

SELECTING WHEAT VARIETIES FOR TORTILLA PRODUCTION

by

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## Abstract

Wheat flour tortillas are the second most consumed bread product after white pan bread. Commercial tortillas are formulated with highly viscoelastic hard wheat flours selected and grown for breadmaking. However, the inherent properties of bread flours require costly formula adjustments to enhance dough extensibility necessary for tortilla production. The objective of this study was to identify the biochemical and physical factors in wheat affecting wheat tortilla quality. Six popular hard winter wheat cultivars (1863, Armour, Clara CL, Denali, Everest, Tiger) were grown in five locations in Kansas. Wheat and flour properties were characterized using approved AACCI methods. Protein composition was determined using size-exclusion high performance liquid chromatography. Flour particle size and starch granule size were measured with laser diffraction. Tortillas were made with a laboratory hot press method. Tortilla shelf-stability over 14 days, opacity, appearance, dough machinability and specific volume were measured. Data collected from flour and tortilla tests were analyzed using ANOVA and means were compared with Tukey-Kramer HSD. In general, the flours did not differ significantly in flour or tortilla properties. Regression analysis (Pearson) showed flour protein content was highly and significantly correlated with tortilla opacity ( $r=-0.81$ ), L color value ( $r=-0.79$ ), a color value ( $r=0.80$ ), and day 14 shelf-stability ( $r=0.76$ ). Flour water absorption showed highly significant correlations with tortilla opacity ( $r=-0.81$ ), L color value ( $r=-0.79$ ), a color value ( $r=0.77$ ) and day 14 shelf-stability ( $r=0.73$ ). Tortilla opacity was highly correlated with B-type starch granules ( $r=-0.83$ ). This study showed that starch granule size, flour protein content and flour water absorption appeared to influence tortilla appearance. However, repeating the study with a larger and more diverse sample set is recommended.

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## **Dedication**

To the memory of my grandfather, C. Marshall Dann. Although he passed away over a decade ago, he remains profoundly influential in my life. Professionally, my grandfather was an accomplished chemist, patent attorney and patent commissioner. Although I knew him more as the as a very occupied retiree. During this time, he was an avid member of his choir, church treasurer, ancient languages teacher, pianist, world traveler and most importantly, bread baker. I am indebted to him for my college education, pursuit of knowledge, love of science and sheer determination. If he were still alive, I know he would be highly interested in my studies. He would rejoice in having a companion to discuss polymers over dinner. Sorry I arrived to the table a bit too late. Though he understood that some reactions take time, patience and dedication.

## Chapter 1 Introduction

Once considered an ethnic specialty, tortillas have become a popular component of mainstream American diets. The highly versatile nature of tortillas contributes to their position as the main competitor to white bread, outselling white sandwich bread in 2010. Sold in retail stores, restaurants and as a component of the frozen food market, tortillas represent a significant market for U.S. wheat utilization. The tortilla market has sustained annual growth of 9% since 1996 with U.S. sales exceeding \$11 billion in 2012. Wheat flour tortillas represent approximately 45% of the total tortilla market, with corn tortillas representing the remainder (Kabbani 2013).

Hard winter wheat, the major wheat class grown in Kansas, has been bred to produce flour that is appropriate for breadmaking. Ideal bread flours produce doughs with high elasticity and good extensibility. The majority of the research in hard winter wheat quality has focused on improving wheat quality for bread production while limited research has focused on the functionality requirements for optimal tortilla quality. Desirable characteristics of tortillas include large diameter, high flexibility, opacity, light color and long shelf stability. Flour for tortillas should produce doughs that are highly extensible with a resilient gluten network that retains its flexibility in the final product (Waniska 1999). The biochemical properties of starch and gluten protein play significant roles in tortilla quality. Starch affects shelf-stability (rollability) and contributes to textural properties while final tortilla diameter is controlled by interactions among gluten proteins (Alviola et al 2008). Thus, desirable tortilla flours produce doughs with greater extensibility, but lower elasticity compared to bread flours. Although the dough requirements are different, the tortilla industry currently uses flours developed for breadmaking. Consequently, costly adjustments in formulation and processing are often needed to achieve desired characteristics. In order to develop wheat lines more suitable for tortilla production and to benefit all sectors of the industry, an understanding of flour chemistry influencing tortilla quality is essential.

## **Objectives**

1. Systematically evaluate the tortilla making performance of several Kansas State University (KSU) developed wheat varieties to identify those that are more desirable for tortilla making.
2. Identify the biochemical and/or physical factor(s) in wheat that affect flour tortilla quality.

## Chapter 2 Literature Review

### Tortilla History

Tortillas are traditionally an unleavened, round flatbread originating from Central America. Tortillas are made from nixtamalized maize or wheat flour. The maize tortilla was developed by Mesoamerican civilizations. The masa was prepared from a coarse cornmeal blended with water, after which, the resulting dough was flattened and steamed or baked on stones (Smith 1999). In contrast, wheat flour tortillas are made from dough consisting of flour, fat and water. The dough is shaped by flattening and hand-stretched before baking. While maize tortillas are more traditional and the older of the two styles, wheat flour tortillas are particularly popular in parts of northern Mexico and the southwestern United States. In the U.S. market, wheat flour tortillas evolved from being a minor ethnic specialty to a highly popular bread product with current sales competing with white pan bread. This review focuses on hot-pressed wheat flour tortillas, the most common commercial tortilla production style. The aim of this review is to elaborate on the evolution and standardization of wheat flour tortillas, their production methods and composition.

Led by Hernán Cortés in 1519, the Spaniards introduced many foods and cooking techniques to the Aztecs. The most important being wheat and frying techniques which would eventually be used to produce wheat flour tortillas (Smith 1999; Guerra 2006). The Spanish acknowledged the maize flatbreads made by the indigenous peoples and later named them tortillas, meaning 'little cake' in Spanish. Spaniards preferred wheat to maize due to its familiar taste, its importance in European history and theology.

Beyond the introduction of wheat to the New World, there is much speculation around the popularization of the wheat tortilla. The first printed recipe for wheat tortillas traces back to 1914 in the *California Mexican-Spanish Cook Book: Selected Mexican and Spanish Recipes* by Bertha Haffner-Ginger (1914), but they existed long before this

printing. One suggestion is that a minority of Jewish families fleeing the Spanish Inquisition settled in northern Mexico and replaced maize with wheat. Whether practicing their faith clandestinely amidst a predominantly Catholic population or preserving their traditions as Catholic *conversos*, Jews prepared tortillas from wheat flour since maize is not considered kosher during Passover. Also, flour tortillas share some similarities with *matzah*, a traditional, unleavened flatbread eaten during the Passover (Guerra 2006). While the true origins of wheat tortillas are vague, their development is most likely due to better availability of wheat at lower costs than maize and a taste preference for wheat in certain areas of Central America, northern Mexico and southwestern United States.

Since the 1970's the availability and the consumption of tortillas in the U.S. expanded beyond the southwestern borders, in conjunction with the distribution and flourishing of the Hispanic population throughout the U.S. The U.S. Census Bureau reported that the Latino population increased from 14.6 million in 1980 to a staggering 52.0 million in 2010 (U.S. Census Bureau 2011). While a majority of this population is highly concentrated in the Sunbelt states in the U.S., the Latino population increased in northern and central U.S. regions as well. Schmidt elaborated on how his U.S. based tortilla operations expanded across the U.S. (Schmidt 1985):

“By the mid-1960's, the Hispanic population in Southern California was growing, and our company's 900-plus home-service salesmen were requesting corn tortillas and wheat flour tortillas, as well as fried corn snacks for distribution in the Hispanic communities. The natural evolution was to purchase a captive Mexican tortilla factory in 1967. The event started my wan long, personal association with the development of the production processes relating to these ethnic products”.

The Latino presence created a demand for ethnic ingredients and prepared foods. Large supermarket chains in the middle-south and southwest installed small flour tortilla lines in their on-premise baking facilities (Janson 1990). Latinos introduced and popularized tortillas in the form of burritos and enchiladas to non-Hispanic consumers as more Mexican fast food and sit-down restaurants were established across the

country. Tortilla sales increased in non-Mexican restaurants and in other food service institutions such as schools and hospitals (Steinberg 1994).

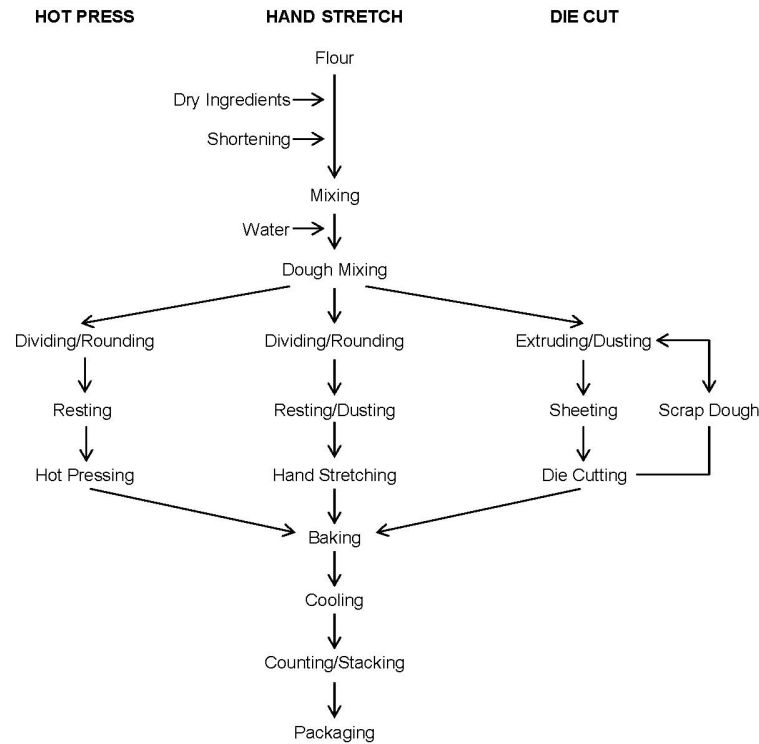
While the tortilla market expanded, the general American perception of the product was misaligned. Most Americans viewed tortillas only as a “spicy Mexican snack food” (Steinberg 1994). American taste buds were not adapting to maize tortillas due the textural and flavor changes maize undergoes during the nixtamalization process. Steinberg mentions, “When someone in this audience figures out how to produce corn tortillas that taste like corn, he or she will find unlimited horizons for growth” (Steinberg 1994). As a fresh bread product, wheat tortillas attracted a bigger audience in the U.S. with American palates more accustomed to white pan bread.

With an increasing demand for wheat tortillas, tortilla formulations, appearance and production methods were altered to increase their supply. The Tortilla Industry Association (TIA) formed in 1989 to promote and represent the interests of the burgeoning tortilla market. The organization addresses management, regulation, quality control, distribution methods and provides educational support for commercial tortilla manufacturers. TIA states that in addition to promoting tortillas, they seek “alternative uses of tortillas in mainstream American fare, such as sandwiches, soups and desserts”. Formulation and appearance of commercial tortillas has evolved from a quite simple to complex system. Most supermarket tortillas contain more than flour, water and fat to create tortillas that are marketed as ‘chewy’ or ‘soft’ with long shelf-stabilities. Tortillas in the grocery aisles tend to be white in color with an even distribution of toast spots, are round in shape and are marketed under vague terms such as “restaurant”, “fajita” and “cantina” style. With tortillas sales exceeding \$11 billion in 2011, American consumers appear to embrace these changes.

## **Production Methods**

The three commercial tortilla production methods include die-cut, hand-stretched and hot-pressed (Figure 2.1). Each technique yields slightly different tortilla qualities and has different end-use applications.





**Figure 2.1 Production Schemes for Hot-Press, Hand-Stretch and Die-Cut Tortillas (Serna-Saldivar et al 1988)**

### ***Hand-Stretching***

Hand-stretched tortillas are the most traditional of the three processes, but also the least common. Despite the inferred name, this method is mostly automated. Dough is divided, rounded and given an intermediate proof. The individual dough balls are sheeted into a thin, circular shape and receive a final hand-stretching prior to the final baking step (Pylar and Gorton 2009b). The tortillas are characterized by a “homemade” or irregular shape, are dense in structure and feel slightly powdery from the additional dusting flour required for manual processing and sheeting (Janson 1990; Kraut 2006). They are often consumed as table tortillas or processed into fried products such as sopaipillas and chimichangas (Pylar and Gorton 2009a). As commercial tortilla production methods increased in the U.S., hand-stretching grew at the slowest rate due to the higher costs associated with a labor-intensive process and an increased need for sanitation (Serna-Saldivar et al 1988).

### ***Die-Cutting***

In contrast, die-cut tortillas have the shortest production time, in addition to lower labor requirements and waste levels. With the die-cut method, the dough is extruded onto a conveyor belt where it is cross-rolled and sheeted to the desired thickness. Then the dough is die-cut into an oblong shape that will shrink back to a circular shape prior to baking (Schmidt 1985; Pyle and Gorton 2009b). Discarded dough can be recycled back into the sheeting step to reduce loss. The resulting tortillas are powdery from dusting flour, but also characteristically tough, chewy, dense and thick. Their sturdiness makes them suitable for fried salad bowls and frozen entrees (Serna-Saldivar et al 1988; Pyle and Gorton 2009a). Die-cut tortilla quality is often considered the most inferior of the three methods.

### ***Hot-Pressing***

As the tortilla industry expanded in the 1980's, the hot-press method grew the fastest. Currently, 95% of commercial tortillas are hot-pressed, compared to 2% hand-stretched and 3% die-cut (Kabbani 2013). In the hot-pressed method, dough is extruded into balls, which are stamped in a heated hydraulic press before baking. These tortillas have higher quality and functionality than those produced from the other methods. Resulting tortillas are distinctively smooth, pliable, sturdy and used in various dishes, sandwich wraps and other portable meals. Hot-pressed tortillas are characterized by their soft, tender, layered texture.

## **Analytical Methods for Determining Quality**

Tortilla quality standards are not as well defined in comparison to other common commercially manufactured bread products. Research on tortilla quality is in its relative infancy, progressing for 30 years only with the majority of research efforts being performed at Texas A&M University. Currently, commercial tortilla quality varies greatly not only among brands, but also within tortillas of the same brand and style (Mao et al 2002). The primary determinants for tortilla quality are flexibility, opacity and size (diameter and thickness). While all quality tests can be performed subjectively, several

objective tests have been developed to quantify common quality attributes such as appearance, texture, flexibility and machinability.

