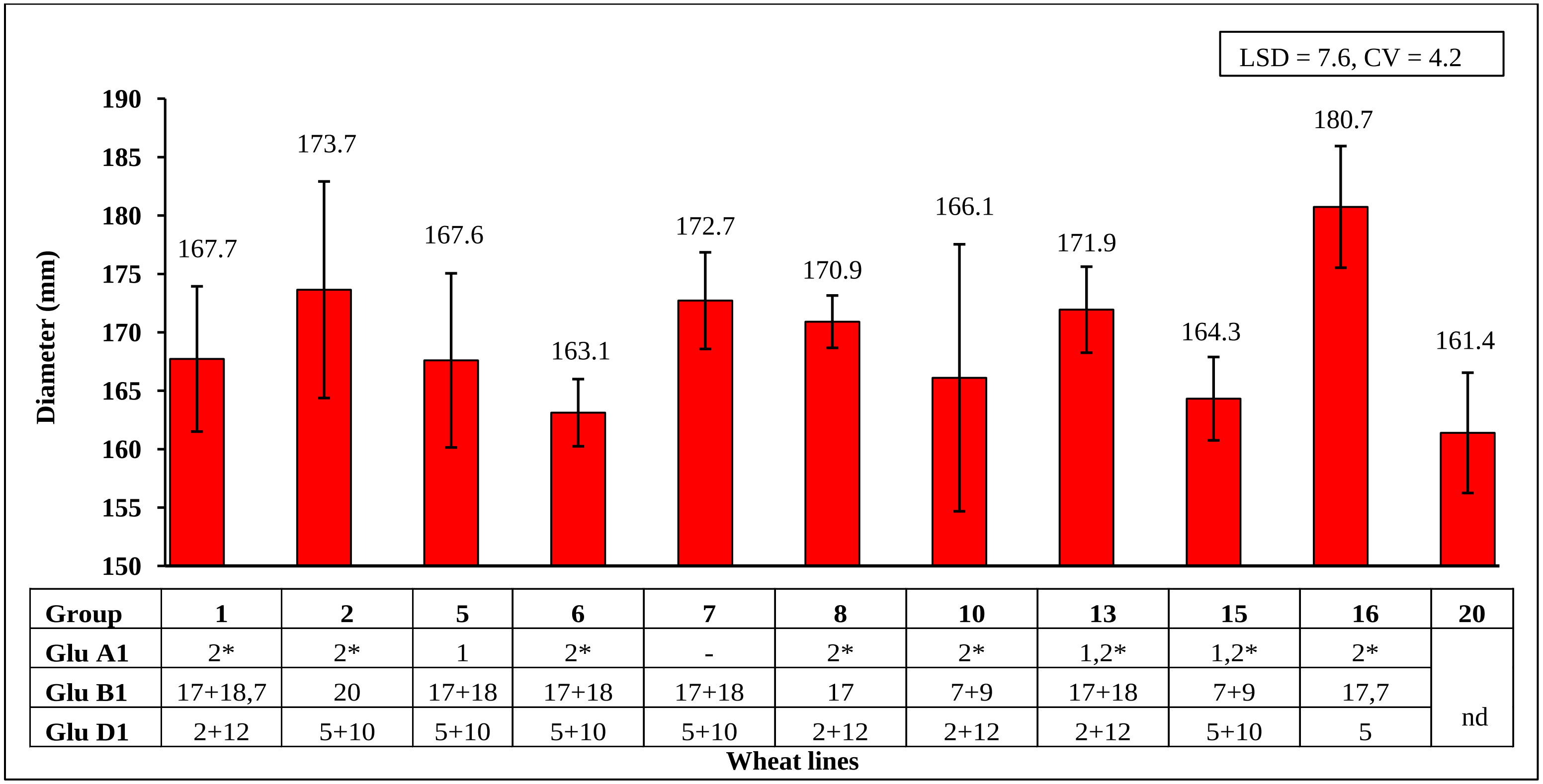
Variations in HMW glutenin allele composition had a significant effect on the diameter of tortillas. ($p < 0.05$)(CV = 4.26 %). Tortilla diameter averaged between 161 and 181 mm (Table VI, Fig 1). Control tortillas had the smallest diameter compared to all the wheat lines, but were not significantly different from wheat lines possessing the following allele composition; (2*, 17+18,7, 2+12), (1, 17+18, 5+10), (2*, 17+18, 5+10), (2*, 7+9, 2+12) and (1,2*, 7+9, 5+10) (Figure 1).

Presence of 2* at GluA1, both 17 & 7 at GluB1 and 5 at GluD1 resulted in very large tortillas (group 16 tortillas) (Table VI). This was due to the absence of sub-unit 10 at the GluD1 loci which provides dough strength; hence the dough was less elastic and did not shrink back during hot-pressing producing tortillas that were 12% larger in diameter compared to control tortillas. Similarly, tortillas produced using lines with (2*, 20, 5+10) had large diameters.

This confirms that the dough strengthening properties conferred by presence of 5+10 at GluD1 was weakened by the presence of 20 on the GluB1 loci and agrees with the subjective dough results. The interactive effect of presence of 17+18 and 5+10 at GluA1 and GluD1 loci coupled with the absence of HMW Glu subunits on GluA1 produced large tortillas hence, the dough strengthening functionality of the presence of 5+10 at GluD1 requires HMW Glutenin sub-unit 2* to be present on GluA1 (Figure 1). Conversely, presence of 2* at GluA1 and 17+18 on GluB1 loci did not counteract the dough strengthening property of 5+10. This is evident by small diameter (163 mm) tortillas produced using lines with (2*, 17+18, 5+10), the dough shrunk back after pressing due to strong gluten matrix.

Tortilla flexibility/ Rollability

Tortilla flexibility scores determined on a 5 point scale over 16 day storage period were significantly ($p < 0.05$) affected by allele variations in the HMW glutenin at the homologous loci of the A, B and D genomes.



Group	1	2	5	6	7	8	10	13	15	16	20
Glu A1	2*	2*	1	2*	-	2*	2*	1,2*	1,2*	2*	nd
Glu B1	17+18,7	20	17+18	17+18	17+18	17	7+9	17+18	7+9	17,7	
Glu D1	2+12	5+10	5+10	5+10	5+10	2+12	2+12	2+12	5+10	5	

Fig. 1. Effect of variations in HMW Glutenin allele composition on tortilla diameter.
nd = not determined, Glu = glutenin

As expected tortilla flexibility significantly decreased over storage at 22 ºC (Table VII).

Flexibility scores varied from 3.5 – 5.0 after 4 days of storage and between 2.6 and 4 after 16 days of storage (Table VII) with CV of 10.3% and 16.7% for day 4 and 16 respectively. Flours from wheat lines with more than 13% protein content produced tortillas that had good flexibility scores (≥ 3.0 flexibility score) over storage points. The line possessing a deletion at A genome, 17+18, and 5+10 produced large diameter tortillas with the lowest flexibility scores during storage compared to tortillas from all other wheat lines and control despite having 13.1% protein content and presence of 5+10 glutenin subunit on GluD1 loci. This can confirms that the absence of HMW glutenin at the GluA1 loci is associated with excessive weakening of gluten strength. On the other hand, tortillas made from lines possessing (1,2*, 7+9, 5+10) and (2*, 17+18, 5+10) had small diameter with highly acceptable flexibility score (4.0) after 16 days of storage. This was due to the elastic nature of doughs that was conferred by the presence of 5+10 on Glu D1 (Mondal et al 2008).

The interactive effect of the presence of (2*, 17+18, 7, 2+12) resulted in small diameter tortillas that had superior flexibility scores compared to control tortillas (Figure 2). This confirms that the presence of 2+12 on Glu D1 is associated with week gluten strength hence the large diameter tortillas and is also indicative that presence of 2* at Glu A1 and sub units 17+18, 7 on Glu B1 play a significant role in tortilla storage stability (Figure 2).

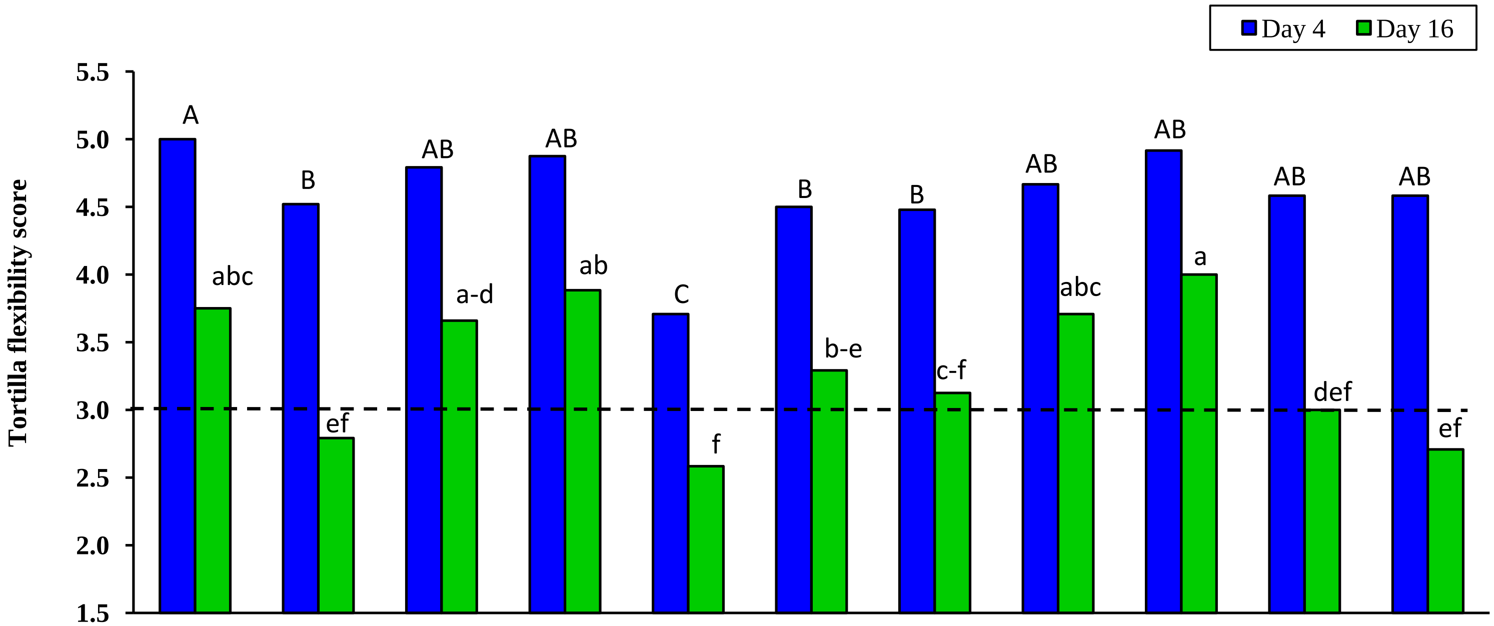
Table VII

Effects of different HMW glutenin allele's composition on the flexibility of tortillas over storage period[1]

HMW Glu Allele composition					Tortilla flexibility score			
GluA1	**GluB1**	**GluD1**	**Entry**	**n**	**Day 4**	**Day 8**	**Day 12**	**Day 16**
2*	17+18,7	2+12	1	12	5.0 a (5.0 – 5.0)	4.9 a (4.5 - 5.0)	4.3 ab (3.5 - 5.0)	3.8 abc (2.5 - 4.5)
2*	20	5+10	2,11	24	4.5 b (2.5 - 5.0)	4.4 c (2.0 - 5.0)	3.6 d (2.0 - 5.0)	2.8 ef (1.0 - 4.5)
1	17+18	5+10	5,9,14,18	48	4.8 ab 3.5	4.4 bc (2.0 - 5.0)	4.0 a-d (2.0 - 5.0)	3.7 a-d (1.5 - 5.0)
2*	17+18	5+10	6	12	4.9 ab (4.5 5.0)	4.6 abc (4.0 - 5.0)	4.4 ab (3.5 - 5.0)	3.9 ab (2.5 - 5.0)
-	17+18	5+10	7	12	3.7 c (3.0 - 5.0)	3.3 d (1.0 - 5.0)	3.0 e (1.0 - 5.0)	2.6 f (1.0 - 4.5)
2*	17	2+12	8	12	4.5 b (3.0 - 5.0)	4.2 bc (2.0 - 5.0)	3.8 bcd (1.5 - 5.0)	3.3 b-e (1.0 - 5.0)
2*	7+9	2+12	10,19	24	4.5 b (3.0 - 5.0)	4.1 c (3.0 - 5.0)	3.7 cd (2.5 - 5.0)	3.1 c -f (2.0 - 5.0)
1,2*	17+18	2+12	13	12	4.7 ab (3.5 - 5.0)	4.5 abc (3.0 - 5.0)	4.2 abc (3.0 - 5.0)	3.7 abc (2.5 - 4.5)
1,2*	7+9	5+10	15	12	4.9 ab (4.0 - 5.0)	4.8 ab (4.0 - 5.0)	4.5 a (3.5 - 5.0)	4.0 a (2.5 - 4.5)
2*	17,7	5	16	12	4.6 ab (3.5 - 5.0)	4.4 bc (3.0 - 5.0)	4.0 a-d (2.5 - 4.5)	3.0 def (2.0 - 4.0)
0	0	0	20	12	4.6 ab (3.5 - 5.0)	4.1 c (3.5 - 5.0)	3.5 de (3.0 - 4.0)	2.7 ef (2.0 - 3.5)
LSD					0.4	0.6	0.6	0.7

1 Average from two trials of lines planted in three locations, Values followed by the same letter in the same column are not significantly different ($\rho < 0.05$).

2 Range for lines with similar HMW Glutenin allele composition. 3 Least significant difference ($\rho < 0.05$). n = number of repetitions from lines with similar allele composition.



Group	**1**	**2**	**5**	**6**	**7**	**8**	**10**	**13**	**15**	**16**	**20**
Glu A1	2*	2*	1	2*	-	2*	2*	1,2*	1,2*	2*	nd
Glu B1	17+18,7	20	17+18	17+18	17+18	17	7+9	17+18	7+9	17,7	
Glu D1	2+12	5+10	5+10	5+10	5+10	2+12	2+12	2+12	5+10	5	

Wheat lines

Fig. 2. Effect of variations in HMW Glutenin allele composition on tortilla flexibility over storage.

nd = not determined, Glu = glutenin. Bars with similar letter indicate similar flexibility scores, Upper case letters for day 4 and lower case for day 16.

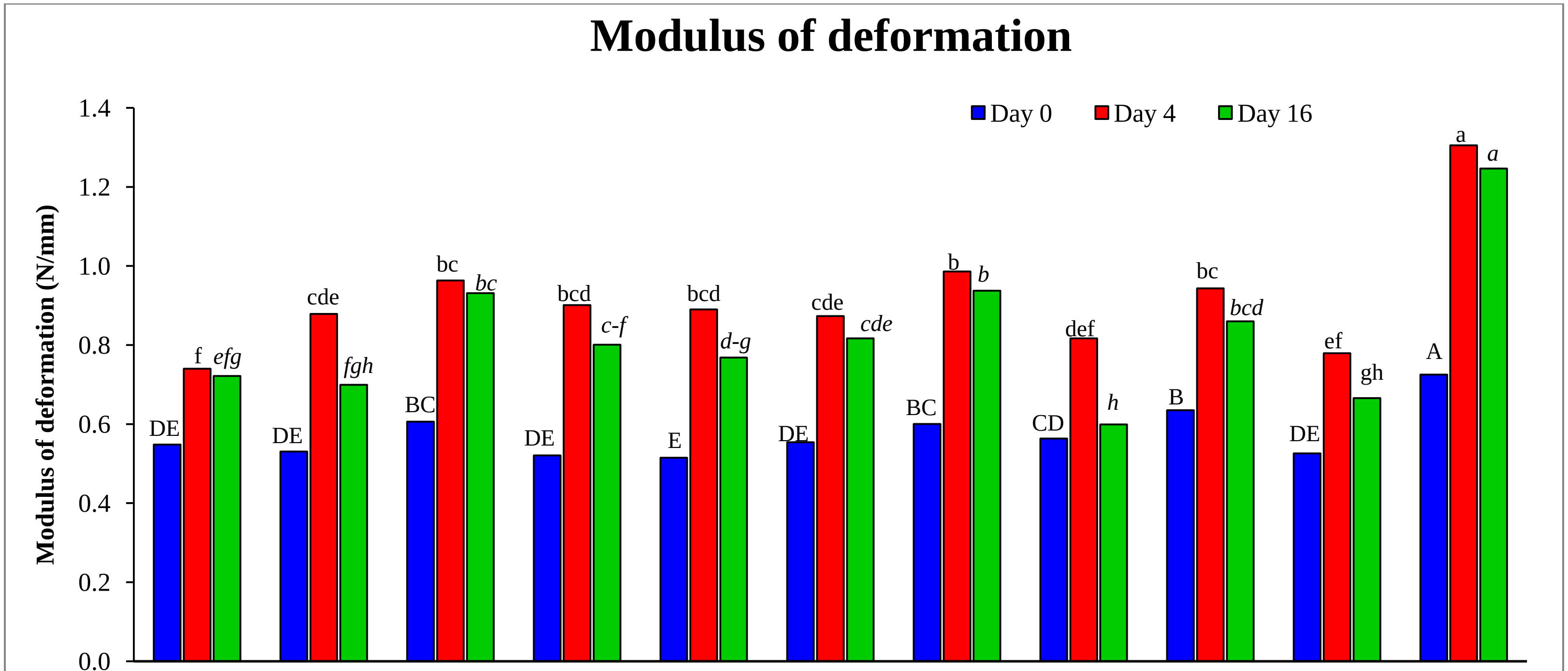
---Ideal flexibility score after 16 days of storage

Two-dimensional tortilla extensibility

Objective tortilla properties; deformation modulus (N/mm), force to rupture (N), distance to rupture (mm) and work to rupture (N.mm), analyzed using a texture analyzer over a period of 16 days, were significantly affected by variations in HMW Glu composition ($p < 0.05$). Large textural differences were noticeable between day 0 and day 4 of storage with smaller changes between day 8 and day. 16 (Figures 3 -4).

The deformation modulus increased significantly after 4 days of storage and remained constant at days 8 and 12, and then slightly decreased after day 16. This is attributed to starch retrogradation over time (Alviola and Waniska 2008). The wheat lines had significantly ($p < 0.05$) lower deformation modulus compared to control at all storage points (Figure 3), meaning that the wheat lines produced softer tortillas compared to control.

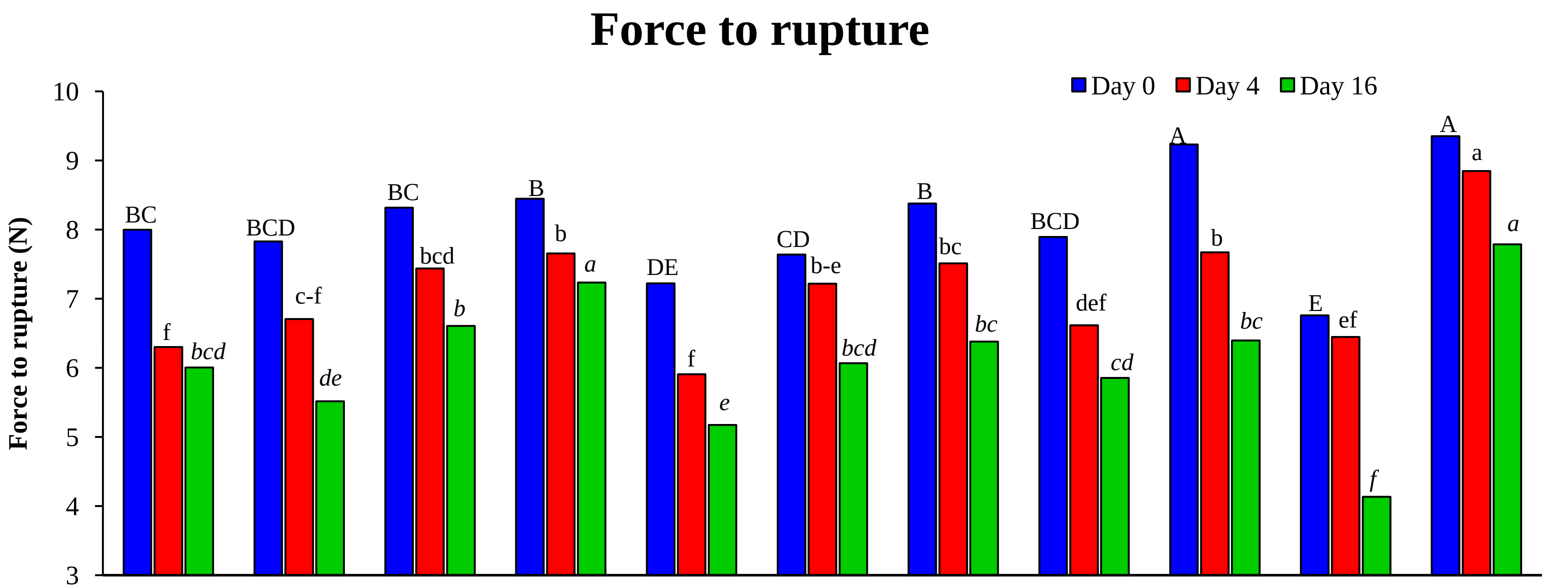
Force, distance and work to rupture significantly decreased over storage (Figure 4). Control tortillas required higher force to rupture than tortillas made using the wheat lines. On the day of production (day 0), tortillas with (1, 2*, 7+9, 5+10) required high force to rupture (9.23N) and was similar to control (9.35N). This agrees with the high dough compression force and resistance to extension (Table V) and agrees with the earlier reports that the 5+10 allele contributes to dough strength (Mondal et al. 2008). Hence, more force was required to rupture these tortillas.