Bello et al (1991) analyzed tortilla dough rheology and handling characteristics on a hedonic scale system rating machinability, pliability, viscosity, stickiness, appearance and texture. They found that tortilla dough requires less water, resulting in drier, firmer dough in comparison to pan bread dough and is characterized as a “pliable dough...capable of being easily molded or shaped” with “a smooth appearance and silky touch or feel” (Bello et al 1991). If the dough is too stiff, it appears to be heterogeneous, dry and often results in small tortillas. In contrast, wet dough is difficult to machine and sticks to the hands and hot platen surfaces during pressing. Methods for determining the optimal amount of water and mix time for dough development specific to tortilla formulation involve using either a modified farinograph method or the mixograph. Water is normally added to center the peak at 500 FU using a farinograph, however for tortillas, the peak is modified with its center at 750 FU (Bello et al 1991).

After tortillas are pressed and baked, the most basic measurements taken to evaluate quality include diameter, thickness and weight. The diameter should be taken across the center of the tortilla in a minimum of two places to account for any irregular symmetry. Thickness can be measured with a digital caliper on individual tortillas or as an average of several tortillas stacked upon one another. Baked weight is important to collect to ensure consistency in scaling, rounding and baking. With these physical measurements, the specific volume of a tortilla can be calculated to quantify spread and height (Figure 2.2). Specific volume values are helpful for comparing tortillas varying in size and among independent studies.

$$\text{Specific Volume } \left(\frac{\text{cm}^3}{\text{g}}\right) = \pi \left(\frac{\text{diameter}}{2}\right)^2 \times \text{height} \times \frac{1000}{\text{weight}}$$

**Figure 2.2 Specific Volume Equation**

Desirable tortillas are opaque, light in color, round, symmetrical and possess smooth edges and uniform toast spots. Opacity and translucency are slight misnomers for describing a common visual trait in tortillas. While those terms actually characterize the amount of light passing through an object, in tortilla ‘translucent’ and ‘opaque’ apply to the spectrum of light to dark coloring seen. A ‘translucent’ tortilla is gummy and dark or yellowish in color, while ‘opaque’ tortillas are bright white. Opacity is considered desirable because consumers perceive translucency as a characteristic of tortillas that are under-baked or high in fat (Alviola and Awika 2010). The color contrast is often subjectively scored on a gradient scale from 0 (translucent) to 10 (opaque). Alviola and Awika (2010) found that the *L* value (whiteness) obtained using a chromameter correlated highly ( $r=0.96$ ) with subjective opacity scores from experienced evaluators, making it a rapid and objective method for measuring opacity in tortillas. In typical flour tortillas, *L* values range between 75 (darker, more translucent) to 90 (whiter, more opaque). Measurements should be taken in multiple places on both sides of the tortilla to account for color variation between the two surfaces. While whiter tortillas are preferred, the presence of toast spots is considered an appealing and distinctive tortilla trait. Toast spots are dark brown patches on the tortilla. They occur because blisters that result from leavening reactions cause certain areas to come into direct contact with hot oven surfaces and therefore, undergo more aggressive browning. Toast spots have been measured using digital visual analysis software (Kim and Flores 1999; Mao and Flores 2001; Mao et al 2002).

Several tests have been designed to quantify tortilla flexibility and elasticity to simulate their performance in food preparation. These tests are commonly performed in combination with shelf-life studies to describe staling rates. The roll test involves wrapping a tortilla around a 1 cm diameter dowel and evaluating its structural integrity

on a scale from 1 (cracks heavily) to 5 (flexible, no cracks) (Friend et al 1995). Suhendro et al (1998) developed an objective roll test for corn tortillas by measuring the amount of force and work to roll the tortilla using a texture analyzer. Assessing the amount of force, distance and work to rupture the tortilla using the texture analyzer has also been applied to quantify elasticity or stretchability (Mao et al 2002). While all three methods describe flexibility and extensibility, only the latter two objectively quantify these properties. The sensitivity of measuring tortilla textural changes over time caused by staling varies among the three methods. Both methods using the texture analyzer were found to effectively measure these changes up to five days after baking, beyond which differences are not as apparent (Bejosano et al 2005). The subjective roll test is more sensitive to staling up to 16 days after baking, yet requires panel training to ensure both consistency and accuracy. Alviola and Awika (2010) compared the roll and extensibility tests. They found that objectively determined rupture distances measured using the extensibility test had a good potential to replace the subjective roll test. Additionally, the extensibility test predicted tortilla shelf-stability after two weeks by using measurements obtained on day four. However, other studies have shown that correlations between objective and subjective rheological tests are not high enough to indicate either method alone is sufficient to characterize the staling process (Suhendro et al 1998; Bejosano et al 2005; Alviola and Awika 2010).

Sensory evaluation using a combination of descriptive analysis and consumer acceptance panels is an alternative method for evaluating tortilla quality. Due to the high cost of training panelists for descriptive analysis, few studies have been conducted and published on tortilla quality. Consumer panels would shed light on which tortilla attributes are essential to consumer acceptance. In combination with descriptive analysis, the levels of these attributes can be measured to set standard targets in tortilla quality.

Chemical analysis of tortillas in regards to quality is also rarely published within the literature unless a novel formulation is being tested. Bello et al (1991) measured pH, moisture, crude fat and crude protein. pH values are crucial for gauging the effects of

leavening and food-grade acidulants throughout baking and shelf-life (Cepeda et al 2000; Adams and Waniska 2002).

Manufacturers must set their own targets for desired attributes to maintain quality and consistency of their product. Some manufacturers have incorporated significantly sophisticated visual inspection systems to quantify, sort and reject tortillas based on physical properties and processing defects including: diameter, toast spots, color, folds, bites and doubles (Montrose Technologies Inc. 2010). Tortilla attributes will likely become more standardized as production continues to increase and as conglomerated bakeries purchase smaller bakeries, in accordance with the current trend in the baking industry. These analytical tests provide standard rapid and easy evaluation methods essential to monitoring tortilla quality within the industry, in addition to providing a common language for describing quality between industry and researchers.

### **Hot-Press Tortilla Preparation**

Tortillas are made by forming a modified colloidal system similar to pan bread. The gluten formed during dough mixing forms an elastic film that holds starch granules together (He and Hosney 1991). Handling, machinability and flexibility hinge upon formation of this continuous network. Low water absorption yields a stiff dough which is difficult to press. High water absorption yields tortillas with a silky, soft texture with many layers, but can cause dough machinability problems due to stickiness (Lallemand 1996). Water content is typically determined with the farinograph by centering the absorption peak at 750 Farinograph Units (FU) (Bello et al 1991), which roughly corresponds to 50-60% water (fwb). Doughs are mixed to optimum development. Under-mixed doughs produce tortillas of varying quality with characteristically small blisters (Bello et al 1991).

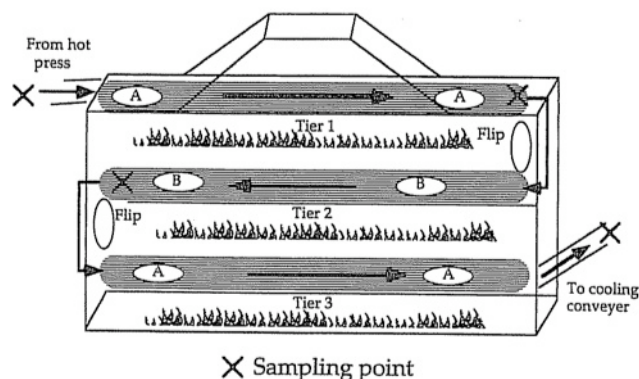
The large dough mass is divided and rounded into balls, often with an extruder. Common dough ball weights are 25 g for fajita or snack size and 60 g for larger style tortillas. A resting period usually follows the dividing and rounding stage. Albeit the reported proofing stage lengths vary greatly, from two minutes to one hour (Whitaker 2004). Bello et al (1991) deemed 20 minutes acceptable in a 60-70% RH, 32°C environment.

The pressing step involves moving the relaxed dough pieces into a heated, hydraulic press. Press plate temperatures are between 177 to 232°C. Tortillas are pressed for approximately one to two seconds. During this stage, the heated plates dehydrate both surfaces of the dough disk and the starch and gluten contract to form a semi-continuous skin on both external surfaces. This skin limits the release of carbon dioxide and steam to enhance puffing during the bake stage (McDonough et al 1996). Dwell time and pressure in the press affect both leavening rates and final tortilla quality (Adams and Waniska 2005). Scanning electron microscopy images of a tortilla dough disk depict intact small and large starch granules after pressing, indicating that water flashes off the dough surface instead of gelatinizing the starch (McDonough et al 1996).

The disk is immediately transferred to a three-tiered oven (Figure 2.3) heated to a range of 190 to 260°C. The tortilla moves from the top to the bottom in the oven within 25 to 40 seconds. After passing through the first tier, the tortilla expands (puffing) as steam and carbon dioxide are released. Starch granules in the interior of the dough disk begin to deform, indicating the onset of gelatinization. Starch granules in the interior and on the surface of disk still possess their birefringence, indicating the granules are not fully gelatinized. When the tortilla flips onto the second tier, the opposite side begins to expand from steam and leavening reactions. Small and large air bubbles create voids in the gluten matrix. The starch granules in the center of the disk continue to gelatinize. On the third tier, the tortilla is flipped back to the first baked side, the starch granules on the surface partially gelatinize and the gluten network firms (McDonough et al 1996). Browning reactions take place through conduction from the hot surfaces of the baking plates or mesh bands, creating “toast points”. The degree of toasting can be controlled by modifying the oven burner setting (Pylar and Gorton 2009a).

After leaving the oven, the tortilla cools to ambient temperature on a multi-tier conveyor, augmented with large fans (Whitaker 2004). The puffed tortilla deflates shortly after leaving the oven. The voids created from leavening reactions and steam yield the characteristic layered, flaky texture found in tortillas. Insufficient cooling before packaging degrades tortilla quality. If moisture condenses within the package, tortillas

tend to stick together (zippering) and the risk of mold growth increases. Commercial tortillas are packaged in plastic bags to limit further moisture loss.



**Figure 2.3 Three-tiered Tortilla Oven** (McDonough et al 1996)

## **Formulation Ingredients and Functionality**

The traditional tortilla formulation includes water, flour, lard and salt. These tortillas tend to have a short shelf-life (2-4 days) and are deemed a daily bread, which is made and consumed on the same day. However, U.S. consumers expect tortillas to last for a week to several months. As a result, industrially manufactured tortillas are formulated with alternative fats, hydrocolloids and mold inhibiting ingredients to enhance the tortilla making process and improve quality over a longer period of time (Dally and Navarro 1999). The following is a summary of common ingredients included in commercial tortillas. Wheat flour, the primary tortilla ingredient, will be thoroughly discussed in the next section.

### **Water**

Water is an important main ingredient needed to process and incorporate dry ingredients into a dough. Hydrating the gluten protein, in addition to adding mechanical energy input, will give rise to a continuous gluten network and will yield the unique rheological properties of the dough. Water levels usually fall between 45-55% (fwb), but vary depending on the hydration requirements of the flour, the amount of shortening and the presence of reducing agents. This range is low in comparison to bread dough, but tortilla doughs should be firmer for easier handling and pressing.



### ***Fats and Shortenings***

Fat primarily functions to lubricate the gluten matrix and soften and tenderize the final tortilla (Mao et al 2002). While lard is the traditional fat, most modern industrial formulations use hydrogenated vegetable shortening due to the undesired taste imparted by lard and health concerns associated with lard's high levels of trans-saturated fats. Levels of shortening usually fall between 3 to 15% (fwb). Increasing the level tends to have a positive effect by improving tortilla dough machinability, increasing tortilla flexibility and diameter (Bello et al 1991; Srinivasan et al 2000). Better dough machinability is the result of lipid-protein or lipid-starch complexes formed during mixing. Longer shelf-stabilities result because staling reactions are delayed through modifications to starch interactions (Dally and Navarro 1999). The use of soybean and palm oils in the formulation produced tortillas comparable to shortening. However, liquid fats produced doughs that were too soft and required further formula readjustments (Bejosano et al 2006).

### ***Salt***

Salt is added to the formula at 1.5 to 3.0% (fwb). Salt enhances the flavor, strengthens the dough, improves dough machinability and lengthens shelf-stability. The water binding capacity of salt lowers water activity rates, thus the free water for microbial reactions is reduced. The strengthening and toughening effects of salt on the dough is presumed to be the result of ionic shielding charges on the gluten forming proteins (Serna-Saldivar et al 1988).

### ***Leavening***

Leavening agents are added to the formula to create the unique tender, layered textures caused by puffing. The gas cell structure imparted by chemical leavening agents is the result of acids neutralizing the salt of a bicarbonate, through which carbon dioxide is released (Heidolph 1996). The solubility of the acid is dependent on a series of conditions including the amount used, hydration rates, dough pH and temperature. Normally 1 to 2% (fwb) leavening agents are included in the formula. The varying effects of leavening agents on tortilla processing and quality were studied by Cepeda et al

(2000), Adams and Waniska (2002) and Bejosano and Waniska (2003). Slow-acting leaveners such as sodium aluminum phosphate (SALP) and sodium aluminum sulfate (SAS) are commonly used because they react during baking. Aluminum bicarbonate (ABC) and sodium bicarbonate (SBC) can be used interchangeably, although ABC was shown to lengthen flexibility over time (Bejosano and Waniska 2003). Simply adding a higher level of leavening agents to the formula was thought to improve opacity because gas cells diffract light. However, research into leavening has shown that optimizing the amount of acid, rather than increasing the levels of leavening agents, indirectly improves opacity (Cepeda et al 2000; Adams and Waniska 2002).

### ***Reducing Agents***

L-cysteine is a reducing agent commonly added at 20 to 30 ppm (fwb) to the formula to shorten mix time and improve dough extensibility. The sulfhydryl group in L-cysteine chemically disrupts disulfide bridges from forming between gluten-forming proteins during mixing and prevents them from reforming during proofing (Wieser 2012). The advantages of incorporating L-cysteine include shorter dough mixing, better handling, larger diameters and thinner tortillas. The disadvantages include shortened shelf-stabilities and the need for stringent controls on water addition due to the effects of enhanced extensibility (Friend et al 1995; Srinivasan et al 2000).