Group	**1**	**2**	**5**	**6**	**7**	**8**	**10**	**13**	**15**	**16**	**20**
Glu A1	2*	2*	1	2*	-	2*	2*	1,2*	1,2*	2*	nd
Glu B1	17+18,7	20	17+18	17+18	17+18	17	7+9	17+18	7+9	17,7	
Glu D1	2+12	5+10	5+10	5+10	5+10	2+12	2+12	2+12	5+10	5	

Fig. 3. Effect of variations in HMW Glutenin allele composition on tortilla modulus of deformation over storage.

nd = not determined, Glu = glutenin. Bars with similar letter indicate similar modulus of deformation, Upper case letters for day 0, lower case for day 4 and *Italic* for day 16



Group	**1**	**2**	**5**	**6**	**7**	**8**	**10**	**13**	**15**	**16**	**20**
Glu A1	2*	2*	1	2*	-	2*	2*	1,2*	1,2*	2*	nd
Glu B1	17+18,7	20	17+18	17+18	17+18	17	7+9	17+18	7+9	17,7	
Glu D1	2+12	5+10	5+10	5+10	5+10	2+12	2+12	2+12	5+10	5	

Wheat lines

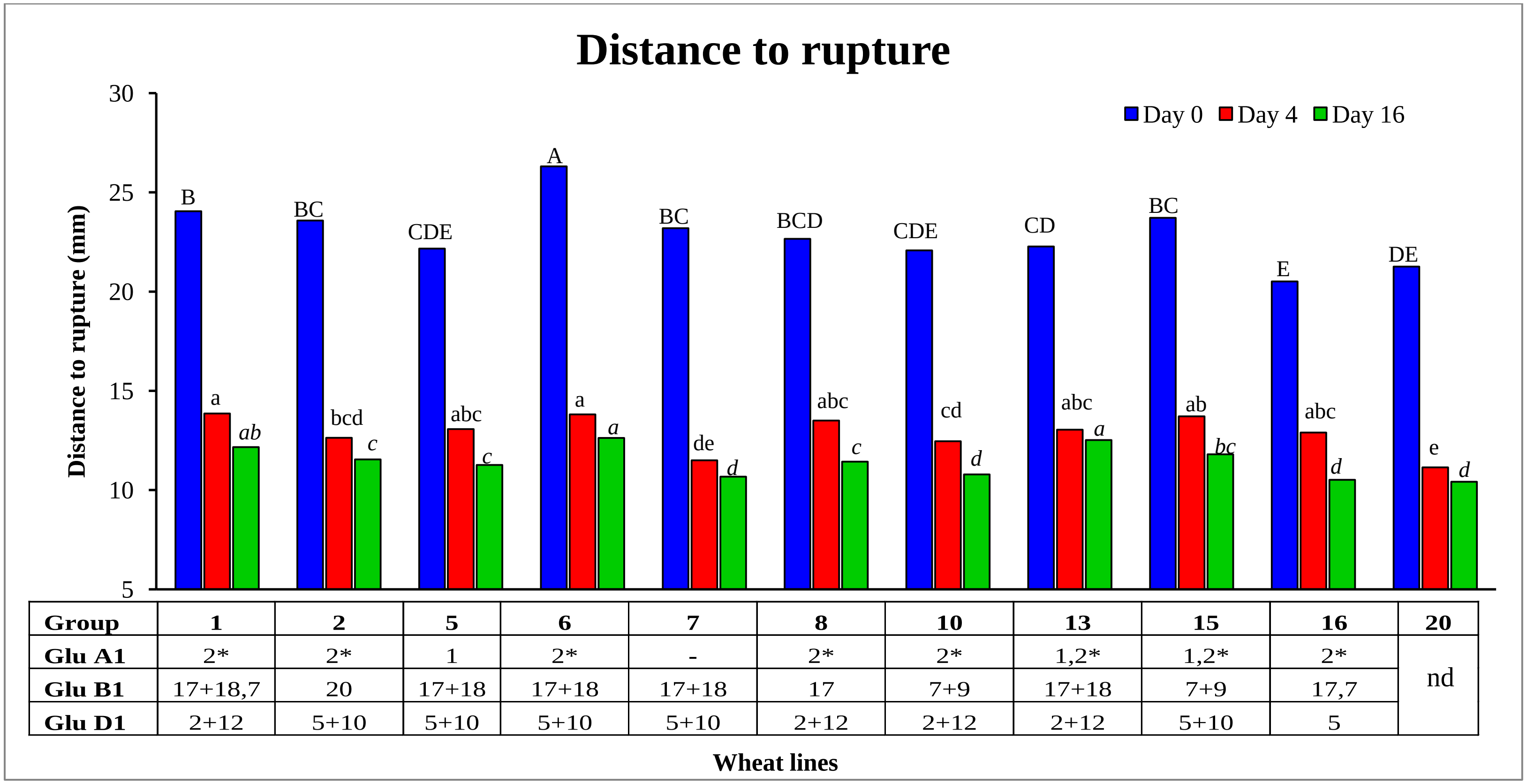
Fig. 4. Effect of variations in HMW Glutenin allele composition on tortilla force to rupture over storage.

nd = not determined, Glu = glutenin. Bars with similar letter indicate similar force to rupture, Upper case letters for day 0, lower case for day 4 and *Italic* for 16

On day 4 of storage, tortillas made using lines lacking an allele at Glu A1, but possessing 17+18 at Glu B1 and and 5+10 at Glu D1 required the least force to rupture (Figure 4) confirming that dough strengthening effect of the presence of 5+10 needs to be complemented with the presence of alleles at GluA1 (Mondal et al. 2008). On the last day of storage (day 16), tortillas with (2*, 17, 7, 5) required the least force to rupture (Figure 4) meaning that they were very brittle and ruptured easily.

Tortillas with (2*, 17+18, 5+10) required the longest distance to rupture on the day of production (Figure 5). This confirms that the elastic nature of gluten is associated with the interactive effect of the 2*, 17+18 and 5+10 alleles on the homologous loci of Glu1 A, B and D respectively. Tortillas from these lines had small diameters due to shrinking back of the gluten after hot pressing.

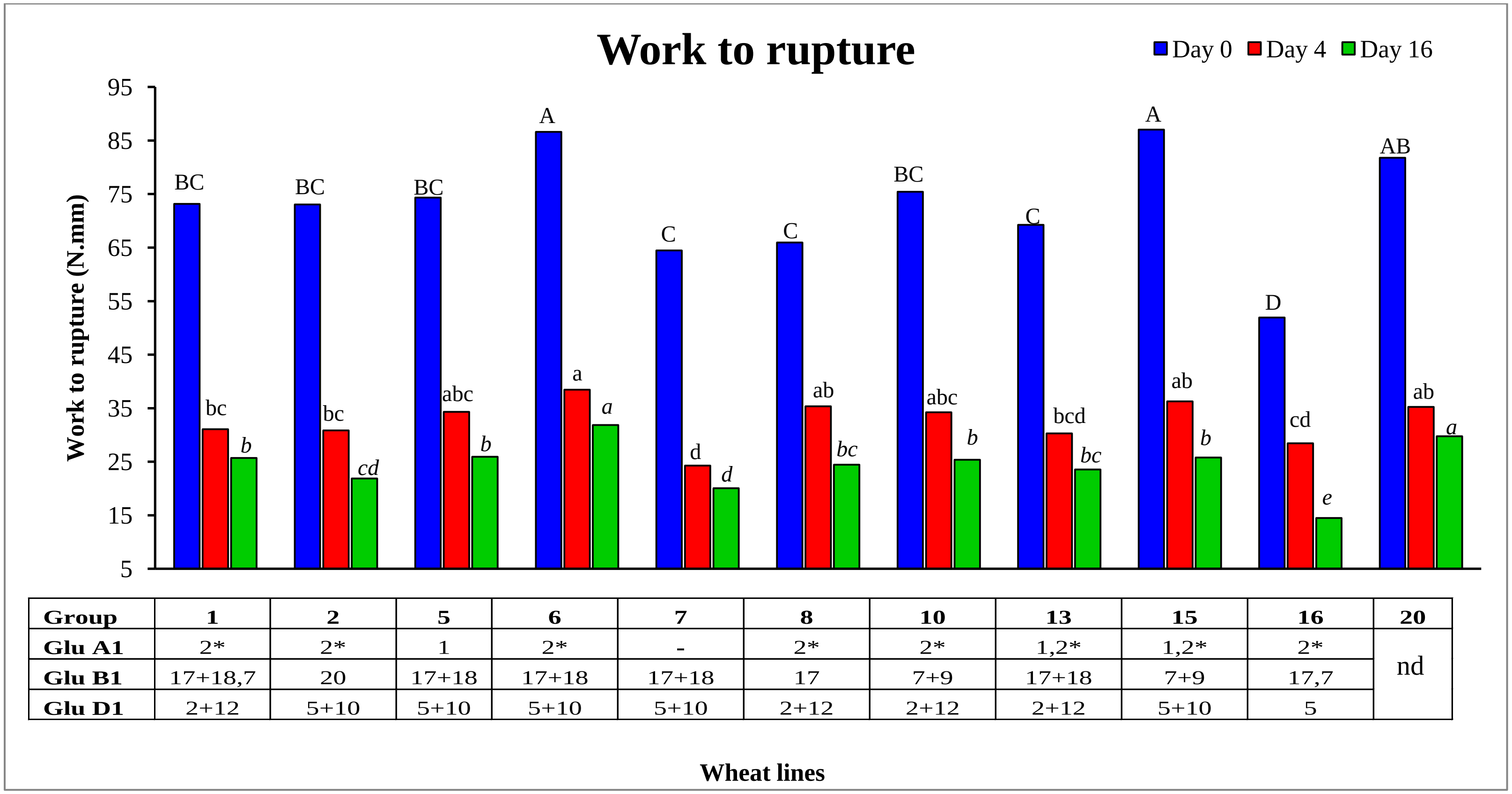
Work to rupture after 16 days of storage was highly negatively correlated (r = -0.94) with tortilla diameter and opacity (r = -0.84). Lines with (2*, 17, 7, 5) required the lowest work to rupture across all storage time points (Figure 6), This confirms the dough subjective and objective test that this allele combination is associated with soft easy to press dough as is evident from the highly opaque and large diameter tortillas produced using this lines.



Group	1	2	5	6	7	8	10	13	15	16	20
Glu A1	2*	2*	1	2*	-	2*	2*	1,2*	1,2*	2*	nd
Glu B1	17+18,7	20	17+18	17+18	17+18	17	7+9	17+18	7+9	17,7	
Glu D1	2+12	5+10	5+10	5+10	5+10	2+12	2+12	2+12	5+10	5	

Fig. 5. Effect of variations in HMW Glutenin allele composition on distance to rupture tortillas over storage.

nd = not determined, Glu = glutenin. Bars with similar letter indicate similar distance to rupture, Upper case letters for day 0, lower case for day 4 and *Italic* for day16



Group	**1**	**2**	**5**	**6**	**7**	**8**	**10**	**13**	**15**	**16**	**20**
Glu A1	2*	2*	1	2*	-	2*	2*	1,2*	1,2*	2*	nd
Glu B1	17+18,7	20	17+18	17+18	17+18	17	7+9	17+18	7+9	17,7	
Glu D1	2+12	5+10	5+10	5+10	5+10	2+12	2+12	2+12	5+10	5	

Fig. 6. Effect of variations in HMW Glutenin allele composition on work to rupture tortillas over storage.

nd = not determined, Glu = glutenin. Bars with similar letter indicate similar flexibility scores, Upper case letters for day 0, lower case for day 4 and *Italic* for day 16

CHAPTER SUMMARY

Good quality tortillas should have large diameters and resist cracking, and breaking during preparation and consumption (Waniska 1999). Despite the negative effects on tortilla palatability and consumer acceptance (Bejosano and Waniska 2004), most commercially produced tortillas still rely on ingredients; reducing agents, emulsifiers, acids and leavening agents to normalize tortilla dough in order to produce ideal quality tortillas from diverse wheat flours (Waniska 1999). This study provide an increased understanding of the role played by different HMW glutenin allele combinations on the loci of theGlu A1, GluB1 and Glu D1 genome of wheat on the quality of tortillas.

Diameter was significantly negatively correlated with the percentage of insoluble polymeric proteins (% IPP), HMW glutenin to LMW glutenin ration, subjective dough force to extend, dough compression force, F100, tortilla moisture content, tortilla deformation modulus, force to rupture and work to rupture after 16 days of storage ($r = -0.86, -0.47, -0.61, -0.73, -0.90, -0.72, -0.70$, and -0.95, respectively at $P < 0.05$). Diameter was positively correlated ($p < 0.05$) with flour protein content (%) ($r = 0.72$), subjective dough extensibility ($r = 0.63$), and tortilla specific volume ($r = 0.93$).

On the other hand tortilla flexibility at the end of storage was significantly negatively correlated with opacity ($r = -0.61$) and positively with %IPP ($r = 0.82$), distance ($r = 0.79$) and work ($r = 0.82$) to rupture tortillas after 16 days.