### ***Mold Inhibitors and Antimicrobial Agents***

Because tortillas have a relatively high water activity, usually values at 0.88, they are susceptible to mold growth (Pyler and Gorton 2009a). The shelf-life of tortillas can be extended with the addition of antimicrobial agents and mold inhibitors. The salts of propionates or sorbates are added at levels of 0.3 to 0.6% (fwb). Both additives are soluble in water and are often applied as an aqueous spray after baking, but can be added to dry ingredients as well. Friend et al (1995) found that these agents perform optimally at a dough pH of 5.5 for calcium propionate and 5.8 for potassium sorbate. Acidulants such as fumaric acid are added to reduce the pH and enhance the effectiveness of antimicrobial agents. Sorbate and propionate levels should be minimized because even at low levels, they impart bitter flavors.

### ***Dough Conditioners***

Emulsifiers, commonly monoglyceride or sodium stearoyl lactylate (SSL), are added to improve dough machinability and processing. SSL is commonly used in bread systems to optimize numerous properties, one of which is delayed staling. The mechanism behind this property consists of the emulsifier binding to proteins during mixing. When proteins denature during baking, the emulsifier complexes with amylose to delay retrogradation (De Stefanis et al 1977). The level of SSL in tortillas often falls between 0.25 and 0.5% (fwb). The maximum level allowed in the U.S. under GRAS status is 0.5% (fwb). Friend et al (1995) studied the effects of both monoglyceride and SSL in tortillas and concluded that while both improved dough machinability, emulsifier type did not improve flexibility over time. High levels of monoglyceride resulted in gummy, translucent tortillas. Akdogan et al (2006) studied emulsifiers in whole wheat tortillas and found SSL increased rollability scores (flexibility) at 0.125% (fwb), but higher levels had deleterious effects on tortilla diameters.

### ***Minor Ingredients***

Many minor ingredients are commonly incorporated into tortilla formulations. Yeast and dry milk are added to enhance flavor. Sugar enhances flavors, in addition to promoting browning or toasting during baking. Hydrocolloids, such as carboxymethyl cellulose (CMC), might improve shelf- and freeze-thaw stabilities (Friend et al 1993). Vegetable powders may be incorporated to impart color and flavor.

Numerous other studies have explored improving tortillas nutritionally by incorporating flours from grains other than wheat including legumes, whole wheat flour and zero trans-fat (Gonzalez-Agramon and Serna-Saldivar 1988; Friend et al 1992; Seetharaman et al 1994; Torres et al 1994; Serna-Saldivar et al 2004; Anton et al 2009; Barros et al 2010). Alternative formulations exhibit mixed results on tortilla quality. However, there is increased consumer interest in novel tortilla products as the tortilla market expands.

## **Wheat and Flour Chemistry and Quality**

Wheat flour constitutes approximately 90% of the tortilla's dry ingredients. Therefore, wheat and flour quality greatly influence tortilla quality. However, wheat composition and chemistry is complex. Minor variations occurring in wheat due to environmental, genetic and physical stresses can greatly alter grain quality and its intended use. Bakers depend on consistency of flour to produce consistent quality end products and obtain flour with certain specifications (ash, protein, falling number, etc.) to ensure such consistency. Specifications are not well defined for consistency in tortilla production. The following summarizes previous research on the effects of wheat and flour composition on tortilla quality.

### ***Wheat Kernel Composition***

*Triticum aestivum* L. is the hexaploid cereal grain commonly known as wheat. The grass bears a single-seeded caryopsis called the kernel. Physically, the wheat kernel is rounded on the dorsal side, possessing a longitudinal crease on the ventral side (Delcour and Hoseney 2010a). Wheat is considered a 'naked' grain because its hull is lost during threshing. Wheat is classified by the amount of force to crush the kernel (indicating hardness or softness), color (red or white) and growing season (winter or spring).

The kernel can be divided into four main anatomical parts: pericarp, aleurone, germ and starchy endosperm. The pericarp consists of multiple compressed, protective layers encompassing the three-layered, pigment-containing seed coat. The hyaline layer is tightly bound to and lies between the seed coat and aleurone. The aleurone, the next inner layer, is a single layer of cells with thick walls. The aleurone surrounds the largest portion of the kernel, the starchy endosperm. The endosperm is a complex matrix of cells containing the storage proteins (gluten) and starch. The shape, size and wall thickness of the cells varies from the subaleurone layer to the center, becoming more irregular towards the heart of the kernel. The cell walls contain arabinoxylans that possess gel-forming properties, which are vital for dough development. Gluten, gliadin and globulins are first deposited as protein bodies. Large lenticular starch granules form

during kernel synthesis first. As the kernel matures, the protein bodies compress the large starch granules and small spherical starch granules form and fill in any voids in the matrix (Delcour and Hosney 2010a). Endosperm texture, either hard or soft, is a genetic trait. Friabilin is a protein complex found on starch granule surfaces in soft wheat. Friabilin contains two isoform polypeptides, puroindoline-a and puroindoline-b. The lack of either puroindoline is assumed to result in hard wheat (Gooding 2009). Hardness affects milling conditions, flour yield, starch damage, particle size and flour end use suitability for cakes, biscuits, bread, noodles, tortillas, etc.

Several studies show that tortillas produced from soft wheat are significantly different from those produced from hard wheat (Wang and Flores 1999a; Wang and Flores 1999c; Waniska et al 2004; Ramirez-Wong et al 2007). Hard winter wheat yields better quality tortillas that exhibit increased flexibility, extensibility and shelf-stability. Hard winter wheat is considered ideal, possessing moderate protein levels and starch damage content optimal for rheological dough formation and baked product quality. Soft wheat flour tortillas tend to be dry, crack easily and have limited extensibility if their formulation is not modified (Wang and Flores 1999a).

### ***Tortilla Flour Specifications and Production***

The baking industry relies on the milling industry to produce a variety of flours that meet specifications to achieve optimal rheological, baking and baked product quality. While flour specifications are fairly well defined for bread, flour properties for optimal tortillas are vague.

Ramírez-Wong et al (2007) evaluated the effect of extraction rate of hard red and white winter wheats on tortilla quality. Their study concluded that flour milled to 74 or 80% extraction produced tortillas with good flexibility and texture. Tortillas produced from flour of 100% extraction were considered acceptable, but were darker in color and less flexible.

The flour particle size is an important factor affecting performance. Reducing the particle size too much during milling can mechanically deteriorate the protein composition of the flour and increase the amount of starch damage, two properties that

affect baking quality (Eliasson and Larsson 1993c). When hard wheat flours were fractionated and reduced to pass through a <38 µm sieve, the resulting tortillas had reduced flexibility and extensibility (Wang and Flores 1999c). Tortillas produced from flour recovered from the second and third break streams exhibited better flexibility and extensibility than the tortillas made from flour from the middling streams (Wang and Flores 1999a).

Flour can be chemically treated to enhance flour for a specific end-use. While common chemical treatments involve flour fortification with minerals and vitamins (thiamin, riboflavin, niacin and iron) that were lost during the milling steps, bleaching and oxidation treatments are also applied to flour to whiten and mature flour at a faster rate than natural air-induced oxidation. Oxidizing agents alter the degree of polymerization of glutenin subunits which relaxes the dough and increases extensibility (Delcour and Hosney 2010b). Waniska et al (2004) observed that tortillas made from flours treated with benzoyl peroxide or azodicarbonamide had improved shelf-stability.

## ***Protein Characterization and Functionality***

### ***Wheat Protein Composition***

The protein content of flour is a primary factor for selecting end-use. Much of the literature implies that suitable flours for tortilla making contain 10 to 12% protein content (Waniska et al 2004). However, the protein composition or quality appears to have an equal or greater influence on tortilla quality.

Within the plant protein classification system developed by Osborne (Osborne 1907), proteins are divided into four categories based on their solubility in different solutions: albumins in water, globulins in saline solution, prolamins in dilute ethanol (60-70%) and glutelins in dilute acids. Wheat proteins contain all four protein fractions at varying amounts and with highly diverse functionalities in dough and bread systems.

Albumins and globulins are concentrated in the aleurone, bran and germ layers and contain metabolic enzymes responsible for seed germination. Both fractions are relatively small and have fast elution rates during SE-HPLC analysis (50-100 peaks) (Eliasson and Larsson 1993a). The level of albumins and globulins appears to be fairly

consistent between wheat cultivars and unaffected by environmental stresses. When nitrogen levels increase as a result of fertilization, the amount of gluten-forming proteins increases and metabolic proteins remain constant (Eliasson and Larsson 1993a). The influence of globulin and albumin fractions on bread and tortilla making is not well understood.

The glutelin and prolamin fractions in wheat are called glutenin and gliadin, respectively. These are the storage proteins (Delcour and Hosney 2010d). Glutenin and gliadin constitute the gluten-forming proteins, imparting the viscoelastic, cohesive dough properties essential for tortilla making. Glutenin contributes to dough elasticity, while gliadin contributes to dough extensibility.

Gliadins are monomeric proteins consisting of single chain polypeptides. These chains are characterized by high concentrations of glutamine, proline and other hydrophobic amino acids (Eliasson and Larsson 1993a). Gliadins account for 30-35% of the total protein content. Approximately 70 different gliadin fractions have been identified, and are further classified as  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\omega$ -gliadins.  $\omega$ -Gliadins are considered sulfur-poor (0-8 cysteine residues per 100,000 g protein).  $\alpha$ -,  $\beta$ - and  $\gamma$ -Gliadins contain more sulfur (17-22 residues per 100,000 g protein) (Eliasson and Larsson 1993a). The molecular weights range from 30,000-40,000 kDa for  $\alpha$ -,  $\beta$ - and  $\gamma$ -gliadins to 60,000-80,000 kDa for  $\omega$ -gliadins.  $\omega$ -Gliadins also form  $\beta$ -turns, in contrast with  $\alpha$ -,  $\beta$ - and  $\gamma$ -gliadins, which form  $\alpha$ -helices and  $\beta$ -pleated sheets. As a result,  $\omega$ -gliadins are stabilized by hydrophobic interactions and hydrogen bonding, while  $\alpha$ -,  $\beta$ - and  $\gamma$ -gliadins form hydrogen and disulfide bonds and are considered more stable due to their helix-forming capabilities. The molecular function of gliadins in baking has not been well established (Eliasson and Larsson 1993a).

Glutenins are polymeric proteins consisting of multiple chains of individual polypeptides linked together by disulfide bonds (Southan and MacRitchie 1999). Glutenins constitute 40-50% of the total protein in wheat flour (Eliasson and Larsson 1993a). Glutenins are considerably larger than gliadins. Glutenins are categorized into low molecular weight glutenin subunits (LMW-GS) and high molecular weight glutenin subunits (HMW-GS) (Shewry et al 1986). Due to their aggregative properties, their

molecular weights are difficult to measure. However LMW-GS weight estimates are between 30,000-51,000 kDa and HMW-GS between 90,000-150,000 kDa (Eliasson and Larsson 1993a). HMW-GS are dominated by a large proportion of hydrophobic amino acid residues such as glycine, proline, glutamine and leucine with small proportions of acidic and basic amino acids (Khan and Bushuk 1978). HMW-GS form  $\beta$ -turns and possibly some  $\beta$ -pleated sheets, while the terminal regions form  $\alpha$ -helices. Glutenin subunits can link together with disulfide bridges, forming large polymeric glutenin (Eliasson and Larsson 1993a).

Protein composition appears to influence tortilla diameter and shelf-stability. Suhendro et al (1993) researched the effect of protein additives in the tortilla formulation and found that only vital wheat gluten improved dough properties and tortilla rollability. This group speculated that vital wheat gluten, in comparison to alternative plant- and dairy-based protein sources, contained the proper amount of sulfur containing amino acids for disulfide interchanges during dough mixing. Alviola et al (2008) demonstrated that when using the protein-modifying enzymes, transglutaminase and protease, that native flour proteins must be well developed and homogeneously distributed for tortillas to retain their flexibility. Transglutaminase catalyzes the formation of covalent cross-links between lysine and glutamine residues, producing less sticky, faster-developing doughs that are able to hold more water and produce better textured bread. Protease functionality is not well understood, but is added to bread dough to reduce mix times and increase extensibility. Both enzymes modify the gluten matrix through enzyme hydrolysis and increased cross-linking, but the resulting matrices were heterogeneous in comparison to the control. This effect reduced tortilla shelf-stability significantly.

### ***Molecular Weight Distribution***

Molecular weight distribution (MWD) is used to characterize the proportion of monomeric and polymeric protein fractions in wheat flour. Size-exclusion high performance liquid chromatography is used to separate protein fractions based on their size and relative mobility through a porous column matrix (Bietz 1984). Larger proteins elute rapidly, while smaller proteins take longer to move through the porous media.



While this method does not measure proteins in their native state, the rapid automatic sampling procedure enables researchers to isolate and compare the molecular weight distribution of polymeric and monomeric wheat proteins (Southan and MacRitchie 1999).

### ***Insoluble Polymeric Protein***

Insoluble polymeric protein (IPP) consists of the largest glutenin polymers, extracted using reducing agents or with acid-base hydrolysis and applying sonication to facilitate extraction. Measuring IPP content in flour is another convenient method for characterizing gluten. Several studies have reported a relationship between IPP and breadmaking quality (Orth and Bushuk 1972; Huebner and Wall 1976; MacRitchie 1978; Chakraborty and Khan 1988; Bean et al 1998). Bean et al (1998) developed a rapid method for quantifying IPP content in flour and found a correlation between the IPP content and dough strength using hard winter wheat. An increase in IPP content corresponds with decreased tortilla diameters and improved shelf-stability (Pascut et al 2004; Mondal et al 2008). Mondal et al (2008) also found that an increase in IPP content correlated positively ( $r=0.749$ ) with shelf-stability.