Gli/ Glu ratio was significantly correlated ($r = -0.94$; $p < 0.05$) with work to rupture on day 16 of storage. Flour protein content significantly negatively correlated (p

< 0.05) with subjective dough smoothness ($r = -0.81$), softness ($r = -0.62$), dough compression force ($r = -0.80$), F100 ($r = -0.82$), tortilla deformation modulus ($r = -0.73$) and force to rupture ($r = -0.65$) after 16 days of storage. Protein content positively ($P < 0.05$) correlated with subjective dough extensibility ($r = 0.77$) and tortilla specific volume ($r = 0.63$).

The line with only 17+18 and 5+10 (group 7) produced tortillas with larger diameters than control, but had poor flexibility compared to control, it yielded more extensible dough compared to control (Figure 2). This could be a result of absence of both 1, and 2* on A genome. Wheat lines in group 16 with (2*, 17, 7, 5) produced tortillas with significantly larger diameters compared to control tortillas. However, these tortillas had inferior flexibility compared to tortillas in groups 1(2*, 17+18, 7, 2+12), 5 (1, 17+18, 5+10), 8 (2*, 17, 2+12) and 13 (1,2*, 17+18, 2+12) that had comparatively larger diameters and were more stable across environments (Figure 2). These tortillas had acceptable flexibility scores (≥ 3.0) after 16 days of storage. Without addition of reducing agents, doughs from these lines were easy to press into a round disc that did not shrink back. Hence, wheat lines possessing these allelic combinations need to be investigated further as identity preserved (IP) lines for tortilla production

CHAPTER III

EFFECTS OF RESISTANT STARCH ON WHEAT FLOUR TORTILLA QUALITY

INTRODUCTION

Tortilla as vehicle to improve dietary fiber consumption

Refined wheat flour tortillas are the most popular tortilla consumed in the USA. Refined flour tortillas have poor nutrition profile and generate a high glycemic response, similar to white wheat bread (Saldana and Brown 1984). However, consumers prefer the sensory attributes of refined wheat tortillas compared to whole wheat tortillas. Tortillas are the fastest growing and most popular bread consumed in North America (Dally and Navarro 1999), Research shows that less than 10% of Americans consume the recommended daily intake (RDI) of fiber. Hence, tortillas can be used to promote healthy eating.

Dietary fibers are food components that are resistant to digestion and absorption in the small intestine. There are two main categories of dietary fiber - soluble and insoluble. Consumption of soluble fiber such as gums, hydrocolloids, most pectins, mucilages and some hemicelluloses reduces cholesterol levels. Insoluble fibers include cellulose, some hemicelluloses, lignin and enzyme-resistant starches; these have been shown to increases transit time in the gut, thus reducing the risk of colon cancer, diverticulitis, colitis and other gastrointestinal ailments (Englyst and Cummings 1985).

Consumption of diets high in whole grains has been linked to lowered cholesterol, blood pressure, cardiovascular disease, cancer risk, and prevention of constipation and other bowel problems (Food Procuct Design 2001; 2009). Low consumption of dietary fiber in the USA is linked to the increased Diabetes Type II. Finding an acceptable way to increase fiber in commercial tortillas would increase total dietary fiber intake.

Like other dietary fibers, resistant starch or by-products of its hydrolysis are not absorbed in the small intestines (Englyst et al. 1993), but are partially or completely fermented in the large intestines (Englyst and Cummings 1985). Resistant starch (RS) slows digestion of carbohydrates and gives a sustained, low elevation of blood sugar, providing a low glycemic load, and delays hunger by acting as a bulking agent (Sajilata et al. 2006). Resistant starch acts as a prebiotic (Seetharaman et al. 1994). Resistant starch can be used to produce high dietary fiber tortillas (Alviola et al. 2010).

Evaluation of physical, chemical and organoleptic effects of adding mixtures of whole and refined red or white wheat flours at different levels (0, 25, 50, 75, 100 whole wheat) in tortilla formulations indicate that consumers dislike whole wheat tortillas compared to refined flour tortillas (Friend et al. 1992). Incorporation of fiber isolated form corn, oat, pea, soy and sugar beet at increasing levels (0, 4, 8, 12, 16 and 20%) significantly decreased dough machinability, tortilla shelf life and negatively affected tortillas color and diameter (Anton 2008; Seetharaman et al. 1994). However, addition of up to 12% fiber gave the best consumer acceptability and minimally affected shelf stability (Seetharaman et al. 1994).

There are alternative ingredients that can be used to improve dietary fiber in wheat flour tortillas. However, the incorporation of different ingredients into the traditional tortilla formulation has significant effects on the shelf stability and organoleptic properties (Anton 2008; Waniska et al. 2002). Use of soluble and insoluble fibers in wheat flour tortillas often leads to poor product quality (Seetharaman et al. 1997). Use of 8% soluble fiber can lead to poor gluten development and extensive gelatinization during baking, producing dense crumbs in tortillas (Seetharaman et al. 2002). Insoluble fibers physically disrupt the gluten matrix resulting in collapse of air bubbles and produces tortillas with decreased shelf-stability (Seetharaman et al. 1997). Hence, significantly improving dietary fiber content in refined wheat tortillas without negatively affecting shelf-stability and shelf-life of tortillas remains a challenge.

Definition of resistant starch

Starch is a major dietary source of carbohydrates. It is the most abundant storage polysaccharide in plants, and occurs as granules in the chloroplast of green leaves and the amyloplast of seeds, pulses, and tubers (Ellis et al. 1998). Starch was traditionally thought to be completely digested in human small intestines by pancreatic and brush border enzymes. However, some forms of starch resist digestion in the small intestines and are fermented by colon bacteria (Englyst et al. 1993). These starches are called “resistant starches.” Extensive studies have shown similarities in their physiological functions and those of dietary fiber (Asp 1994; Eerlingen and Delcour 1995). Resistant starch (RS) was a term used to describe a small fraction of starch that was resistant to hydrolysis by exhaustive amylase and pullulanase treatment *in vitro* (Englyst et al. 1982; Sajilata et al. 2006). . Thus, resistant starch is the fraction of dietary starch, which escapes digestion in the small intestine (Sajilata et al. 2006). Resistant starch is currently measured chemically as the difference between total starch (TS) obtained from a homogenized and chemically treated sample and the sum of rapidly digestible starch (RDS) and slowly digestible starch (SDS), after enzyme digestion of non-homogenized food samples (Sajilata et al. 2006). This is summarized in formula below:

$$RS = TS - (RDS + SDS)$$

Resistant starch has fine particles and bland taste/flavor, and can be added to various food formulations to produce products with better consumer acceptability than those made with traditional fibers (Sajilata et al. 2006). The diversity of the food industry coupled with the numerous varieties of food products require starches that tolerate a wide range of processing techniques (Visser et al. 1997). The ingredient industry is working to meet these new trends and demands by modifying native starches using chemical, physical, and enzymatic methods (Betancur and Chel 1997). These modifications lead to the formation of high resistant starch and indigestible residues (Sajilata et al. 2006).

Classes of resistant starches

There are four classes of resistant starch: RS1, RS2, RS3, and RS4 or I, II, III, and IV starches (Sajilata et al. 2006).

RS1

This refers to starch that is physically protected and inaccessible to pancreatic amylases. These are found in whole grains, partially milled grains, seeds and in dense processed starchy foods (Sajilata et al. 2006). RS1 is heat stable and can be used in a wide variety of thermally processed food products.

RS2

These are intact ungelatinized/ raw starch granules. The granular dehydrated structure makes it less accessible to digestive enzymes. Hence, it is slowly digested. Examples include raw banana and potato (Sajilata et al. 2006).

RS3

This is mainly retrograded starch. It is found in all starch containing foods. It is formed when gelatinized starch is cooling, when amylose leaches out of the fully hydrated starch granules and reassociates as double helices that are stabilized by hydrogen bonds (Wu 1978). RS 3 can only be dispersed with potassium hydroxide or dimethyl sulphoxide (Asp and Bjorck 1992; Sajilata et al. 2006).

RS4

These are chemically modified starches obtained by cross linking or etherification or esterification processes. It is inert to enzyme digestion, food processing conditions and has minimal or no effect on functionality of other food ingredients.

Modification of starches to produce resistant starches

Plant breeding techniques can be used to alter the proportions of amylopectin and amylose in starchy grains. Various techniques have emerged that can be used to genetically modify crops to produce starches with specific functionality (Regina et al. 2006). Waxy (high amylopectin) and high amylose cornstarches have been produced and provide different nutritional and functional properties. High amylose starches have higher gelatinization temperature; they easily retrograde and form complexes with lipids. Such properties can be utilized in formation of foods with high-resistant starch content

(Cummings and Stephen 2007). Starches can also be chemically modified to provide functional properties that impart desired qualities in various food products such as decreased viscosity and to improve gel stability, mouth feel, appearance and texture, and resistance to heat treatment (Cummings and Stephen 2007).

Various chemical procedures have been developed and are used in starch modification, these include substitution and crosslinking (Cummings and Stephen 2007). Substitution is the etherification or esterification of some of the hydroxyl groups on the glucose units of amylose and amylopectin. This reduces retrogradation leading to slow staling of bread. Substitution also lowers gelatinization temperature, provides freeze–thaw stability and increases viscosity (Cummings and Stephen 2007). Crosslinking is a chemical process that involves introduction of a controlled number of links between the amylose and amylopectin chains. The links reinforce the hydrogen bonding within the starch granules. Crosslinking increases gelatinization temperature, improves acid and heat stability, inhibits gel formation and controls viscosity during processing. Altering the chemical nature of starch can make it resistant to digestion (Cummings and Stephen 2007).

MATERIALS AND METHODS

Tortilla formulation and processing

The control formulation included 500 g either refined wheat flour (RF: ADM Inc.) or whole white wheat (WW) flour (Farmer Direct Foods, Inc. Atchison, KS), 30 g of shortening (Sysco Corporation, Houston, TX), 7.5 g salt (Morton International, Inc., Chicago, IL), 3 g sodium bicarbonate (Arm and Hammer, Church and Dwight Company, Inc., Princeton, NJ), 2.9 g sodium aluminum sulfate (Budenheim USA Inc., Plainview, NY), 2.5 g sodium steroyl lactylate (Caravan Ingredients, Lenexa, KS), 2.5 g sodium propionate (Niacet Corp., Niagara Falls, NY), 2 g potassium sorbate (B. C. Williams, Dallas, TX), 1.65 g encapsulated fumaric acid (Balchem Corp., New Hempton, NY) and distilled water. The tortillas were baked from refined wheat flour substituted at three levels (5%, 10% and 15%) with each of the four commercially produced resistant starches two from wheat Fibersym^{TM} and FiberRite^{TM} (MGP Ingredients, Atchison, KS) and corn starches Hi-Maize 260^{TM} and Hi-Maize^{TM} Corn Flour 150 (National Starch Inc. Bridgewater, NJ). Fibersym and FiberRite are both cross linked and stabilized wheat starches. However, FiberRite is hydrothermally treated (pre-gelatinized) and has larger particle (10-60 μm) size compared to Fibersym (10-30 μm). FiberRite is reversibly swellable. These two RS are classified as resistant starch type 4. On the other hand Hi-Maize 260 and Hi-Maize Flour 150 are both natural and derived from high amylose corn. M260 has small particle size (10 – 15 microns) whereas M150 is a coarse powder and yellow in color. They fall under the RS2 classification of resistant starches. Tortillas were processed as described in Chapter II.

Evaluation of dough properties

Subjective and objective dough evaluations

Control and treated doughs were subjectively evaluated for smoothness, softness, extensibility, force to extend and press rating following method described in Chapter III. Objective tests, namely: stress relaxation, dough extensibility test and dough compression test were also conducted. All these tests were done as described in Chapter II.

Evaluation of tortilla physical properties

Tortilla properties: weight, diameter, height, opacity, color, moisture, rollability/ flexibility, and two-dimensional extensibility were measured following methods described in Chapter II.

Organoleptic evaluation of tortilla properties

Seventy three untrained panelists from Texas A&M University were asked to evaluate the tortillas made with refined flour (control), whole wheat flour, 5 – 10% Fibersym and FiberRite for overall appearance, texture, color and flavor acceptability on a 9-point hedonic scale where 1= extremely dislike and 9= extremely like (Bejosano et al. 2005). Each sample was randomly assigned a three digit code and presented to each of the panelist in a random order. Information on age, gender, ethnicity and tortilla consumption frequency was gathered from the panelists using the sensory evaluation ballot (See Appendix A, Figure A1).

Total dietary fiber (TDF) analysis

Total dietary fiber of the tortillas, resistant starch and tortilla flour was evaluated using the AOAC Official Method 985:43.

Statistical analysis

Microsoft office excel 2007 (Microsoft Corporation, Redmond, WA) was used to derive means, standard deviations and plots. Data analysis was done using SPSS version 16.0 (SPSS Inc. Chicago, Il) and SAS version 9.2 (SAS Institute, Cary, NC). Analysis of variance (ANOVA) and determination of least significant difference (LSD) were performed at $\alpha = 0.05$ significance level to determine differences among the samples and treatments.

RESULTS AND DISCUSSION

Subjective dough properties

The type and level of RS used significantly affected ($P < 0.05$) dough smoothness, softness, and extensibility. Increasing levels of Fibersym (Fsym), Hi-Maize 260 (M260) and Hi-Maize150 did not affect dough smoothness, softness and were similar to refined flour (RF), whereas increase in level of FiberRite produced firmer and less extensible doughs compared to RF (Table VIII). Whole wheat (WW) dough was slightly smooth and was similar to doughs with 10 and 15% FRite. This can be due to the large particle size of WW and FRite. Doughs with 10-15% Frite and WW had similar softness but were hard compared to RF dough. This is due to the high moisture absorption properties and large particle size of whole wheat flour and FRite (Table VIII).

Table VIII

Effects of resistant starch fortification on dough physical properties[1]

Treatments	Smoothness[2]	Softness[2]	Extensibility[2]	Force to extend[2]	Pressrating[2]	Hardness (N)	Resistance to Extension (N)	Extensibility (mm)	Water used (%)
Control, Refined Wheat	2.0 c	2.0 c	3.8 a	1.8 d	2.3 c	113 d	0.31 d	55.9 a	52
Whole wheat	3.3 a	3.3 a	1.8 d	3.3 ab	3.5 a	229 a	0.65 abc	17.3 d	56
Fibersym + %RF									
5	2.0 c	1.8 c	3.8 a	1.8 d	2.3 c	142 cd	0.60 bcd	26.8 bcd	52
10	2.3 bc	2.0 c	3.0 abc	2.0 d	2.5 bc	122 cd	0.62 bcd	39.3 abc	53
15	2.0 c	1.8 c	3.3 ab	2.0 d	2.3 c	112 d	0.45 cd	41.6 ab	54
FiberRite + %RF									
5	2.0 c	2.0 c	3.0 abc	2.0 d	2.5 bc	124 cd	0.50 cd	39.7 ab	53
10	2.8 ab	2.8 ab	2.3 cd	3.0 abc	3.3 ab	130 cd	0.54 bcd	32.3 bcd	56
15	3.3 a	3.0 a	1.5 d	3.5 a	3.8 a	221 a	0.60 bcd	20.6 d	58
Hi-Maize260 + %RF									
5	2.0 c	2.0 c	3.3 ab	2.0 d	2.3 c	124 cd	0.55 bcd	18.9 bcd	61
10	2.3 bc	2.0 c	3.0 abc	2.3 cd	2.5 bc	155 bcd	0.69 abc	18.4 d	61
15	2.3 bc	2.3 bc	3.0 abc	2.3 cd	2.3 c	115 d	0.68 abc	26.5 cd	62
Hi-Maize Flour 150									
5	2.3 bc	1.8 c	3.5 ab	2.0 d	2.0 c	164 bc	0.82 ab	19.8 d	60
10	2.0 c	2.0 c	3.3 ab	2.0 d	2.0 c	137 cd	0.95 a	18.2 d	62
15	2.5 bc	2.3 bc	2.8 bc	2.5 bcd	2.3 c	196 ab	0.74 abc	22.6 d	63
LSD[3]	0.5	0.5	1.0	0.9	0.8	47.1	0.31	17.1	

[1] Means followed by the same letter in the same column are not significantly different ($\rho < 0.05$)

[2] 5 - point Subjective dough evaluation scales:- Smoothness: (1 = rough and 5 = smooth), Softness : (1 = very soft, 5 firm), Extensibility: (1 = not extensible and 5 = very extensible), force to extend: (1 = less force and 5 = much force), Pressrating: (1 = easy to press and 5 = much force to press).

[3] Least significant difference ($\rho < 0.05$).

Subjective dough extensibility scores decreased with increased substitution and ranged between 1.5 and 3.8 (Table VIII). Treatments with FSym and RF had similar extensibility. Dough with 10-15% FRite and WW dough were the least extensible and required higher force to extend compared to RF dough ($p<0.05$). Doughs with M260, M150 and RF dough required similar force to extend (Table VIII).

Doughs with 5-15% FSym, M260, M150 required less force to press compared to doughs with 10-15%Frite and WW, which required more force to press compared to RF (Table VIII). Doughs with FiberSym and RF had similar water absorptions (Table VIII). The amount of water required for dough formation was dependent on the type of RS and level of substitution used.

Refined flour (RF) and Fibersym doughs generally required less amount of water and were most machinable (Table VIII). This was due to limited presence of water binding components in these doughs. Fibersym is inert and doesn't participate in intermolecular bonding in food systems (Woo et al. 2009).

Whole wheat (WW) and FiberRite had higher water absorption than refined flour (RF), because pentosans in whole wheat bran have high water binding capacity, the swelling capacity of FiberRite led to the high amount of water required for this RS. This made these doughs less machinable than RF dough.

The Hi-Maize starches (M260 and M150) required the most water. These are physically modified starches and can absorb significant quantities of water. This also negatively impacted their machinability and handling Doughs with FRite and WW had required equal amounts of water to form. (Table VIII). Compared to RF, Doughs made with M260 and M150 required significantly high water absorption and required more water for functionality with increase in substitution (Table VIII). Doughs with FRite, WW, M260 and M150 required more water to have soft and machinable doughs. Subjective tests showed that dough with FiberSym was the most machinable, and required less water and force to extend and low press rating (Table VIII) at all substitution levels. Hence, they were easy to form large discs during dough pressing

Objective dough properties

Dough hardness (N) measured using the dough compression test on a TATX2i was significantly affected by the level of resistant starch ($p < 0.05$). Dough hardness ranged between 113 N (refined wheat dough) and 229 N (whole wheat dough). RF dough and doughs with 5 and 10% RS were similar in hardness (Table VIII). Whole wheat dough and doughs with 15% FibeRite and 15% Hi–Maize 150 were hardest (Table VIII). This agrees with dough subjective evaluation. No clear trend was observed for hardness, probably due to the large error rate associated to this test.

Increased levels of wheat based RS; FiberSym and FibeRite, did not affect resistance to extension. However, increased level of fortification with maize based RS M260 and M150 increased dough resistance to extension (Table VIII). Dough extensibility (mm) was significantly ($p < 0.05$) affected by the type and level of resistant starch. The RS treatments had lower dough extensibility compared to refined wheat flour (RF) dough (Table VIII).

Whole wheat dough was the least extensible of all the treatments as a result of bran particles disrupting the gluten matrix. Pregelatinized RS4 (FRite) had high water holding capacity and hence required larger amounts of water for functionality compared to non-pregelatinized RS4 (Fibersym) which is insoluble and did not compete with gluten for water giving more extensible doughs.

The type of RS and level of fortification used modified the dough properties and markedly affected the tortilla properties as described below.

Tortilla properties

Tortilla properties were significantly affected by addition of resistant starch. Whole wheat tortillas were significantly thinner than tortillas from the other treatments. Refined flour tortillas were thicker due to retention of the gas (Bejosano and Waniska 2004) that was generated during hot-pressing and baking due to formation of a semi-continuous seal across the tortilla surfaces (McDonough et al. 1996), Whole wheat tortillas were thinner because bran particles disrupted the semi-continuous seal across the tortilla surface releasing the gas formed during baking. Tortilla moisture content ranged between 29 – 35.5%. No trends were observed for the moisture content. Whole wheat tortilla and tortillas with 10 – 15% fiberRite had similar specific volume (Table IX). Resistant starch type and level affected the L-value of tortillas. Tortillas with WW had low L-values due to the pigmentation from the pericarp and bran. Tortillas with 15% FSym were lighter than RF tortillas this is due to the white color of FSym, hence at higher substitution level the tortillas were lighter (high L- value). FRite tortillas had similar lightness at all fortification levels (Table IX). This was because the large particle size of FRite broke the gluten matrix releasing the air bubble hence, creating a translucent tortilla surface. L-value for tortillas with M260 increased with increased substitution. Generally RS tortillas had L-values comparable to RF tortillas indicating limited effect of RS substitution on tortilla appearance. M150 tortillas had low L-values that decreased with increased substitution, due to the yellow color of M150 that comes from corn.

Tortilla opacity for the treated tortillas was generally not different from control. However, tortillas with FSym had the highest opacity scores that increased with increase in substitution, this agrees with specific volume that shows that these tortillas had good gas retention and hence high opacity and L-values. Low opacity and L-value scores for whole wheat tortillas were a result of both translucency caused by retention of less air bubbles (Adams and Waniska 2002) and incorporation of light brown bran components in the tortillas.

Table IX

Effects of resistant starch fortification on the physical properties of tortillas[1]

Treatments	**Diameter (mm)**	**Moisture (%)**	**Weight (mm)**	**Height (mm)**	**Opacity %**	**Specific Volume**	**L-Value**
Control, Refined Wheat	164 d	34.1 ab	40.5 b-e	3.00 a	75 cde	1.57 abc	81.5 bc
Whole wheat	160 ef	33.4 ab	39.5 de	2.39 d	49 f	1.25 e	64.3 f
Fibersym + RF							
5	169 b	33.6 ab	40.9 b-e	3.00 a	85 abc	1.64 abc	82.0 ab
10	177 a	31.0 ab	40.2 cde	2.82 bc	88 ab	1.72 ab	82.3 ab
15	176 a	33.6 ab	39.3 e	2.82 bc	90 a	1.74 a	83.4 a
FiberRite + RF							
5	161 e	34.4 ab	41.3 a-d	2.96 ab	74 cde	1.46 cd	81.8 abc
10	158 f	29.0 b	41.9 abc	2.80 bc	84 abc	1.32 de	81.0 bc
15	158 f	35.5 a	43.0 a	2.84 abc	77 b-e	1.29 de	81.3 bc
Hi-Maize260 + RF							
5	167 bcd	33.9 ab	40.3 b-e	2.84 abc	75 cde	1.53 bc	81.5 bc
10	166 cd	34.2 ab	40.2 cde	2.81 bc	69 de	1.51 c	82.1 ab
15	165 cd	34.7 a	40.7 b-e	2.90 abc	80 a-d	1.53 c	82.4 ab
Hi-Maize Flour 150							
5	167 bc	34.4 ab	40.5 b-e	2.96 ab	80 a-d	1.60 abc	80.2 cd
10	166 cd	34.1 ab	40.7 b-e	2.77 c	72 de	1.47 cd	78.6 de
15	165 cd	33.9 ab	42.2 ab	2.85 abc	68 e	1.46 cd	78.0 e
LSD[3]	2.1	5.5	1.9	0.2	12	0.2	1.8

[1] Means from two trials, Values followed by the same letter in the same column are not significantly different ($\rho < 0.05$).