### ***Genetics***

Gluten proteins are genetically coded on nine loci. Three *Glu-1* loci (*Glu-A1*, *Glu-B1* and *Glu-D1*) are on the long arms of the chromosomes 1A, 1B and 1D and code high molecular weight glutenin subunits (HMW-GS). Three *Gli-1/Glu-3* loci (*Gli-A1/Glu-A3*, *Gli-B1/Glu-B3* and *Gli-D1/Glu-D3*), on the short arms of the chromosomes 1A, 1B and 1D, code for  $\omega$ - and  $\gamma$ -gliadins and low molecular weight glutenin subunits (LMW-GS). Three *Gli-2* loci (*Gli-A2*, *Gli-B2* and *Gli-D2*) code for  $\alpha$ - and  $\beta$ -gliadins on the short chromosome arms of homoeologous group 6 (MacRitchie and Lafiandra 2001).

The development of near-isogenic wheat lines, wheats with entire or portions of their chromosomes silenced, enable researchers to study the varying effects of protein fractions on bread and tortilla making quality. Mondal et al (2008) and Jondiko et al (2012) studied the functionality of HMW-GS subunits on tortilla quality using near-isogenic wheat lines and found contrasting results. Mondal et al (2008) found the

presence of *Glu-D1* HMW-GS improved shelf-stability, of which subunit combinations on *Glu-D1*, 5 + 10 accrued better rollability scores than subunits 2 + 12. Jondiko et al (2012) found subunits 2 +12 produced tortillas with better diameters and flexibility than did subunits 5 + 10. *Glu-B1* HMW-GS increased tortilla diameters, but to the detriment of shelf-stability. Deletions on all three loci affected tortilla diameter. In particular, the deletion of *Glu-D1* HMW-GS produced an extensible dough and large diameter tortillas with acceptable flexibility up to 16 days of storage (Jondiko et al 2012). Larger diameter tortillas with similar shelf-stabilities to parent lines were made from lines possessing 1, 5 + 10 (on *Glu-A1* and *Glu-D1*, respectively) and a deletion on *Glu-B1* (Mondal et al 2008).

Monomeric protein (gliadin) functionality has also been explored using near-isogenic wheat lines (Mondal et al 2009). Gliadin content is speculated to enhance the extensible properties of wheat flour. Deletions were made to the loci encoding gliadins on loci *Gli 1* and *Gli 2*. Deletions on *Gli A2* reduced the amount of  $\alpha$ - and  $\beta$ -gliadins in comparison to  $\omega$ -gliadins. Because  $\alpha$ - and  $\beta$ -gliadins are able to form disulfide bonds, the resulting doughs were extensible and produced tortillas with similar rollability scores to parent lines. Deletions to *Gli 1* loci increased the amount of cross-linking and polymeric protein content. The resulting doughs were stronger and tortilla diameters were smaller.

### ***Starch Characterization and Functionality***

Wheat flour contains 63-70% starch on a 14% moisture basis (Atwell 2001). It is assumed that starch is partially responsible for inherent bread characteristics including texture, volume, appearance, moisture retention and staling. However, starch behavior in breadmaking is not well understood due to difficulties in analyzing starch within limited water systems. This problem is further accentuated when analyzing starch functionality in tortillas for two primary reasons: the formula requires 10-15% (fwb) less water than bread does and tortillas are baked for a shorter time at higher temperatures. Much of the following research on starch functionality in bread and tortillas is inferred from the analysis of isolated starch fractions in aqueous solutions.

In its native state, starch is present as granules within the endosperm. Wheat starch granules are classified into two, sometimes three, size distribution categories (Wilson et al 2006). A-type granules are the largest in size ( $>10\ \mu\text{m}$ ) and lenticular in shape. B-type granules are spherical and smaller ( $5\text{-}10\ \mu\text{m}$ ). C-type granules are the smallest in size ( $<5\ \mu\text{m}$ ). Each granule is composed of the two polysaccharide polymers, amylose and amylopectin. Amylose is primarily a straight-chain glucose polymer linked by  $\alpha$ -(1,4) bonds. Amylopectin is a highly branched glucose polymer with a long backbone of  $\alpha$ -(1,4) glycosidic linkages, connected by  $\alpha$ -(1,6) glycosidic branch points. The periodic organization of amylopectin clusters within the granule gives rise to crystalline regions (Eliasson and Larsson 1993a). In normal wheat starch, amylose constitutes 25-30% and amylopectin 75-80% of the starch granule (BeMiller and Huber 2010). Mutant wheat strains containing high amylose or high amylopectin (waxy) exist and are commercially available. The starch granule contains 1% or less of minor components such as lipids, proteins and phosphates (Eliasson and Larsson 1993a). Variations in the starch composition such as amylose to amylopectin ratio, chain-length, starch granule size distribution, presence of phospholipids, crystalline structures affect the functional properties of starch in baking (Wilson et al 2006).

The two primary roles starch plays in baking are by its gelatinization and retrogradation. Intact starch granules absorb about 30% of their dry weight in water at room temperature (Delcour and Hosney 2010c). However, when starch is heated in the presence of excess water, the granule absorbs water and swells, followed by amylose leaching out of the granule to form an amorphous gel. Gelation increases the viscosity of the dough and helps “set” the structure of the baked product (Eliasson and Larsson 1993b). Retrogradation and starch recrystallization occur when the amorphous gelatinized starches become rigid. In this process, amylopectin regains its crystallinity within a continuous amylose matrix. Both retrogradation and starch recrystallization are assumed to be responsible or highly linked to staling in bread products. It is important to note that amylose and amylopectin play two different roles. Amylose crystallization determines the initial hardness of a starch gel, while amylopectin retrogradation affects

the development of a gel structure and crystallinity in starch systems over time (Delcour and Hosney 2010c).

Variances in starch composition and condition affect the rheological and staling properties in tortillas. Starch damage incurred in milling influences water absorption, amylose redistribution and results in sticker doughs (Eliasson and Larsson 1993a). Mao and Flores (2001) investigated the effect of mechanical starch damage on tortillas by excessively milling flours until their particle size was greatly reduced. Tortillas with increased starch damage levels up to 16.57%, produced less stretchable, yet more flexible fresh tortillas. However, their shelf-stability after four days was diminished in comparison to tortillas formulated from flour with a normal starch damage level and flour particle size.

$\alpha$ -Amylase, a starch-hydrolyzing enzyme, is commonly used in bread production to increase the amount of fermentable sugars and decrease staling rates. Alviola and Waniska (2008) found that tortillas containing  $\alpha$ -amylase retained excellent flexibility after 28 days of storage, thus exhibiting a decreased staling rate.

The role of amylose (or the effect of reduced amylose content) in tortilla making and quality was examined by preparing tortillas with waxy and partially waxy wheat flours. Guo et al (2003) evaluated tortillas made from waxy and wild-type wheat flours with amylose content ranging from 0 to 29%, while Waniska et al (2002) used wild-type and partial waxy wheat flour. In both studies, lower amylose content was associated with higher water absorption, reduced dough elasticity, earlier puffing during baking and darker, more translucent tortillas. Lower amylose flours possess different pasting properties than normal flours by forming more viscous gels over a shorter time range. Initially, this accounts for faster puffing, but the lack of air cell structure after baking resulted in diminished opacity and height (Waniska et al 2002).

### ***Minor Flour Components***

#### ***Non-Starch Polysaccharides***

Non-starch polysaccharides such as arabinoxylans, cellulose and  $\beta$ -glucans found in the cell walls in the endosperm matrix constitute 2-2.5% of the flour.

Arabinoxylans are the major component among the three non-starch polysaccharides. The total arabinoxylan content is divided into 65% water-soluble and 35% water-insoluble constituents. Arabinoxylans, like damaged starch, can absorb several times their weight in water, form bonds with gluten proteins and increase dough viscosity (Stauffer 1999). Wang and Flores (1999b) studied the effects of wheat starch and gluten on tortilla texture using fractionated and reconstituted flour. The resulting tortillas possessed poor characteristics. They attributed the diminished quality as a result of altering the normal protein-starch ratio through reconstitution and the loss of water-solubles during the fractionation process. It is speculated that arabinoxylans are responsible for dough drying and strengthening during dough fermentation (Delcour and Hosney 2010d). Yet the lack of fermentation in the tortilla making process indicates that the role of non-starch polysaccharides in this process needs further study.

### ***Lipids***

Lipids constitute approximately 1-2.5% of the endosperm. Flour lipids are categorized into free and bound, depending on their extractability. They can also be classified as nonpolar (predominantly triglycerides, monoglycerides and sterol esters) and polar (glycolipids and phospholipids). Polar lipids react with carbohydrates and proteins, influencing dough properties, baking behavior and bread staling (Pylar and Gorton 2009c). The role of native flour lipids in tortilla making has not been explored.

### ***Environmental Effects on Flour Quality***

Environmental factors lead to significant differences in wheat milling properties and flour quality for a given cultivar. Factors influencing starch and protein fractions in wheat include growth climate, light exposure, extreme hot and cold temperatures, amount of water, soil type, presence of and type of fertilizer.

Sulfur and nitrogen are commonly included in fertilizer treatments to improve yield and the nutritional profile of seed and grain crops. The amount of sulfur in the fertilizer changes the frequency of disulfide bond formation between cystine residues. As a result, sulfur levels alter the molecular weight distribution of protein fractions. When sulfur is limiting, the amount of sulfur-poor proteins ( $\omega$ -gliadins) increases in comparison

with sulfur-rich ( $\alpha$ - and  $\beta$ -gliadins, albumins and globulins). Therefore, the amount of sulfur-poor HMW-GS increases to the detriment of LMW-GS. A high molecular weight distribution improves dough strength, increases mixing requirements and reduces dough extensibility (Wrigley et al 1984). High nitrogen levels can increase the amount of total protein in the grain. However, the gliadin content increases at a faster rate than the glutenin, causing a decrease in polymeric-to-monomeric protein ratio (Gupta et al 1992).

Elevated temperatures during grain filling affect protein and starch content. While increasing temperatures up to 30°C increased dough strength, temperatures exceeding 30°C decreased dough strength (Randall and Moss 1990). Heat-shock affects protein content by reducing the unextractable polymeric protein (UPP), lowering the molecular weight distribution and weakening dough properties (Ciaffi et al 1994). High temperatures interfere with starch synthesis. Temperatures above 30°C shorten the duration of starch accumulation, possibly by suppressing the genes responsible for activating starch biosynthesis enzymes (Dupont and Altenbach 2003). The proportion of A to B size granules also decreases with higher temperatures (Blumenthal et al 1995).

The development of the wheat kernel can be affected by genetic x environment interaction. Genetic variation is primarily thought responsible for kernel texture and hardness. Grain moisture, damage, pesticide content and presence of foreign material is attributed to environmental growing, harvest and handling conditions. Genetic x environment interaction are reported to influence test weight, protein content, sprouting and flour ash content (Bequette 1989; Blumenthal et al 1993). However, several studies have proposed that environment or genetics influence grain and flour quality individually, in contrast to a combined effect of genetic x environment interaction (Lukow and McVetty 1991; Peterson et al 1992). Understanding how environment, genetics and genetic x environment interaction influence grain, flour and end-use quality would be highly advantageous for wheat breeders and growers to introduce more stability in quality and marketability of a particular cultivar.

## Chapter 3 Materials and Methods

### Wheat Samples

Ten hard winter wheat varieties were evaluated. Hard red wheat varieties included 1863, Armour, Denali, Everest, Fuller, Jagger and Tam111. Hard white varieties included Clara CL, RonL and Tiger. Wheat was grown in crop year 2011 in five locations in Kansas: Ford County, Hays, Pawnee County and two Ellis County locations. One location (Ellis County) was irrigated, while the rest were dryland. Not all varieties were available from each location (Table 3.1). Data from varieties that were available from three or more locations were statistically analyzed. Data for the varieties available from only one or two locations that were not included in the statistical analysis are listed in the Appendix. All varieties grown in Pawnee County had unusually high flour protein contents, ranging from 15.24 to 17.29% and small kernel diameters. These varieties were removed from statistical analyses. In total, 22 variety and location combinations were evaluated.

**Table 3.1 Wheat Variety and Growth Location Combinations**

Variety	Ford (Dryland)	Hays (Dryland)	Ellis (Dryland)	Ellis (Irrigated)	Pawnee (Dryland)
1863	✓	✓	✓	✓	✓
Armour	✓	■	✓	✓	✓
Denali	✓	✓	✓	✓	✓
Everest	✓	✓	✓	✓	■
RonL	■	✓	■	■	■
Tam111	■	■	✓	■	✓
Tiger	✓	✓	✓	✓	✓
Clara CL	✓	✓	✓	■	✓
Jagger	■	✓	■	■	■
Fuller	■	✓	■	■	■

included in statistical analysis  
 sample not available  
✓ sample available

## **Wheat Physicochemical Tests**

Wheat samples were cleaned with a Carter Dockage tester (Carter-Day Company, Minneapolis, MN). The test weight (lb/bu) was measured using AACCI Approved Method 55-10.01 (AACCI International 2010). The Single Kernel Characterization System was used to measure the mean kernel weight (mg), diameter (mm) and hardness index (SKCS 4100, Perten Instruments, Springfield, IL) (AACCI Approved Method 55-31.01). Wheat protein and moisture content was determined by NIR (DA 7200 Perten, Springfield, IL).

### **Wheat Milling**

Wheat was tempered to 15% moisture content overnight (AACCI Approved Method 26-95.01). Approximately 1000 g samples were milled to ~70% extraction on a Quadrumat Senior Experimental Mill (C.W. Brabender, Duisburg, Germany) with modification (accommodate use of the Senior Mill over the Junior Mill) to the AACCI Approved Method 26-50.01. The feed rate was 150 g/min. All samples were milled to straight grade flour.

### **Flour Characteristics**

#### ***Flour Physicochemical Tests***

Flour protein and moisture content were determined with a DA 7200 NIR (Perten Instruments, Springfield, IL) (AACCI Approved Method 39-11.01). The amount of starch damage in the flour was measured using the SDmatic (Chopin Technologies, Villeneuve La Garenne, France) (AACCI Approved Method 76-33.01). Flour particle size distribution was measured by laser diffraction analysis using the LS 13320 tornado dry module (Beckman-Coulter, Miami, FL). All tests were run in duplicate.

#### ***Water Absorption***

Optimum water absorption was estimated using the 10 g Mixograph (National Manufacturing Co., Lincoln, NE) following AACCI Approved Method 54-40.02. The predicted optimum water absorption was estimated based on the equation,  $y = 1.5x +$



43, where  $x$  is the flour protein content on a 14% moisture basis. Each flour sample was tested as a series of mixograms with 2% incremental lower and higher water absorption levels from the predicted optimum to determine dry, optimum and wet dough conditions. Dry dough conditions were characterized by wild, jagged pen strokes. Wet dough conditions were indicated by a swayback in development and a narrow band after peak development.

The water addition to process the dry tortilla ingredients into a dough was determined by subtracting 10% on a flour weight basis from the optimum water absorption. For example, a flour sample with 68% optimum water absorption would require 58% water to process into a tortilla dough. New mixograms of each sample were obtained using the modified water level to measure the optimum mix time for tortilla doughs.

### ***Dough Evaluation***

The strength and extensibility of each dough (at the optimized water level) was measured using the TA.XTPlus Texture Analyzer (Stable Micro Systems/Texture Technologies, Surrey, UK) in conjunction with the Kieffer test rig using the SMS/Texture Technologies testing protocol. Doughs were mixed 30 sec short of optimum in a mixograph using 10 g of flour, 0.2 g sodium chloride, and optimum water absorption. The dough piece was gently molded into a rectangle with minimal handling and set on the grooved section of the Teflon former lubricated with mineral oil. A lamella strip was placed in each groove to aid dough strip removal. The cover block was placed on top of the former and a clamp compressed the blocks together. The excess dough, forced out the sides of the former, was trimmed off with a knife and discarded. Following a 30 min rest, the former was removed from the clamp and the top block slid back to expose an individual dough strip. Only full-length dough strips were tested. Lifting the lametta strip ends protruding out of the former enabled transfer of the dough onto the sample plate without deformation. The lametta strip was peeled off prior to positioning the sample plate in the testing rig with the probe hook centered beneath the exposed dough strip. During testing the dough hook extended the strip upward until it broke. The test

procedure used a 5 kg load cell and measured force in tension with a 5 g trigger force, 2.0 mm/sec pre-test speed, 3.3 mm/sec test speed and 10.0 mm/sec post-test speed for a total distance of 75 mm. The maximum force and extension limit correlate with resistance to extension and degree of extensibility, respectively. Three doughs were mixed per treatment and at least five strips per dough were tested.

## **Tortilla Making**

Tortillas were prepared using the following laboratory scale hot press method. All ingredients (Table 3.2) were mixed in a 100 g pin mixer (National Manufacturing Co., Lincoln, NE) until dough was fully developed. Each tortilla dough was processed from the flour of a single hard winter wheat cultivar. A control flour was also randomly processed into tortillas between treatments to ensure consistency throughout the tortilla making process. Tortilla dough was scaled into three 25 g dough balls and manually rounded. Dough balls were rested in covered, lightly greased bowls at room temperature (27°C) for 20 min. Each ball was individually hot-pressed with top and bottom platen temperatures at  $162 \pm 5^\circ\text{C}$  for 15 sec (DoughPro, Dual/Heat, Propress Corp., Paramount, CA). The gap between the platens was set to 1.5 mm. Pressed dough was baked on a griddle (Gold Medal 8200, Cincinnati, OH) set to a surface temperature of  $190 \pm 5^\circ\text{C}$ . The tortillas were baked for a total of 1.5 min (flipped at 30 sec, 30 sec, 20 sec and 10 sec intervals). Tortillas were cooled on a wire rack for 10 min, then packaged in zippered polyethylene bags and stored at room temperature (28°C) for 14 days. Tortilla making was performed in triplicate.

**Table 3.2 Laboratory Scale Hot Press Tortilla Formula**

<b>Ingredient</b>	<b>Formulation (fwb%)</b>
Wheat Flour	100.00
Water	Variable <sup>a</sup>
Salt	1.50
Calcium Propionate	0.50
Potassium Sorbate	0.40
Shortening	6.00
Sodium Bicarbonate	0.60
Fumaric Acid (encapsulated)	0.24
Sodium Aluminum Phosphate	0.58

<sup>a</sup>%Water = (% mixograph optimum absorption – 10)

### **Tortilla Evaluation**

Each dough was evaluated and given a subjective dough score on a scale of 1 (very extensible) to 5 (firm, elastic) for its handling properties. Final tortilla diameter was measured in three separate locations (with the second and third measurements 45° and 90° from the first measurement, respectively) across the tortilla, then averaged. Each tortilla was weighed on an analytical balance. Tortilla thickness was measured with a digital caliper in three separate locations around the tortilla (approximately 3 cm from the edge, 120° from one another) and averaged. Tortilla color was measured with a CR-310 Chromameter (Minolta, Osaka, Japan) that was calibrated with a standard calibration plate. Three color measurements were taken from the top and bottom surfaces of each tortilla and averaged. Opacity was subjectively evaluated on a scale from 0 (completely translucent) to 10 (fully opaque) (Adams and Waniska 2002; Adams and Waniska 2005) (Figure 3.2). Shelf-stability was characterized by measuring the tortilla flexibility on days 1, 7 and 14 after baking. Tortillas were wrapped around a 1 cm dowel and subjectively scored on degree of cracking using a scale of 1 (breaks easily) to 5 (very flexible, no cracks) (Figure 3.1) (Friend et al 1995; Adams and Waniska 2002; Adams and Waniska 2005).

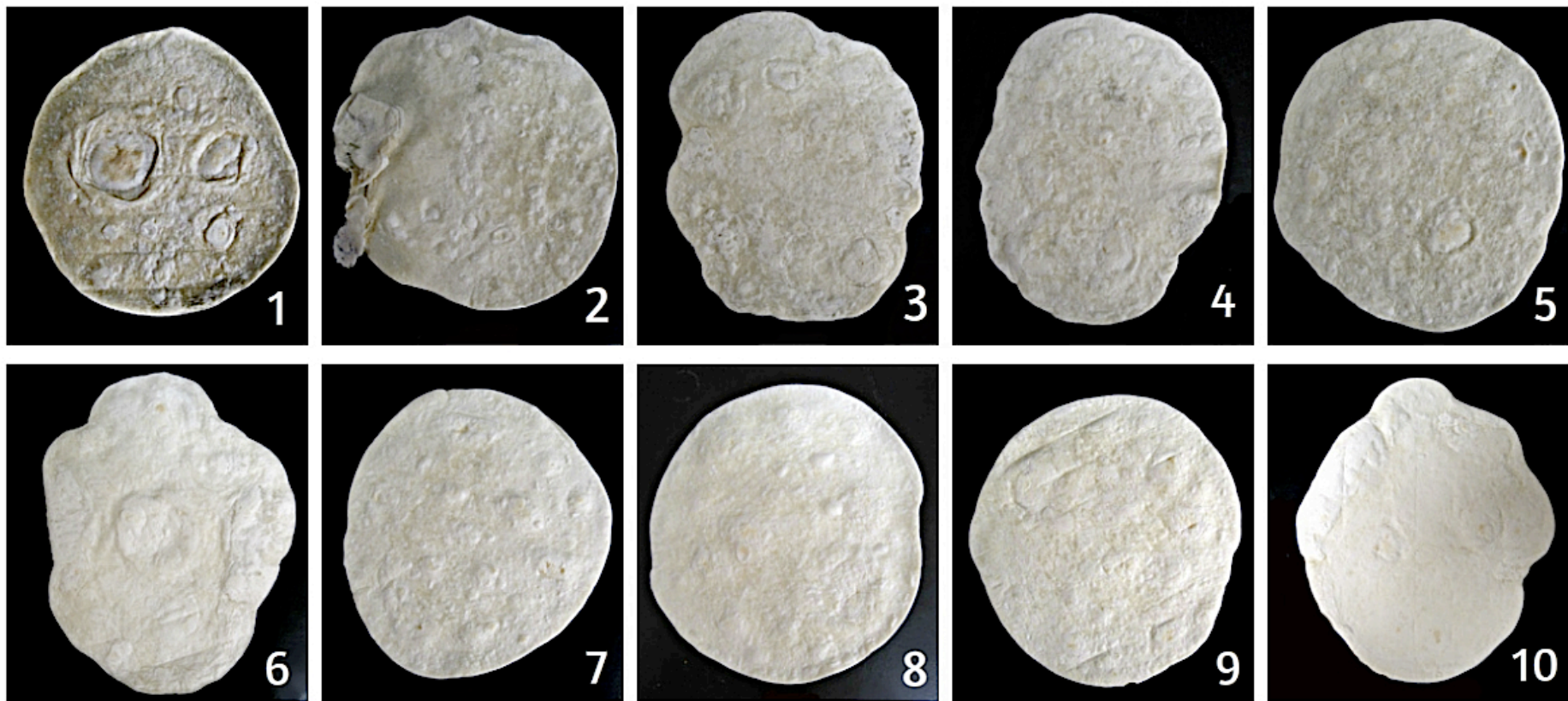


**Figure 3.1 Scoring Scale for Determining Tortila Flexibility**

1= cracks easily, brittle

3= moderate flexibility, minor tearing on surface

5= very flexible, no cracking



**Figure 3.2 Key for Scoring Opacity**

1= completely translucent

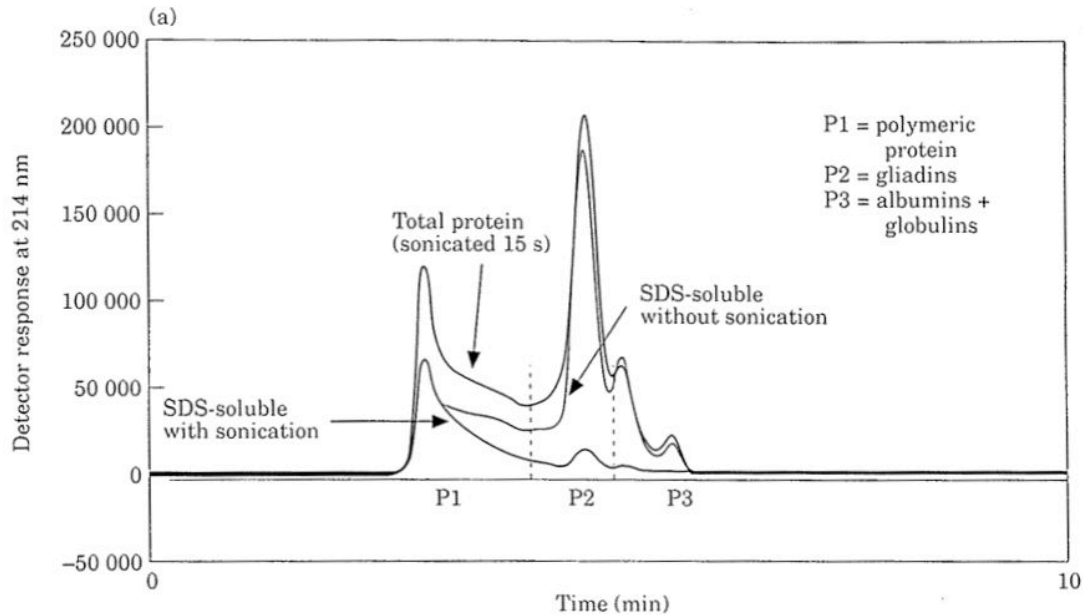
5= moderately translucent

10= completely opaque

## Protein Analysis

### *Molecular Weight Distribution*

The molecular weight distribution of glutenin, gliadin and globulin/albumin fractions was characterized using size-exclusion high performance liquid chromatography (SE-HPLC) according to a method used by Singh et al (1990) and Batey et al (1991). Flour ( $10 \pm 0.1$  mg) was weighed into a microcentrifuge tube followed by the addition of 1 mL of 50 mM disodium phosphate + 0.5% SDS buffer at pH 6.9 and solubilized by vortex agitation for 5 min. This extracted the total and extractable polymeric protein fractions. The tubes were then centrifuged at 12,000 rpm for 20 min. The supernatants were filtered twice for 5 min through 0.45  $\mu$ m filter tubes then transferred to HPLC vials and sealed. Vials were heated in an 85°C water bath for 10 min. After heating, the vials were cooled to room temperature for 30 min before being analyzed with SE-HPLC. Aliquots (20  $\mu$ l) of each sample were fractionated in a Biosep SEC-4000 column (Phenomenex, Torrance, CA) in a SE-HPLC system (Agilent 1100, Agilent Technologies, Santa Clara, CA) with a binary pump and automated sample injector. The mobile phase consisted of a 50/50 ratio of deionized water + 0.1% trifluoroacetic acid (TFA) and acetonitrile + 0.1% TFA. ChemStation software (Agilent Technologies, Santa Clara, CA) was used for pump control, data acquisition and to integrate chromatograms. Peak areas were automatically determined as the area under the curve between the valleys to determine the proportion of protein constituents (Figure 3.3). The area under peak 1 of the total protein extraction/total area of total protein extraction x 100 indicated the proportion of polymeric glutenin proteins in the wheat flour sample. Using the same formula, peaks 2 and 3 indicated the proportion of monomeric gliadins and monomeric albumin/globulin fractions, respectively. The polymeric to monomeric ratio was calculated by dividing the area of peak 1 by the area peak 2. All samples were run in duplicate.



**Figure 3.3 Chromatogram of Total Protein Fractionation** (Wesley et al 2001)

### ***Insoluble Polymeric Protein***

Flour ( $100 \pm 1$  mg) and 1 ml 50% 1-propanol were vortex mixed for 5 min in a microfuge tube. Tubes were centrifuged for 5 min at 12,000 rpm. The supernatant was discarded and the remaining insoluble polymeric pellet was macerated with a toothpick. This step was repeated twice to ensure all soluble protein was extracted. The final remaining pellet was lyophilized. Protein content of the pellet was determined following AACCI Approved Method 46-30.01, using a LECO FP-428 nitrogen determinator (LECO Co., St. Joseph, MI). Insoluble polymeric protein percentage (%IPP) was calculated by multiplying the nitrogen values by 5.7, then dividing by the total flour protein content (Bean et al 1998). All samples were run in duplicate.