[2] Least significant difference ($\rho < 0.05$)

Tortilla diameters were significantly affected by the type and level of resistant starch used and ranged between 158mm and 177mm (Figure 7). Generally, increase in substitution with FSym resulted in an increase in tortilla diameters by between 2% and 8% compared to control (RF) tortillas (Table IX). This is due to dilution of the gluten matrix. Fibersym does not participate in intermolecular network during mixing and hence, dough made using FSym was easy to spread into larger discs during pressing. Other RS types had minimal effect on tortilla diameter compared to RF tortillas (Figure 7). On the other hand, tortillas with FRite had small diameters and were not significantly different from WW tortillas (Table IX). This could be due their high water absorption properties and formation of hydrogen bond networks that made the dough resistant to extension hence, small diameter tortillas.

Tortilla flexibility/ rollability

The effects of resistant starches on shelf-stability was determined subjectively on a 5 point flexibility scale where 1 = breaks easily and 5 = flexible. Texture analyzer was used to objectively determine the effects of the starches on the texture of the tortillas over 16 day’s storage period.

As expected, tortillas from all the treatments decreased in flexibility over storage time (Figure 8). Increase in RS substitution resulted in rapid decreased in tortilla flexibility due to the decrease in protein content hence, weaker gluten structure. The type of starch used significantly affected tortilla flexibility after 16 days of storage (Figure 8). However, tortillas with 15% FiberSym were still acceptable (≥ 3.0 flexibility scores) after 16 days of storage compared to 15% Hi-Maize tortillas (Figure 8).

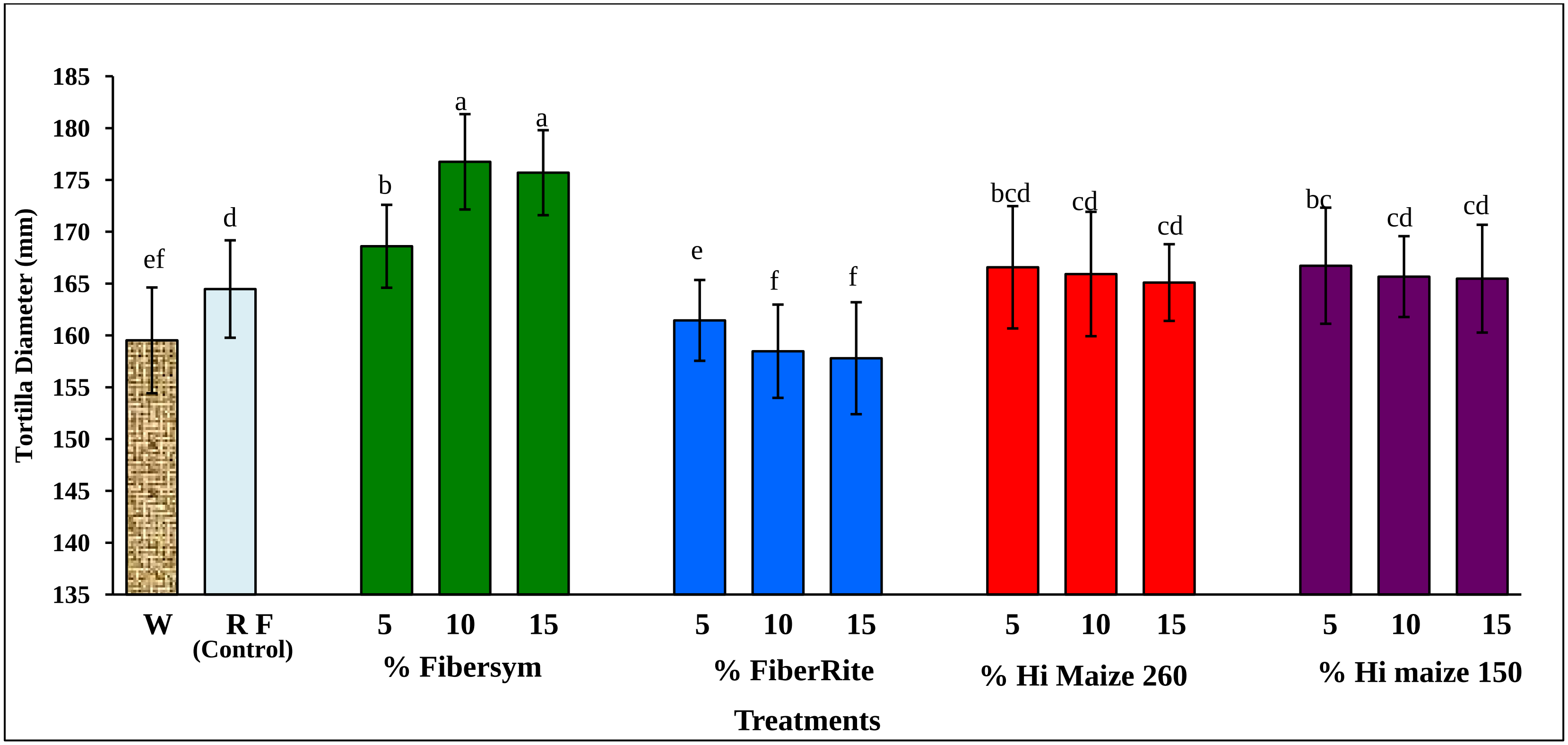


Figure 7. Effect of resistant - starch fortification on tortilla diameters.
Error bars represent ± standard deviation. W = Whole wheat tortillas and RF = Refined wheat flour tortillas (control) .
Bars with the same letter are not significantly different ($p < 0.05$)

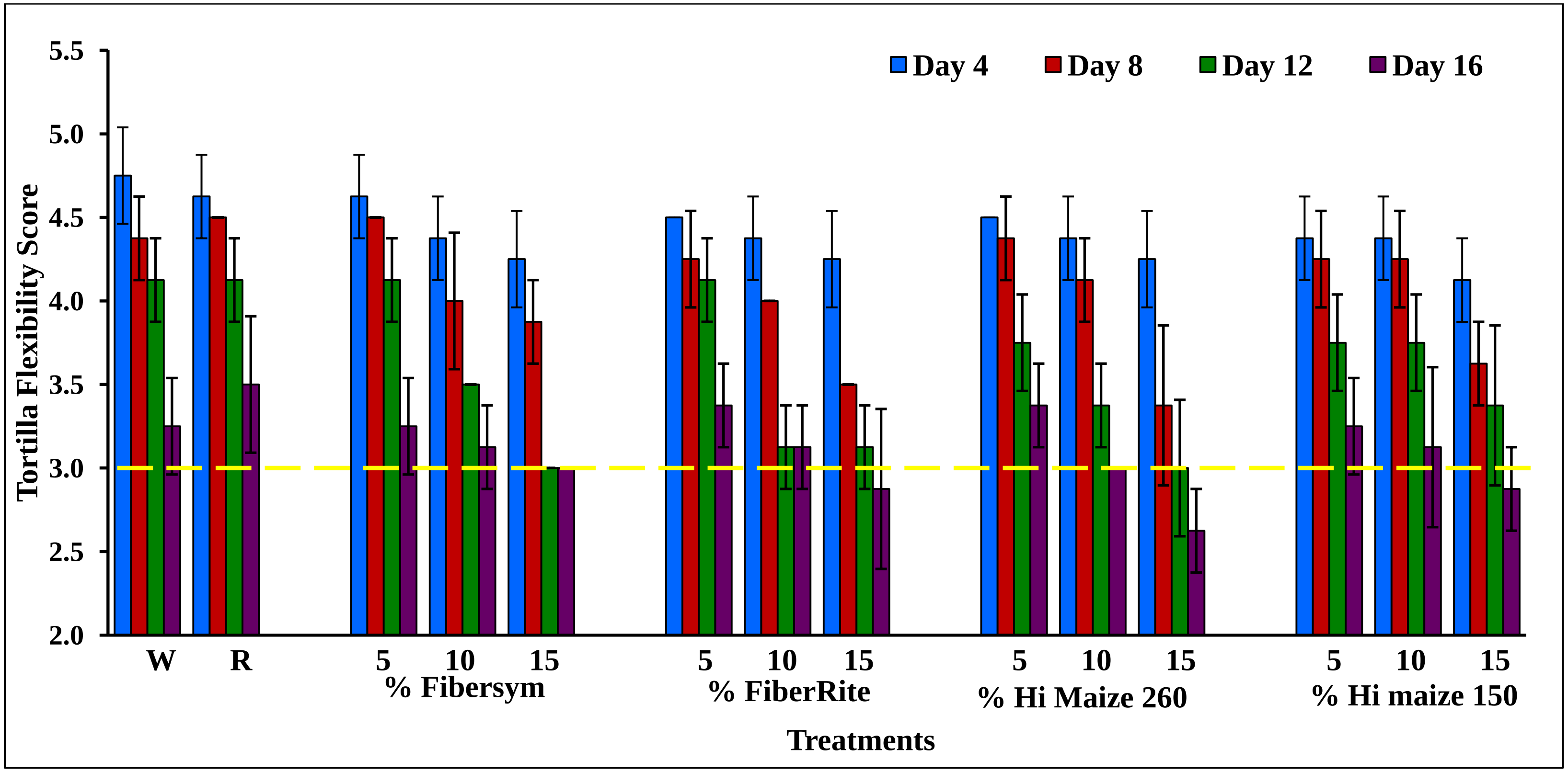


Figure 8. Subjective tortilla flexibility scores for control tortillas and tortillas with resistant starch over storage period (1 = not flexible and breaks easily,5 = very flexible with no cracks). Error bars represent ± standard deviation. Dotted line represents acceptable tortilla flexibility ratings after 16 days of storage. W = Whole wheat tortillas and RF = Refined wheat flour tortillas (control).

Two - dimensional tortilla extensibility

TA-TX2i texture analyzer was used to objectively determine the tortilla deformation modulus, force, distance and work to rupture over 16 days of storage.

Deformation modulus (N/mm) was significantly affected by storage time, resistance starch type and level of substitution ($p < 0.05$). Deformation modulus increased over storage time for all treatments meaning that it required less force to rupture the tortillas overtime due to starch retrogradation. RF and WW tortillas had significantly higher deformation modulus compared to all the RS treatments. This is attributed to the disruptive effect of RS on the gluten matrix hence, easy to rupture (Table X).

The force (N) required to rupture tortillas was significantly affected by the level and RS type over storage (Table XI). Tortillas with 10% and 15% FSym required lower force to rupture than RF on day 0 and day 4 of storage ($p<0.05$) (Table XI). Tortillas with FRite were similar to WW on at both days 0 and 4 during storage (Table XI). On day 8 of storage, 5-10% M260 and 5%M150 were similar to WW. After 12 days of storage 5-15% FRite and 10% M260 required similar force to rupture compared to WW ($p<0.05$) (Table XI).

Table X

Effects of resistant starch fortification on tortilla modulus of deformation[1]

Deformation Modulus (N/mm)										
Treatment	**Day 0**		**Day 4**		**Day 8**		**Day 12**		**Day 16**	
Control, Refined Wheat	0.76	bcd	1.38	a	1.24	a	1.17	abc	1.16	abc
Whole wheat	0.89	a	1.12	b-e	1.17	ab	1.34	a	1.36	a
Fibersym+ RF										
5%	0.66	fg	0.92	f	1.05	abc	1.14	abc	0.95	cd
10%	0.64	g	0.99	ef	1.14	abc	0.85	d	0.95	cd
15%	0.67	fg	0.74	g	0.96	c	0.88	d	0.85	d
FiberRite + RF										
5%	0.71	def	1.20	bcd	1.21	ab	1.24	abc	1.12	abcd
10%	0.77	bc	1.26	ab	1.22	ab	1.27	ab	1.33	ab
15%	0.81	b	1.10	b-e	1.20	ab	1.25	abc	1.09	abcd
Hi-Maize 260 + RF										
5%	0.81	b	1.10	cde	1.12	abc	1.15	abc	1.08	abcd
10%	0.69	efg	1.24	abc	1.11	abc	1.02	cd	1.17	abc
15%	0.77	bc	1.07	def	1.21	ab	1.12	abc	1.03	bcd
Hi-Maize Flour 150 +RF										
5%	0.67	fg	1.07	def	1.03	bc	1.06	bcd	1.05	bcd
10%	0.70	def	1.13	b-e	1.05	abc	1.16	abc	1.06	abcd
15%	0.74	cde	1.13	b-e	1.10	abc	1.05	bcd	1.22	abc
LSD	0.06		0.16		0.20		0.24		0.30	

[1] Each value is the mean of two trials; Values followed by the same letter in the same column are not significantly different ($\rho < 0.05$).

Table XI

Effects of resistant starch fortification on force required to rupture tortillas[1]

Force to Rupture (N)										
Treatment	**Day 0**		**Day 4**		**Day 8**		**Day 12**		**Day 16**	
Control, Refined Wheat	9.7	ab	10.9	a	7.6	ab	7.0	a-d	8.6	a
Whole wheat	9.1	a-d	7.5	bcd	10.9	a	8.2	a	7.9	abc
Fibersym+ RF										
5%	8.9	bcd	6.7	cde	8.1	ab	7.3	ab	8.1	ab
10%	7.1	f	5.8	ef	6.9	b	5.8	cde	6.4	bcde
15%	7.2	ef	4.9	f	5.0	b	5.7	cde	5.9	cde
FiberRite + RF										
5%	9.2	a-d	8.4	b	8.3	ab	8.3	a	8.7	a
10%	9.6	abc	8.0	bc	7.3	b	7.4	ab	8.0	abc
15%	8.7	bcd	6.2	def	6.5	b	7.0	abc	6.2	bcde
Hi-Maize 260 + RF										
5%	10.5	a	6.7	cde	7.8	ab	8.2	a	7.7	abcd
10%	8.2	c-f	8.1	bc	6.5	b	6.3	b-e	6.8	abcde
15%	8.9	bcd	6.6	cde	7.1	b	5.6	de	5.3	e
Hi-Maize Flour 150 +RF										
5%	8.1	def	8.1	bc	6.4	b	6.5	b-e	7.7	abcd
10%	8.4	b-f	6.9	b-e	6.7	b	6.7	b-e	6.1	bcde
15%	8.6	b-e	6.7	cde	5.9	b	5.5	e	5.7	de
LSD	1.5		1.6		3.6		1.4		2.2	

[1] Each value is the mean of two trials; Values followed by the same letter in the same column are not significantly different ($\rho < 0.05$).

Table XII

Effects of resistant starch fortification on distance required to rupture tortillas[1]

Treatment	Distance to Rupture (mm)									
	Day 0		Day 4		Day 8		Day 12		Day 16	
Control, Refined Wheat	19.6	abc	12.5	a	10.3	bcd	10.5	abc	11.3	ab
Whole wheat	14.9	g	11.0	bcd	8.5	e	10.2	abc	10.0	de
Fibersym+ RF										
5%	20.1	ab	12.0	ab	12.1	a	10.8	abc	11.7	a
10%	17.2	def	10.6	cd	10.4	bcd	11.1	a	10.7	abcd
15%	17.1	ef	10.5	cd	9.8	cde	10.7	abc	10.6	bcd
FiberRite + RF										
5%	19.3	a-d	11.7	abc	11.2	abc	10.9	ab	11.3	ab
10%	18.2	b-e	11.3	a-d	10.3	bcd	10.4	abc	10.1	cde
15%	15.8	fg	10.1	d	10.4	bcd	10.2	abc	9.8	de
Hi-Maize 260 + RF										
5%	20.5	a	10.9	bcd	11.3	ab	11.2	a	10.7	abce
10%	18.0	bce	11.0	bcd	10.2	bcd	10.5	abc	9.9	de
15%	17.7	c-f	10.9	bcd	10.4	bcd	9.5	c	9.4	ef
Hi-Maize Flour 150 +RF										
5%	18.5	a-e	12.2	ab	10.7	a-d	10.1	abc	11.1	abc
10%	18.5	b-e	10.3	d	11.0	a-d	10.2	abc	10.1	cd
15%	17.7	def	10.4	cd	9.7	de	9.7	bc	8.7	f
LSD	2.0		1.3		1.4		1.3		1.0	

[1] Each value is the mean of two trials; Values followed by the same letter in the same column are not significantly different ($\rho < 0.05$).

Distance to rupture (mm), the distance over which the tortilla was extended before it ruptured, decreased with increase in RS level, It significantly decreased from day 0 to day 4; but thereafter did not change to the end of storage period (from day 8 to day 16) (Table XII).

RF tortillas required the longest distance to rupture (20 mm) on day 0 while 15% FRite required the least distance to rupture (15.8 mm) at the same timepoint. At the end of storage 10% M150 tortillas required the least distance to rupture (9mm) (Table XII). This means that the tortillas with 15% FRite and 10% M150 were brittle and broke easily at these storage points.

Work to rupture the tortilla (N.mm) was significantly affected by the type and level of starch used (Table XIII). Like force and distance to rupture work significantly decreased between day 0 and day 4 ($P< 0.05$). On the day of production all treatments required high work to rupture because the tortillas were soft and had not undergone excessive retrogradation hence, had an extensive network of gelatinized starch which produced stronger intermolecular bonding among starch, proteins and other components in the tortillas.. Tortillas with 15 % resistant starches, were similar to WW and required similar work to rupture at all storage points ($p < 0.05$) (Table XIII).

Structural differences of the resistant starches significantly contributed to the differences in tortilla properties. The large particle size of Hi-maize corn starches (M150) and M260 contributed to high water holding capacity. However, tortillas with these starches lost moisture at a faster rate compared to RF tortillas. Hence, lower flexibility scores. The corn based starches competed with gluten for water leasing to inferior gluten structure formation. Hence, low tortilla flexibility scores over storage.

Pregelatinized RS4 (FRite) has high water holding capacity and hence required high amounts of water for functionality compared to Non-pregelatinized RS4 (Fibersym) which is insoluble and did not compete with gluten for water. Hence, ease of dough formation and eventually better tortilla structure.

Sensory evaluation

Treatments with fibersym and fiberRite were selected for organoleptic evaluation because these treatments had good dough characteristics, machinability; tortilla flexibility and superior dietary fiber contents compared to Hi-Maize 150 and Hi – Maize 260 which required more water to form dough. An untrained 73 member consumer panel (40 female and 33 males, 40% Caucasians, 23% Hispanics, aged between 18-70 years old with 67% falling in the 18-40 age brackets) evaluated one-day-old tortillas made using refined wheat (control), whole wheat, FiberSym and FibeRite. The consumer evaluated the tortillas for overall acceptability, appearance, flavor and texture on a 9-point hedonic scale 1 = dislike, 5 = neither like nor dislike, and 9 = like.

Table XIII

Effects of resistant starch fortification on work to rupture tortillas[1]

Treatment	Work to Rupture (N.mm)									
	Day 0		Day 4		Day 8		Day 12		Day 16	
Control, Refined Wheat	75	ab	49	a	27	abc	26	a-e	34	a
Whole wheat	56	cd	31	bcd	33	a	31	a	29	abc
Fibersym+ RF										
5%	67	bc	26	cde	33	a	27	abc	32	ab
10%	47	d	22	e	24	a-d	21	c-f	22	bcd
15%	51	d	18	e	17	d	20	def	20	cd
FiberRite + RF										
5%	68	bc	34	bc	32	ab	31	ab	33	a
10%	69	bc	32	bcd	26	a-d	26	abcd	27	abcd
15%	58	bcd	21	e	23	a-d	24	bcdef	20	cd
Hi-Maize 260 + RF										
5%	90	a	25	de	29	abc	32	a	27	abcd
10%	57	cd	32	bcd	25	a-d	22	c-f	22	bcd
15%	63	bcd	25	de	22	bcd	18	f	18	d
Hi-Maize Flour 150 +RF										
5%	61	bcd	35	b	23	a-d	22	cdef	28	abc
10%	64	bcd	25	de	25	a-d	23	c-f	20	cd
15%	61	bcd	25	de	20	cd	18	ef	18	d
LSD	18		9.1		10.2		7.3		9.6	

[1] Each value is the mean of two trials; Values followed by the same letter in the same column are not significantly different ($\rho < 0.05$).

Table XIV

Effects of resistant starch fortification on sensory properties of tortillas*, **

Mean Acceptability Scores n = 73	Refined wheat (RF)	Whole wheat	% FiberSym + RF			% FiberRite + RF		
			5	10	15	5	10	15
Overall Acceptability	6.6	5.5*	6.3	7.0	7.5*	6.8	6.9	6.5
Appearance	7.2	5.9*	7.1	7.2	7.6	7.2	7.2	6.9
Flavor	6.7	5.3*	6.5	6.8	7.3	6.6	7.2	6.7
Texture	6.7	5.8*	7.1	7.8	8.2*	6.5	7.0	6.4

** Values based on a 9 – point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely). Each value is the average from two trials. Each value is a mean from two trials.

* Means are significantly different from control ($\rho < 0.05$).

Whole wheat tortillas had significantly ($p < 0.05$) less acceptable overall appearance, flavor, and texture (Table XIV) similar to previous findings (Friend et al. 1992).This confirms consumer preference of highly refined products relative to whole grain, and hence the need to enrich refined products to provide dietary fiber to consumers. Compared to refined wheat,