## **Starch Analysis**

### ***Starch Size Distribution***

Wheat starch was isolated by an adaptation of the sorghum starch isolation method used Park et al (2006). The following procedure was used to isolate the starch. Wheat flour (2.5 g) was mixed with a buffer (50 ml) in a 1:20 ratio. The buffer was composed of 0.1 M sodium phosphate, 0.5% sodium dodecyl sulfate (SDS) and 0.5%

sodium metabisulfite diluted in distilled water to pH 7. After gentle mixing, the solution was sonicated for 100 sec using Model VCF-1500 (Sonic & Materials, Newton, CT) equipped with a 25.4 mm probe positioned 5 mm from the bottom of the beaker, which was immersed in an ice water bath to prevent sample heating. The sonication amplitude was set to 75%. The solution was transferred to a 50 ml plastic tube, centrifuged for 10 min at 4000 rpm and decanted. Approximately 40 ml of distilled water was used to wash the precipitated starch through a 62  $\mu\text{m}$  mesh screen. The resulting solution was centrifuged for 5 min at 4000 rpm. The precipitate was suspended in 40 ml of distilled water using a vortex mixer and decanted twice. The extracted starch was lyophilized, then ground with a mortar and pestle and suspended in 1% sodium azide solution for particle size distribution determination using a LS 13320 laser diffraction particle size analyzer with the universal liquid module (Beckman/Coulter, Miami, FL). Granule size distributions were categorized as described by Park et al (2004), where A granules were  $>15 \mu\text{m}$ , B granules were 5-15  $\mu\text{m}$  and C granules were 0-5  $\mu\text{m}$ .

## **Data Evaluation**

Data from wheat varieties grown in three or more locations (Table 3.1) were analyzed by Proc Mixed (SAS v. 9.3 Institute, Cary, NC) using a completely randomized block design. Analysis of variance (ANOVA) was conducted on samples using Proc GLM and means were compared using Tukey-Kramer HSD. Wheat and flour data were correlated to tortilla parameters using Pearson's correlation coefficients. All models were analyzed at the  $p < 0.05$  level of significance. The appendix contains data not included in the statistical analyses (varieties grown in less than three locations).



## Chapter 4 Results and Discussion

### Wheat Properties

Armour and Tiger had the lowest test weights, kernel weights and kernel diameters (Table 4.1). 1863, Denali, Everest and Clara CL had similar test weights and kernel diameters. Kernel hardness index values fell within the standard target range for hard red and white winter wheat varieties (60-90 SKCS-Hardness Index Units) (USDA/ARS Grain Marketing and Production Research Center 2014). Kernel moisture was typical (~11.5%) for all samples. Kernel protein was high for hard winter wheat and ranged from 13.82% (Tiger) to 15.76% (Clara CL). The remaining varieties did not vary greatly. Their protein levels averaged ~14%.

**Table 4.1 Wheat Kernel Properties**

Variety	Test Weight (lb/bu)	Kernel Weight (mg)	Kernel Diameter (mm)	Kernel Hardness	Kernel Moisture (%)	Kernel Protein (% 12 mb)
1863	60.78 ± 1.11	28.29 ± 0.94	2.46 ± 0.04	75.52 ± 3.14	11.50 ± 0.36	14.57 ± 0.35
Armour	56.27 ± 0.41	23.99 ± 1.36	2.27 ± 0.06	78.92 ± 5.02	11.61 ± 0.60	14.17 ± 0.43
ClaraCL	59.92 ± 1.15	26.05 ± 0.83	2.44 ± 0.05	84.15 ± 2.56	11.76 ± 0.50	15.76 ± 1.16
Denali	60.13 ± 0.50	28.38 ± 0.51	2.46 ± 0.02	74.43 ± 0.61	11.83 ± 0.38	14.07 ± 0.71
Everest	60.47 ± 0.99	27.52 ± 1.99	2.47 ± 0.08	78.12 ± 3.62	11.63 ± 0.36	14.10 ± 0.60
Tiger	56.59 ± 1.82	24.24 ± 1.67	2.32 ± 0.04	74.27 ± 3.10	11.30 ± 0.33	13.82 ± 0.70

### Flour Properties

Flour protein ranged from 11.39% (Tiger) to 12.18% (Clara CL) and did not differ significantly among varieties (Table 4.2). Flour moisture was similar among all varieties (~14%). Although Armour contained significantly higher starch damage levels, the levels were typical of hard winter wheat using the SDMatic amperometric method (4-8% starch

damage levels for hard winter wheat). The average flour particle size was representative of hard wheat flour (~90  $\mu\text{m}$  or less) and did not differ significantly among varieties.

**Table 4.2 Flour Properties <sup>a</sup>**

<b>Variety</b>	<b>Flour Protein (%, 14 mb)</b>	<b>Flour Moisture (%)</b>	<b>Starch Damage (%)</b>	<b>Mean Flour Particle Size (<math>\mu\text{m}</math>)</b>
1863	12.09 $\pm$ 0.67 a	14.00 $\pm$ 0.23 a	4.01 $\pm$ 0.16 b	94.27 $\pm$ 2.00 a
Armour	11.48 $\pm$ 0.32 a	13.70 $\pm$ 0.20 a	4.52 $\pm$ 0.16 a	91.36 $\pm$ 2.26 a
ClaraCL	12.18 $\pm$ 0.90 a	14.05 $\pm$ 0.05 a	4.09 $\pm$ 0.16 b	94.31 $\pm$ 2.26 a
Denali	11.56 $\pm$ 0.72 a	14.01 $\pm$ 0.16 a	4.09 $\pm$ 0.16 b	86.68 $\pm$ 2.00 a
Everest	11.67 $\pm$ 0.67 a	13.85 $\pm$ 0.20 a	4.18 $\pm$ 0.16 ab	91.22 $\pm$ 2.00 a
Tiger	11.39 $\pm$ 0.66 a	13.87 $\pm$ 0.19 a	4.08 $\pm$ 0.16 b	88.48 $\pm$ 2.00 a

<sup>a</sup> different letters in a column indicate significant differences at  $p < 0.05$

### **Dough Properties**

Water absorption levels and mix times were measured using the mixograph (Table 4.3). The actual mix times to process each flour into a cohesive dough on a larger scale for tortilla making were slightly longer (requiring between 15 to 75 sec more time than the values estimated with the mixograph) for all varieties except Denali. Armour had a significantly lower water absorption level (65.0% fwb) than did the other varieties, despite having the highest level of starch damage. The tortilla dough absorption levels showed the same varietal trends as did the mixograph water absorption levels. This is because the amount of water added to process ingredients into a dough was calculated from the mixograph water absorption level. Flours with higher levels of starch damage often require more water due to higher amounts of water-soluble components (Evers and Steven 1985). Water absorptions levels can also be determined by protein quality and quantity as well. Although varietal differences in the water absorption levels were found, the range (65-67%) was narrow. Tiger took the

longest time to reach its peak time (4.50 min) in the mixograph, while Everest's development was significantly shorter (2.50 min).

The Kieffer test measures the force to extend the dough (strength) and the distance the dough is stretched before rupturing (extensibility). Everest required the least force to extend the dough (23.41 g), indicating it was weaker than the other varieties. While Armour required the highest extension force (30.90 g), it did not differ significantly in rupture distance compared to the other varieties (60.69 mm). Thus varietal differences in dough elasticity and extensibility were not observed. The subjective dough scores, used for characterizing dough handling properties during tortilla making, were similar among varieties. Scores fell between 3.37 and 4.27, indicating all varieties produced extensible, yet moderately elastic doughs.

Table 4.3 Dough Properties <sup>a</sup>

Variety	Mixograph Water Absorption (%)	Tortilla Dough Water Absorption (%)	Mixograph Peak Time (min)	Tortilla Dough Mix Time (min)	Kieffer Force (g)	Kieffer Distance (mm)	Subjective Dough Score
1863	67.00 ± 1.02 a	57.00 ± 1.02 a	3.15 ± 0.15 b	3.45 ± 0.25 b	26.80 ± 3.18 ab	77.36 ± 6.42 a	3.40 ± 0.37 a
Armour	65.00 ± 1.02 c	55.00 ± 1.02 c	3.75 ± 0.17 a	4.30 ± 0.28 a	30.90 ± 3.55 a	60.69 ± 7.18 ab	3.37 ± 0.40 a
ClaraCL	67.00 ± 1.02 a	57.00 ± 1.02 a	3.45 ± 0.17 a	3.75 ± 0.28 ab	29.18 ± 3.55 ab	69.09 ± 7.18 ab	4.02 ± 0.40 a
Denali	66.00 ± 1.02 b	56.00 ± 1.02 b	3.45 ± 0.15 a	3.45 ± 0.25 ab	30.57 ± 3.18 ab	70.76 ± 6.42 ab	3.60 ± 0.37 a
Everest	67.00 ± 1.02 a	57.00 ± 1.02 a	2.45 ± 0.15 b	3.00 ± 0.25 b	23.41 ± 3.55 b	66.23 ± 7.18 ab	4.30 ± 0.40 a
Tiger	66.00 ± 1.02 b	56.00 ± 1.02 b	4.45 ± 0.15 a	4.75 ± 0.25 a	29.88 ± 3.18 ab	59.54 ± 6.46 b	4.27 ± 0.37 a

<sup>a</sup> different letters in a column indicate significant differences at p<0.05

## Tortilla Properties

Diameter, opacity, thickness and day 14 rollability were deemed the most important parameters defining tortilla quality. Overall, none of these tortilla properties were found to be significantly different among the varieties (Table 4.4). Only final tortilla weight showed significant differences among the varieties. The tortilla weight is measured to gauge moisture loss during baking, as initial dough weight was held constant. Armour produced the heaviest (21.28 g) and Denali made the lightest tortillas (20.69 g). Although differences in weight were observed, this range was narrow and was considered normal for tortillas processed from 24 g dough balls. The tortillas were around 130 mm in diameter, 2 mm thick, 21 g in weight and 1.35 cm<sup>3</sup>/g in specific volume.

**Table 4.4 Tortilla Dimensions <sup>a</sup>**

Variety	Diameter (mm)	Weight (g)	Thickness (mm)	Specific Volume (cm <sup>3</sup> /g)
1863	133 ± 1.76 a	20.78 ± 0.12 ab	2.09 ± 0.12 a	1.37 ± 0.06 a
Armour	127 ± 2.03 a	21.28 ± 0.14 a	2.25 ± 0.09 a	1.32 ± 0.06 a
ClaraCL	130 ± 2.03 a	21.11 ± 0.14 ab	2.15 ± 0.06 a	1.30 ± 0.06 a
Denali	132 ± 1.76 a	20.69 ± 0.12 b	2.17 ± 0.09 a	1.35 ± 0.06 a
Everest	133 ± 1.76 a	20.88 ± 0.12 ab	2.02 ± 0.06 a	1.30 ± 0.06 a
Tiger	129 ± 1.76 a	21.14 ± 0.12 ab	2.24 ± 0.06 a	1.34 ± 0.06 a

<sup>a</sup> different letters in a column indicate significant differences at p<0.05

All varieties produced tortillas that were moderately light in color and mostly opaque with minor areas of translucence. Opacity scores fell around 7 (Table 4.5). L color values ranged from 83.26 to 84.31, indicating they were slightly darker than pure white. The a color values ranged from -0.83 to -0.89, meaning all tortillas were neutral on the red-green spectrum. The b color values ranged from 6.40 to 7.63, indicating tortillas had a slight yellowish cast on the yellow and blue spectrum.

**Table 4.5 Tortilla Appearance <sup>a</sup>**

Variety	L color value	a color value	b color value	Opacity Score
1863	82.53 ± 0.77 a	-0.84 ± 0.16 a	15.74 ± 0.40 a	6.40 ± 0.57 a
Armour	84.21 ± 0.89 a	-0.89 ± 0.18 a	16.23 ± 0.45 a	7.63 ± 0.66 a
ClaraCL	83.35 ± 0.89 a	-0.83 ± 0.18 a	16.14 ± 0.45 a	7.10 ± 0.66 a
Denali	83.56 ± 0.77 a	-0.83 ± 0.16 a	15.16 ± 0.40 a	7.30 ± 0.57 a
Everest	83.26 ± 0.77 a	-0.86 ± 0.16 a	16.26 ± 0.45 a	6.92 ± 0.57 a
Tiger	84.31 ± 0.77 a	-0.89 ± 0.16 a	16.24 ± 0.40 a	7.51 ± 0.57 a

<sup>a</sup> different letters in a column indicate significant differences at p<0.05

Rollability scores reflect tortilla flexibility over time, thereby characterizing shelf-stability. Tortillas processed from all of the varieties started out very flexible with no cracks (scores close to 5) on day one, but progressively became more rigid (staled) and reached an approximate score of 2 over fourteen days (Table 4.6). A score of 2 indicates that the tortillas showed significant signs of cracking and breaking. Due to the trend of consumers buying and storing tortillas over long periods of time, varieties yielding scores of ≥3 on the fourteenth day are considered ideal. A score of 3 indicates that despite diminished flexibility, the tortillas remain cohesive and can withstand rolling and stuffing.

**Table 4.6 Tortilla Shelf-Stability <sup>a</sup>**

Variety	Day 1 Rollability Score	Day 7 Rollability Score	Day 14 Rollability Score
1863	4.83 ± 0.10 a	3.19 ± 0.74 a	2.07 ± 0.34 a
Armour	4.67 ± 0.10 a	3.30 ± 0.93 a	1.92 ± 0.38 a
ClaraCL	4.74 ± 0.14 a	3.41 ± 0.62 a	2.36 ± 0.39 a
Denali	4.69 ± 0.10 a	2.94 ± 0.91 a	1.75 ± 0.33 a
Everest	4.81 ± 0.05 a	3.08 ± 0.47 a	2.00 ± 0.34 a
Tiger	4.83 ± 0.06 a	2.97 ± 0.76 a	2.03 ± 0.33 a

<sup>a</sup> different letters in a column indicate significant differences at p<0.05

## Starch Analysis

The average yield of the isolated wheat starch was 67.4%. Wheat starch granules were sorted into three categories based upon size: A-type (>15 µm), B-type (5-15 µm) and C-type (<5 µm) (Table 4.7). The distributions of A- and C-type starch granules were similar among all varieties. Denali, 1863 and Tiger contained significantly higher levels of B-type starch granules than did Armour, Clara CL and Everest.