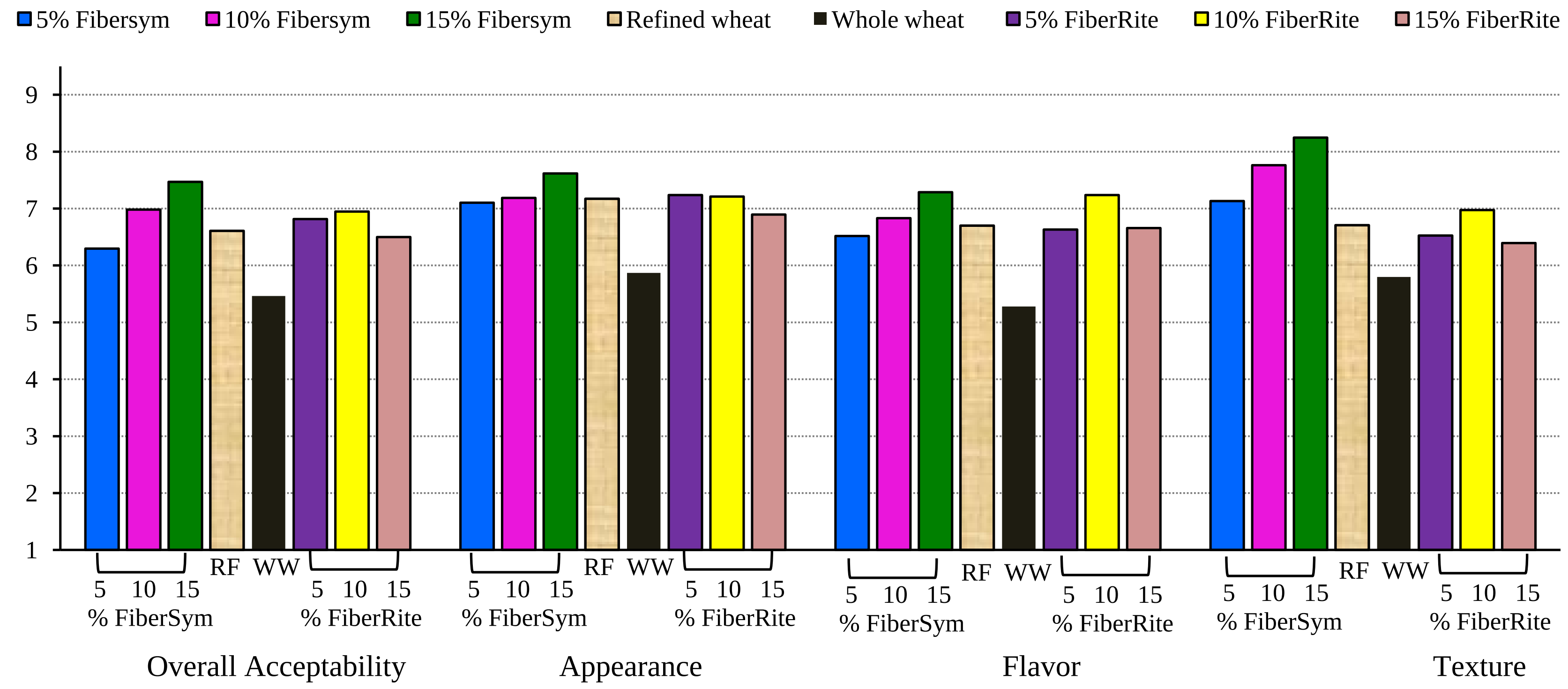


Figure 9 . Sensory evaluation scores for control and resistant – starch fortified tortillas: Based on a 9 – point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely). Each bar represents the average from two trials. Bars with * are significantly different from control ($p < 0.05$). Control treatments: WW = Whole wheat tortillas, RF = Refined wheat flour tortillas.

Tortillas with Fibersym had better texture scores (Figure 9). Tortillas with 15% FiberSym had significantly higher overall acceptability and texture scores than RF tortillas (Table XIV) and hence, good source of dietary fiber in refined flour tortillas. Tortillas with FiberRite had similar sensory properties as for refined flour tortillas. Consumer evaluation confirms that RS substitution did not produce any negative sensory properties in tortillas; this means that resistant starches are a viable means to increasing dietary fiber consumption.

Total dietary fiber (TDF) content

The TDF content of the tortillas varied depending on the RS type used in the formulation. The starches can provide between 43 - 99% TDF. The TDF for tortillas with Fibersym and FiberRite were evaluated using the AOAC 991-43 method whereas for tortillas containing M260 and M150 were calculated based on the manufactures specifications. The TDF values were significantly affected by the type of RS used (Table XII). TDF analysis of the tortilla indicated that 15% Fibersym and FiberRite increased the dietary fiber content in tortillas from 1.9% in RF to 14.3 and 13.6% respectively; these were comparable to the fiber content of whole wheat flour tortillas which had 12.6% dietary fiber (Table XV).

Consumption of tortilla with fiber at 15% substitution provide 3.5, 3.0, 2.4 and 2.0 g / 30 g tortilla respectively for Fibersym^{TM}, FiberRite^{TM} , Hi-Maize 260^{TM} and Hi-Maize^{TM} Corn Flour 150.

Table XV

Total Dietary Fiber (TDF) content of flour and tortillas*

Treatment	Total Dietary Fiber (TDF)	
	Calculated (g/40g tortilla)	AOAC 991:43 (% db)
Refined flour	nc	1.9
Fibersym powder	nc	79.2
Tortillas		
Refined wheat (RF)	2.0	2.8
Whole wheat	4.0	12.6*
% Fibersym + RF		
5	1.2	nd
10	2.3	nd
15	3.5	14.3*
% FiberRite + RF		
5	1.0	nd
10	2.0	nd
15	3.0	13.6
% Hi-Maize 260 + RF		
5	0.8	nd
10	1.6	nd
15	2.4	nd
% Hi-Maize Flour 150 + RF		
5	0.6	nd
10	1.3	nd
15	2.0	nd

[*] Means are significantly different from control ($p < 0.05$, Dunnett’s test).
nd, not determined; nc, not calculated

CHAPTER SUMMARY

Hot pressed tortillas with added resistant starches were consistently round, puffed, white in color. The most important quality attributes of good quality tortilla are large diameter, high opacity (≥70%) and sustained flexibility (≥ 3.0) after 16 days of storage (Waniska et al. 2004). Incorporation of resistant starch produced good diameter tortillas compared to refined flour tortillas (control). Tortillas with Fibersym had the largest tortillas at 15% substitution level.

Increase in substitution with M150 and M260 did not have negative effects on tortilla diameter compared to control. Tortillas made using FiberRite were smaller than control and increased use of FiberRite resulted in reduced tortilla diameter (Figure 7). However, tortilla flexibility was consistently negatively affected by increase in substitution levels for all the four resistant starch sources after 16 days, compared to control. Up to 10% substitution with all the resistant starches produced tortillas that had good flexibility after 16 days of storage. However, at higher level of substitution (15%), Tortillas made using FiberRite, Hi Maize 260 and Hi Maize 150 had inferior flexibility scores (< 3.0) (Figure 8). Tortillas with Fibersym were the most flexible of the treatments with resistant starches. Up to 10% of refined flour can be substituted with RS2 and RS4 without significant changes to tortilla quality. At this level of substitution the TDF per 40 g tortillas: 2.4, 2.1, 1.6 and 1.3g for Fibersym^{TM}, FiberRite^{TM} , Hi-Maize 260^{TM} and Hi-Maize^{TM} Corn Flour 150 respectively. These values are higher than (1.2g) TDF of control (refined wheat flour). However, these levels do not meet the “good fiber source” levels as recommended by the FDA.

Sensory evaluation indicates that the consumers preferred tortillas with 15% Fibersym. Consumers did not detect any negative effects on flavor, texture and appearance. This agrees with shelf stability tests that high levels of FiberSym can be incorporated in tortillas with minimal effects on tortilla flexibility. The Organoleptic evaluation agrees with studies that RS4 can be used to increase TDF in tortillas without negatively affecting tortillas properties (Woo et al. 2009).

CHAPTER IV

CONCLUSIONS

Variations in HMW-GS allele composition affected the protein quality and contributed to differences in the tortilla structure. Wheat lines possessing the following allelic combinations (2*, 17+18, 7, 2+12), (1, 17+18, 5+10), (2*, 17, 2+12) and (1, 2*, 17+18, 2+12) produced flour with 12.8 – 13.3% protein content. These lines produced extensible doughs, which were not elastic resulting in large diameter tortillas (larger than control tortillas) that had superior flexibility after 16 days of storage. This reveals the contribution played by variations in HMW-GS composition on dough extensibility, tortilla diameter and flexibility. Thus, sub-units 1 and 17+18 weakened dough strengthening property of 5+10. Dough weakening effect of 2+12 was strengthened by presence of HMW-GS (2* and 17), (2*,7 and 17+18), and (1,2 and 17+18), on Glu-A1 and B1 respectively. This improved tortilla diameter and flexibility. Wheat lines possessing these allele combinations should be optimized for the production of wheat flour tortillas. On the other hand, wheat lines with (17+18 and 5+10) and (2*, 17+7, 5) produced extensible doughs, large, but less flexible tortillas than control. This means that absence of HMW-GS at GluA1 and HMW-GS 10 at GluD1 is associated with decrease in flexibility over storage.

Wheat lines with (2*, 17+18, 5+10) and (1,2*,7+9,5+10) produced small diameter tortillas with superior flexibility than control. This agrees with findings by Mondal et al. (2008) that the presence of 5+10 is associated with increased dough elasticity hence small diameter tortillas. Presence of HMW-GS’s (1, 2*) and (17+18, 7+9) at Glu A1 and B1, does not decrease dough elasticity, these HMW-GS's provide improved tortilla flexibility over storage.

RS2, whole wheat, and cross-linked; pre-gelatinized RS4 (FiberRite) produced hard, less-extensible doughs and thinner tortillas compared to control, due to high water absorption. Cross-linked RS4 (Fibersym) dough and tortillas were comparable to control; this RS has very low water absorption, thus is physically inert in the dough system, it does not compete with protein and other flour components for water

15% of RS2 and RS4 increase DF in control by 4% and 11.5% respectively, compared to control tortillas (2.8%). Whole wheat tortillas were less acceptable than control in appearance, flavor and texture, while tortillas with 15% Fibersym had higher overall acceptability than control. RS2 negatively affect dough machinability and tortilla shelf stability. However, 15% RS4 improved the DF in refined flour tortillas to meet the Food and Drug Administration (FDA) requirement for "good source of fiber," providing between 10-19% of the recommended daily intake for fiber per serving, without negatively affecting dough or tortilla quality. However, use of RS4 in tortillas may result in increased production costs, this is normal for healthy food products. Cost implications need to be investigated. Cross-linked RS4 has good potential as a vehicle to improve dietary fiber intake in refined wheat flour tortillas.

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APPENDIX A

CONSUMER QUESTIONNAIRE FOR HIGH FIBER FLOUR TORTILLA STUDY

Panelist #_______

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 ☐ Over 80

Gender:
☐ Male ☐ Female

Ethnicity
☐ Caucasian
☐ African American/Black
☐ Hispanic
☐ Asian
☐ Native American
☐Other

About how often do you eat flour tortillas? (soft tacos, burritos, wraps, etc.)
☐ Everyday
☐ At least once a week
☐ Once every two weeks
☐ Once a month
☐ Once a year
☐ Never
Do you suffer from any food allergies?
☐ Yes
☐ No
If you have any food allergies, you cannot participate in this study. Thank you for your willingness to help.

<u>Instructions</u>:
You will be testing <u>five samples</u> of tortillas.
Make sure to use the ballot with the sample number that matches the number of the sample.
Drink water before you evaluate each sample and as needed throughout testing.
Please be sure to answer the questions completely and honestly.

SAMPLE: *Random 3 digit number*

Please check one box that represents your response

1. Please rate your OVERALL ACCEPTABILITY of this sample

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike Extremely	Neither like nor dislike	Like Extremely

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

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike Extremely	Neither like nor dislike	Like Extremely

3. How much do you like or dislike the FLAVOR (taste and aroma) of this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike Extremely	Neither like nor dislike	Like Extremely

4. How much do you like or dislike the TEXTURE (mouth feel) of this sample?

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

Dislike Extremely	Neither like nor dislike	Like Extremely

Additional Comments: ____

Figure A1: Sensory evaluation ballot

VITA

Tom Odhiambo Jondiko received his Bachelor of Science degree in food science and technology from Egerton University-Njoro, Kenya in 2005. He joined the Texas A&M University’s Master of Science program in food science & technology in August, 2008. Prior to which he was a Master of Science student at the University of Missouri, between January and June 2008.

Mr. Jondiko has presented his research work on effects of cross-linked resistant starches and genetic deletions on the quality of wheat tortillas at various international forums including AACC Int. and IFT annual meetings. At the University of Missouri, he researched the cholesterol lowering properties of cereal grains. He worked at the Muscle Foods Processing Laboratory gaining experience in slaughtering and processing of specialty meats.

Mr. Jondiko has worked at established food companies both in USA and in Kenya: Quality innovation team at Kellogg Company from January 2010 to July 2010. Prior to which, He was an innovation product development food science intern at Frito Lay in Plano, Texas in 2009.

In Kenya, he was a research assistant with JOJI consultants. He was a quality assurance employee at the Lake Basin Development Authority (LBDA) rice factory and at Coca cola bottling company in Kenya.

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