**Table 4.7 Starch Granule Size Distribution (Volume %) <sup>a</sup>**

Variety	A Starch Granules	B Starch Granules	C Starch Granules
1863	45.96 ± 2.13 a	28.91 ± 1.09 a	25.14 ± 0.72 a
Armour	48.93 ± 2.33 a	27.79 ± 1.11 b	22.96 ± 0.27 a
ClaraCL	49.85 ± 2.33 a	26.24 ± 1.11 b	24.52 ± 0.71 a
Denali	50.43 ± 2.13 a	28.66 ± 1.09 a	20.92 ± 1.00 a
Everest	50.23 ± 2.13 a	27.02 ± 1.09 b	22.75 ± 1.11 a
Tiger	46.65 ± 2.13 a	28.08 ± 1.09 a	25.27 ± 1.57 a

<sup>a</sup> different letters in a column indicate significant differences at p<0.05

## Protein Analysis

Higher polymeric to monomeric ratios and %IPP content indicate that more high molecular weight, gluten-forming polymers are present in the flour. While previous studies have found moderately negative correlations between higher %IPP content and tortilla diameters (Mondal et al 2008; Pascut et al 2004), this study found no significant differences between tortilla diameters processed from Tiger or Clara CL, although Tiger (51.76%) and ClaraCL (47.89%) had significantly higher %IPP than the other varieties. Tiger had significantly higher polymeric to monomeric protein ratio (0.77) compared to the other varieties (Table 4.8).

**Table 4.8 Protein Analyses <sup>a</sup>**

<b>Variety</b>	<b>Polymeric: Monomeric</b>	<b>IPP (%)</b>
1863	0.61 ± 0.02 b	44.92 ± 0.82 b
Armour	0.59 ± 0.03 b	47.06 ± 2.20 ab
ClaraCL	0.64 ± 0.03 b	47.89 ± 0.89 a
Denali	0.63 ± 0.02 b	45.65 ± 1.38 b
Everest	0.60 ± 0.02 b	43.54 ± 0.80 b
Tiger	0.77 ± 0.02 a	51.76 ± 1.34 a

<sup>a</sup> different letters in a column indicate significant differences at p<0.05

### **Pearson's Correlations**

Pearson's correlations coefficients and respective p-values between flour, dough and tortilla properties are listed in Table 4.9.

#### ***Flour Particle Size***

Flour particle size can affect baked product performance. Tortillas processed from aggressively milled flour or flour with high starch damage levels can be inflexible and dark in appearance (Wang and Flores 1999c; Ramirez-Wong et al 2007). Increased flour particle size significantly correlated with higher flour water absorption ( $r=0.50$ ,  $p=0.01$ ) and flour protein content ( $r=0.48$ ,  $p=0.01$ ). However, no significant correlations were found between flour particle size and dough properties including mix time, Kieffer force, Kieffer distance and dough score. Tortilla specific volume ( $r=-0.47$ ,  $p=0.01$ ) and opacity ( $r=-0.43$ ,  $p=0.03$ ) were poorly and negatively correlated with increased flour particle size. Day 7 ( $r=0.40$ ,  $p=0.04$ ) and day 14 ( $r=0.42$ ,  $p=0.03$ ) rollability scores were positively, but poorly correlated to flour particle size.

#### ***Starch Damage***

Excessive amounts of starch damage can lead to sticky dough and diminished bread quality (Eliasson and Larsson 1993a). However some starch damage is considered good for promoting water holding capacity and extensible dough formation,



which can result in larger tortillas. Starch damage had a significant negative correlation with flour protein content ( $r=-0.64$ ,  $p=0.00$ ) and water absorption ( $r=-0.62$ ,  $p=0.00$ ). The reason behind this observed relationship is unclear. Normally, damaged starch increases water absorption rates (Evers and Steven 1985). No significant correlations were found between starch damage and mechanical dough tests including mix time, Kieffer force and Kieffer distance. Starch damage had significant, but low correlations with dough score ( $r=0.40$ ,  $p=0.04$ ) and opacity ( $r=0.46$ ,  $p=0.02$ ). The strength of the relationship between starch damage levels and rollability scores increased over time: day 1 ( $r=-0.40$ ,  $p=0.04$ ), day 7 ( $r=-0.44$ ,  $p=0.02$ ) and day 14 ( $r=-0.48$ ,  $p=0.00$ ), however the correlations were poor. Mao and Flores (2001) observed that tortilla flexibility increased with higher amounts of starch damage and smaller flour particle size initially, but tortillas became more rigid at faster rates during shelf-stability studies. They attributed the initial increased flexibility to increased moisture content due to higher water requirements, which was not a trend observed in this study.

## ***Protein***

### ***Flour Protein Content***

Flour protein content is known to highly influence dough and tortilla properties, affecting texture, appearance and shelf-stability. Increased flour protein content had a strong positive correlation with water absorption ( $r=0.94$ ,  $p=0.00$ ). Interestingly, subjective dough scores had a negative, but moderate significant correlation with flour protein ( $r=0.61$ ,  $p=0.00$ ). This would indicate that as protein content increased, the dough was stronger, more difficult to mould into balls and did not relax between the mixing and resting steps. However, the correlations between Kieffer force and distance tests and flour protein content were insignificant, indicating mechanical measurements did not detect any differences between the force and distance to extend dough.

In regards to tortilla appearance, increased flour protein content showed adverse relationships with diameter, specific volume, color and opacity. Both tortilla diameter ( $r=-0.64$ ,  $p=0.00$ ) and specific volume ( $r=-0.65$ ,  $p=0.00$ ) were shown to have significant moderate inverse relationships with flour protein content. Opacity ( $r=-0.86$ ,  $p=0.00$ ) and

L color values ( $r=-0.75$ ,  $p=0.00$ ) were significant and highly correlated with flour protein content, indicating that higher protein flours produced tortillas with increased translucency and darker color. Red to green color values (a) are not often reported to characterize tortilla appearance; however, a significant and high relationship was observed with protein content ( $r=0.80$ ,  $p=0.00$ ). While this relationship would indicate that as protein content increases, tortillas develop a red color (a color values become more negative). Although it should be noted that the a color values fell at the center of the red-green (gray area) within a very narrow range (minimum=-1.58 and maximum=0.89), indicating that only small variations contributed to this correlation.

Flour protein content is considered to influence tortilla flexibility initially and over time. The strength of the correlation between flour protein content and rollability scores increased during the 14 day shelf-stability test: day 1 ( $r=0.53$ ,  $p=0.01$ ), day 7 ( $r=0.63$ ,  $p=0.00$ ), day 14 ( $r=0.76$ ,  $p=0.00$ ).

### ***High Molecular Weight Glutenins***

An increased concentration of high molecular weight glutenin subunits (characterized by higher polymeric to monomeric ratios and insoluble polymeric protein content) appears to adversely affect dough and tortilla properties such as extensibility, diameter, color and flexibility.

Dough properties including mix time, Kieffer force and Kieffer distance indicated that increased high molecular weight protein content in the flour enhanced dough elasticity. Mix time was moderately and significantly correlated with %IPP ( $r=0.67$ ,  $p=0.00$ ), while the polymeric to monomeric protein ratio ( $r=0.41$ ,  $p=0.03$ ) was poorly correlated. A significant, yet moderate relationship was found between %IPP and Kieffer force ( $r=0.53$ ,  $p=0.00$ ) and Kieffer distance ( $r=0.56$ ,  $p=0.00$ ).

Many studies suggest that flours with higher protein content and higher glutenin composition negatively affect tortilla appearance including tortilla diameters (Pascut et al 2004; Alviola et al 2008; Mondal et al 2008; Jondiko et al 2012), in addition to opacity and color (Pascut et al 2004; Alviola and Awika 2010). In contrast to their findings, a significant, but poor relationship was observed between the polymeric to monomeric

ratio and opacity ( $r=0.41$ ,  $p=0.03$ ) in this study. No significant correlations were found between diameter and %IPP or polymeric to monomeric protein ratio.

In almost all published studies including shelf-stability tests, flour protein content and in particular, gluten-forming protein affect staling rates to the same or greater extent than does starch. However, the correlations between shelf-stability and parameters characterizing high molecular weight protein fractions were low to moderate here, indicating this positive relationship was not strong. %IPP moderately correlated with day 1 rollability scores only ( $r=0.44$ ,  $p=0.02$ ). Polymeric to monomeric protein ratio displayed an inverse and poor relationship with rollability scores on day 7 ( $r=-0.49$ ,  $p=0.01$ ) and on day 14 ( $r=-0.53$ ,  $p=0.00$ ). The results from previous studies examining the role of high molecular and low molecular weight fractions in tortilla quality are not always in agreement (Waniska et al 2004; Mondal et al 2008; Mondal et al 2009; Pierucci et al 2009; Jondiko et al 2012). Alviola et al (2008) even suggested that the protein molecular weight distribution is a minor determinant of staling, in comparison to the proper formation and distribution of the gluten network throughout the dough and the retention of it after baking.

### ***Water Absorption***

Water absorption levels were moderately correlated with tortilla appearance properties including opacity ( $r=-0.81$ ,  $p=0.00$ ), L value ( $r=-0.79$ ,  $p=0.00$ ) and a value ( $r=0.77$ ,  $p=0.00$ ). These trends indicated that tortillas were darker in appearance when made from flours with increasing water absorption levels. These observations are similar to the correlations observed between flour protein content and the same tortilla appearance properties.

Tortilla appearance might be dependent on flour protein content, water absorption levels or both. A strong correlation was found between flour water absorption and flour protein content ( $r=0.94$ ,  $p=0.00$ ). The phenomenon in which flours with greater protein content require more water to hydrate and develop a gluten matrix is well established. Covariance analysis is required to understand whether the correlations

between tortilla appearance and flour protein content or water absorption are the result of collinear or independent relationships.

### ***Starch Granule Size Distribution***

In this study, the concentration of B-starch granules was 26-29% (by volume) of the starch content and showed moderate correlations with water absorption ( $r=0.70$ ,  $p=0.00$ ), diameter ( $r=-0.63$ ,  $p=0.00$ ), 14 day rollability ( $r=0.68$ ,  $p=0.00$ ) and L color value ( $r=-0.61$ ,  $p=0.00$ ). A high correlation was observed between B-starch granules and opacity ( $r=-0.83$ ,  $p=0.00$ ). A-starch granules correlated moderately with 14 day rollability ( $r=-0.59$ ,  $p=0.00$ ) and opacity ( $r=0.62$ ,  $p=0.00$ ). C-starch granules showed no significant correlations with tortilla properties (data not shown). Most studies exploring the effects of starch granule size on pan bread quality indicate that variations in starch granule size influence bread crumb structure, water absorption and loaf volume (D'Appolonia and Gilles 1971; Kulp 1973; Hayman et al 1998). Soulaka and Morrison (1985) found that a 25-35% (by weight) concentration of B-starch granules correlated with larger loaf volumes, yet other studies found no significant correlations between bread properties (Hoseney et al 1971). Varying conclusions on the effect of starch granule size in bread quality could be the result of variations in starch isolation techniques and baking methods between studies (Park et al 2004). Differently sized starch granules could vary in their swelling behavior or interactions, giving rise to different tortilla properties. Due to the differences in the tortilla making process and bread structure, it appears there is a relationship between starch granule size and tortilla properties including diameter, opacity, flexibility and staling rates.

Table 4.9 Pearson's Correlation Coefficients and p-Values for Flour, Dough and Tortilla Properties <sup>ab</sup>

	Mix Time	Water Abs	Kieffer Force	Kieffer Dist	Dough Score	Diam	Spec Vol	Opac	L value	a value	Day 1 Roll	Day 7 Roll	Day 14 Roll	Starch Dam	Flour Part Size	A Starch	B Starch	Poly: Mono	%PP
FP	-0.26	<b>0.94</b>	0.20	-0.35	<b>-0.61</b>	<b>-0.64</b>	<b>-0.65</b>	<b>-0.86</b>	<b>-0.75</b>	<b>0.80</b>	<b>0.53</b>	<b>0.63</b>	<b>0.76</b>	<b>-0.64</b>	<b>0.48</b>	<b>-0.60</b>	<b>0.79</b>	<b>-0.47</b>	-0.04
	0.19	<b>0.00</b>	0.32	0.07	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.01</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.01</b>	<b>0.00</b>	<b>0.00</b>	<b>0.01</b>	0.85
Mix Time	1.00	-0.37	<b>0.57</b>	<b>0.67</b>	0.30	-0.17	0.08	0.03	0.24	-0.04	0.28	-0.10	-0.21	0.22	-0.24	0.01	-0.09	<b>0.41</b>	<b>0.67</b>
		0.06	<b>0.00</b>	<b>0.00</b>	0.13	0.40	0.70	0.90	0.24	0.84	0.16	0.61	0.29	0.27	0.22	0.97	0.65	<b>0.03</b>	<b>0.00</b>
Water Abs		1.00	-0.02	<b>-0.44</b>	<b>-0.67</b>	<b>-0.53</b>	<b>-0.54</b>	<b>-0.81</b>	<b>-0.79</b>	<b>0.77</b>	<b>0.43</b>	<b>0.57</b>	<b>0.73</b>	<b>-0.62</b>	<b>0.50</b>	<b>-0.54</b>	<b>0.70</b>	<b>-0.38</b>	-0.08
			0.91	<b>0.02</b>	<b>0.00</b>	<b>0.01</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.03</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	<b>0.01</b>	<b>0.00</b>	<b>0.00</b>	<b>0.05</b>	0.69
Kieffer Force			1.00	<b>0.60</b>	0.08	-0.31	<b>-0.46</b>	-0.30	-0.03	0.33	0.27	<b>0.42</b>	0.28	0.00	0.10	-0.17	0.23	-0.23	<b>0.53</b>
				<b>0.00</b>	0.71	0.11	<b>0.02</b>	0.13	0.88	0.10	0.18	<b>0.03</b>	0.16	0.99	0.63	0.39	0.24	0.26	<b>0.00</b>
Kieffer Dist				1.00	<b>0.56</b>	-0.04	-0.03	0.27	<b>0.52</b>	-0.15	-0.01	0.10	-0.19	0.26	-0.05	0.16	-0.36	0.11	<b>0.56</b>
					<b>0.00</b>	0.85	0.88	0.18	<b>0.01</b>	0.46	0.98	0.61	0.34	0.19	0.80	0.43	0.07	0.60	<b>0.00</b>
Dough Score					1.00	0.22	0.25	<b>0.64</b>	<b>0.67</b>	<b>-0.46</b>	-0.28	-0.21	<b>-0.51</b>	<b>0.40</b>	-0.36	<b>0.51</b>	<b>-0.60</b>	0.39	0.12
						0.28	0.21	<b>0.00</b>	<b>0.00</b>	<b>0.02</b>	0.16	0.29	<b>0.01</b>	<b>0.04</b>	0.07	<b>0.01</b>	<b>0.00</b>	0.05	0.55
Diam						1.00	<b>0.45</b>	<b>0.52</b>	0.28	<b>-0.46</b>	<b>-0.61</b>	-0.34	<b>-0.43</b>	0.28	-0.16	<b>0.50</b>	<b>-0.63</b>	0.11	-0.27
							<b>0.02</b>	<b>0.01</b>	0.17	<b>0.02</b>	<b>0.00</b>	0.09	<b>0.03</b>	0.15	0.44	<b>0.01</b>	<b>0.00</b>	0.58	0.17
Spec Vol							1.00	<b>0.55</b>	<b>0.54</b>	<b>-0.62</b>	-0.22	<b>-0.54</b>	<b>-0.51</b>	0.22	<b>-0.47</b>	0.18	-0.35	<b>0.41</b>	-0.09
								<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	0.27	<b>0.00</b>	<b>0.01</b>	0.26	<b>0.01</b>	0.38	0.08	<b>0.04</b>	0.66
Opac								1.00	<b>0.70</b>	<b>-0.87</b>	<b>-0.44</b>	<b>-0.54</b>	<b>-0.68</b>	<b>0.46</b>	<b>-0.43</b>	<b>0.62</b>	<b>-0.83</b>	<b>0.41</b>	-0.01
									<b>0.00</b>	<b>0.00</b>	<b>0.02</b>	<b>0.00</b>	<b>0.00</b>	<b>0.02</b>	<b>0.03</b>	<b>0.00</b>	<b>0.00</b>	<b>0.03</b>	0.96
L value									1.00	<b>-0.72</b>	-0.23	<b>-0.39</b>	<b>-0.55</b>	0.35	-0.31	0.32	<b>-0.61</b>	0.24	0.10
										<b>0.00</b>	0.25	<b>0.05</b>	<b>0.00</b>	0.08	0.12	0.11	<b>0.00</b>	0.23	0.63
a value										1.00	<b>0.43</b>	<b>0.59</b>	<b>0.64</b>	<b>-0.43</b>	0.30	<b>-0.41</b>	<b>0.69</b>	-0.26	0.09
											<b>0.03</b>	<b>0.00</b>	<b>0.00</b>	<b>0.02</b>	0.13	<b>0.03</b>	<b>0.00</b>	0.19	0.64

<sup>a</sup> Within each cell, the correlation coefficient (r) is listed first and the p-value below.

<sup>b</sup> Bold values are significant at a p < 0.05 level.

(table continued on next page)

Pearson's Correlation Coefficients and p-Values for Flour, Dough and Tortilla Properties (continued)

	Mix Time	Water Abs	Kieffer Force	Kieffer Dist	Dough Score	Diam	Spec Vol	Opac	L value	a value	Day 1 Roll	Day 7 Roll	Day 14 Roll	Starch Dam	Flour Part Size	A Starch	B Starch	Poly: Mono	%IPP
Day 1 Roll											1.00	0.28	<b>0.38</b>	<b>-0.40</b>	-0.07	-0.33	<b>0.48</b>	0.02	<b>0.44</b>
												0.16	<b>0.05</b>	<b>0.04</b>	0.73	0.10	<b>0.01</b>	0.94	<b>0.02</b>
Day 7 Roll												1.00	<b>0.89</b>	<b>-0.44</b>	<b>0.40</b>	<b>-0.49</b>	<b>0.52</b>	<b>-0.49</b>	0.15
													<b>0.00</b>	<b>0.02</b>	<b>0.04</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	0.46
Day 14 Roll													1.00	<b>-0.48</b>	<b>0.42</b>	<b>-0.59</b>	<b>0.68</b>	<b>-0.53</b>	0.09
														<b>0.01</b>	<b>0.03</b>	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	0.67
Starch Dam														1.00	<b>-0.42</b>	<b>0.65</b>	<b>-0.48</b>	0.21	-0.12
															<b>0.03</b>	<b>0.00</b>	<b>0.01</b>	0.30	0.55
Flour Part Size															1.00	<b>-0.45</b>	0.15	-0.36	-0.07
																<b>0.02</b>	0.46	0.07	0.75
A Starch																1.00	<b>-0.74</b>	0.19	-0.17
																	<b>0.00</b>	0.33	0.40
B Starch																	1.00	-0.35	0.02
																		0.07	0.94
Poly: Mono																		1.00	0.34
																			0.09

## Chapter 5 Conclusion

The objectives of this study were to 1) evaluate the performance of Kansas State University developed wheat varieties in tortilla making and 2) to identify the biochemical properties within the varieties contributing to optimal tortilla characteristics.

Parts of this study failed to meet those objectives for various reasons. The results from the analysis of variance showed that there were few significant differences in the biochemical properties among the wheat varieties. Also, there were almost no significant differences in the resulting tortillas processed from the wheat varieties.

The lack of differences could possibly be explained as a result of the following occurrences. The original study was designed to contain at least 50 samples, representing ten variety and five growth location combinations. However, some samples failed to thrive at certain growing locations, while other wheat samples failed to produce enough flour for all testing procedures and wheat grown at Pawnee County was removed from the study on the basis of the abnormally high protein contents. Growing conditions were not as diverse as originally expected with three of the five locations being in Ellis County. Therefore, the sample size was reduced to 22 samples, which severely lowered the diversity and statistical power of this study. The biochemical properties among selected flour samples were found to be mostly homogenous, all resulting in adequate tortilla quality. Analytical pup loaf bread tests, performed independent of this study, revealed that the wheat varieties used had similar and moderate breadmaking properties as well (Miller and Lingenfelter 2013).

While the quality of the varieties for tortilla making did not differ significantly, regression analysis showed some correlations between tortilla quality and flour biochemical properties. Increased flour protein and polymeric protein contents corresponded with smaller tortilla diameters, better flexibility scores over time and darker, more transparent tortillas. Higher concentrations of B-starch granules corresponded negatively with tortilla diameters and opacity, but positively with extended flexibility. A-starch granules shared a positive relationship with tortilla opacity, yet a negative one with extended tortilla flexibility. Starch damage and flour particle size

showed low moderate correlations with tortilla properties, but stronger correlations with dough properties.

The industry seeks tortilla flours that form extensible doughs that spread during pressing to produce wider diameter tortillas, yet have elasticity to provide long shelf stability. This study and previous research have demonstrated the difficulty in identifying flour properties that give rise to these tortilla properties. It is apparent that protein and starch play a dichotomous role in tortilla making. Higher total protein content, increased levels of glutenin-forming fractions and B-starch granules often yield very flexible tortillas, but at the sacrifice of opacity and size.



## Chapter 6 Future Work

- Extend the study with a larger number of varieties, varying widely in breadmaking quality and grown in more diverse locations. This will increase the statistical power and decrease experimental error.
- In addition to the testing methods used in this study, more objective tests to measure tortilla quality could be added. For example, using the texture analyzer to measure tortilla flexibility during shelf-stability tests. There also might be some value to using C-Cell to measure color, transparency, puffing and symmetry.
- Perform multivariate analysis using multiple qualitative predictors to determine which and to what extent biochemical fractions impact tortilla quality parameters.
- The moderate to high correlations between B-starch granule content and tortilla properties such as opacity and L color values suggest that starch fractions may effect appearance. In order to explore this relationship further, a more in-depth analysis of A- and B-starch granule starch fractions on tortilla qualities is needed. Testing could include measuring their gelatinization properties with DSC. In addition to the effects of starch granule size, starch functionality in tortilla making is not well understood. There are many areas and techniques for which the effects of starch in tortilla quality could be studied.

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## Appendix A Summary of Omitted Data

The following data includes varieties grown in fewer than three locations and varieties grown at Pawnee County. Varieties grown in fewer than three locations were omitted due to their lack of statistical power. All samples from Pawnee County had excessively high and unusual protein content. Therefore, they were strong statistical outliers and were omitted.

**Table A.1 Wheat Properties**

<b>Variety</b>	<b>Location</b>	<b>Test Weight (lb/bu)</b>	<b>Kernel Weight (mg)</b>	<b>Kernel Diameter (mm)</b>	<b>Kernel Hardness</b>	<b>Kernel Protein (% , 12 mb)</b>
<b>1863</b>	<b>Pawnee</b>	55.90	26.00	2.37	69.61	20.64
<b>Armour</b>	<b>Pawnee</b>	55.28	24.33	2.33	75.92	19.40
<b>Clara CL</b>	<b>Pawnee</b>	56.82	25.91	2.41	79.05	20.79
<b>Denali</b>	<b>Pawnee</b>	55.57	26.64	2.38	72.43	21.42
<b>Fuller</b>	<b>Hays</b>	61.87	32.96	2.70	77.82	15.02
<b>Jagger</b>	<b>Hays</b>	58.29	29.77	2.56	87.76	10.68
<b>RonL</b>	<b>Hays</b>	61.79	30.55	2.53	85.64	13.47
<b>Tam 111</b>	<b>Ellis</b>	60.55	29.08	2.49	78.41	13.58
<b>Tam 111</b>	<b>Pawnee</b>	55.95	28.83	2.48	72.00	21.63
<b>Tiger</b>	<b>Pawnee</b>	56.42	30.27	2.50	66.59	20.06

**Table A.2 Flour Properties**

<b>Variety</b>	<b>Location</b>	<b>Flour Protein (%, 14 mb)</b>	<b>Starch Damage (%)</b>	<b>Mean Flour Particle Size (<math>\mu\text{m}</math>)</b>
1863	Pawnee	17.29	3.50	96.19
Armour	Pawnee	15.39	3.42	98.30
Clara CL	Pawnee	16.39	3.64	100.58
Denali	Pawnee	16.34	4.10	91.02
Fuller	Hays	12.15	4.21	95.02
Jagger	Hays	10.06	5.14	99.25
RonL	Hays	10.90	4.40	97.19
Tam 111	Ellis	11.23	4.00	86.30
Tam 111	Pawnee	16.69	3.11	97.92
Tiger	Pawnee	15.24	3.35	97.56

**Table A.3 Dough Properties**

<b>Variety</b>	<b>Location</b>	<b>Water Absorption (%)</b>	<b>Mixograph Peak Time (min)</b>	<b>Kieffer Force (g)</b>	<b>Kieffer Distance (mm)</b>
1863	Pawnee	66.00	2.50	17.90	98.68
Armour	Pawnee	64.00	2.75	23.57	73.05
Clara CL	Pawnee	63.00	3.25	33.58	62.75
Denali	Pawnee	51.00	4.25	48.91	55.55
Fuller	Hays	55.00	4.25	29.62	57.63
Jagger	Hays	60.00	4.00	28.24	49.42
RonL	Hays	58.00	4.75	31.26	58.48
Tam 111	Ellis	54.00	3.50	24.96	85.65
Tam 111	Pawnee	68.00	3.00	31.33	77.53
Tiger	Pawnee	61.00	3.25	36.24	60.08

**Table A.4 Tortilla Properties**

<b>Variety</b>	<b>Location</b>	<b>Diameter (mm)</b>	<b>Weight (g)</b>	<b>Thickness (mm)</b>	<b>L color value</b>	<b>Opacity Score</b>	<b>Day 1 Rollability Score</b>	<b>Day 7 Rollability Score</b>	<b>Day 14 Rollability Score</b>
<b>1863</b>	<b>Pawnee</b>	121	21.35	1.33	81.69	7.67	4.89	3.33	2.00
<b>Armour</b>	<b>Pawnee</b>	130	20.35	1.88	82.37	4.89	4.89	3.78	2.67
<b>Clara CL</b>	<b>Pawnee</b>	127	21.20	1.86	85.53	7.56	4.89	3.67	3.67
<b>Denali</b>	<b>Pawnee</b>	123	21.21	1.88	80.53	4.78	5.00	4.56	3.00
<b>Fuller</b>	<b>Hays</b>	126	21.39	2.24	84.61	8.89	4.56	2.11	1.00
<b>Jagger</b>	<b>Hays</b>	130	21.03	2.02	84.77	8.78	4.67	2.78	1.67
<b>RonL</b>	<b>Hays</b>	138	20.31	1.90	84.90	6.56	5.00	2.78	2.56
<b>Tam 111</b>	<b>Ellis</b>	128	20.78	2.59	86.91	8.22	4.89	2.67	1.66
<b>Tam 111</b>	<b>Pawnee</b>	133	20.45	1.71	82.84	4.78	4.89	3.33	2.00
<b>Tiger</b>	<b>Pawnee</b>	123	21.40	2.06	82.89	6.83	5.00	3.67	3.00

**Table A.5 Total Protein Analyses**

<b>Variety</b>	<b>Location</b>	<b>Polymeric: Monomeric Proteins</b>	<b>%IPP</b>
1863	Pawnee	0.63	41.82
Armour	Pawnee	0.60	47.54
Clara CL	Pawnee	0.56	46.46
Denali	Pawnee	0.53	50.01
Fuller	Hays	0.65	48.04
Jagger	Hays	0.83	49.81
RonL	Hays	0.54	47.33
Tam 111	Ellis	0.68	47.69
Tam 111	Pawnee	0.53	47.33
Tiger	Pawnee	0.67	52.50

**Table A.6 Starch Granule Size Distribution (Volume %)**

<b>Variety</b>	<b>Location</b>	<b>A Starch Granules</b>	<b>B Starch Granules</b>	<b>C Starch Granules</b>
1863	Pawnee	39.20	33.96	26.84
Armour	Pawnee	46.90	30.07	23.03
Clara CL	Pawnee	47.59	27.34	25.07
Denali	Pawnee	44.24	33.58	22.17
Fuller	Hays	51.35	26.15	22.50
Jagger	Hays	48.49	26.69	24.82
RonL	Hays	52.00	26.67	21.33
Tam 111	Ellis	47.20	26.83	25.97
Tam 111	Pawnee	46.66	29.93	23.40
Tiger	Pawnee	43.31	31.32	25.36